Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

3 June 2016

CLH report

Proposal for Harmonised Classification and Labelling

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

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

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

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

Index Number: **006-044-00-7**

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Version number: 2 **Date: November 2015**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Isoproturon; 3-(4-isopropylphenyl)-1,1-dimethylurea
EC number:	251-835-4
CAS number:	34123-59-6
Annex VI Index number:	006-044-00-7
Degree of purity:	> 98.0% w/w
Impurities:	See confidential Annex

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Carc. 2; H351 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M = 10
Current proposal for consideration by RAC	Repr. 2; H361f STOT RE 2; H373 M-acute = 10 M-chronic = 10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 2; H351 Repr. 2; H361f STOT RE 2; H373 Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M-acute = 10 M-chronic = 10

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.2.	Flammable gases	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.3.	Flammable aerosols	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.4.	Oxidising gases	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.5.	Gases under pressure	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.6.	Flammable liquids	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.7.	Flammable solids	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.10.	Pyrophoric solids	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.13.	Oxidising liquids	none	Not applicable	Not classified	Data conclusive but not sufficient for classification

2.14.	Oxidising solids	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.15.	Organic peroxides	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	none	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	none	Not applicable	Not classified	Hazard class not assessed in this dossier
	Acute toxicity - dermal	none	Not applicable	Not classified	Hazard class not assessed in this dossier
	Acute toxicity - inhalation	none	Not applicable	Not classified	Hazard class not assessed in this dossier
3.2.	Skin corrosion / irritation	none	Not applicable	Not classified	Hazard class not assessed in this dossier
3.3.	Serious eye damage / eye irritation	none	Not applicable	Not classified	Hazard class not assessed in this dossier
3.4.	Respiratory sensitisation	none	Not applicable	Not classified	Hazard class not assessed in this dossier
3.4.	Skin sensitisation	none	Not applicable	Not classified	Hazard class not assessed in this dossier
3.5.	Germ cell mutagenicity	none	Not applicable	Not classified	Hazard class not assessed in this dossier
3.6.	Carcinogenicity	none	Not applicable	**Carc. 2; H351**	
3.7.	Reproductive toxicity	**Repr. 2;** **H361f**	Not applicable	Not classified	
3.8.	Specific target organ toxicity – single exposure	none	Not applicable	Not classified	Hazard class not assessed in this dossier *
3.9.	Specific target organ toxicity – repeated exposure	**STOT RE 2;** **H373**	Not applicable	Not classified	
3.10.	Aspiration hazard	none	Not applicable	Not classified	Hazard class not assessed in this dossier
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400 Aquatic Chronic 1; H410**	**M-acute = 10** **M-chronic= 10**	**Aquatic Acute 1; H400 Aquatic Chronic 1; H410**	
5.1.	Hazardous to the ozone layer	none	Not applicable	Not classified	Data lacking*

1) Including specific concentration limits (SCLs) and M-factors
2) Data lacking, inconclusive, or conclusive but not sufficient for classification
* This endpoint is not addressed by this proposal.

Labelling:	Signal word:	Warning
	Pictograms:	GHS08 GHS09
	Hazard statements:	H351 - Suspected of causing cancer H361f - Suspected of damaging the fertility H373 - May cause damage to organs (blood) through prolonged or repeated oral exposure H410 - Very toxic to aquatic life with long lasting effects
Precautionary statements:		(P102) Keep out of reach of children. P260 Do not breathe dust. P273 Avoid release to the environment P280 Wear protective gloves/protective clothing. P308 + P313 IF exposed or concerned: Get medical advice/attention. P391 Collect spillage P405 Store locked up. P501 Dispose of contents/container to …

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Until 4 November 2015 no registrations dossiers are available.

2.2 Short summary of the scientific justification for the CLH proposal

Isoproturon was evaluated in the context of the work programme for review of existing active substances provided for in Article 8(2) of Directive 91/414/EEC concerning the placing of plant protection products on the market, with a view to the possible inclusion of this substance in Annex I to the Directive (Isoproturon, SANCO/3045/99-final, 12 March 2002).

For isoproturon the effects on human health and the environment have been assessed in accordance with the provisions laid down in Regulation (EEC) No 3600/92 for a range of uses proposed by the notifiers. Under Regulation (EC) No 933/94, Germany was designated as Rapporteur Member State (RMS). The RMS submitted the relevant assessment reports and recommendations to the Commission on 30 July 1999 in accordance with Article 7(1)(c) of Regulation (EEC) No 3600/92. This assessment report has been reviewed by the Member States and the Commission within the Standing Committee on Plant Health. The review was finalised on 7 December 2001 in the format of the Commission review report for isoproturon. The review did not reveal any open questions or concerns, which would have required a consultation of the Scientific Committee on Plants (Isoproturon, SANCO/3045/99-final, 12 March 2002).

Article 5(5) of Directive 91/414/EEC provides that the inclusion of an active substance can be renewed. Germany is the designated RMS for the renewal of the approval of the active substance isoproturon according to Regulation (EU) No 1141/2010 (AIR 2). The RMS Germany and the Co-Rapporteur Member State (Co-RMS) Czech Republic are preparing a Renewal Assessment Report (RAR) to deliver for this process.

Regarding health hazards, isoproturon has a legal classification (regulation (EC) No 1272/2008) for the toxicological endpoint carcinogenicity (Carc. 2; H351).

During the renewal procedure of isoproturon under directive 91/414/EC, it was noted that this current legal classification should be amended to include a classification for reproductive toxicity (Repr. 2; H361f), based on the evidence of impaired fertility from results in appropriate animal studies, and for specific target organ toxicity – repeated exposure (STOT RE 2; H373), based on signs of toxic haemolytic anaemia observed in appropriate animal studies. The existing classification for the toxicological endpoint carcinogenicity and the non-classification regarding the remaining toxicological endpoints was considered appropriate. Therefore only the toxicological data relevant for the evaluation of the newly proposed hazards were reported in this CLH dossier.

Regarding environmental hazards, isoproturon has a legal classification (regulation (EC) No 1272/2008) for the aquatic ecotoxicological endpoints as very toxic to aquatic life with long lasting effects for acute (Aquatic Acute 1; H400) and chronic (Aquatic Chronic 1; H410) endpoints and M-factor of 10. For separation and determination of acute and chronic M-factors the relevant ecotoxicological studies were reported.

Concerning the evaluation of study results with regard to the proposal for harmonised classification and labelling the relevant assessment reports of the previous review (Monograph, 27 July 1999) and the RAR of the recent re-evaluation (Renewal Assessment Report, 18 September 2013, not finalised), provide background documents for the CLH report.

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 the outcome 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. Carcinogenisity was not considered as part of the Annex XV CLH proposal. In conclusion, RAC cannot provide an opinion on the carcinogenicity of isoproturon. However, the DS is invited to submit a new CLH proposal including carcinogenicity and all relevant mechanistic data, if they consider a revision of the current classification justified.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Isoproturon is an active substance in the meaning of Regulation (EC) No. 1107/2009 (replaces Directive 91/414/EEC) and all hazard classes are subject to harmonised classification at Community level and no other justification is needed.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

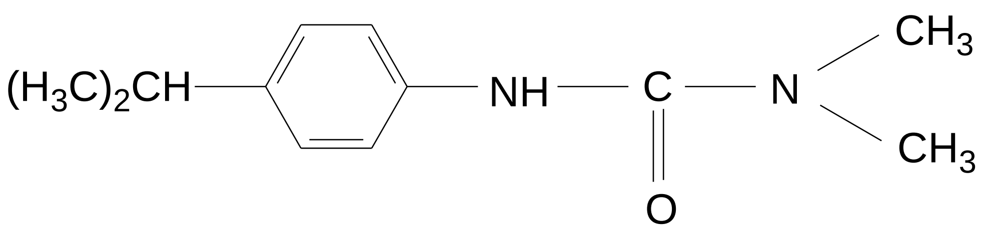
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	251-835-4
EC name:	3-(4-isopropylphenyl)-1,1-dimethylurea
CAS number (EC inventory):	34123-59-6
CAS number:	
CAS name:	Urea, N,N-dimethyl-N`-[4-(1-methylethyl)phenyl]-
IUPAC name:	3-(4-isopropylphenyl)-1,1-dimethylurea
CLP Annex VI Index number:	006-044-00-7
Molecular formula:	$C_{12}H_{18}N_2O$
Molecular weight range:	206.3 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
3-(4-isopropylphenyl)-1,1-dimethylurea		≥ 970 g/kg* 970 g/kg** 95 - >99%***	* FAO specification (AGP:CP/250 (1990))

			** Minimum purity of the active substance as manufactured *** Purity of toxicological studies (Purity or batch number in many toxicological studies was not reported.)

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	White, solid, odourless powder (at 22°C)	Sinning, D.J; 2002	Visual inspection, respectively inspection
Melting/freezing point	Approx. 160°C	Sydney, P, 2008a	EEC Method A.1 (DSC)
Boiling point	Not determinable as the test substance decomposed before boiling; decomposition above approx. 210°C	Sydney, P, 2008a	EEC Method A.2 (DSC)
Relative density	D_4^{20} 1.15	Turner, B., 2009a	EEC Method A.3 (pyknometer method)
Vapour pressure	1.29×10^{-5} Pa (25°C) 6.1×10^{-6} Pa (20°C)	Turner, B., 2009b Weiss, A.; Görg, J.; 2010	EEC Method A.4 (vapour pressure balance)
Surface tension	67.0 mN/m (90% aqueous solution; 0.047 g/L)	Sydney, P, 2008b	EEC method A.5 (ring method)
Water solubility	52 mg/L at 25°C As isoproturon does not dissociate, the water solubility is not pH dependent	Sinning, D.J; 2002	OPPTS Guideline 830.7840 in accordance with EEC method A.6 (Flask method)
Partition coefficient n-octanol/water	log POW = 2.6 (25°C)	Sinning, D.J; 2002	OPPTS Guideline 830.7570 in accordance with EEC method A.8 (HPLC)
Flash point		BAM, 2013	The flash point does not need to be tested because the substance is a solid.
Flammability	not highly flammable	Sydney, P., 2008c BAM, 2013 BAM, 2013	Flammability upon ignition (solids): In the preliminary test according to EU Method A.10, the test substance melted and burned locally with a yellow flame which extinguished 10 seconds after removal of the heat source. There was no propagation along the powder train. As a negative result was obtained in the preliminary test, a definitive burning rate test was not required. Flammability in contact with water: The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not to be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).

Explosive properties	no explosive properties	BAM, 2013	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive properties.
Self-ignition temperature	There was no exothermic reaction of the test substance, indicating that it does not self-ignite up to the melting point (154.8 °C at ambient pressure). Decomposition occurred above approx. 210 °C, value derived from DSC measurement.	Sydney, P., 2008c	EEC Method A:16
Oxidising properties	no oxidising properties	BAM, 2013	The classification procedure needs not to be applied because the organic substance contains oxygen, which is chemically bonded only to carbon.
Granulometry			
Stability in organic solvents and identity of relevant degradation products			
Dissociation constant	isoproturon does not dissociate	Sinning, D.J; 2002	OPPTS Guideline 830.7370 (Titration method) There were no isobestic points found at a suitable wavelength (i.e. above 250 nm) Therefore a dissociation constant could not be determined.
Viscosity	Substance is a solid.		

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Herbicides containing isoproturon are used in agriculture for the control of a range of mono- and dicotyledonous weeds in cereals. They are systemic selective foliar applied herbicides. The application is possible pre- and postemergence.

Isoproturon is a biocidal active substance listed in Regulation 1062/2014, ANNEX II, PART 1. Active substance/product-type combinations have been supported from 4 August 2014. Additional information can be obtained here[1].

1 http://echa.europa.eu/web/guest/information-on-chemicals/biocidal-active-substances
http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1062&from=EN

Products are used as film preservatives and construction material preservatives.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification is proposed. All physical and chemical properties and physical hazard classes are considered in the CLH report.

Isoproturon has no explosive properties (BAM, 2013), is a solid and has no autoignition properties, is not flammable in contact with water and the molecular structure does not indicate oxidizing properties (Table 8). Therefore, no classification of isoproturon for physico-chemical properties is required according to CLP.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

This endpoint is not addressed by this proposal.

4.2 Acute toxicity

This endpoint is not addressed by this proposal.

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

This endpoint is not addressed by this proposal.

4.4 Irritation

This endpoint is not addressed by this proposal.

4.5 Corrosivity

This endpoint is not addressed by this proposal.

4.6 Sensitisation

This endpoint is not addressed by this proposal.

4.7 Repeated dose toxicity

Subacute and subchronic studies with isoproturon (IPU) were performed in rats, dogs, mice and monkeys by oral, dermal or inhalation administration. A summary of the repeated dose toxicity assessed during the EU review appears in Table 11 and Table 12.

Following oral administration of IPU, the liver and the blood (red blood cells) were found to be the main target organs. Haemolytic anaemia (associated with Heinz bodies, methemoglobinaemia, hyperplastic bone marrow, extramedullary hematopoiesis and increased hemosiderin in liver, kidneys and bone marrow) was seen at or above dosages of approximately 800 ppm in rats, 500 ppm in dogs and 150 mg/kg bw/d in monkeys. The findings in the liver (increased weight, bile duct proliferation, degeneration of hepatocytes, basophilic foci) were associated with increased enzyme activities (AP, ALT, AST) and reductions in total protein or albumin. There was evidence that the effects seen were reversible. The overall NOAEL was 80 ppm (about 5.6 mg/kg bw/d) in rats, 50 ppm (about 3.2 mg/kg bw/d) in dogs, and 50 mg/kg bw/d in monkeys.

Following dermal administration, single decedents were seen at 500 and 1000 mg/kg bw/d while other studies gave no evidence for systemic or local toxicity at 1000 or 2000 mg/kg bw/d. In a subacute inhalation study in rats, no local or systemic effects were noted at 0.25 mg/l. In a second subacute inhalation study an interstitial pneumonitis in rats of the high dose group (0.6 mg/l) was reported. However, rats in a subchronic inhalation study showed only respiratory irritation at a concentration of 6.32 mg/l

Table 9: Summary table of relevant repeated dose toxicity studies

Study	**Dose levels**	**NOAEL**	**Target/main effects at LOAEL (when relevant)**	**Reference**
30 day dietary rat	0-500-1250-3200-8000-20000 ppm	500 ppm (43 mg/kg bw/d)	Liver (increased relative liver weight)	Scholz and Weigand 1973 § TOX9551871
4 week dietary Mouse	0-80→4000-400-2000 ppm	2000 ppm (307-378 mg/kg bw/d)	Liver (increased relative liver weight)	Hunter et al 1979 § TOX9551872
4 week dietary Dog	0-50-160-500→1250 ppm	50 ppm (3.3 mg/kg bw/d)	Liver and bone marrow	Kramer and Brunk 1975 § TOX9551873
5 day dermal Rabbit	400-800 mg/kg bw/d	400 mg/kg bw/d	Single death at 800 mg/kg bw/d	Hollander and Weigand 1975 § TOX9551877
21 day dermal Rat	0-250-500-1000 mg/kg bw/d	250 mg/kg bw/d	Single death at 500 mg/kg bw/d	Dikshith 1982 § TOX9551878
21 day dermal Rabbit	0-500-1000-2000 mg/kg bw/d	2000 mg/kg bw/d	No systemic or local toxicity	Bhide 1996 § TOX9651094
14 day inhalation Rat	0-0.01-0.05-0.25 mg/l	0.25 mg/l	No systemic or local toxicity	Owen and Glaister 1982 § TOX9551876
14 day inhalation Rat	0-0.173-0.664 mg/l	0.173 mg/l	Interstitial pneumonitis	Anonym 1985 § TOX9651095
13 wk dietary rat	0-80-400-2000-10000→20000 ppm	400 ppm (35 mg/kg bw/d)	Reduced feed intake and body weight gain, anaemia, methaemoglobin	Leuschner et al 1973 § TOX9551874
13 wk gavage rat	0-85-250-750 mg/kg bw/d	85 mg/kg bw/d	Liver, bone marrow, anaemia, methaemoglobin	Bhide 1984 § TOX9651092
13 wk dietary rat	0-400-1500-5000 ppm	400 ppm (20-40 mg/kg bw/d)❶	Reduced body weight, liver weight increased	Dickhaus and Heisler 1987 § TOX9550729
13 wk dietary rat	0-400-1200-2400 ppm	400 ppm (20-40 mg/kg bw/d)❶	Reduced body weight, liver, bone marrow, methaemoglobin	Bhide 1990 § TOX9550326

❶ Value calculated by means of a conversion factor of 0.05-0.1 (Anonym, 2000; ASB2013-4646)
* Acceptable according evaluation of the DAR dated 27 July 1999, ASB2010-10305
§ Supplementary according evaluation of the DAR dated 27 July 1999, ASB2010-10305

Mean daily intake calculated by the applicant, if the here appropriate conversion factor of 13 (Anonymous, 2000; ASB2013-4646) is used, 3.8 mg/kg bw/day would result.

→ In view of the lack of effects the respective dose level was increased half way through the study.

Table 10: Summary table of relevant repeated dose toxicity studies (continued)

Study	Dose levels	NOAEL	Target/main effects at LOAEL (when relevant)	Reference
13 wk dietary Rat	0-80-800-8000 ppm	80 ppm (5.6 mg/kg bw/d)	Reduced feed intake and body weight, liver, methaemoglobin, anaemia, extramedullary haemopoiesis	Wragg et al 1991 *& TOX9300281
13 wk dietary dog	0-50-160-500→800 ppm	50 ppm (3.2 mg/kg bw/d)#	Liver (increased weight), bone marrow, anaemia	Scholz and Brunk 1973 § TOX9551875
13 wk dietary dog	0-50-150-500 ppm	50 ppm (3.8 mg/kg bw/d) #	Reduced feed intake and body weight, bone marrow, anaemia, methaemoglobin	Bhide 1990 § TOX9500341
13 wk gavage monkey	0-50-150-450 mg/kg bw/d	50 mg/kg bw/d	Reduced feed intake and body weight, liver, bone marrow, anaemia, methaemoglobin	Bhide 1984 § TOX9651093
13 wk dermal rabbit	0-1000 mg/kg bw/d	1000 mg/kg bw/d	No systemic or local toxicity	Bhide 1990 *& TOX9500343
13 wk inhalation rat	0-6.32 mg/l	6.32 mg/l	Respiratory irritation; no systemic toxicity	Bhide 1990 § TOX9500342

* Acceptable according evaluation of the DAR dated 27 July 1999, ASB2010-10305
§ Supplementary according evaluation of the DAR dated 27 July 1999, ASB2010-10305
& The study is claimed to be in compliance with GLP and the OECD guidelines.
# Mean daily intake calculated by the applicant, if the here appropriate conversion factor of 13 (Anonymous, 2000; ASB2013-4646) is used, 3.8 mg/kg bw/day would result.

→ In view of the lack of effects the respective dose level was increased half way through the study.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Oral 28-day toxicity

Rat

Scholz and Weigand (1973; TOX9551871): 30-day range-finding-test with SPF-Wistar rats. No test guideline is quoted, inhouse methodology was used. The method was largely similar to that of OECD guideline 407 (14/28 day repeat dose oral toxicity: rodent). The test material stability and achieved concentrations in the rodent diet was not tested or reported. No statistical treatment of results was reported. The test guideline requires at least 5 male and 5 females to be treated at each dose level. This was complied with and additionally a further 5 male and 5 females were sacrificed after a 30 day recovery period. At the time of the study GLP compliance was not compulsory and the study is not claimed to be compliant. The study is considered to be supplementary.

Material and methods:

The test substance was administered orally in the feed to groups of 10 male and 10 female SPF-Wistar rats bred in house, for a 30 day period at concentrations of 0, 500, 1250, 3200, 8000 and 20000 ppm. The control group received only the basal diet. The test material (isoproturon 95-96 % purity, batch number not quoted) was incorporated into pulverised Altromin 1324 diet (Altromin GmbH Lage/Lippe) and subsequently pressed into a pelleted form. Both the diet and tap water were available *ad libitum*. Rats were housed in plastic cages on wood shavings with 5 rats of the same sex and group per cage.

Behaviour and general state of health were assessed throughout the experiment. Feed consumption was checked continuously throughout the experiment and body weight was recorded twice a week. After 30 days administration dosing was stopped. 50% of the rats (5 male and 5 female rats per group) were sacrificed 24 hours after the cessation of treatment and the other 50 % were retained without treatment for a further 14 days. All rats were sampled for laboratory investigations immediately after the treatment period. In addition enzyme activity-values in the serum were determined in animals sacrificed 14 days after the cessation of treatment.

Prior to the beginning and after 30 days of treatment, haematological examinations and urinalysis were performed in all rats. Analytes measured were as follows. For haematology:- haemoglobin, RBC, WBC, haematocrit, differential white cell count and Heinz bodies. For urinalysis:- appearance, colour, pH-value, bilirubin, albumin, haemoglobin, glucose and sediment. For clinical chemistry:- alkaline phosphatase, ALT and AST.

Each animal was subjected to necropsy. The liver and kidneys were weighed and were subjected to histological examinations.

Findings:

No treatment-related adverse clinical signs were noted in the treatment groups other than 20000 ppm. In the top treatment group (20000 ppm) all male and female rats died between days 6 and 18 of the dosing period. They showed marked emaciation and cannibalism. Body weight gains were significantly reduced ($p < 0.05$) in all treatment groups except for the females in the 500 ppm group. At 500 ppm in males, body weight gain was reduced by 8.7 % over the entire treatment period. Body weight gain returned to normal in all treatment groups during the 14-day follow-up period.

Achieved test substance intakes were as follows:

Table 11: Achieved dosages and body weight change

Group Number	Dietary concentration (ppm)	Achieved dose (mg/kg bw/d)		Body weight change (g)	
		male	female	male	female
1	20000	911.2	602.2	-	-
2	8000	538.5	523.1	16.8*	3.4*
3	3200	258.8	254.6	90.0*	35.8*
4	1250	105.3	105.6	100.8*	39.0*
5	500	42.8	43.4	111.6*	49.2
6	control	0	0	123.4	53.0

(* P <0.05)

There was a dose dependent depression of feed consumption during the first half of the experiment which returned to normal during the second half of the study.

There were no treatment related changes in either haematology or clinical chemistry parameters. Urinalysis revealed only slightly positive urinary albumin in two females receiving 8000 ppm.

There were no treatment related gross pathological changes. 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 increase was not evident after 14 days of recovery. Kidney weights were unaffected.

Table 12: Group mean liver weights at 24 hours and 14 days after treatment

Group/sex	Dose (ppm)	Mean liver weight 24 hrs after treatment		Mean liver weight 14 days after treatment	
		absolute (g)	relative (%)	absolute (g)	relative (%)
2 / male	8000	10.71	5.75*	9.75	4.21
3 / male	3200	11.87	4.93*	12.38	4.16
4 / male	1250	10.88	4.26	12.01	3.89
5 / male	500	11.32	4.12	11.78	3.85
6 / male	control	11.24	3.93	12.83	4.08
2 / female	8000	8.07	5.70*	7.16	4.22
3 / female	3200	7.63	4.69*	7.15	4.09
4 / female	1250	7.16	4.13*	6.08	3.40
5 / female	500	5.77	3.40	7.14	3.75
6 / female	control	6.95	3.81	7.88	4.01

(* P <0.05)

With the exception of siderosis in the liver of rats treated at 20000 ppm (probably resulting from the much reduced feed intake) there were no other histological changes attributed to treatment.

Conclusion:

It was concluded that the approximate NOEL was 500 ppm (approximately 43 mg/kg bw/d) based on reduced body weight gain and increased relative liver weights.

Oral 28-day toxicity

Mouse

Hunter et al. (1979; TOX9551872): Preliminary assessment of isoproturon toxicity to mice by dietary administration for 4 weeks.

No test guideline is quoted, however the method used was similar to that of OECD guideline 407 (14/28 day repeat dose oral toxicity: rodent) and complied with the exception that the test material stability and achieved concentrations in the rodent diet was not tested or reported. Also, no clinical examinations (haematology, clinical chemistry and urinalysis) were made. In view of the lack of effects at the top dose level, the dose level from the low dose group was increased from 80 ppm to 4000 ppm half way through the study. Since the study was designed to determine dose levels for following sub-chronic studies, this action was consistent with achieving the required information using the available animals. The study is considered to be of supplementary scientific value. At the time of the study GLP compliance was not compulsory and the study is not claimed to be compliant.

Material and methods:

The test material was administered orally in the feed to groups of 8 male and 8 female CD 1 mice (Charles River Laboratories UK) for a 28 day period at concentrations of 0, 80/4000 (after 2 weeks of treatment the dietary level of 80 ppm was increased to 4000 ppm), 400 and 2000 ppm. The control group received only the basal diet. No batch number or purity is given for the test substance but diets were prepared weekly. Both the diet and tap water were available *ad libitum*. The mice were housed in polypropylene cages on autoclaved sawdust with 4 of the same sex and group per cage.

Behaviour and general state of health were assessed throughout the experiment. Feed consumption was checked weekly and body weight was recorded twice a week.

At the end of the study, the animals were sacrificed and subjected to necropsy. Selected organs were weighed and subjected to microscopic examinations if considered necessary.

No special statistical analysis was applied to the data. Student's 't' test was used to assess the significance of intergroup differences in body weight.

Findings:

There were no overt signs of reaction to treatment and consequently the isoproturon concentration of the diet of the lowest treatment group (80 ppm) was increased to 4000 ppm for the final half (two weeks) of the study. The mice remained asymptomatic throughout the study period. Feed consumption was unaffected by treatment. Mice receiving 4000 ppm for the second half of the study showed slightly reduced body weight gain in males and females (statistically significant in males only).

There was no gross pathological change attributable to treatment. Organ weight analysis revealed a slight increase in liver weight when adjusted for body weight in animals receiving 4000 ppm and 2000 ppm however the changes were minor and the authors did not attribute them to a test substance effect.

Table 13: Achieved dosages and liver weight change

Group	**Achieved dosage (mg/kg bw/d) m / f**	**Dose (ppm)**	**Male liver weights**		**Female liver weights**	
			abs (g)	**adj[1] (g)**	**abs (g)**	**adj[1] (g)**
1	12 / 13	control	1.9	1.72	1.5	1.46
2	59 / 69	80 → 4000	2.0	2.13**	1.7	1.68
3	307 / 378	400	2.0	1.96	1.5	1.47
4	585 / 796	2000	1.9	2.01*	1.6	1.62

1 Organ weights were adjusted for final body weight as covariate
* P < 0.05 ** P < 0.01

No tissues were processed for histological examination as no macroscopic findings were found.

Conclusion:

The NOEL is 2000 ppm (equivalent to a daily intake of 307 mg/kg bw/d in males and 378 mg/kg bw/d in females) based on effects on body weight gain.

Oral 28-day toxicity

Dog

Kramer and Brunk (1975; TOX9551873): 30-Day Feeding Trial with Beagle dogs.

No test guideline is quoted, inhouse methodology was used as described below. With the exception of the reduced animal numbers (only two male and two females per group) and the lack of test formulation analysis, the method followed was similar to contemporary guideline studies for such a range finding exercise. No special statistical analysis was applied to the data. At the time of the study GLP compliance was not compulsory and the study is not claimed to be compliant. The study is considered to be supplementary.

Material and methods:

The test material (95 - 96 % purity) was fed daily to 2 male and 2 female pure bred English beagle dogs (bred in-house) for a 28-30 day period at concentrations of 0, 50, 160 and 500 (increased to 1250 after 15 days) ppm in the diet (Maize meal, Latz Purina GmbH). Males received a ration of 850 g and females 650 g per day. The control group received only the basal diet. Water was freely available at all times. Clinical signs were observed daily and physical examinations and reflex excitability were performed weekly. Body weights were recorded weekly. Eye examinations and a hearing test were performed prior to the first treatment and at the end of the study.

Blood samples for haematology, serum analyses and blood glucose determinations and urine samples were collected prior to commencement and at the end of the experiment. Enzyme investigations were performed prior to the experiment, 24 hours, 7 and 14 days after the commencement of the study and at the end of the experiment. Haematology samples were assayed for: RBC, Hb, clotting time, haematocrit, ESR, reticulocytes, Platelets, Heinz bodies and differential blood picture. Clinical chemistry samples were assayed for:- Ca, Na, K, inorganic phosphorous, uric acid, creatinine, bilirubin, chloride, urea nitrogen, glucose, ALT, AST, AP, LDH and GDH. Urine samples were monitored for: SG, pH, appearance, colour, sediment, protein, glucose, bilirubin and haemoglobin.

The animals were sacrificed one day following the last treatment day and subjected to necropsy. Selected organs from each animal in each group were weighed and fixed for histological examinations.

Findings:

There were no deaths and dogs receiving 50 or 160 ppm per day showed no clinical symptoms or adverse reaction. 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 (i.e. when animals received 1250 ppm), showed a reduction of feed intake ranging from 26.2 to 72.8 %. An exception was 1 dog whose feed intake had already fallen by 48.6 % during the first half of the trial (i.e. when animals received 500 ppm).

There were no treatment related observations in reflex excitability, ophthalmoscopy and hearing or on dental check.

At 500/1250 ppm signs of haemoconcentration were observed in the icteric bitch and signs of anaemia (especially a decreased haemoglobin concentration) in the other bitch.

The icteric bitch presented a strongly increased serum bilirubin concentration, increase in GOT and GPT activities, a transient increase in GDH activity and an increase in the AP activity at the end of the trial. One dog showed an increase activity of GPT after 2 weeks and at the end of the experiment.

A distinctly positive urinary bilirubin reaction was recorded for the icteric bitch at the end of the trial. There were no significant changes in dogs treated at 160 ppm or less. There were no significant changes in organ weights. One female receiving 500/1250 ppm displayed generalised icterus, strongly exsiccotic skin, induration of the liver and numerous retractions of the liver surface. The icteric bitch (500/1250 ppm) revealed necrosis of the liver, bileduct proliferation and pericholangitis.

The examination of the bone-marrow in two dogs in the 160 ppm dose group and a total of 3 dogs in the 500/1200 ppm dose group revealed pronounced deposition of haemosiderin in the reticular cells with increased erythrophagocytosis. In addition, some dogs at the high dose level showed reduction of erythrocytic and granulocytic precursors.

Conclusion:

The NOAEL was 50 ppm, based on effects in the bone marrow at 160 ppm and more extensive liver and haematological changes at 500/1250 ppm. In the original report, no conversion of this dietary concentration to a mean daily intake has been made. Since a moist, semi-solid diet had been fed, it seems appropriate to use a conversion factor of 13 following the recommendation as given in the ”Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticide Residues” (Anonymous, 2000, ASB2013-4646) in its Appendix I, ”Approximate relation of parts per million in the diet to mg/kg bw per day”. Thus, a mean daily intake of 3.8 mg/kg bw should be assumed. In the previous evaluation, however, 3.3 mg/kg bw/day was mentioned. This figure had been provided by the applicants and seems realistic but the mathematical calculation on the basis of actual food intake and body weight is not available.

Oral 90-day toxicity

Rat

Leuschner et al. (1973; TOX9551874): 13-week oral (dietary administration) toxicity study of HOE 16,410 OH in Sprague-Dawley rats, with subsequent 2-week recovery period.

No test guideline is claimed, however the protocol followed is largely similar to the current OECD test guideline 408 (Sub-chronic oral toxicity - rodent 90 day study). The reported study deviated in respect of lack of test formulation analysis, increased test substance incorporation at the top dose level after 6 weeks due to lack of toxicological response and a slightly shorter list of tissues taken at necropsy for subsequent histopathological examination (notably trachea, aorta, salivary glands, pancreas, oesophagus, peripheral nerve and sternum). At the time of the study GLP compliance was not compulsory and the study is not claimed to be compliant. The study is considered to be supplementary.

Material and methods:

Groups of 20 male and 20 female Sprague Dawley rats (S. Ivanovas, 7967 Kiblegg/Wurtt., P.O. Box 7, Germany, 40-41 days old at commencement of treatment) received diets containing 0, 80, 400, 2 000 and 10 000 (for 6 weeks and which was then increased to 20 000) ppm of Hoe 16 410 OH (isoproturon) for a 13 week period. The batch number and purity of the test material was not specified in the report. The control group received only the basal diet. (Altromin R, Altromin GmbH, 4910 Lage/Lippe). Behaviour and general state of health were assessed throughout the experiment. Feed consumption was checked continuously throughout the experiment and body weight was recorded once a week. Prior to sacrifice eyes, hearing and teeth were appropriately examined.

Prior to the beginning, after 6 and 13 weeks, haematological examinations and urinalysis were performed in 10 animals per group. The enzyme activity-values in the serum were determined in 10 animals per group at the end of the experiment and 2 weeks later.

Haematology samples were assayed for: RBC, Hb, WBC, clotting time, haematocrit, reticulocytes, platelets and differential blood picture. Heinz bodies and methaemoglobin were determined only at the end of the treatment and recovery period. Clinical chemistry samples were assayed for: Ca, Na, K, inorganic phosphorous, uric acid, bilirubin, chloride, urea nitrogen, glucose, SGOT, SGPT, AP, total protein and CO_2. Urine samples were monitored for: - SG, pH, colour, sediment, protein, glucose, bilirubin ketone bodies and haemoglobin.

Either 24 hours or 14 days after termination of the experiment respectively, 50 % of the animals (10 male and 10 female rats per group) were sacrificed and subjected to necropsy. Selected organs were weighed and subjected to histological examinations.

Statistics: Student's 't' test was used to analyse general data.

Findings:

Rats treated at 80, 400 or 2000 ppm for 13 weeks showed no clinical signs nor did 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. Reactions were difficult to elicit and grooming activity decreased. During the 13th week, 1 male and 2 females died at this high dose level. 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 significantly lower mean body weight in both sexes in weeks 6 and 13. After two additional weeks on untreated diet, male animals had nearly recovered whereas, in females, body weight was still lower than in the control group. 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 (see Table B.6.3 6). During the recovery phase weights rebounded somewhat in both sexes but clearly did not regain the deficit.

Table 14: Achieved doses and body weight change

Group	Dose level	Achieved dosage	Group mean body weight (g)							
			predose		week 6		week 13		week 15	
	ppm	mg/kg bw/d	male	female	male	female	male	female	male	female
1	80	6.9 / 7.0	116.3	113.0	309.5	215.5	401.3	229.7	411.8	252.5
2	400	36.6 / 34.4	116.6	113.1	306.4	215.4	402.8	240.6	419.0	250.4
3	2000	171/175	116.6	113.0	286.0*	198.3*	368.6*	215.1*	396.1	223.2*
4	10000[1] 20000	990/1032[1] 1491/1557	116.4	113.1	247.4*	153.3*	198.5*	124.3*	255.3*	192.3*
4	control	0	116.7	112.8	309.1	212.8	402.7	239.3	415.7	249.6

[1]dose increased after 6 weeks
* P < 0.01 (Student t-test)

Feed consumption closely followed body weight gain and showed a decrease in groups 3 and 4 receiving either 2000 or 10000/20000 ppm. When the intake was expressed in terms of relative to body weight intake was similar in all groups. Estimated water consumption was similar in all groups.

Rats receiving 10000/20000 ppm showed a statistically significant increase in the methaemoglobin (Met-Hb) content and in the number of Heinz bodies at the end of the dosage period. The haemoglobin at least in male rats and the erythrocyte concentration (Red blood cell count, RBC) in both sexes also tended to be reduced at this dose level (although statistical significance was not achieved) with all parameters returning approximately to normal value by the end of the 14-day follow-up period.

At 10000/20000 ppm, deviations from physiological normality were further reflected by reduced glucose value, increased blood urea nitrogen, marginally increased enzymatic activities (ALAT, ASAT, AP) and decreased serum albumin level (significant only in males). These findings proved, to a large extent, reversible during the recovery period. In contrast to haematological effects, these changes are considered rather a reflection of the poor nutritional and health status of these animals and not of a primary toxic effect. In particular with regard to enzyme activities, this assumption was mainly due to the observation that the liver was apparently no target of isoproturon-related toxicity in this study. Alterations in haematological and clinical chemistry parameters are summarised in

Table 15**Error! Reference source not found.**. No abnormalities were detected in the urinalysis parameters.

Table 15: 90-day study in rats: Altered hematological and selected clinical chemistry findings after 13 weeks (mean values with SD, presumed treatment-related changes in bold)

Parameter	**Males**					**Females**				
	0 ppm	**80 ppm**	**400 ppm**	**2000 ppm**	**10000/ 20000 ppm**	**0 ppm**	**80 ppm**	**400 ppm**	**2000 ppm**	**10000/ 20000 ppm**
Heinz bodies (in % of RBC)	0.1 ± 0.3	0.1 ± 0.3	0	**0.9 ± 1.5**	**41.1* ± 38.2**	0	0.2 ± 0.6	0	**1.2 ± 1.4**	**41.8* ± 27.9**
Met-Hb (g/100 mL serum)	0.29 ± 0.07	0.26 ± 0.09	0.29 ± 0.07	0.28 ± 0.08	**0.78* ± 0.19**	0.29 ± 0.07	0.28 ± 0.08	0.33 ± 0.09	0.31 ± 0.05	**0.77* ± 0.19**
RBC (10^6/µL)	7.7 ± 0.4	7.9 ± 0.4	7.9 ± 0.4	7.7 ± 0.4	**7.1 ± 0.3**	7.7 ± 0.5	8.0 ± 0.4	7.8 ± 0.5	7.7 ± 0.5	**7.1 ± 0.5**
Hemoglobin (g/100 mL blood)	15.4 ± 0.8	15.9 ± 0.7	15.8 ± 0.8	15.3 ± 0.9	**14.2 ± 0.7**	15.1 ± 1.0	15.9 ± 0.8	15.5 ± 0.9	14.3 ± 3.4	14.4 ± 0.8
ALAT (mU/mL serum)	8.6 ± 1.8	9.0 ± 1.8	8.9 ± 2.1	9.0 ± 1.6	**13.7* ± 4.9**	8.3 ± 1.7	8.5 ± 2.0	8.5 ± 1.5	8.2 ± 1.6	**12.1 ± 4.2**
ASAT (mU/mL serum)	68.7 ± 9.7	70.2 ± 10.3	70.6 ± 15.2	71.3 ± 10.2	**85.4* ± 15.2**	70.5 ± 11.6	73.9 ± 13.2	68.8 ± 11.6	70.9 ± 12.9	**89.2* ± 15.7**
Glucose (mg/100 mL serum)	133.7 ± 15.1	129.4 ± 19.0	125.5 ± 17.3	127.6 ± 20.1	**97.5* ± 25.7**	129.9 ± 16.0	130.7 ± 15.2	129.7 ± 17.4	127.0 ± 18.2	**94.8* ± 14.3**
AP (mU/mL serum)	146.0 ± 18.0	142.5 ± 13.2	142.9 ± 16.5	155.0 ± 14.3	158.3 ± 21.1	133.6 ± 21.5	128.9 ± 16.8	124.1 ± 18.6	126.1 ± 18.9	**153.0 ± 21.9**

* $p < 0.01$ (Student t-test)Rats that died during treatment showed pulmonary changes, haemorrhagic rhinitis and also stomach ulceration.

In animals receiving 10000/20000 ppm, many organs were found to be involuted after 13 weeks, evidently as a result of cachexia. The relative liver weight (ratio to body weight) was increased (+ 88 % for males and + 78 % for females). Mean relative liver weight subsided during the recovery period but was still superior to those in the control groups at the end of the 2 week recovery period.

The microscopic findings in the test animals were few. They did not differ from those for the control animals except to reflect macroscopic observations.

Conclusion:

The NOAEL was 400 ppm (35 mg/kg bw/d), based on reduced feed intake and lower body weight gain as well on slight haematological changes observed at the next higher dose level of 2000 ppm. The high dose of 10000 or 20000 ppm was clearly toxic. The blood findings might be indicative of toxic hemolytic anaemia.

Bhide (1984; TOX9651092): Subacute oral toxicity for 90 days in rats of isoproturon (technical).

The study is not claimed to be GLP compliant. No test guideline is quoted, inhouse methodology was used as described below. In comparison to the current OECD guideline (408) for a subchronic oral toxicity (Rodent 90-day Study) the reported study has a lot of deficiencies. The purity of the test substance, test material stability and achieved concentrations in the rodent diet were not tested or reported. No statistical analysis was applied to the data. There was only a limited program of clinical and pathological examinations. No ophthalmological examination was made. There was no electrolyte balance investigated. Necropsy of the jejunum, caecum, rectum and a peripheral nerve was not carried out. There were no data summarised in tabular form of the results of the gross necropsy and histopathology, showing for each test group the number of animals with lesions, the typ of lesions and the percentage of animals displaying each type of lesion. The study is considered to be supplementary.

Material and methods:

The study included a dose range-finding study. In this study rats were treated with isoproturon orally at doses ranging from 128 to 4100 mg/kg bw/d for 14 days. Cage-side observations, body weight and organ weights were noticed. In the main study 10 male and female Wistar rats (bred and reared in the animal house of the testing facility) were administered isoproturon (technical) in 0.2 % agar solution in water by gavage once daily seven days a week for 90 days at dose levels of 0, 85, 250 and 750 mg/kg bw/d. A supplementary group of 10 male and female 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. All the animals were observed daily for toxicological symptoms. The quantity of feed consumed by a group of five rats was recorded daily and the mean daily intake for each week was calculated for each group. The weight of each rat was recorded on 0 day and at weekly intervals throughout the course of the study. The group mean body weights were calculated. Laboratory investigations were done on day 0, 46, 91 and 121, in animals fasted overnight. Blood samples were collected in the morning. Haematology samples were assayed for: Hb, PCV, RBC, WBC, differential white blood cell count, platelets, reticulocytes, prothrombin time and Heinz bodies. Clinical chemistry samples were assayed for: BUN, AlP, ALT, total serum protein and glucose. Urin samples were monitored for: pH, glucose, protein, ketones, blood and microscopy of the sedimentation. All the animals (except reversal group) were sacrificed on 91st day. Necropsy of all the internal organs of each animal was carried out and the weights of following organs were recorded: liver, kidneys, heart, spleen, adrenals, testes, ovaries. The organ weights were recorded as absolute values and their relative values (i.e. percent of the body weight) were calculated. Pieces from the following organs were taken and preserved for histopathological examination: adrenal, aorta, bone with marrow, brain, colon, duodenum, eyes, gall bladder, heart, ileum, kidneys, liver, lungs, lymphnodes, oesophagus, ovaries, pancreas, pituitary, salivary gland, seminal vesicles, skin, spleen, stomach, testes, thymus, thyroid, urinary bladder, uterus.

Findings:

Animals receiving the highest dose had a decrease in locomotor activity, diarrhoea, chromodacryorrhea in both male and female rats. No mortality was observed at any dose levels. A slight gradual reduction in body weight gain was noticed in animals which received a dose of 250 mg/kg bw/d. After the treatment the reduction in body weight gain was reversed. A significant gradual reduction in body weight gain was seen in animals of the high dose group. Feed intake was reduced in animals which received doses of 250 and 750 mg/kg bw/d. After the withdrawal of treatment, the reduction in feed intake was reversed. A slight reduction in Hb, PCV and RBC was seen in both male and female rats which received 250 mg/kg bw/d. The reduction in Hb, PCV and RBC was marked in animals of the high dose group. This group also showed an increase in reticulocyte and the presence of Heinz bodies both in male and female animals. Gradual increase in Hb, PCV and RBC were noted in animals of the supplementary group (250 mg/kg bw/d) after stopping the treatment. An increase in the methaemoglobin content and a slight increase in the weight of spleen were noticed in animals treated with the high dose. In a few animals of the medium dose group slight degenerative changes in liver were noticed. The animals of the high dose group showed slight to moderate degenerative changes in the liver, the heart and the kidneys. Haemosiderosis of slight to moderate degree and pigmentation in the spleen as well as hyperplasia of bone marrow cells was observed in some of the animals treated with 750 mg/kg bw/d.

Conclusion:

The NOEL is 85 mg/kg bw/d, based on reduced feed intake and body weight gain and anaemia.

Dickhaus and Heisler (1987; TOX9550729): Three months subacute toxicity isoproturon techn. as feeding study in the species rat.

No international test guideline is claimed, however the protocol followed is largely similar to the current OECD test guideline 408 (Sub-chronic oral toxicity - rodent 90 day study). The reported study deviated in respect of the lack of the test formulation analysis. Purity of the test substance was not given. There was limited information about housing and feeding conditions. There was a shorter list of clinical biochemistry determinations on blood (calcium, phosphorus, chloride, sodium, potassium, urea nitrogen, blood creatine, total bilirubin, total serum protein measurements) and there was a shorter list of tissues taken at necropsy for subsequent histopathological examination (notably trachea, aorta, salivary glands, oesophagus, jejunum, ileum, caecum, rectum, urinary bladder and sternum with bone marrow). The study is not claimed to be GLP compliant. The study is considered to be supplementary.

Material and methods:

Groups of 20 male and 20 female Wistar rats (delivered by Winkelmann, Borchen, Germany) received diets containing 0, 400, 1500 and 5000 ppm of isoproturon techn. for a 90 day period. The batch number and purity of the test material was not specified in the report. The control group received only the basal diet (Altromin, Lage/Germany). 10 male and 10 female Wistar rats per group and dose level scheduled for follow-up observations were kept for a further 30 day period without treatment. Clinical examinations were done in intervals of 4 weeks. Laboratory and pathological examinations were done after 90 and 120 days. Haematology samples were assayed for: leucocytes, hematocrit, hemoglobin, erythrocytes and differential blood picture. Clinical chemistry samples were assayed for: glucose, total albumin, AST, ALT, GGT, LDH, AlP. Urine samples were monitored for: specific gravity, pH, urobilinogen, erythrocytes, bilirubin, ketone, glucose, nitrite, protein and leucocytes. After 90 days treatment and 30 days after termination of the experiment respectively, 50 % of the animals (10 male and 10 female rats per group) were sacrificed and subjected to necropsy. Selected organs were weighed and subjected to histological examinations (organ weights: brain, heart, liver, kidneys, adrenal glands, spleen, ovaries, testicles, epididymis; histology: liver, heart, kidneys, spleen, brain, thyroid glands, stomach, duodenum, colon, pancreas, hypophysis, testicles, uterus, ovaries, adrenal glands, lung, N. ischiadicus, urinary bladder). Weight changes and feed consumption were evaluated by calculation of three-factorial variance analysis. Comparison of group averages is performed according to the method of Tukey. Hematological and biochemical analysis were calculated by determination of mean values and standard deviations. Organ weights were calculated by three-factorial variance analysis.

Findings:

All over the test there were no essential differences concerning appearance and behaviour between control and test animals. The clinical examination and special examination of eyes, ears, oral cavity and reflexes did not point to test specific variations. All animals survived the cumulative administration of isoproturon techn. until the test was finished. Over the whole experiment no significant differences between controls and lowest dosage group occurred. Male and female animals which received a dose of 1500 ppm isoproturon techn. showed during the loading period significant decreased weight gains. During the reversal time, 90 till 120 days, a dose dependent increase of weight gains could be observed, which was significant ($p > 0.05$) in male animals of the high dose group. Male and female animals of the highest dose group showed during the loading period a highly significant decreased feed consumption. During the reversal period no essential differences in comparison to the control occurred. During the loading period feed efficacy in male and female animals of highest dosage group was clearly diminished. During the reversal period an increase of feed efficacy occurred, which was more evident in males than in females. In the highest dosage group 90 days leucocytes blood values of male and female animals were significantly increased. Organ weights of the liver were significantly increased in males of the middle and high dosage group after 90 days. After 120 days organ weights of liver in males of the high dosage group were only significant increased. Organ weights of the spleen were significantly increased in females of the high dosage group after 90 days only. Histopathological examination could not detect test-substance dependent alterations.

Conclusion:

The NOEL is 400 ppm (approximately equivalent to 20-40 mg/kg bw/d), based on decreased body weight gains and increased weights of liver.

Bhide (1990; TOX9550326): Subchronic oral toxicity study (90 day) in rats with isoproturon.

It is claimed by the author that the study was performed in compliance with OECD principles of GLP. The protocol followed is largely similar to the current OECD test guideline 408 (Subchronic Oral Toxicity - Rodent: 90 day Study). The reported study deviated in respect of the lack of test formulation analysis. Although the author claimed a statistical treatment of the results there was no statistical significance of effects indicated in the tabular form of the summarised data. The study is considered to be supplementary.

Material and methods:

Groups of 10 male and 10 female Wistar rats (I.I.T. Animal House; 5 to 6 weeks old at the inition of the treatment) received diets containing 0, 400, 1200 and 2400 ppm of isoproturon (purity 98 %; supplied by Monatari Industries Ltd., New Delhi; a batch number was not given) for a period of 90 days. A supplementary high dose group was conducted 28 days after the termination of the treatment. The control group received only the basal diet (pelleted rat feed supplied by Lipton India Ltd., Bangalore). All clinical signs including time of onset, intensity and duration were recorded once daily during the acclimation, treatment and recovery periods. Feed consumption was recorded once a week. The eyes of control and high dose animals were examined prior to the starting of the treatment and in week 13 of the study.

Blood samples for haematology and clinical biochemistry were collected from all animals of the 4 dose groups at the termination of the treatment (91. day) and from all animals of a supplementary high dose group at the termination of the recovery period (119. day). Haematology samples were assayed for: RBC, Hb, HCT/PCV, platelet count, leucocyte count and leucocyte differential count. Clinical chemistry samples were assayed for: - Ca, Na, K, phosphorous, bilirubin, chloride, urea nitrogen, creatinine, glucose, ALT, AST, gamma GT, total protein, albumin and methaemoglobin.

All animals were necropsied at the end of the treatment period (day 91) and at the end of the 28 day post treatment recovery period (day 119, supplementary group). Selected organs were weighed and subjected to histological examinations.

The author claimed that Dunnett-test-(many to one t-test) was applied for the comparison between the treated groups and the control group for each sex.

Findings:

No mortality was observed in any group of animals during the period of study. Intermittent diarrhea and loss of fur were observed in both male and female animals receiving the dose of 2400 ppm. Female animals treated at the dietary dose level 1200 ppm exhibited intermittent diarrhea. Opthamological examination did not reveal any treatment related changes. The male and female animals treated at the dietary level of 2400 ppm showed reduction in body weight gain. After the termination of the treatment the animal of the supplementary group receiving the dose of 2400 ppm showed significant gain in body weight. Slight reduction in body weight gain was seen in animals receiving the dose of 1200 ppm. Slight reduction in the quantity of feed consumed was seen in male and female animals receiving the dose of 2400 ppm. The reduction in feed consumption was found to be reversed during the period of observation for 28 days.

The values of Hb, PCV and RBC were found to be lowered in both male and female animals treated at the dose levels of 2400 ppm. The presence of Heinz bodies was also seen in these animals. The level of total protein in blood was found to be slightly lowered while the level of methaemoglobin was found to be elevated in the animals treated at the dose level of 2400 ppm. After the termination of the treatment, the levels of total protein and methaemoglobin were found to be within the normal limits.

The increase in liver weights was observed in both male and female animals receiving the high dose. No such increase in liver weights was recorded in animals 28 days after termination of the treatment. However, congestion in spleen was found in these animals.

Haemosiderosis with congestion or with proliferation of histiocytes in the spleen and also hyperplasia of the bone marrow cells were observed in some animals receiving 1200 ppm and 2400 ppm. No such structural changes were seen in animals of the supplementary group sacrificed 28 days after the termination of the treatment.

Conclusion:

On the basis of intermittent diarrhea, reduction in body weight gain, haemosiderosis and hyperplasia of the bone marrow cells the NOEL was found to be 400 ppm (approximately equivalent to 20-40 mg/kg bw/d).

Wragg, Blackwell and Brooks (1991; TOX9300281): Isoproturon: Ninety day sub-chronic oral (dietary) toxicity study in the rat.

It is claimed by the authors that the study was performed in compliance with GLP and the OECD guideline for Testing of Chemicals „Subchronic Oral Toxicity - Rodent: 90-day Study“ (No. 408). The protocol followed is largely similar to the current OECD test guideline. The study is considered to be acceptable.

Material and methods:

The test material (IPU isoproturon technical, Batch number 0033/91; purity: 99,2 %) was administered by dietary admixture to three groups, each of ten male and ten female Sprague-Dawley CD strain rats, for ninety consecutive days, at dietary concentrations of 80, 800 and 8000 ppm. A further group of ten males and ten females was exposed to basel laboratory diet to serve as a control. Clinical signs, body weight, feed and water consumptions were monitored during the study. Haematology and blood chemistry were evaluated for all animals at the end of the study. Ophthalmoscopic examination was also performed. On completion of the dosing period all animals were killed and were subjected to a full external and internal examination. Data were processed to give group mean values and standard deviations where appropriate. Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating F-max test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruska Wallis non-parametric analysis of variance and Mann Whitney U-Test. Histopathology data were analysed using Chi squared analysis and Kruskal-Wallis one way non-parametric analysis of variance.

Findings:

Two high dose females showed pallor of the extremities and incidents of hunched posture and piloerection were apparent in animals of both sexes. A reduction in bodyweight gain and feed consumption was seen in high dose animals throughout the treatment period. A possible dose-related less pronounced reduction in bodyweight gain was also apparent for intermediate dose females. High dose animals of both sexes showed a reduction in RBC, Hb and Hk, together with an increase in the MCV and, particularly in females, an elevated reticulocyte count. Intermediate dose females also showed a slight reduction in RBC together with increases in both MCV and reticulocytes. The relative amount of methaemoglobin present was elevated in high dose animals of both sexes. In intermediate dose females the increase was only slight. High dose animals also showed an increase in prothrombin time. High dose females showed a slight increase in K and AlP. Total protein, albumin, glucose and urea were reduced in high dose animals of both sexes due to the reduction in the dietary intake or associated with hepatic changes. One high dose male had a darkened liver at necropsy. A further six males from this dose group showed small seminal vesicles and a small prostate gland. Organ weight changes were noted in high dose animals. Intermediate dose females showed only a slight increase in relative brain weight. Treatment-related changes were observed in the liver, spleen, adrenal glands, kidneys, ovaries, seminal vesicles, and prostate. In the liver scattered deposits of haemosiderin pigment were observed for both male and female rats dosed at 8000 ppm and 800 ppm. Bile duct proliferation was recorded for both sexes dosed at 8000 ppm and eosinophilic degeneration of hepatocytes was noted for male rats dosed at 8000 ppm. Foci of basophilic hepatocytes were also noted for a few high dose rats and for one 800 ppm male rat. The severity of haemosiderin pigment accumulation was increased for both sexes dosed at 8000 ppm and there were also indications of an increased severity of splenic extramedullary haemopoiesis at this dose level. Histopathological changes including vacuolation of zona glomerulosa cells, a greater severity of haemosiderin pigment deposition in the zona reticularis, an increased incidence of vacuolation of the zona reticularis cells and a reduced incidence of vacuolation of zona fasciculata cells were observed in the adrenal glands of the high dose animals. Deposits of haemosiderin pigments were observed in the renal proximal tubular epithelium of male and female rats at all treatment levels. Proliferation and/or vacuolation of stromal cells were observed for female rats dosed at 8000 ppm. Reduced secretory contents of the seminal vesicles and prostate was recorded amongst male rats of the high dose group and one male rat receiving 800 ppm showed reduced secretory contents of the seminal vesicles.

Table 16: Hematological parameters

Dose level (ppm) Sex	0 m/f	80 m/f	800 m/f	8000 m/f
RBC (T/l)	7.89/7.49	7.82/7.34	7.82/7.04**	6.99***/6.42***
MCV (fl)	52/55	52/55	53/57**	57***/59***
Methemoglobin (%)	1.73/1.08	1.20/1.61	1.76/2.69**	5.70***/5.50***
Reticulocytes (%)	4/2	4/3	4/7***	5/11***

* p <0.05; ** p <0.01; *** p <0.001

Table 17: Body weight, relative organ weights

Dose level (ppm) Sex	0 m/f	80 m/f	800 m/f	8000 m/f
Body weight (g) on day 90	537/313	562/306	532/282	303/220
Rel. brain weight (% of bw)	0.3876/0.5923	0.3734/0.6179	0.3977/0.6454*	0.6380**/0.8413***
Rel. liver weight (% of bw)	3.3132/3.1219	3.3347/3.0873	3.3452/3.1541	4.4844***/4.2327***
Rel. kidney weight (% of bw)	0.5487/0.5885	0.5481/0.5884	0.5619/0.6023	0.6673***/0.6773***
Rel. adrenal weight (% of bw)	0.0129/0.0254	0.0106**/0.0228	0.0123/0.0252	0.0102**/0.0182***

* p <0.05; ** p <0.01; *** p <0.001

Table 18: Histopathological findings (No. of animals)

Dose level (ppm) Sex	0 m/f	80 m/f	800 m/f	8000 m/f
Kidney, pigment deposition: absent	10/10	9/4	8/2	0/0
Kidney, pigment deposition: minimal	0/0	1/6	2/8	10/1
Kidney, pigment deposition: slight	0/0	0/0	0/0	0/9
Liver, pigment deposition: absent	10/10	10/10	9/6	0/0
Liver, pigment deposition: minimal	0/0	0/0	1/4	5/8
Liver, pigment deposition: slight	0/0	0/0	0/0	5/2
Liver, basophilic foci: absent	10/10	10/10	9/10	3/9
Liver, basophilic foci: minimal	0/0	0/0	1/0	7/1

Conclusion:

A NOAEL has been established at 80 ppm (5.6 mg/kg bw/d) on the base of a reduction in RBC, an elevated relative amount of methaemoglobin, deposits of haemosiderin pigment in the liver and foci of basophilic hepatocytes. Changes detected at 80 ppm were confined to haemosiderin deposition in the kidneys and were considered not to be indicative of serious damage to the health of the animals.

One further study, not considered influential, has been identified by literature search. The study design was deficient compared to guideline and non-GLP. Doses tested do not influence the NOAEL of IPU as concluded in the DAR, and results are supportive of the generally low toxicity of IPU. This study is allocated a reliability score of “3” by the notifier and summarised in brief detail only:

Reference:	IIA 5.3.2
Report:	Raizada, R., Srivastava, M.K., Kaushal, R.A., Singh, R.P., Gupta, K.P. (2001): Subchronic oral toxicity of a combination of insecticide (HCH) and herbicide (ISP) in male rats. J.Appl.Toxicol 21, 75-79. Published, ASB2012-14785
Guidelines:	None stated
Deviations:	Not applicable
GLP:	No
Acceptability:	The study is considered to be acceptable.

Executive Summary

The subchronic toxicity of a mixture of HCH and IPU was assessed relative to each chemical separately. Groups of 10 male rats were dosed by gavage in peanut oil; with respect to IPU (Hoechst India, purity 97.5 %) doses of 22.5, 45 or 90 mg/kg bw/day were tested. Limited parameters were assessed (symptoms, organ weights, haematology, enzyme activity in hepatic samples; histology limited to 8 tissues); bodyweight and food consumption are not described.

There were very few findings attributed to IPU (hepatic LDH activity appeared impaired at 45 mg/kg bw/day; WBC counts decreased at all dose levels without dose relationship). Decreased WBC values reported in this study are not key findings for IPU in other studies and from dose-response appear false (attributable to high control value). Although haematology was conducted, typical IPU-related mild RBC deficits were not reported. No histological change attributable to IPU was reported. No interaction of IPU with HCH was detected. This study design was deficient compared to guideline and non-GLP. A reliability score of “3” is attributable to this study by the notifier.

Dog

Scholz and Brunk (1973; TOX9551875): Toxicological test, 90-day dietary administration in Beagle dogs.

No test guideline is claimed, however the protocol followed is largely similar to the current OECD test guideline 409 (Subchronic oral toxicity - non-rodent 90 day study). The reported study deviated in respect to lack of tests of formulation analysis, increased test substance incorporation at the top dose level after 15 doses due to lack of toxicological response and a slightly shorter list of tissues taken at necropsy for subsequent histopathological examination (notably aorta, salivary glands, oesophagus, duodenum, ileum, caecum, rectum and a representative lymph node). No statistical analysis was applied to the data. At the time of the study GLP compliance was not compulsory and the study is not claimed to be compliant. The study is considered to be supplementary.

Material and methods:

The test material (isoproturon 96 % batch not quoted) was administered daily to groups of 4 male and 4 female beagle dogs for a 90 day period at concentrations of 0, 50, 160 or 500 (increased to 800 ppm after 15 days) in the diet. The control group received only the basal diet (Latz FF, Lat-Purina GmbH). Males received a daily food ration of 850 g and females 650 g. In addition each dog received 150 g per day of minced meat. Water was freely available at all times. Clinical signs were observed daily; reflex excitability and physical examinations were performed weekly. Body weights were recorded weekly. Eye examinations and a hearing test were performed prior to the first treatment and at the end of the study.

Blood samples for haematology and urine samples were collected prior to commencement of the experiment, after 4 weeks in the 500 ppm group, after 6 weeks and at the end of treatment period for all groups. Serum analyses and blood glucose determinations were performed prior the commencement and before the end of the experiment. Enzymes studies were performed prior to treatment and after 24 hours, 1, 2, 4 and 8 weeks during the study and at the end of the treatment period. Haematology samples were assayed for: RBC, WBC, Hb, clotting time, haematocrit, ESR, reticulocytes, Platelets, Heinz bodies and differential blood picture. Clinical chemistry samples were assayed for: Ca, Na, K, inorganic phosphorous, uric acid, creatinine, bilirubin, chloride, urea nitrogen, glucose, SGOT, SGPT, AP and GDH. Urine samples were monitored for:- SG, pH, appearance, colour, sediment, protein, glucose, bilirubin and haemoglobin.

The animals were sacrificed one day following the last administration of the test substance and subjected to necropsy. Selected organs in each animal in each group were weighed and fixed for histological examinations.

Findings:

There were no unscheduled deaths and few clinical signs of reaction to treatment. Dogs receiving 500/800 ppm showed slightly impaired general condition. Six of the 8 animals in this group vomited occasionally during the first days after dose increase. At 160 ppm three dogs showed pale mucosae from 4, 6 and 10 week respectively onwards.

Dogs receiving 500/800 ppm showed a decrease in body weight, particularly after the increase of dose which was most marked for four animals (mean body weight loss during the study: -3.4 kg). Feed consumption in these animals before the dose increase (up to day 15) was decreased in 1 male and 1 female. After the increase of dose level, 5 dogs revealed a distinctly reduced feed intake (ranging from 16.8 to 66.1 %). There were no other reported changes in the other monitored parameters.

In the top dose group, 6 of the 8 animals revealed toxic haemolytic anaemia with concomitant formation of Heinz bodies in 4 cases (after 4 weeks duration of the test). There were no other changes considered to be treatment related in either clinical chemistry or urinalysis.

Of the animals receiving 500/800 ppm, 5/8 showed impaired nutritional state. Small prostate glands were observed in 2 dogs (inadequate development of the gland tubes was noted at microscopic examination). Among dogs receiving 500/800 ppm and 160 ppm mean liver weights were dose-dependently increased in males and females.

Table 19: Hematological parameters

Dose level (ppm) Sex	0 m/f	50 m/f	160 m/f	500-->800 m/f
Hb (g/l)	169/170	163/165 (4 ↓ / 3 ↓)	162/157 (5 ↓ / 8 ↓)	134/136 (21 ↓ / 20 ↓)
Heinz bodies (No. of animals)	0/0	0/0	0/0	2/2

(./) Change in percent of the control; ↓ Decrease

Table 20: Liver weight, histopathological findings in the liver

Dose level (ppm) Sex	0 m/f	50 m/f	160 m/f	500-->800 m/f
Liver weight (g)	427/389	483/412	507/467	550/569
Siderosis, Kupffer cells (No. of animals)	2/1	1/2	4/4	4/4

Histopathology: 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 siderosis of Kupffer cells of the liver; all those elements reflecting the form of haemolytic anaemia.

Conclusion:

The NOAEL was 50 ppm based on microscopic findings noted in the blood and liver and increases in liver weights. In the original report, no conversion of this dietary concentration to a mean daily intake has been made. Since apparently a moist, semi-solid diet had been fed, it seems appropriate to use a conversion factor of 13 following the recommendation as given in the ”Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticide Residues” (Anonymous, 2000, ASB2013-4646) in its Appendix I, ”Approximate relation of parts per million in the diet to mg/kg bw per day”. Thus, a mean daily intake of 3.8 mg/kg bw should be assumed. In the previous evaluation, however, 3.2 mg/kg bw/day was given. This figure had been provided by the notifier and seems realistic but the mathematical calculation on the basis of actual food intake and body weight is not available.

Bhide (1990; TOX9500341): Subchronic oral toxicity study (90 days) with isoproturon in dogs;

The study is claimed to be in compliance with OECD principles of GLP and the current OECD guideline (Subchronic Oral Toxicity - Non-rodent; 409).

In comparison to the OECD guideline the reported study has the following deficiencies: The breed of the experimental animals was not defined. The test material stability and achieved concentrations in the non-rodent diet were not tested or reported. Clinical laboratory investigations (haematology, clinical biochemistry) were carried out only once at the end of the test period. Necropsy did not include an examination of a peripheral nerve. Although the author claimed a statistical treatment of the results there was no statistical significance of effects indicated in the tabular form of the summarised data. The study is considered to be supplementary.

Material and methods:

The test material (isoproturon 98 % batch not quoted) was administered daily to groups of 4 male and 4 female dogs for a 90 day period at concentrations of 0, 50, 150 or 500 ppm in the diet. A supplementary group of 4 male and 4 female rats was treated with 500 ppm for 90 days and observed for reversibility of toxic effects for a post-treatment period of 28 days. The control group received only the basal diet (boiled mutton and vegetables, milk and bread). Tap water was freely available at all times. Feed consumption and body weights were recorded weekly. Eye examinations were performed prior to the first treatment and in week 13 of the study. Blood samples for haematology and clinical biochemistry were collected at the end of the test period for all groups. Haematology samples were assayed for: RBC, WBC, diff. WBC count, Hb, haematocrit, PCV, platelets and Heinz bodies. Clinical chemistry samples were assayed for: Ca, Na, K, inorganic phosphorous, chloride, urea nitrogen, creatinine, bilirubin, glucose, AST, ALT, GGT and methaemoglobin. Urinalysis was not carried out. The animals were sacrificed one day following the last administration of the test substance or 28 day post treatment and subjected to necropsy. Selected organs in each animal in each group were weighed and fixed for histological examinations. Body weight, feed consumption, organ weights and clinical laboratory data were claimed to be analysed with statistical methods. It is reported that the Dunnett-test-(many to one t-test) was applied for the comparison between the treated groups and the control group for each sex.

Findings:

There were no unscheduled deaths. Dogs receiving 500 ppm showed emesis, intermittent diarrhea and reduced locomotor activity. One male animal receiving the dose of 150 ppm exhibited emesis and diarrhea. Dogs receiving 500 ppm showed a decrease in body weight gain. After the termination of the treatment the animals of the high dose group were found to regain the body weight gain. A slight reduction in body weight gain was observed in animals treated at the dietary dose level of 150 ppm. Feed consumption of the animals of the high dose group was found to be reduced. After the termination of the treatment no such reduction in the feed intake was seen in these animals. The values of Hb, PCV and RBC were found to be lowered in both male and female animals of the high dose group. The presence of Heinz bodies was also seen in these animals. After the recovery time these changes were found to be reversed. The values of total protein in blood were found to be slightly lowered while the level of methaemoglobin was found to be elevated in animals of the high dose group. After the recovery time the values were found to be in a normal range. Haemosiderosis (1 animal), lymhoid depletion (1 animal) and hyperplasia of bone marrow (1 animal) were observed in animals receiving the high dose.

Table 21: Body weight, feed consumption

Dose level (ppm) **Sex**	**0** **m/f**	**50** **m/f**	**150** **m/f**	**500** **m/f**
Body weight, wk 0 (kg)	6.67/6.90	6.68/6.65	6.65/6.65	6.72/6.58
Body weight, wk 13 (kg)	8.39/8.65	8.69/8.39	8.09/7.86	7.55/7.22
Feed consumption, wk 0 (g/day)	484/479	480/478	483/474	471/479
Feed consumption, wk 13 (g/day)	486/480	477/472	452/442	402/370

Table 22: Hematological parameters

Dose level (ppm) **Sex**	**0** **m/f**	**50** **m/f**	**150** **m/f**	**500** **m/f**
Hb (g/l)	136/131	132/130 (3 ↓ / 1 ↓)	134/124 (1.5 ↓ / 5.4 ↓)	114/105 (16.2 ↓ /20 ↓)
RBC (T/l)	6.58/6.05	6.35/6.23 (3.5 ↓ / 2.9 ↑)	6.08/6.08 (7.6 ↓ / 0.5 ↑)	5.15/5.03 (21.8 ↓ / 6.9 ↑)
Methemoglobin (%)	0.30/0.35	0.30/0.28 (0 / 20 ↓)	0.28/0.28 (6.7 ↓ / 20 ↓)	1.20/1.18 (400 ↑ / 337 ↑)

(./.): Change in percent of the control; ↓ Decrease; ↑ Increase

Conclusion:

The NOAEL was 50 ppm, based on clinical signs and the reduction in body weight gain. In the original report, no conversion of this dietary concentration to a mean daily intake has been made. The description of the diet is not sufficient to find out whether it was a moist, semi-solid one that had been fed or a dry one but the amount of consumed food points to the first option. Thus, it seems appropriate to use a conversion factor of 13 following the recommendation as given in the ”Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticide Residues” (Anonymous, 2000, ASB2013-4646) in its Appendix I, ”Approximate relation of parts per million in the diet to mg/kg bw per day”. Thus, a mean daily intake of 3.8 mg/kg bw may be calculated. In the previous evaluation, 1.25 mg/kg bw/day had been roughly calculated, apparently not taking into account the composition and structure of the diet.

Monkey

Bhide (1984; TOX9651093): Subacute oral toxicity for 90 days in monkey of isoproturon (technical).

The study is not claimed to be GLP compliant. No test guideline is quoted, inhouse methodology was used as described below. In comparison to the current OECD guideline (409) for a subchronic oral toxicity (Non-rodent 90-day Study) the reported study has a lot of deficiencies. The purity of the test substance, test material stability and achieved concentrations in the diet were not tested or reported. There was a limited program of clinical and pathological examinations. There was no electrolyte balance investigated. An ophthalmological examination prior to the administration of the test substance was not done or reported. Necropsy of the jejunum, rectum and a peripheral nerve was not carried out. There were no data summarised in tabular form of the results of the gross necropsy and histopathology, showing for each test group the number of animals with lesions, the type of lesions and the percentage of animals displaying each type of lesion. No special statistical analysis was applied to the data. The study is considered to be supplementary.

Material and methods:

The study included a dose range-finding study. In this study monkeys were treated with isoproturon orally at dose levels of 0, 80, 160, 320 and 640 mg/kg bw/d for 14 days. Cage-side observations, body weight, organ weights and gross pathology were noticed. In the main study isoproturon (technical) in 0.2 % agar solution in water was administered by gavage to groups of 3 male and 3 female monkeys (Macaca mulatta; obtained from Lucknow) once daily seven days a week for 90 days at dose levels of 0, 50, 150 and 450 mg/kg bw/d. A supplementary group of 3 male and 3 female monkeys was treated with 150 mg/kg bw/d for 90 days and observed for reversibility of toxic effects for a post-treatment period of 30 days (reversal group). All the animals were observed daily for toxicological symptoms. The quantity of feed consumed by each monkey was recorded daily and the mean daily intake for the week was calculated for each monkey and each group. The weight of each monkey was recorded on 0 day and at weekly interval throughout the course of the study. The group mean body weights were calculated. Laboratory investigations were done on day 0, 46, 91 and 121 in animals fasted overnight. Blood samples were collected in the morning. Haematology samples were assayed for: Hb, PCV,RBC, WBC, differential white blood cell count, platelets, reticulocytes, prothrombin time and Heinz bodies. Clinical chemistry samples were assayed for: BUN, ALP, ALT, total serum protein and blood glucose. Urine samples were monitored for: pH, glucose, protein, ketones, blood and microscopy of the sedimentation. All the animals (except reversal group) were sacrificed on 91st day. Necropsy of all the internal organs of each animal was carried out and the weights of following organs were recorded: liver, kidneys, heart, spleen, adrenals, testes, ovaries, thyroids. The organ weights were recorded as absolute values and their relative values (i.e. percent of the body weight) were calculated. Pieces from the following organs were taken and preserved for histopathological examination: adrenal, aorta, bone marrow, brain, colon, duodenum, eyes, gall bladder, heart, ileum, kidneys, liver, lungs, lymphnodes, oesophagus, ovaries, pancreas, pituitary, salivary gland, seminal vesicles, skin, spleen, stomach, testes, thymus, thyroid, urinary bladder, uterus.

Findings:

A decrease in locomotor activity, diarrhoea was observed in some animals treated with 150 mg/kg bw/d. Animals treated with 450 mg/kg bw/d exhibited a decrease in locomotor acivity, diarrhoea, haemorrhagic patch on the upper eyelids and gradual cachexia. A monkey of the highest dose group died on 74th day of treatment. A gradual reduction in body weight gain and slight reduction in the feed intake was observed in animals treated with doses of 150 and 450 mg/kg bw/d. This reduction in body weight gain was more in the high dose group. After the treatment animals of the reversal group regained the increase in body weight and a reduction in feed intake was not seen. A slight reduction in Hb, PCV and RBC was noted in both male and female animals treated with doses of 150 mg/kg bw/d. In animals treated with 450 mg/kg bw/d, there was a marked reduction in Hb, PCV and RBC, and an increase in reticulocyte count. Heinz bodies were also observed in this group. The reduction in Hb, PCV and RBC was found to be reversed after the treatment in the case of the reversal group. Levels of methaemoglobin were found to be elevated in animals of the high dose group. The relative weight of the spleen and the liver of animals treated with doses of 150 and 450 mg/kg bw/d was found to be elevated. A few animals of the high dose group indicated hepatosis of slight to moderate degree in addition to chronic congestion. This was accompanied by congestion and lymphoid hyperplasia with an increased number of histiocytes in the spleen. Hyperplasia of bone marrow was evident. Above changes in liver, spleen and bone marrow were seen in animals treated with 150 mg/kg bw/d to a considerable lesser degree.

Table 23: Body weight, feed consumption

Dose level (mg/kg bw/d) **Sex**	**0** **m/f**	**50** **m/f**	**150** **m/f**	**450** **m/f**
Body weight, wk 0 (kg)	2.52/2.50	2.42/2.53	2.72/2.52	2.60/2.32
Body weight, wk 13 (kg)	4.80/4.57	4.63/4.53	4.25/3.65	3.20/3.27
Feed consumption, wk 1 (g/day)	480/497	488/493	483/477	487/487
Feed consumption, wk 13 (g/day)	492/483	495/493	323/323	225/240

Table 24: Hematological parameters

Dose level (mg/kg bw/d) **Sex**	**0** **m/f**	**50** **m/f**	**150** **m/f**	**450** **m/f**
Hb (g/l)	150/148	151/149	118/121 (21.4 ↓ / 18.2 ↓)	66/63 (56 ↓ / 57 ↓)
RBC (T/l)	5.6/5.2	5.4/5.4	3.9/4.0 (30 ↓ / 23 ↓)	3.0/3.0 (46 ↓ / 42 ↓)
Reticulocytes (%)	0.9/1.3	1.3/2.1 (144 ↑ / 162 ↑)	2.4/2.8 (267 ↑ / 215 ↑)	9.7/11.1 (1078 ↑ / 854 ↑)
Methemoglobin (%)	0.6/0.6	0.7/0.6	0.8/0.7 (133 ↑ / 117 ↑)	1.3/1.4 (217 ↑ / 233 ↑)

(./.): Change in percent of the control; ↓ Decrease; ↑ Increase

Table 25: Organ weights

Dose level (mg/kg bw/d) Sex	0 m/f	50 m/f	150 m/f	450 m/f
Liver weight (g)	119/128	121/129	116/110	93/99
Relative liver weight (% of bw)	2.48/2.81	2.60/2.84	2.73/3.01	2.93/3.03
Spleen weight (g)	7.2/6.8	6.3/6.5	7.4/6.9	6.9/6.8
Relative spleen weight (% of bw)	0.15/0.15	0.14/0.14	0.17/0.19	0.22/0.21

Conclusion:

The NOAEL is 50 mg/kg bw/d, based on a reduction in body weight gain, Hb, PCV and RBC and changes in the liver, spleen and bone marrow.

4.7.1.2 Repeated dose toxicity: inhalation

Adequate data to assess inhalation toxicity were evaluated in the form of 14 day and 90 day inhalation studies in the rat.

Owen and Glaister (1982; TOX9551876): 2 week inhalation toxicity study in the rat.

It is claimed by the authors that the study was performed to the Indian Pesticide Guidelines and that the study is GLP compliant. The test protocol compares favourably with the current OECD test guideline (412) for repeat dose inhalation toxicity, with only very minor deficiencies in the range of clinical biochemistry tests performed and a lack of testes weight at necropsy. The stability of the test substance was not determined. No special statistical analysis was applied to the data. The study is considered to be supplementary.

Material and methods:

Groups of 5 male and 5 female Sprague Dawley rats (Crl:CD(SD)BR Strain, Charles River UK Ltd) were exposed, whole body, 6 h/day, 5 day/week for 2 weeks to a respirable dust, generated from the undiluted test substance, (isoproturon 98.7 % Batch OP 82.020) at the target concentrations of 0 (control), 10, 50 or 250 mg/m3. The absolute exposure chamber concentrations were measured hourly. Particle sizes were determined once each day.

Clinical signs were recorded before and after each daily exposure. Body weights were recorded weekly. Feed consumption was recorded over the 14-day period.

Clinical laboratory studies (haematology, urinalysis and blood chemistry) were performed pre-test, during treatment and prior to termination for all animals.

Haematology samples were assayed for WBC, RBC, Hb, MCH, PCV, MCHC, MCV, Reticulocytes platelets and prothrombin time. Clinical chemistry samples were assayed for: ALAt, AST, AP, Na, K, Cl, glucose, blood urea nitrogen, total protein and protein electrophoresis. Urine samples were monitored for; volume, pH, SG, protein, blood, bilirubin, glucose, ketones, urobilinogen and microscopy of the spun sediment.

At termination, a gross necropsy was performed and selected organs were weighed. Selected organs and tissues from the control and high dose group were examined.

Findings:

Table 26: Achieved atmospheric concentrations

Group Number	Target concentration (mg/m^3)	Mass median aerodydamic diameter (µm)	Mean measured concentrations (mg/m^3)
1	0	-	0
2	10	2.66 ± 0.76	11.1
3	50	2.88 ± 0.82	51.1
4	250	4.18 ± 1.45	223.8

There were no abnormalities attributable to treatment. Three animals (two controls and one low dose group) died as a result of the terminal bleed. Animals in all groups exhibited similar clinical signs. Body weight increase and feed consumption was similar in all groups.

There were no significant intergroup differences in any of the laboratory studies.

3 animals that died during the routine orbital sinus bleed procedure had reddened lung lobes. 1 of these animals (a control) had reddened liver lobes. These effects were not considered attributable to treatment.

Males receiving 250 mg/m^3 showed increases in absolute and relative (to body weight) mean liver weight. No effect was observed in females. In the absence of histopathological changes the effect in males was not considered to be toxicologically significant.

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. There was no evidence to suggest of any local effects in the lungs or systemic effects produced by inhalation of the test substance.

Conclusion:

No local or systemic toxicity were produced by inhalation of the test substance for 2 weeks. Therefore the NOEL is >250 mg/m^3 (>0.25 mg/l).

Anonym (1985; TOX9651095): Subacute inhalation toxicity study of avanon (isoproturon) technical in albino rats (14 days nose only inhalation exposure).

It is quoted that the study was based on the guidelines laid in the „recommendations of Dr. Gaitonde`s subcommittee on Pesticide toxicology". The study is not claimed to be GLP compliant. In comparison to the current OECD guideline for a 28 day or 14 day inhalation study (Test No. 412) the reported study has many deficiencies. The origin and the breed of the experimental animals were not exactly specified. No information about the inhalation equipment was given. Exposure data were not completely reported. Clinical biochemistry determinations in blood were limited (AlP, ALT, glucose, total protein, urea nitrogen). There was no detailed description of gross and histopathological individual findings. No special statistical analysis was applied to the data. The study is considered to be supplementary.

Material and methods:

Two groups of 8 male and 8 female rats were exposed to dust aerosols of isoproturon technical (origin, purity or batch number not reported) at the concentration level of 173.61 mg and 664.49 mg of active ingredient per cubic metre of air inhalation route (nose only exposure) over a period of 6 hours per day, 5 times a week for 14 days exposure. A third group of 8 rats per sex was also exposed to an atmosphere of filtered air under otherwise identical exposure conditions and duration. Body weights, signs of reaction to exposure and mortalities were observed. Clinical examinations included haematology and serum biochemistry. Urinalysis was performed using a multistix. After 14 days of exposure all animals were killed and subjected to detailed macroscopic examination. Lungs, adrenals, gonads, kidneys and liver were weighed. Microscopic examination from rats of the control, low and high dose groups were carried out.

Findings:

The mean body weights of male and female animals all groups were depressed during the first week of exposure and continued to decrease throughout the rest of the exposure period in case of rats from high dose groups. No compound related histomorphological changes were noticed in any of the tissues collected from the control and treated groups except the lung tissues of 3 males and 2 females from the high dose group revealed interstitial pneumonitis with mononuclear cell infiltration.

Conclusion:

It was concluded that under the condition of this study repeated exposure by inhalation, of isoproturon technical dust at the level of 173.61 mg/m^3 (0.173 mg/l) of air for 14 days did not produce any observable toxicity in albino rats, hence may be considered as NOEL.

90-day inhalation toxicity

Bhide (1990; TOX9500342): Subchronic inhalation toxicity study (90 - days) with isoproturon in rats.

It is claimed by the author that the study was performed with the OECD guideline for the testing of chemicals (Subchronic Inhalation Toxicity: 90-day Study; Test No. 413) and that the study is GLP compliant. The test protocol showed some deviations from the current guideline. The breed of the experimental animals was not exactly defined. The stability of the test substance was not determined. Only one exposure concentration was used. Necropsy did not include an examination of a peripheral nerve. Although the author claimed a statistical treatment of the results there was no statistical significance of effects indicated in the tabular form of the summarised data. The study is considered to be supplementary.

Material and methods:

One group of 10 male and 10 female Wistar rats (I. I. T. animal house) was exposed, 6 h/day, 5 day/week for 13 weeks to a respirable dust of the test material (isoproturon technical of Montari Industries limited, purity: 98 %) at the exposure level of 6.32 mg/L. An additional control group of 10 male and 10 female rats was exposed to filtered air. The animals were exposed to the test material by mouth and nose in a dynamic chamber. Particle sizes were determined twice each day.

Clinical signs were recorded once daily. Body weights were recorded weekly. Feed consumption was recorded weekly.

Clinical laboratory studies (haematology, and blood chemistry) were performed at the end of the study. Haematology samples were assayed for WBC, diff. WBC count, RBC, Hb, PCV, platelets. Clinical chemistry samples were assayed for: Ca, phosphorus, Na, K, Cl, glucose, AST, ALT, GGT, blood urea nitrogen, albumin, total protein, creatine, and bilirubin.

At termination, a gross necropsy was performed and selected organs were weighed. Selected organs and tissues from the control and dose group were examined. It was claimed that the students-t-test was used to examine the statistical significance of body weight, feed consumption, organ weights and clinical laboratory data.

Findings:

Table 27: Summary of exposure chemical environment

Group	Chemical concentration nominal (mg/l)	Chemical concentration measured (mg/l)
Control	0	0
Isoproturon	6.32 ± 0.52	0.73 ± 0.12

The mass median aerodynamic diameter of all atmospheres were within the respirable range of 0 - 7 µm on all occasions. Nasal secretion, lacrimation, wet fur and a slight temporary reduction in respiratory rate were observed in treated animals.

Conclusion:

Isoproturon did not exhibit any observable local or systemic toxicity except respiratory irritation at the nominal concentration of 6.32 mg/l when exposed in a dynamic chamber for 6 hour a day for 5 days a week for 13 consecutive weeks. Based on these results the NOAEL is 6.32 mg/l.

4.7.1.3 Repeated dose toxicity: dermal

Adequate data to assess percutaneous toxicity were evaluated in the form of 21 day (rat, rabbit) and 90 day (rabbit) studies.

Rat

Dikshith (1982; TOX9551878): Report on dermal subacute (21 days) toxicity of isoproturon technical in male and female albino rats.

An in-house method was used. In comparison to the current OECD guideline (410) for a 21 day dermal study the reported study has deficiencies. The starting weight and the breed of the rats were not specified, the test substance was applied as a solution in acetone with no ‘negative’ control to investigate the effects of this solvent on the test system. During the study there was no attempt to limit the oral ingestion of the test substance by covering the application site or restraining the animals, although they were individually housed. The group size was four per sex per dose and the laboratory and terminal investigations do not meet current requirements. No special statistical analysis was applied to the data. The study was not GLP compliant. The study is considered to be supplementary.

The results of this study were reported in Dikshith et al, 1990 (TOX9550730).

Material and methods:

Isoproturon, (no details specified) dissolved in acetone was applied to the clipped (approximately 4x4 cm) dorsal surface of albino rats (strain not specified) at doses of 0 (control), 250, 500 or 1000 mg/kg bw/d. Test material was applied to the clipped dorsal surface by ‘painting’ with various concentrations of test substance dissolved in acetone to achieve final doses in terms of mg/kg body weight/day. Controls were painted with acetone only. Treatment was daily for 3 weeks. Pelleted diet (Hind, Lever, India) and water were available *ad libitum*. Body weight, feed and water intake were measured weekly. Signs of toxicity were also observed and recorded.

At termination, all animals were sacrificed. A blood sample was taken and haematological (RBC, WBC and Hb determinations) parameters were evaluated. Serum bilirubin and blood sugar were estimated. Serum and liver homogenate were used for the estimation of GOT, GPT, AP and protein.

Selected organs and tissues were removed, weighed and examined histopathologically.

Findings:

There were 2 mortalities, one male from each of the groups receiving 1000 and 500 mg/kg bw/d. There were no other signs on toxicity. A decrease in body weight was noted in females at all 3 dose levels. However this effect was not clearly dose related and was quoted as being not highly significant by the authors. 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 of males and females and at 500 mg/kg bw/d a reduction in feed consumption in males only. Again, these effects were not considered to be biologically significant by the report authors.

No effects on water consumption were observed.

Haematological alterations were considered to be minimal. RBC counts were decreased in males at all dose levels but in females only at 1000 mg/kg bw/d. Haemoglobin concentrations were decreased in males and females at all dose levels. Neutrophil and lymphocytes were decreased and increased respectively at all 3 dose levels.

Alterations in biochemical parameters both in the serum and livers were considered to be minimal and did not reflect test substance toxicity.

Gross pathology did not reveal any treatment related changes. Marginal increases of relative (to body weight) liver, kidney and adrenal weights were noted in females receiving 1000 mg/kg bw/d. At 500 mg/kg bw/d there were marginal increases in relative liver weight of females and relative kidney and brain weights of males.

No histopathological abnormalities were detected.

Conclusion:

The NOEL is 250 mg/kg bw/d based on a single, possibly treatment related, death at 500 mg/kg bw/d.

Rabbit

Hollander and Weigand (1975; TOX9551877): Acute dermal toxicity in rabbits, 5 treatments.

No test guideline is quoted. The inhouse methodology used (as described below) is non standard. A limited number of parameters were investigated. Clinical (haematology, clinical chemistry and urinalysis) and pathological examinations were not done. No statistcal analysis was applied. Therefore the results should be treated as a range finding study capable of indicating topical and clinical response. The NOEL derived should be interpreted accordingly. At the time of the study GLP compliance was not compulsory and the study is not claimed to be compliant. The study is considered to be supplementary.

Material and methods:

Isoproturon was applied in the form of a 40 % suspension in sesame seed oil at dose levels of 400 or 800 mg/kg bw onto the depilated nape skin of groups of 5 yellow/silver breed rabbits. The animals were treated 5 times on successive days. Oral exposure was avoided by placing a plastic sleeve around each animal's neck. Five hours after each treatment, the nape skin of the rabbits was washed with tap water and a sponge to remove any residual test material. Following the treatment, the surviving animals were kept under observation for a period of 10 to 17 days. Animals were fed on Standard Altromin K diet (Altromin GmbH, Lage/Lippe).

Findings:

The area of treated skin of all rabbits was slightly reddened, chapped and slightly squamous during the treatment period.

One mortality occurred in the 800 mg/kg bw dose group (3 day after the end of treatment). No clinical symptoms were shown by this or any other animal.

Body weights decreased in rabbits of both treatment groups over the treatment period. The animal that died showed a loss of 204 g and the remainder were in the range 22 - 168 g. At the end of the 17-day observation period all surviving animals had regained or exceeded their initial weight.

Conclusion:

The NOEL for dermal toxicity in this study was 400 mg/kg bw/d based on a single, possibly treatment related death in the 800 mg/kg bw/d group.

Bhide (1996; TOX9651094): Subacute dermal toxicity (for 21 days in rabbits) of isoproturon (tech.).

No test guideline is quoted, inhouse methodology was used. The study is not claimed to be GLP compliant. In comparison to the current OECD guideline for a 21 day dermal study (Test No. 410) the reported study has certain deficiencies. The breed of the experimental animals was not exactly specified. Only 3 female and 3 male animals were used at each dose level. There was no electrolyte balance investigated. Pathological examinations were made on all animals 14 days after the treatment period. No special statistical analysis was applied to the data. The study is considered to be supplementary.

Material and methods:

A range-finding study was carried out to assess the toxicity of daily dermal application with isoproturon technical (no details specified) for 5 days and 21 days to rabbits. This study included the following examinations: symptoms, body weight gain, organ weights and gross pathology of selected organs. In the main study isoproturon technical moistened with physiological saline was applied to the clipped area of skin of rabbits (Albino NZW, bred at the testing facility) at doses of 0 (control), 500, 1000 or 2000 mg/kg bw/d. The test material was applied on each of 5 days per week for 6 hours per day for 3 consecutive weeks. The medication was stopped and the animals were observed for a further period of 2 weeks. At the end of this rest period of 2 weeks, all the animals were sacrificed and subjected to necropsy including macroscopic pathology, organ weight analysis and histopathology. The quantity of feed consumed was recorded daily and the mean weekly intake was calculated for each rabbit and each group. The weight of each rabbit was recorded at weekly interval throughout the study. Skin irritation readings were recorded and scored daily according to the method of Draize. Signs of toxicity were also observed and recorded. Laboratory investigations were done on day 0, 21 and 35 in all rabbits.

Findings:

No symptoms of toxicity, skin reactions or mortality were observed at any dose levels. Body weight change and feed consumption of treatment groups were comparable with control groups. Few findings of gross pathology and histopathology randomly distributed in animals of different groups were seen within the normal limits and not attributable to isoproturon treatment.

Conclusion:

The NOEL is 2000 mg/kg bw/d, based on no relevant findings at any dose level.

Percutaneous 90-day toxicity

Bhide (1990; TOX9500343): Subchronic dermal toxicity study (90 - days) with isoproturon in rabbits.

It is claimed by the author that the study was performed in compliance with the OECD guideline for the testing of chemicals (Subchronic Dermal Toxicity: 90-day Study; Test No. 411) and OECD principles of GLP. The method followed was similar to contemporary guideline studies with the exception that the breed of the experimental animals was not exactly defined and necropsy did not include the examination of a peripheral nerve. The study is considered to be acceptable.

Material and methods:

Isoproturon, (98 % purity, supplied by Montari Industries Ltd., New Dehli) was applied to the clipped, dorsal surface (approximately 10 % of the body surface) of 10 male and 10 female NZW rabbits after moistening it with physiological saline at doses of 0 (control) and 1000 mg/kg bw/d. Controls received only saline. Treatment was 6 hours per day, 5 days a week for 13 consecutive weeks. Pelleted diet (Lipton India Ltd., Bangalore) and water were available *ad libitum*. Body weight, feed and water intake were measured weekly. Signs of toxicity were also observed and recorded. At termination, all animals were sacrificed. Blood samples were taken and haematological and clinical biochemistry parameters were evaluated. Organs and tissues were removed, weighed and examined histopathologically. It is claimed by the author that body weight, feed consumption, organ weights and clinical laboratory data were analysed with statistical methods and that the Dunnett-test-(many to one t-test) was applied.

Findings:

Application of isoproturon to the intact skin of rabbits was not found to produce any observable effects in the following parameters: clinical observations, skin reaction at the site application, gain in body weight, feed consumption, haematology, clinical biochemistry and histology.

Conclusion:

The NOEL was found to be 1000 mg/kg bw/d, because there were no observed effects.

4.7.1.4 Repeated dose toxicity: other routes

No other routes were applied.

4.7.1.5 Human information

There is no human information available.

4.7.1.6 Other relevant information

There is no other relevant information available.

4.7.1.7 Summary and discussion of repeated dose toxicity

In subchronic oral studies, the liver and the blood were the main target organs. Anaemia was observed at or above dietary concentrations of approximately 800 ppm (62 mg/kg /day) in rats (Wragg et al 1991; TOX9300281), 500 ppm (12.5 mg/kg bw/d) in dogs (Scholz & Brunk 1973; TOX9551875 Bhide 1990; TOX9500341) and 150 mg/kg bw/d in monkeys (Bhide 1984; TOX9651093). 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. Liver effects were confined to organ weight increases in the rat, dog and monkey beginning at 500 ppm (12.5 mg/kg bw) in dogs (Scholz & Brunk 1973; TOX9551875). In higher doses histopathological liver changes were due to haemolytic anaemia.

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

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

No human data are available for IPU to indicate significant toxicity in humans. However, there are observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed.

For animal studies the evaluation presented in *Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD* can be followed.

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

Liver effects were confined to organ weight increases in the rat, dog and monkey beginning at 500 ppm (12.5 mg/kg bw) in dogs (Scholz & Brunk 1973; TOX9551875). The findings in the liver (increased weight, bile duct proliferation, degeneration of hepatocytes, basophilic foci) were associated with increased enzyme activities (AP, ALT, AST) and reductions in total protein or albumin. There was evidence that the effects seen were reversible. In higher doses histopathological liver changes were due to haemolytic anaemia. Liver toxicity was not observed after dermal and inhalation exposure. The relative weight of the spleen and the liver of monkeys treated with doses of 150 and 450 mg/kg bw/d was found to be elevated. A few animals of the high dose group indicated hepatosis of slight to moderate degree in addition to chronic congestion. This was accompanied by congestion and lymphoid hyperplasia with an increased number of histiocytes in the spleen. The changes in liver, spleen and bone marrow were seen in monkeys treated with 150 mg/kg bw/d to a considerable lesser degree. The hepatocellular changes are not considered significant or severe enough, may be adaptative, and were not observed at dose levels below Guidance Values to warrant classification for STOT RE 2 according to CLP criteria.

Signs of toxic haemolytic anaemia were seen below the equivalent guidance value for oral 90-day studies (≤ 100 mg/kg bw/d). According to the review by Muller et al. 2006 (ASB2010-1656) on Hazard classification of chemicals inducing haemolytic anaemia, haematotoxic effects such as:

- a decrease in Hb levels by at least 20% in a 90-day study
- a decrease in Hb levels by at least 20% due to a combination of Hb reduction and MetHb increase
- haemoglobinuria that is not limited to the first three days of treatment in the repeated dose study in combination with other changes indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%).

A combination of Hb decrease and haemosiderosis may be regarded as sufficiently severe for consideration of classification/labelling.

Haematotoxicity (decrease in Hb by 20 % along with haemosiderin deposition in reticular cells and Kupffer cells of the liver), reported at the mid and high dose level at the end of the oral 90-day dog study (essentially at ≈ 4 or 12.5/20 mg/kg bw/d), is considered to sufficiently fulfil criteria for severity to warrant classification for STOT RE 2 (oral) according to CLP criteria.

Table 28: Toxicological results (at dose levels below the guidance values) in comparison with criteria of specific target organ toxicity – 28 day repeated exposure

Toxicological result	CLP criteria for STOT RE
Subacute oral studies: In the subacute oral studies the liver was found to be the main target organ in all three species. Effects were confined to organ weight increases. In rats the effect of liver weight increase at ≥ 105.6 mg/kg bw/d was shown to be completely reversible (Scholz & Weigand 1973; TOX9551871). In mice increased relative liver weight was observed at 307 mg/kg bw/d (Hunter et al 1979; TOX9551872). In dogs there were severe signs of weight loss and liver toxicity at the highest dietary level of 500 ppm that had been increased to 1250 ppm (mean daily intake > 30 – ca. 60 mg/kg bw)15 days. However, liver effects (generalized icterus, increase of serum bilirubin concentration, pathological increase of serum enzyme activities, positive bilrubion reaction in the urine and necrosis of the liver, bile-duct proliferation and pericholangitis) were recorded in one bitch only. In addition anaemia was observed in one animal (Kramer & Brunk 1975; TOX9551873).	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. **Equivalent guidance value for STOT RE 1:** **Oral, rat: 28-day: ≤ 30 mg/kg bw/d** Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. **Equivalent guidance value for STOT RE 2:** **Oral, rat: ≤ 300 mg/kg bw/d**

Table 29: Toxicological results (at dose levels below the guidance values) in comparison with criteria of specific target organ toxicity – 90-day repeated exposure

Toxicological result	CLP criteria for STOT RE

Subchronic oral studies: Oral administration of IPU for up to 13 weeks was associated with changes in the liver and in the blood. The major change in the blood was anaemia, seen at or above dietary concentrations of approximately 800 ppm (62 mg/kg /day) in rats (Wragg et al 1991; TOX9300281), at 500/800 ppm (38 – 61 mg/kg bw/d, Scholz & Brunk 1973; TOX9551875) or 500 ppm (38 mg/kg bw/d; Bhide 1990; TOX9500341) in dogs and 150 mg/kg bw/d in monkeys (Bhide 1984; TOX96-51093). The severity of the anaemia appeared to be distinctly dose-related. Increasing doses revealed toxic haemolytic anaemia associated with Heinz bodies, methemoglobinaemia, hyperplastic bone marrow, extramedullary hematopoiesis and increased hemosiderin in liver, kidneys and bone marrow. Dogs were most sensitive. Liver effects were confined to organ weight increases in the rat, dog and monkey beginning at 500 ppm (32 mg/kg bw) in dogs (Scholz & Brunk 1973; TOX9551875). In higher doses histopathological liver changes were due to haemolytic anaemia.	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. **Equivalent guidance values for STOT RE 1:** **Oral, rat: ≤ 10 mg/kg bw/d** Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. **Equivalent guidance values for STOT RE 2:** **Oral, rat: 90-day: ≤ 100 mg/kg bw/d**

Table 30: Toxicological results (at dose levels below the guidance values) in comparison with criteria of specific target organ toxicity – dermal and inhalation repeated exposure

Toxicological result	CLP criteria for STOT RE
Dermal administration: Following dermal administration, single decedents were seen at 500 and 1000 mg/kg bw/d (Hollander & Weigand 1975; TOX9551877; Dikshith 1982; TOX9551878) while other studies gave no evidence for systemic or local toxicity at 1000 or 2000 mg/kg bw/d (Bhide 1996; TOX9651094; Bhide 1990; TOX9500343). Inhalation studies: In a subacute inhalation study there were no signs of reaction to treatment of rats at the highest dose of 0.25 mg/l (Owen & Glaister 1982; TOX9551876). In a second subacute inhalation study an interstitial pneumonitis in rats of the high dose group (0.6 mg/l) was reported (Anonym 1985, TOX9651095). However, rats in a subchronic inhalation study showed only respiratory irritation at a concentration of 6.32 mg/l (Bhide 1990; TOX9500342).	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. **Equivalent guidance values for STOT RE 1:** **Dermal, rat: 90-day: ≤ 20 mg/kg bw/d** **Inhalation (dust/mist/fume), rat: 28-day: ≤ 0,02 mg/L** Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. **Equivalent guidance values for STOT RE 2:** **Dermal, rat: 90-day: ≤ 200 mg/kg bw/d** **Inhalation (dust/mist/fume), rat: 28-day: ≤ 0,2 mg/L**

❷ Value calculated by means of a conversion factor of 0.025 (Anonym, 2000; ASB2013-4646)

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

It is concluded that the guidance criterion for severity and potency, as outlined in the Guidance on the Application of the CLP criteria, is regarded as being satisfied for blood effects (anaemia) occurring in the oral 90-day repeated exposure studies in rats and dogs below the Guidance Value of 100 mg/kg bw/d.

There were no haematological effects in other long-term/repeated dose toxicity studies such as carcinogenicity, neurotoxicity or reproductive toxicity studies that could provide evidence of specific target organ toxicity.

Repeated dose toxicity studies by inhalation and dermal routes conclusively demonstrate no haematoxicity that would deserve classification.

Hence, it is proposed to classify IPU for STOT-RE 2 (oral) ("H373: May cause damage to organs (blood) through prolonged or repeated oral exposure").

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 isoprouton 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 haematoxicity 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 precence 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 diarrhea, 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 releative 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 high dose 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 siderosis 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 diarrhea and reduced locomotor activity was 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 diarrhea. 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), lymhoid 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 Bhide, 1984	Cat 1: C ≤ 10 mg/kg bw/d	N/A	Cat 2: 10 < C ≤ 100 mg/kg bw/d	None.
13-week dietary Rat 0-400-1500-5000 ppm Similar to OECD TG 408, not GLP	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).

Dickhaus and Heisler 1987				
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. on the basis of 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. RAC does however not agree with specifying a route of exposure.

4.9 Germ cell mutagenicity (Mutagenicity)

This endpoint is not addressed by this proposal.

4.10 Carcinogenicity

This endpoint is not addressed by this proposal. There are no new data available. The current entry in Annex VI (CLP regulation) is supported.

4.11 Toxicity for reproduction

Summary of the reproductive toxicity endpoints assessed during the EU review:

Table 31: Summary table of relevant reproductive toxicity studies

Study	Dose levels	NOEL (mg/kg bw/d)	Target/main effects	Reference
2-generation, Wistar rat	0-80-400-2000 ppm	par: 5-10 [80 ppm] rep: 5-10 [80 ppm]	Feed intake, bw; implantations, litter size, pup weight ↓	Becker et al. 1989 *& TOX9551913
2-generation, Wistar rat	0-100-200-400 ppm	par: ~10❷ [100 ppm] rep: ~10❷ [100 ppm]	Bw; pregnancy rate, pup weight ↓, retarded spermatogenesis	Bhide 1990; § TOX9300293
2-generation, Wistar rat	0-100-200-400 ppm	par: ~10❷ [100 ppm] rep: ~10❷ [100 ppm]	Feed intake, bw; mating index, pup weight ↓, retarded spermatogenesis	Bhide 1991; *& TOX9651099
2-generation, Wistar rat	0-100-200-400 ppm	par: ~10❷ [100 ppm] rep: ~10❷ [100 ppm]	Feed intake, bw; pregnancy rate, pup weight ↓, retarded spermatogenesis	Bhide 1991; § TOX9500349
10 weeks, effect of IPU on male reproductive system, male albino rats	0, 200, 400 or 800 mg/kg bw/day, 6 days/ week	400 200	Epididymal sperm counts and motility ↓, abnormal sperm ↑, damaged seminiferous tubules ↑, impaired formezan deposition from glucose-6-phosphate and ß-hydroxysteroid dehydrogenase	Sarkar, S. et al, 1997 $ ASB2012-14739
Teratogenicity, CD rat	0-25-100-200 mg/kg bw/d	maternal: 25 developmental: 25	Bw, feed intake ↓; retarded ossification	Fritz 1978 § TOX9551914
Teratogenicity, Wistar rat	0-90-500 mg/kg bw/d	maternal: 90 developmental: 500	Mortality ↑, bw ↓	Sengupta 1985❶ TOX9651089
Teratogenicity, Wistar rat	0-20-80-320 mg/kg bw/d	maternal: 80 developmental: 80	Bw gain, foetal weight ↓; resorption index ↑	Dickhaus & Heisler 1987 * TOX9550735
Teratogenicity, Wistar rat	0-125-250-500 mg/kg bw/d	maternal: unclear developmental: 125	Clinical signs, feed intake ↑; resorption index ↑	Katdare 1991 § TOX9500350
Teratogenicity, Wistar rat	0-45-90-180 mg/kg bw/d	maternal: unclear developmental: 180	No developmental effects	Srivastava, Raizada, 1995 § TOX1999505
Teratogenicity, Chinchilla rabbit	0-12.5-50-100 mg/kg bw/d	maternal: 50 developmental: 100	Bw, feed intake ↓	Fritz et al. 1978 * $ TOX9551915
Teratogenicity, NZW rabbit	0-10-40-160 mg/kg bw/d	maternal: 40 developmental: 40	Bw, feed intake, foetal weight ↓	Dickhaus & Heisler 1987 *& TOX9550736

par: parental toxicity; rep: reproduction toxicity
❶ Study was considered not acceptable, but provided additional information
❷ Value calculated by means of a conversion factor of 0.1 (Anonym, 2000; ASB2013-4646)
* Acceptable according evaluation of the DAR dated 27 July 1999 (ASB2010-10305)
§ Supplementary according evaluation of the DAR dated 27 July 1999 (ASB2010-10305)
$ Supplementary according current evaluation
& The study is claimed to be in compliance with GLP and the OECD guidelines.

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In the two-generation reproduction toxicity studies in rats using dietary dose levels up to 2000 ppm, parental toxicity (reduced body weight gain and feed consumption) and reproductive toxicity (reduced mating index, pregnancy rate, number of implantations, litter size, pup weight) were seen at dose levels of 400 ppm or above. There was evidence of histopathological changes in testes (retarded spermatogenesis) in few F1 animals at 200 ppm and above. The parental, reproductive and offspring NOAEL was 100 ppm (about 10 mg/kg bw/d). Histological changes were already seen at 400 mg/kg bw/day and above.

4.11.1.2 Human information

There is no human information available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In the rat developmental toxicity studies using dose levels up to 500 mg/kg bw/d, 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 or above. There was no evidence of teratogenicity. The maternal and developmental NOAEL was 80 mg/kg bw/d.

In the rabbit developmental toxicity studies using dose levels up to 160 mg/kg bw/d, maternal toxicity (reduced body weight gain and feed consumption) were seen at 100 mg/kg bw/d and above and embryo/foetotoxicity (reduced foetal weight) at 160 mg/kg bw/d. There was no evidence of teratogenicity. The lowest relevant developmental NOAEL was 40 mg/kg bw/d. No further studies are planned.

4.11.2.2 Human information

There is no human information available.

4.11.3 Other relevant information

In a newly submitted published study (Sarkar et al, 1997, ASB2012-14739) decreased epididymal sperm counts and motility, and increased percentage of abnormal sperm were observed at 800 mg/kg bw/day in rats treated 6 days/ week for 10 weeks. Histological changes were already seen at 400 mg/kg bw/day and above.

4.11.4 Summary and discussion of reproductive toxicity

Taking into account the unavailability of epidemiological data there is no evidence to establish a causal relationship between human exposure to the substance IPU and reproduction toxicity. However, there is evidence of impaired fertility from results in appropriate animal studies.

In a two-generation reproduction toxicity study in rats the mean number of implantation sites and the corresponding number of living pups per litter were significantly decreased at a dietary dose level of

2000 ppm (about 134-263 mg/kg bw/day). Feed consumption and body weights of the F0 and F1 generations were significantly reduced at this dose level during all periods (Becker et al. 1989; TOX9551913).

Other two-generation reproduction toxicity studies in rats demonstrated a lower pregnancy rate (Bhide 1990; IIT No.1001; TOX9300293; Bhide 1991; IIT No. 1096; TOX9500349) and a lower mating index of F1 females (Bhide 1991; IIT No. 1088; TOX9651099) at 400 ppm (about 40 mg/kg bw/day), histopathological changes in the testes (retarded spermatogenesis) of F1 animals at 200 ppm (about 20 mg/kg bw/day) and above (Bhide 1991; IIT No. 1096; TOX9500349) or at 400 ppm (about 40 mg/kg bw/day) (Bhide 1990; IIT No.1001; TOX9300293; Bhide 1991; IIT No. 1088; TOX9651099). Parental toxicity was seen in these studies at dose levels of 400 ppm (about 40 mg/kg bw/day) on the basis of reduced feed consumption and body weight gains. Histopathological changes in the liver (hydropic degeneration) and the spleen (lymphoid hyperplasia) of F1 animals were seen at 200 ppm (20 mg/kg bw/day) and above, too (Bhide 1990; IIT No.1001; TOX9300293; Bhide 1991; IIT No. 1096; TOX9500349; Bhide 1991; IIT No. 1088; TOX9651099).

In a published study about the effect of isoproturon on male reproductive system decreased epididymal sperm counts and motility, and increased percentage of abnormal sperm were observed at 800 mg/kg bw/day in rats treated 6 days/ week for 10 weeks. Histological changes of the testes and histochemical activity of selected enzymes in testicular tissue were seen at 400 mg/kg bw/day and above (Sarkar, S. et al, 1997; ASB2012-14739).

4.11.5 Comparison with criteria

Taking into account the unavailability of epidemiological studies, clinical data and case reports there is no evidence to establish a causal relationship between human exposure to the substance IPU and reproduction toxicity. However, there is evidence of impaired fertility from results in appropriate animal studies.

Adverse effects on sexual function and fertility:

Table 32: Toxicological results concerning adverse effects on sexual function and fertility

Toxicological result	**CLP criteria**
In the two-generation reproduction toxicity studies in rats using dietary dose levels up to 2000 ppm, reproductive toxicity (reduced mating index, pregnancy rate, number of implantations, litter size, pup weight) were seen at toxic parental dose levels of 400 ppm or above. 2000 ppm (F0: 138-174 mg/kg bw/day; F1: 134-263 mg/kg bw/day): mean number of implantation sites and the corresponding number of living pups per litter ↓; feed consumption and bw (F0 and F1) during all periods ↓; (Becker et al. 1989; TOX9551913) ≥ 200 ppm (20 mg/kg bw/day❷): histopathological changes in the testes (retarded spermatogenesis), liver (hydropic degeneration) and spleen (lymphoid hyperplasia) of F1 animals; 400 ppm (40 mg/kg bw/day❷): bw gain ↓ in F1 parental animals and in F2 pups; (Bhide 1990; IIT No.1001; TOX9300293) ≥ 200 ppm (20 mg/kg bw/day❷): histopathological changes in liver (hydropic degeneration) and spleen (lymphoid hyperplasia) of F1 animals; 400 ppm (40 mg/kg bw/day❷): bw gains ↓ in F1 parental animals and in F2 pups, mating index ↓ of F1 females, retarded spermatogenesis in F1 males; (Bhide 1991; IIT No.1088; TOX9651099) ≥ 200 ppm (20 mg/kg bw/day❷): histopathological changes in testes (retarded spermatogenesis), liver (hydropic degeneration) and spleen (lymphoid hyperplasia) of F1 animals; 400 ppm (40 mg/kg bw/day❷): bw gains ↓ in F1 parental animals and in F2 pups; pregnancy rate ↓ of F1 females; (Bhide 1991; IIT No. 1096; TOX9500349). ≥ 400 mg/kg bw/day (40 mg/kg bw/day❷): incidence of damaged seminiferous tubules ↑, impaired formezan deposition from glucose-6-phosphate and ß-hydroxysteroid dehydrogenase ↑; 800 mg/kg bw/day (80 mg/kg bw/day❷): epididymal sperm counts ↓ and motility ↓; percentage of abnormal sperm ↑; (Sarkar, S. et al, 1997; ASB2012-14739).	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - and where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

❷ Value calculated by means of a conversion factor of 0.1 (Anonym, 2000; ASB2013-4646)

Adverse effects on development:

Table 33: Toxiclogical results concerning adverse effects on development

Toxicological result	**CLP criteria**
In the rat developmental toxicity studies using dose levels up to 500 mg/kg bw/day, maternal toxicity and embryo/foetotoxicity were seen at 100 mg/kg bw/day or above. There was no evidence of teratogenicity. ≥ 100 mg/kg bw/day: feed intake ↓ and bw gain ↓ in the dams; retardation of skeletal development (ossification) in foetuses; (Fritz 1978; TOX9551914) 500 mg/kg bw/day: mortality ↑; bw gain ↓; (Sengupta 1985; TOX9651089) 320 mg/kg bw/day: bw gain ↓ in the dams; bw ↓ of foetuses; index of resorptions (not statistically significant) ↑; (Dickhaus & Heisler 1987; TOX9550735) 125 mg/kg bw/d: clinical signs, feed intake ↑; ≥ 250 mg/kg bw/d: index of resorptions ↑; (Katdare 1991; TOX9500350) In the rabbit developmental toxicity studies using dose levels up to 160 mg/kg bw/day, maternal toxicity was seen at 100 mg/kg bw and above and embryo/foetotoxicity at 160 mg/kg bw/day. There was no evidence of teratogenicity. 100 mg/kg bw/d: feed intake ↓; bw gain ↓; (Fritz et al. 1978; TOX9551915) 160 mg/kg bw/d: bw gain ↓ and feed consumption ↓ in the dams; (Dickhaus & Heisler 1987; TOX9550736)	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

↓ : decreased; ↑ : increased; bw : body weight

4.11.6 Conclusions on classification and labelling

There is evidence that reproduction toxicity seen in rats is due to reduced male fertility. In the two-generation reproduction toxicity studies histopathological changes in the testes revealed retarded spermatogenesis. The results of a supplementary published study confirm an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses. Reproductive toxicity was observed at clear parental toxicity. However, there is no clear evidence to conclude that the observed reproductive toxicity is solely produced as a non-specific secondary consequence of parental toxicity.

Overall, seeing “some evidence” for adverse effects on reproduction, it is proposed to classify IPU for reproductive toxicity in category 2 (H361f: Suspected of damaging fertility) according to the CLP criteria.

Taking into account available epidemiological data there is no sufficient evidence to establish a causal relationship between human exposure to IPU and subsequent developmental toxic effects in the progeny.

In the rat and rabbit developmental toxicity studies using dose levels up to 500 mg/kg bw/d and up to 160 mg/kg bw/d, respectively, there was no evidence of teratogenicity. Hence no classification and labelling is proposed for developmental toxicity.

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	0-80-400-2000 ppm, groups of 25/sex, oral	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.

days prior to pairing) GLP-compliant Study considered acceptable. Becker *et al*., 1989	exposure via diet (Doses corresponding to daily intake ranging from 0-275 mg/kg bw/d)	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 Study considered supplementary. No guideline, not GLP Sarkar *et al*., 1997	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 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 whether 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, 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.

4.12 Other effects

This endpoint is not addressed by this proposal.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 34: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aqueous hydrolysis at pH 5, 7 and 9	hydrolytically stable DT_{50} > 30 d	--	Gorman, M.; (1995, Rep. No. 42508)
Photo degradation in sterile water at pH 7	DT_{50} = 72 – 88 d	--	Bürkle; Jordan (1992; Rep. No. WAS95-00197)
Ready biodegradation	Not readily biodegradable (3% after 28 days)	--	Fiebig, S.; (2010, Rep. No. AST13326)
Biodegradation in water/sediment systems (Bury pond & Emperor lake)	DT_{50} = 124.4 – 280.7d (whole system) DT_{50} = 68.6 – 86.6 d (water)	SFO/Level P-I (new calculated by O'Brian 2012)	Girkin, R. (2002, AGM113/014083)
Biodegradation in water/sediment systems (Waldwinkel & Rückhaltebecken)	DT_{50} = 50.7 – 299.6 d (whole system) DT_{50} = 27.2 – 198.9 d (water)	SFO/Level P-I (new calculated by Callow & Jarvis 2011)	Fischer, H. (1995, A&M 016/94)
Biodegradation in water/sediment systems (pond & creek)	DT_{50} = 173.5 – 204.8 d (whole system) DT_{50} = 57.6 – 61.7 d (water)	SFO/Level P-I	Baluff , M. (1994, 93078/01-CWS)
Biodegradation in water/sediment systems (Gravel Pit & River Nidda)	DT_{50} = 62.8 – 65.9 d (whole system) DT_{50} = 37.7 – 45 d (water)	SFO/Level P-I (new calculated by Callow & Jarvis 2011)	Bürkle & Mehler (1993, Rep. No. A51489)

5.1.1 Stability

Hydrolytic degradation:

Author: Gorman, M.
Title: Hydrolysis of 14C-Isoproturon as a function of pH at 25°C and 50°C.
Date: 26.10.1995
Doc ID: Report no. 42508, WAS95-00302
Guidelines: Subdivision N -161-1 and EEC Guideline C. 7, Hydrolysis
GLP: yes
Acceptability: yes

Materials and Methods

The hydrolysis of ^{14}C-Isoproturon, labelled in the phenyl ring, was investigated in aqueous buffer solutions of pH 4, 5, 7, and 9 under sterile conditions, in the dark and at 25 and 50°C. The duration of the tests was 30 days. Liquid scintillation counting was used to determine the total ^{14}C- activity in solution at each sample point. Isoproturon and its hydrolysis breakdown products were quantified by HPLC using UV-detection.

Results

The hydrolysis rate of isoproturon at 25°C was very slow at all pH values with half-lives > 539 days. The hydrolytic breakdown of isoproturon was more rapid at 50°C with half-lives of 27.6 to 116 days. Seven hydrolysis products were observed. The major metabolite, identified as 4-isopropylaniline, did not exceed 10 % of applied amount in any of the buffer systems at 25°C. In the case of the 50°C study the amount of 4-isopropylaniline increased during the test at any tested pH to more than 10 % (18-62 % after 30 days). A second unidentified metabolite reached up to 15.7 % and 10.6 % 23 days after incubation at pH 7 and pH 9, respectively. The mean ^{14}C mass balance was > 99% for each test system. The calculated half-lives are listed in Table 34. Based on these results isoproturon is considered as hydrolytically stable (DT_{50} > 30 d).

Table 35: Half-lives of isoproturon in aqueous solution at different pH values

Hydrolysis medium	Temperature (°C)	Half-life (days)
pH 4 buffer	25	1230
pH 5 buffer	25	1210
pH 7 buffer	25	1560
pH 9 buffer	25	540

Conclusion

The study was already accepted in the Monograph (1999) of isoproturon. Based on these results isoproturon is considered as hydrolytically stable (DT_{50} > 30 d).

Photochemical degradation

Author:	Bürkle, W. L. and Jordan, H. J.
Title:	Direct photolysis of the 14C-labelled active ingredient in aqueous solution, degradation kinetics and quantum yield.
Date:	1992
Doc ID:	WAS95-00197
Guidelines:	Subdivision N § 161-2 "Photodegradation Studies in Water and Phototransformation of Chemicals in Water, Part A: Direct Phototransformation" (Draft Guideline)
GLP:	yes
Acceptability:	yes

Materials and Methods

The direct photo transformation of 14C-isoproturon, radio labelled in the aromatic ring system, was investigated. Therefore sterile water solutions containing 11.17 mg as/L were continuously irradiated for 167 hours with two different relative light intensities (equal to 498 hours of sunlight or 41.5 days and 605 hours, 50 days, resp.) with a xenon lamp (spectrum close to the natural light) in glass vessels, covered with a quartz plate, at 25 ± 2°C. The pH of the solution was adjusted to 7 with a phosphate buffer solution. Samples were taken for analysis at 0, 4, 7, 24, 48, 72, and 167 hours after spiking. Volatile degradation products were collected in absorption traps. Dark control samples were stored under same conditions and analysed at the final sampling time. Photo breakdown products were identified using HPLC technique with reference compounds.

Results

In the dark control samples no degradation was observed (material balance: 99 – 106 %). The recovery rates of all irradiated samples were in the range of 98 – 104 %. A small tendency to volatilisation was detected (1.7 - 2.5 % of applied radioactivity). Four photo degradation products were observed and identified during the test. None of the degradation products accounted for more than 5.4% of the applied radioactivity. The amount of $^{14}CO_2$ was less than 1.3%. At the end of the tests at least 80% of unchanged isoproturon was quantified. In relation to sunlight intensity in June at 52° latitude north the photochemical half-life times were calculated to be 72 and 88 days, respectively. According to these results, the direct photolysis in aqueous solution will probably be of minor importance for the degradation of isoproturon in the environment.

Conclusion

In relation to sunlight intensity in June at 52° latitude north the photochemical half-life times were calculated to be 72 and 88 days, respectively. None of the 4 degradation products were identified in concentrations of more than 5.4 % of applied radioactivity. The quantum yield was $2.1 – 2.6 \cdot 10^{-6}$ at pH 7. Based on the results of this study, direct photo transformation in surface water is not a relevant process under environmental conditions.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

ready biodegradability study:

Author:	Fiebig, S
Title:	Isoproturon technical: Ready biodegradability modified sturm test.
Date:	05. March 2010
Doc ID:	BVL Doc.ID 2356692, AST13326
Guidelines:	OECD 301 B
GLP:	yes
Acceptability:	yes

Materials

The biodegradability of isoproturon (technical isoproturon, purity 98.4 %, Batch Number – IPU95T1603AS) was investigated in a 5-liter brown-glass bottle incubated at low light conditions at 20-23°C over a time period of 28 days at a isoproturon concentration of 15 mg/L in duplicates.

Duplicate samples of mineral medium were inoculated with non-adapted municipal activated sludge (at 107 to 108 CFU/L) and isoproturon added to give a concentration in the medium of 15 mg/L (equivalent to 10.3 mgC/L). Degradation in the test system was then followed over 28 days by determining the CO2 produced. Evolved CO2 was trapped in Ba(OH)2 and the quantity of evolved CO2 was determined by titration. Any degradation was stopped on day 28 by acidification of the test systems and the last titration made on day 29 after any residual CO2 had been purged from the system over a 24 hour period. The percentage degradation was determined by comparison with the theoretical CO2 production of the test item.

Incubations with sodium benzoate were included as functional controls. Toxicity controls were also incubated including both isoproturon and sodium benzoate.

Results

The percentage degradation of sodium benzoate in the functional controls was 60 % after 7 days and was 89 % after 28 days. The validity criterion of ≥ 60 % degradation after 14 days is therefore fulfilled.

Considering replicate No. 1 the rate of degradation was 0 % throughout the whole study. The rate of degradation of replicate No. 2 was 2 % after 11 days and 6 % after 25 days until the end of the test. The mean biodegradation of isoproturon after 28 days was 3 %.

Isoproturon was not biodegradable under the test conditions within 28 days.

In the toxicity controls biodegradation of 44 % of total $ThCO_2$ (sodium benzoate and isoproturon) was observed after 14 days and 50 % after 28 days. This is equivalent to 94 % biodegradation if it is assumed that all CO_2 was derived from sodium benzoate demonstrating that isoproturon had no inhibitory effect.

Conclusion

The study is acceptable and shows that isoproturon is not readily biodegradable.

5.1.2.3 Simulation tests

<u>Biodegradation in water/sediment systems</u>:

Four laboratory studies on the rate of degradation of isoproturon (Bürkle & Mehler, 1993; Baluff, 1994; Fischer, 1995; Girkin, 2002) and re-calculation of results of Bürkle & Mehler (1993), Fischer (1995) and Girkin (2002) according to FOCUS Degradation Kinetics (2006) (Girkin, 2002; Callow & Jarvis, 2011; O'Brien, 2012) were considered acceptable to derive degradation half-lives for risk assessment.

The rates of degradation for parent and metabolites (re-)calculated are based on the original raw data and data from newly submitted studies using the current FOCUS kinetics guidelines.

In the water/sediment system, degradation is slow and isoproturon degrades between 50.7 and 299.6 days at 20°C. The half-lives of the active substance isoproturon resulting from the re-evaluation of the water/sediment studies are summarized in the Table below. A geometric mean SFO-DT_{50} of 129.3 days was determined for isoproturonfor the whole system. Degradation of isoproturon in sediment could not be determined.

Table 36: Summary of half-lives of isoproturon resulting from the re-evaluation of the water/sediment studies

Parent	**Distribution (Max. sed. 72.9 % after 65 d)**									
Water / sediment system	pH water phase	pH sed.	t. °C	DegT50 - DegT90 whole sys.	Error (χ2)	DissT50 - DissT90 water	Error (χ2)	DT50 - DT90 sed.	Error (χ2)	Method of calculation
River Nidda	8.7	6.7	20	62.8/208.7	9.1	37.7/125.2	5.15	-	-	SFO*/ Level P-I
Gravel Pit	8.3	7.2	20	65.9/219.0	3.2	45/149.5	6.82	-	-	SFO*/ Level P-I
Pond	7.88	7.2	20	204.8/680.3	5.11	61.7/205	8.97	-	-	SFO/ Level P-I
Creek	7.84	7.3	20	173.5/576.4	1.7	57.6/191.2	7.08	-	-	SFO/ Level P-I
Waldwinkel	7.46	6.97	20	299.6/995.4	5.0	198.9	4.63 (FOMC)	-	-	SFO*/ Level P-I
Rückhalte-becken	7.85	7.10	20	50.7/168.5	8.13	27.2/90.5	4.05	-	-	SFO*/ Level P-I
Bury Pond	8.16 (start) 7.22 (end)	8.16 (start) 7.22 (end)	20	124.4/413.2	6.65	68.6/228	2.6	-	-	SFO**/ Level P-I
Emperor Lake	7.89 (start) 5.24 (end)	7.89 (start) 5.24 (end)	20	280.7/932.6	2.82	86.6/284.3	5.08	-	-	SFO**/ Level P-I
Geometric mean/median of DT_{50}				129.3/149		61/59.7		-		

* re- calculation from study Callow & Jarvis (2011)

**re- calculation from study O'Brian (2012)

Study 1

Author: Callow, B. & Jarvis, T.

Title: Determination of rates of decline for isoproturon in Sediment-Water studies according to the guidance within the FOCUS Kinetics Guidance Document

Date: 14. November 2011

Doc ID: 0804202.UK1/EWC0008; BVL.Doc.ID 2356693

Guidelines: Yes (FOCUS Kinetics Guidance Document, 2006)

GLP: Not applicable

Acceptability: Yes

Material and Methods

Rates of degradation were calculated according to the guidance of the FOCUS Degradation Kinetics Workgroup1, using KinGui Version 1.1 (Bayer CropScience 2006).

The approach used followed that given in Chapter 10 of the FOCUS Kinetics Guidance Document. The suitability of the fit of the models was evaluated both visually and statistically by calculating the minimum % error required to pass the chi2 test at a probability of 0.05 (acceptability criteria chi2 error < 15 %).

Two levels of kinetics are proposed for the determination of rates of degradation from sediment/water studies. P-I is for single compartment approaches and P-II is proposed for multi-compartment approaches where degradation in both the water and sediment is considered. In this exercise only the single compartment (P-I) approach was used and DT50s for the total system were determined.

Results

The detections of isoproturon in water/sediment systems of Bürkle and Mehler (1993) and Fischer (1995) are given in Table 37 and Table 38.

Table 37: Detection of isoproturon in water/sediment system of Bürkle and Mehler (1993) [% AR]

Days after treatment	System	
	River Nidda System	Gravel Pit System
0	98.0	103.9
0.25	99.2	98.3
1	111.9	98.1
2	100.8	101.3
7	99.4	98.5
14	93.5	90.5
30	98.0	71.3
60	54.4	49.6
120	13.9	33.5

Table 38: Detection of isoproturon in water/sediment system of Fischer (1995) [% AR]

Days after treatment	System	
	Rückhaltebecken	Waldwinkel
0	95.3	92.6
0	95.5	96.5
1	98.0	97.3
1	96.7	97.0
2	100.9	96.4
2	96.8	91.1
8	96.4	97.4
8	94.4	106.1
14	92.9	91.9
14	95.2	91.6
30	83.4	90.1
30	80.0	87.2
65	23.0	89.7
65	48.0	97.2
100	26.2	87.9
100	12.2	82.9
224	<2.9	49.7
224	5.1	52.3

The new calculated DT_{50} are given in table below.

Table 39: Calculated DT_{50} in the water/sediment systems

Parameter	**Bürkle and Mehler (1993)**		**Fischer (1995)**	
	River Nidda	**Gravel Pit System**	**Rückhaltebecken**	**Waldwinkel**
	SFO	**SFO**	**SFO**	**SFO**
DT_{50} (days) (persistence and modelling)	62.8	65.9	50.7	299.6
DT_{90} (days)	208.7	219.0	168.5	995.4
χ^2 error (%)	9.1	3.2	8.13	5.0

Conclusion

The SFO model satisfactorily described the decline of isoproturon with an acceptable statistical and visual fit obtained in all systems. The DT_{50}s were 50.7 to 299.6 days (DT_{90}s 168.5 to 995.4 days).

Study 2

Author: O'Brien, K.
Title: Calculations of environmental fate endpoints in water-sediment systems for Isoproturon according to recommendations of the FOCUS working group on degradation kinetics.
Date: 26.09.2012
Doc ID: 249755-A2- 070803-01; BVL.Doc.ID 2357617
Guidelines: Yes (FOCUS, 2006)
GLP: Not applicable
Acceptability: Yes

Materials and methods

The kinetic evaluation of the data from the study by Girkin (2002) was performed using the model software KinGUI version 1.1. Input data from Girkin (2002) for the modelling study are shown in Table 40.

Table 40: Concentration of isoproturon in the water /sediment system for kinetic modelling

	Bury Pond		**Emperor Lake**	
DAT	Water [%AR]	Total [%AR]	Water [%AR]	Total [%AR]
0	92.5	95.9	98.8	101.3
2	61.2	76	71.8	93.1
7	53.8	85.6	60.2	95.8
14	46.4	84.6	49	88.1
30	40.3	79.7	38.6	85.7
61	32.1	69.3	33.3	82.9
100	19.3	45	26.6	76

Results

The $\chi 2$ values of SFO fit was > 15 %, so SFO could not be applied. Additionally more than 10 % of initial isoproturon was still present after 100d (end the study) so DT_{90} was not reached within study period. Therefore no FOMC kinetic was considered as well. Eventually biphasic kinetic (DFOP) was applied according to FOCUS (2006). The results of the statistic evaluation of DFOP kinetic are shown in Table 41 and Table 42. DT_{50} and DT_{90} values of the active substance isoproturon are summarized in Table 43.

Table 41: Kinetic evaluation of degradation rates of isoproturon in water/sediment system

Girkin (2002) Bury Pond				
Kinetic model	DFOP (SFO-slow phase used)		**SFO**	
Compartment	Water (Diss)		Total (Deg)	
Parameter	M0 k_deg_1 k_deg_2 g	χ^2 R^2	M0 k	χ^2 R^2
Estimation	M0: 92.5 k_deg_1: 0.8528 k_deg_2: 0.0101 g: 0.3976	$\chi^2 = 5.08$ $R^2 = 0,9845$	M0: 89.31 k_deg: 0.0056	$\chi^2 = 6.65$ $R^2 = 0.8182$
DT_{50} (d)	68.6 (slow phase)		124.4	
DT_{90} (d)	228 (slow phase)		413.2	

Table 42: Kinetic evaluation of degradation rates of isoproturon in water/sediment system

Girkin (2002) Emperor Lake				
Kinetic model	**DFOP (SFO-slow phase used)**		**SFO**	
Compartment	Water (Diss)		Total (Deg)	
Parameter	M0 k_deg_1 k_deg_2 g	χ^2 R^2	M0 k	χ^2 R^2
Estimation	M0: 97.70 k_deg_1: 0.3626 k_deg_2: 0.0080 g: 0.4398	$\chi^2 = 5.08$ $R^2 = 0,9845$	M0: 95.62 k_deg: 0.0025	$\chi^2 = 2.82$ $R^2 = 0,8388$
DT_{50} (d)	86.6 (slow phase)		280.7	
DT_{90} (d)	284.3 (slow phase)		932.6	

Table 43: Summary of degradation rates of isoproturon in water/sediment system after kinetic evaluation

water/ sediment system	**pH H_2O**	**pH Sed**	**T (°C)**	**water**		**Error (χ2)**	**total**		**Error (χ2)**	**kinetic**
				$DissT_{50}$	**$DissT_{90}$**		**$DegT_{50}$**	**$DegT_{90}$**		
active substance isoproturon										
Bury Pond	8.16 (start) 7.22 (end)	8.16 (start) 7.22 (end)	20	68.6	228	2.6	124.4	413.2	6.65	SFO (slow phase of DFOP for water phase)
Emperor Lake	7.89 (start) 5.24 (end)	7.89 (start) 5.24 (end)	20	86.6	284.3	5.08	280.7	932.6	2.82	SFO (slow phase of DFOP for water phase)

Conclusion

Dissipation rates for the water phase of all systems (Bury Pond, Emperor Lake) are acceptable. The DT_{50}s were 124.4 to 280.7 days (DT_{90}s 413.2 to 932.6 days).

5.1.3 Summary and discussion of degradation

The study on ready biodegradability according to OECD 301 B (Modified Sturm test) shows that isoproturon is not readily degradable (only 3 % degradation at 28 days).

In water/sediment systems it was shown that isoproturon was not rapidly degradable with DT_{50} values of 50.7 - 299.6 days (whole system) and DT_{50} values of 27.2 – 198.9 days (water).

Isoproturon is hydrolytically stable under acidic and neutral conditions. Aquatic photolysis is not considered to be an important transformation route for isoproturon in the environment with DT_{50} of 72 -88 days.

The results of the test on the biodegradation of isoproturon in the water/sediment system and abiotic degradation show that isoproturon is considered not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

5.2 Environmental distribution

Not relevant for this dossier.

5.2.1 Adsorption/Desorption

5.2.2 Volatilisation

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

Table 44: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD 305E (flow through)	$BCF_{steady\ state}$: 2.6 -3.6 (whole fish, parent)	Not lipid normalized	Douglas, M.T. (1990, RNP 320/90161)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log $K_{o/w}$ of isoproturon is 2.6 at 25°C. So there is no indication for bioaccumulation potential of isoproturon.

5.3.1.2 Measured bioaccumulation data

Author: Douglas, M.T.
Title: Assessment of bioaccumulation of isoproturon in rainbow trout
Date: 1990
Doc ID: RNP 320/90161; 95-00190
Guidelines: OECD 305E
GLP: Yes
Validity: Yes

Summary

The bioaccumulation of isoproturon technical (99.7 % purity) by fish has been studied following the general procedure outlined in OECD Guideline No. 305 E. Rainbow trout (*Oncorhynchus mykiss*, former *Salmo gairdneri*) were exposed to nominal test concentrations of 0.1 and 1.0 mg/l for 6 days under continuous flow conditions followed by a 12 day depuration period. The uptake was rapid. After 12 hours BCF values of 2.6 and 3.6 were reached. Depuration was also rapid. Consequently, isoproturon has a low potential to bioconcentrate in fish.

Table 45: Bioconcentration factors of isoproturon technical

Time	**Low level (0.1 mg/l)**			**High level (1.0 mg/l)**		
(Day)	Cf- Cb	Cw	BCF	Cf- Cb	Cw	BCF
	(ppm)	(mg/l)		(ppm)	(mg/l)	
0.5	0.25	0.108	2.3	4.16	0.989	4.2
1	0.16	0.115	1.4	2.28	1.036	2.2
2	0.39	0.113	3.5	3.66	1.029	3.6
4	0.33	0.110	3.0	3.95	1.028	3.8
6	0.30	0.108	2.8	4.48	1.028	4.4
Overall Mean	0.286	0.108**a**	2.6	3.706	1.028**a**	3.6

a = Mean value includes data from Days 3 and 5.

Cf = Mean concentration in test fish.

Cb = Mean concentration in control fish.

Cw = Mean concentration in water

5.3.2 Summary and discussion of aquatic bioaccumulation

Isoproturon has a log $K_{o/w}$ of 2.6 (25°C). The experimentally derived steady state BCF of 2.6 -3.6 for isoproturon related to parent and whole fish is below the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008) for not rapidly degradable substances.

5.4 Aquatic toxicity

Table 46: Summary of relevant information on aquatic toxicity

Group, species	**Time-scale (Test type)**	**Endpoint**	**Toxicity (mg a.s./L)**	**Reference**
Fish				
Oncorhynchus mykiss	96 h (static)	Mortality, LC_{50}	37.22 nom 23.83 mm	Ritter (1989; Rep.No. 233403)
Cyprinus carpio	96 h (static)	Mortality, LC_{50}	41.0 mm	Scheerbaum (2002; Rep.No. FAK87492)
Oncorhynchus mykiss	21 d (semi static)	growth, NOEC	1.0 nom	Douglas (1989; Rep.No. 298/89577)
Aquatic invertebrates				
Daphnia magna	48 h (static)	Immobility, EC_{50}	0.58 mm	Vial (1989; Rep.No. 891405)
Daphnia magna	21 d (semi static)	Offspring production, parental body length, NOEC	0.12 nom	Mc Elligott (1999; Rep.No. SA99481)
Algae /aquatic plants				
Pseudokirchneriella subcapitata	72 h (static)	Biomass, E_bC_{50} Growth rate E_rC_{50} $NOE_{b/r}C$	0.025 mm 0.098 mm 0.018 mm	Scheerbaum (2002; Rep.No SPO87491)
Navicula pelliculosa	72 h (static)	Biomass, E_bC_{50} NOE_bC Growth rate E_rC_{50} NOE_rC	0.013 nom 0.0025 nom 0.046 nom 0.0064 nom	Hoberg (1998; Rep.No. 98-5-7319)
Lemna gibba	14 d (semi static)	Biomass, E_bC_{50} NOE_bC Frond number E_rC_{50} NOE_rC	0.037 mm 0.0052 mm 0.045 mm 0.0019 mm	Hoberg (1998;Rep.No. 98-5-7326)
Other aquatic organisms				
Chironomus riparius	28 d (static, spiked water)	Emergence, NOEC	0.344 mm 0.5 nom	Suteau (1997; Rep. No. SA96316)

mm…mean measured
nom...nominal

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1

Author:	Ritter, A.
Title:	Isoproturon, substance technical (Hoe 016410 00 ZD99 0004): 96-hour acute toxicity study (LC50) in the rainbow trout
Date:	1989
Doc ID:	233403
Guidelines:	OECD 203
GLP:	Yes
Validity:	Yes

Executive Summary

The acute toxicity of isoproturon technical (98.5 %) to rainbow trout (*Oncorhynchus mykiss*) was investigated under static conditions over 96 h at nominal concentrations of 9.5; 17.1; 30.9; 55.6 and 100 mg/L. Tween 80 (100 µl/L) was used as vehicle for solution of isoproturon and also tested as solvent control. Groups of 5 fish each were exposed to each test concentration and controls. The analytical results show that the concentration of isoproturon in the fish tank water ranged between 50.9 % and 79.7 % of the nominal test concentration at the start and between 51.0 and 92.0 % after 96 hours. Toxicological results were evaluated based on mean measured concentrations since average recovery of isoproturon was 65.44 % of nominal at analysed concentration levels.

The LC_{50} (96 h) was 23.83 mg as/L based on mean measured concentrations (nominal concentration: 37.22 mg as/L).

Table 47: Mortalities of rainbow trout exposed to technical isoproturon (nominal concentration)

Concentration	Mortality			
mg/L	24 hours	48 hours	72 hours	96 hours
control	0/10	0/10	0/10	0/10
solvent control	0/10	1/10	1/10	1/10
9.5	0/10	0/10	0/10	0/10
17.1	1/10	1/10	1/10	1/10
30.9	0/10	2/10	2/10	3/10
55.6	0/10	5/10	7/10	8/10
100	4/10	9/10	10/10	10/10

Study 2

Author:	Scheerbaum, D.
Title:	Isoproturon Technical Fish (Common carp), Acute Toxicity Test, Static, 96 h
Date:	2002
Doc ID:	2357618 /Report No. FAK87492
Guidelines:	OECD-Guideline No. 203 (1992)
GLP:	yes
Validity:	yes

Executive Summary

The acute toxicity of the test item isoproturon technical (98.3 %) to fish (*Cyprinus carpio*) was investigated under static conditions over a duration of 96 h at analytically verified mean measured concentrations of 3.6, 7.0, 14.6, 29.1, 57.9 mg as/L. Seven organisms were exposed to each test concentrations and control. No vehicle had to be used to dissolve the test item.

The LC_{50} after 96 h was determined to be 41.0 mg as/L.

Table 48: Mortalities of common carp exposed to technical isoproturon (mean measured concentration)

Concentration	Mortality			
mg/L	24 hours	48 hours	72 hours	96 hours
control	0/7	0/7	0/7	0/7
3.6	0/7	0/7	1/7	1/7
7.0	0/7	0/7	0/7	0/7
14.6	0/7	0/7	0/7	0/7
29.1	0/7	0/7	0/7	0/7
57.9	0/7	0/7	7/7	7/7

5.4.1.2 Long-term toxicity to fish

Author: Douglas, M.T.
Title: The prolonged toxicity of isoproturon to rainbow trout (Salmo gairdneri)
Date: 1989
Doc ID: RNP 298/89577
Guidelines: OECD 204
GLP: Yes
Validity: Yes

Executive Summary

The prolonged toxicity of isoproturon technical (99.7 %) to rainbow trout (*Oncorhynchus mykiss*) was investigated under semi static (daily renewal) conditions over 21 days at nominal concentrations of 1.0; 3.2; 10; 32 and 100 mg/L. Tween 80 (100 µl/L) was used as vehicle for solution of isoproturon and also tested as solvent control. All nominal concentrations of the test substance were verified by chemical analysis (HPLC) at days 0, 4, 7, 11, 14, 18 and 21 in the test media. The measured values at each sampling time were very close to the nominal (95-105 % recovery). Nominal concentrations have been used for all calculations and estimations.
There were no mortalities or other adverse effects observed in groups of 10 fish exposed to test concentrations ranging from 0.10 to 10 mg/l and in the controls for a period of 21 days.

Although there were no obvious reactions to exposure exhibited by the fish during the exposure period, on study termination, statistical analysis (Williams-Test) of the length and weight data of fish indicated that inhibition of growth had occurred at concentrations of 3.2 mg/L and above hence the NOEC of 1.0 mg/L was determined.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Author: Vial, A.
Title: Test for Acute toxicity to Daphnia magna Straus
Date: 1989
Doc ID: Report No. 891405
Guidelines: EPA 850.1010; FIFRA 72-2
GLP: Yes
Validity: Yes

Comment: Although in the RAR under KIIA 8.2.4 the study Doc ID was stated 891403 the correct study Doc ID is 891405. Also the tested concentration range was not reported right in the RAR (nominal 2.6, 3.5, 4.5, 5.9, 7.7, 10, 13, 17 and 22 mg/L) instead of the true test concentration (nominal 0.1, 0.18, 0.32, 0.58, 1.0, 1.8, 3.2, 5.8 and 10 mg/L) by a mistake.

Executive Summary

The acute immobilisation with the test item isoproturon technical was investigated under static conditions over a duration of 48 h. Isoproturon (purity: 97.7 %) was tested at nominal concentrations of 0.1, 0.18, 0.32, 0.58, 1.0, 1.8, 3.2, 5.8 and 10 mg/L (mean measured 0.12, 0.24, 0.31, 0.52, 0.91, 1.6, 3.3, 5.6 and 8.4 mg/L). Twenty test organisms (2 replicates with 10 daphnia) were exposed to each concentration and control.

The EC_{50} after 48 h was determined to be 0.58 (0.49 – 0.71) mg/L based on mean measured concentrations.

Table 49: Immobilisation of daphnia exposed to technical isoproturon (mean measured)

Average measured concentration	Immobilisation	
mg/L	24 hours	48 hours
control	0/20	1/20
0.12	0/20	0/20
0.24	0/20	1/20
0.31	0/20	1/20
0.52	2/20	11/20
0.91	0/20	15/20
1.60	1/20	19/20
3.30	0/20	20/20
5.60	3/20	20/20
8.40	11/20	20/20

5.4.2.2 Long-term toxicity to aquatic invertebrates

Author:	Mc Elligott, A.
Title:	Isoproturon Daphnia magna reproduction test under static renewal conditions
Date:	1999
Doc ID:	Report SA 99481
Guidelines:	OECD Guideline 211
GLP:	Yes
Validity:	Yes

Executive Summary

A GLP-compliant reproduction study with Daphnia magna conducted according OECD Guideline 211 under static renewal conditions over a period of 21 days is available. Isoproturon technical (purity: 1000 g/kg) was tested at nominal concentrations of 0.12, 0.37, 1.1, 3.3 and 10 mg/L. The nominal concentrations of the test substance in the dilution water were verified by chemical analysis at the beginning and end of the 1st, 2nd, 6th and 9th test solution renewals. The measured values at the beginning (T0) of the exposure cycles were very close to the nominal (89-109 % recovery). The values measured at the end of these exposure periods were very close to the initial measured values (91-108% recovery) indicating stability of test substance in dilution water during exposure periods. The mean measured concentrations were 0.12, 0.36, 1.1, 3.3 and 9.8 mg/L. A control and solvent control (0.1 mL/L DMF) were tested additionally. Ten individually held daphnids were exposed per treatment and control.

Statistical analysis of the reproductive variable showed that the number of live young produced per parent daphnid alive on day 21 of the test was significantly different (reduced) compared to the solvent control group at the four highest test concentrations of 0.36 to 9.8 mg/L. Statistical analysis of the length variable also showed that mean length of parent daphnids alive on day 21 of the test was significantly different (reduced) compared to the solvent control group at the four highest test concentrations of 0.36 to 9.8 mg/L. Based on nominal concentrations of isoproturon the NOEC for reproduction and growth (mean length of parents) was determined to be 0.12 mg/L.

5.4.3 Algae and aquatic plants

Study 1

Author:	Scheerbaum, D.
Title:	Isoproturon technical: Alga, Growth inhibition test with Pseudokirchneriella subcapitata, 72 h (formerly Selenastrum capricornutum)
Date:	2002b
Doc ID:	2357623/ Report No. SPO87491
Guidelines:	OECD-Guideline No. 201 (1984)
GLP:	Yes
Validity:	Yes

Study Design and Methods

Test material	Isoproturon technical
Lot/Batch #:	D-3511
CAS No.:	34123-59-6
Description:	White powder
Active substance(s)/Content:	98.3% (w/w)
Storage condition:	7 ± 2°C, protected moisture and light
Density:	1.2 g/cm^3
Solvent:	none
Vehicle and/or positive control:	Reference item: Potassium dichromate (100%)
Test organisms	
Species:	*Pseudokirchneriella subcapitata* HINDAK, strain: SAG 61.81
Source:	Pflanzenphysiologisches Institut der Universität Göttingen, Germany
Culture medium:	Nutrient medium Z according to Lüttge et al. (1994, Botanica Acta, Journal of the German Botanical Society, No. 3 Volume 107 page 111-186 (June 1994), THIEME-VERLAG)
Test design	
Test vessel/volume:	20 mL plastic cuvettes (Ø 50 mm)/10 mL volume
Test medium:	Double concentrated OECD medium
Duration:	72 h
Environmental conditions	
Test temperature:	23°C ± 2°C
pH:	7.92 - 8.52
Lighting:	24 h/d light at 60 – 120 µE/m^2×s

Experimental treatments

The study was conducted under static conditions with an initial cell density of nominally 10^4 cells/mL. Algae of *Pseudokirchneriella subcapitata* were exposed to 7 concentration levels which were made by diluting a saturated stock solution of 100 mg isoproturon technical/L in a geometrical series with a dilution factor of 2. The following mean measured concentrations were the resulting concentration levels tested: 0.0043, 0.0088, 0.018, 0.032, 0.067, 0.14 and 0.27 mg isoproturon/L. Three replicates were tested for each concentration level and 6 replicates for control. Water quality parameters of pH-value were measured at 0 and 72 h, room temperature was measured continuously and light intensity was measured prior to test start.

Observations

Cell density was measured via Chlorophyll-a-fluorescence at the beginning of the test and every 24 h. Each replicate was measured 6-fold. After 72 h 0.5 mL alga suspension from the mean measured concentration levels 0.032 - 0.27 mg a.i/L and control were transferred to 10 mL untreated dilution water and allowed to grow for further 3 d to determine whether the effect of the test item was reversible. Microscopic evaluation of the cells was determined at the start and at the end of the incubation. Test item concentrations of each concentration level and control were analytically verified at the beginning and end of the test. Separate replicates for the test item analysis after 0 and 72 h were prepared (test beginning provided without alga and test end with same density of alga as the test replicates) and incubated under test conditions.

Statistics

The EC_{50} values and confidence intervals after 72 h were calculated by Probit analysis. The NOEC and LOEC were determined by calculation of statistical significance of biomass integrals and growth rates. One Way Analysis of Variance (ANOVA) and DUNNETT's test (growth rates) and the BONFERRONI t-test (biomass integrals) were carried out for the determination of statistically significant differences compared to control replicates.

Results and Discussion

The percentage inhibition of growth rates and biomass integral is presented in the tables below:

Table 50: Mean values at each concentration of isoproturon for biomass integral at 72h and relevant endpoints

Mean measured concentrations of Isoproturon (mg/L)	**Mean biomass integral 0 – 72 hrs**	**Percentage inhibition**
Control	1403250	0
0.0043	1535780	-9.44
0.0088	1639120	-16.81
0.018	1286076	8.35
0.032	610593	56.49
0.067	198684	85.84
0.14	88464	93.7
0.27	31148	97.78
EbC50 mg isoproturon /L	0.025 mg/L	
(95% confidence limits)	0.021 - 0.030 mg/L	
NOEC	0.018 mg/L	
LOEC	0.032 mg/L	

Table 51: Mean values at each concentration of isoproturon for growth rate at 72 h and relevant endpoints

Mean measured concentrations of Isoproturon (mg/L)	**Mean growth rate (1/day) 0 – 72 hrs**	**Percentage inhibition**
Control	1.77	0
0.0043	1.80	-1.76
0.0088	1.84	-4.05
0.018	1.77	0.14
0.032	1.40	20.74
0.067	1.02	42.19
0.14	0.76	57.29
0.27	0.41	76.63
ErC50 mg isoproturon /L	0.098 mg/L	
(95% confidence limits)	0.081 - 0.12 mg/L	
NOEC	0.018 mg/L	
LOEC	0.032 mg/L	

Conclusions

The test on inhibitory effects on *Pseudokirchneriella subcapitata* fulfils the validity criteria of the guideline. Isoproturon Technical was found to inhibit the growth of *Pseudokirchneriella subcapitata* at mean measured concentrations of $\geq$ 0.032 mg a.s./L (biomass and growth rate). The E_bC_{50} and E_rC_{50} values after 72 h were 0.025 and 0.098 mg a.s./L, respectively (based on mean measured concentrations).

Study 2

Author: Hoberg, J.
Title: Isoproturon- Toxicity to the freshwater Diatom, Navicula pelliculosa
Date: 1998
Doc ID: Report No. 98-5-7319
Guidelines: OECD-Guideline No. 201, EEC Directive C.3, Alga Growth Inhibition Test
GLP: Yes
Validity: Yes

Executive Summary

The growth inhibition effect of isoproturon was tested with the diatomea *Navicula pelliculosa*, at 5 concentrations ranging from 0.0026 to 0.1 mg /L and differing by a geometric factor of 2.5. Significant inhibitory effects were observed at concentrations from 0.016 to 0.1 mg/L after 72 h for both biomass and growth rate. EC values were determined on the basis of nominal concentrations, since the analysed content of test material was within 80 and 120 % of the nominal test concentration. The E_bC_{50} and ErC_{50} were calculated to be 0.013 and 0.046 mg a.s./L, respectively.

Study Design and Methods

Test material	Isoproturon
Lot/Batch #:	9603161
CAS No.:	34123-59-6
Description:	White powder
Active substance(s)/Content:	99.8% (w/w)
Storage condition:	ambient temperature (> 2°C, < 30°C), dark
Density:	Not stated
Solvent:	DMF, 0.1 ml per 1000 ml medium
Test organisms	
Species:	Navicula pelliculosa, strain: 153045
Source:	Carolina Biological supply company, Burlington, North Carolina
Test design	
Test vessel/volume:	250 mL Erlenmeyer flasks/100 mL volume
Duration:	72 h
Environmental conditions	
Test temperature:	24 – 25°C
pH:	7.4 – 8.4
Lighting:	24 h/d light, Illumination from the top (Duro-Test Vita-Lite fluorescent bulbs), approx. 3200 - 5400 lux

Experimental treatments

Algae were exposed at initial cell concentrations of 1×10^4 cells/mL to isoproturon at concentrations of 0.0026, 0.0064, 0.016, 0.04 and 0.1 mg a.s./L. From a stock solution of isoproturon in DMF containing 1.0 mg/mL, 5 dilutions in acetone from 0.1 to 0.0026 were prepared. From each stock solution 0.1 mL was added to 100 mL test solution containing algae at an initial cell density of 1×10^4 cells/mL. Hence, the maximum concentration of solvent (0.1 mL/L) as recommended in the guideline OECD 201 was used.

Each concentration was tested in 3 replicates. The control was tested in 3 parallel cultures; three additional solvent controls (DMF) were also tested.

Observations

Samples for chemical analysis were taken from each exposure solution and the controls after initiation of the test and thereafter at 72 h. Analysis of the samples was carried out by means of HPLC

After 24, 48 and 72 hours of growth, cell density was determined by counting cell numbers using a Neubauer chamber and a microscope and growth inhibition was evaluated by calculation.

The pH was measured at the start and at the end of the test in each test concentration and the control. The water temperature was measured daily in a flask incubated under the same conditions as the test flasks.

Statistics

The EbC50 and ErC50 values, the theoretical concentration of test substance which would cause a 50 % reduction in biomass and growth rate, respectively, and the 95 % confidence limits, were determined by linear regression of response (percent reduction of biomass or growth rate as compared with the pooled control) versus mean measured test concentration. The EC values were calculated using four linear regression curves based on (a) untransformed data, (b) untransformed response versus logarithm-transformed concentration, (c) probit-transformed response versus untransformed concentration, and (d) probit-transformed response versus logarithm-transformed concentration. The regression line which provided the best fit of the untransformed or transformed data was selected based on the highest coefficient of determination, r^2. This regression equation was then applied to calculate each EC value and its 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981). A computer program was used to assist in these computations.

Results and Discussion

The analytical results of the test solutions were within 80 and 120 % of the nominal test concentrations, hence the ecotoxicological endpoints were evaluated using the nominal concentrations.

The percentage inhibition of growth rates and biomass integral is presented in the tables below:

Table 52: Mean values at each concentration of isoproturon for the biomass integral at 72 h and relevant endpoints

Isoproturon Nominal concentration [mg/L]	**Biomass integral Percentage inhibition**
Control	0
Solvent control	0
0.0026	4.4
0.0064	15
0.016	55
0.042	93
0.1	104
EbC50 mg a.s. /L	0.013 mg/L
(95% confidence limits)	0.0073 - 0.023 mg/L
NOEC	0.0025 mg/L
LOEC	0.0064 mg/L

Table 53: Mean values at each concentration of isoproturon for the growth rate at 72 h and relevant endpoints

Isoproturon	**Growth rate**

Nominal concentration [mg/L]	Percentage inhibition
Control	0
Solvent control	0
0.0026	0.93
0.0064	2.4
0.016	23
0.042	59
0.1	110
ErC50 mg a.s. /L	0.046 mg/L
(95% confidence limits)	0.028 - 0.064 mg/L
NOEC	0.0064 mg/L
LOEC	0.016 mg/L

Conclusions

Significant effects were observed from 0.016 to 0.1 mg/L after 72 h for the biomass integral and for the growth rate. Based on nominal concentrations, the E_bC_{50} and the E_rC_{50} were determined to be 0.013 and 0.046 mg/L, respectively.

Study 3

Author: Hoberg, J.
Title: Isoproturon- toxicity to the duckweed, Lemna gibba
Date: 1998
Doc ID: Report No. 98-5-7326
Guidelines: US EPA, FIFRA Guideline 122-2 and 123-2
GLP: Yes
Validity: Yes
Comment: Although in the RAR under KIIA 8.6 the study was reported without GLP by a mistake, the study was carried out with GLP (certificate available).

Executive Summary

The effect of isoproturon on the growth of *Lemna gibba* was determined. Static renewal exposure over 14 days was carried out, with renewal on day 3, 6, 9 and 12. Based on a preliminary test, 7 nominal concentration levels were tested in a geometrical series with a dilution factor of 2.5: 0.002, 0.005, 0.013, 0.032, 0.08, 0.2 and 0.5 mg a.i./L. For the definitive test three replicates were investigated for each test concentration, for solvent control and for the control. Frond numbers were assessed on days 0, 3, 6, 9, 12 and 14 for the definitive test. Environmental parameters as water temperature and pH-value were within the acceptable limits. The concentrations of isoproturon were analysed using HPLC-UV. The measured concentrations were generally consistent between sampling intervals and maintained the expected concentration gradient. Based on mean measured concentrations, the treatment levels were defined as 0.0019, 0.0052, 0.014, 0.034, 0.076, 0.20 and 0.44 mg a.i./L, which ranged from 89 to 110% of nominal concentrations. Isoproturon was found to inhibit the growth of the monocotyledon *Lemna gibba* after 14 d. The EC_{50}-values for E_rC_{50} and E_bC_{50} were 0.045 (0.015-0.13) mg/L and 0.037 (0.019-0.071) mg/L, respectively. The related NOEC values were 0.0019 mg/L for growth and 0.0052 mg/L for biomass.

Study Design and Methods

Test material	Isoproturon
Lot/Batch #:	9603161
CAS No.:	34123-59-6
Description:	White powder
Active substance(s)/Content:	99.8% (w/w)
Storage condition:	ambient temperature (> 2°C, < 30°C), dark
Density:	Not stated
Solvent:	DMF, 0.1 ml per 1000 ml medium

Test organisms

Species:	Duckweed *(Lemna gibba)* strain G3
Source:	University of California (Los Angeles), USA

Test design

Method of cultivation:	After 3 days, plants were transferred to freshly prepared growth medium. Growth media and breeding vessels were autoclaved before use to enable the breeding of axenic cultures. Continuous lighting, 3200 to 5400 lux, was used
Culture medium:	Hoagland`s-medium, pH-value 5.0 ± 0.1
Test vessel/volume:	Crystallizing dishes of 270 mL capacity, covered with glass lids and filled with 100 mL test solution
Duration:	14 d

Environmental conditions

Test temperature:	24 – 25°C
pH:	4.9 – 6.4
Lighting:	24 h/d light, Illumination from the top (Duro-Test Vita-Lite fluorescent bulbs), approx. 3200 - 5400 lux

Experimental treatments

The effect of isoproturon on the growth of *Lemna gibba* was determined. Static renewal exposure over 14 days was carried out, with renewal on day 3, 6, 9 and 12. Based on a preliminary test, 7 nominal concentration levels were tested in a geometrical series with a dilution factor of 2.5: 0.002, 0.005, 0.013, 0.032, 0.08, 0.2 and 0.5 mg a.i./L. For the definitive test three replicates were investigated for each test concentration, for solvent control and for the control. Approximately 30 minutes after the test solutions were prepared and added to the test vessels, an inoculum of five plants with three fronds each was aseptically introduced into each vessel. Test vessels were then randomly placed, based on computer-generated random numbers, on a shelf within an environmental chamber.

Observations

At each 3-day interval (days 3, 6, 9 and 12) and at test termination (day 14), fronds were counted and observations were made. Following the day 3, 6, 9 and 12 observations, the fronds were transferred to newly prepared solutions of the appropriate test, control and solvent control concentrations. The test vessels were assigned new random positions in the environmental chamber at each observation period.

At test termination (day 14), frond densities for each replicate treatment, control and solvent control vessel were determined. Fronds were counted and then removed from each vessel, blotted dry and transferred to pre weighed aluminum pans. Fronds were dried at 100°C for three days prior to dry weight determination. pH-values were measured on start, at each renewal of media and end of the test. The water temperature was measured continuously and recorded daily. Light intensity was determined before experimental starting and on each day during the study with a light meter (General electric type 214).

Statistics

Means and standard deviations of frond densities were calculated for each treatment level, the control and the solvent control at each observation interval. Means and standard deviations for biomass were also calculated for each treatment level, the control and the solvent control and were based on the dry plant weight determined at test termination. A t-Test (Sokal and Rohlf, 1981) was used to compare the 14-day control and solvent control growth rate and biomass data. If control and solvent control data were not significantly different ($p \leq 0.05$), these data were pooled for use in statistical evaluation of the data for treatment effects. Additionally, percent inhibition of the 14-day mean frond density and biomass of the treatment data were calculated relative to the pooled control data.

The EC_{25} and EC_{50} values (concentrations of test substance which caused 25 and 50 % reduction, respectively, in 14-day frond density and biomass) and the 95 % confidence limits, were determined by linear regression of response (percent reduction of frond density or biomass as compared with the pooled control) versus mean measured test concentration. The EC values were calculated using four linear regression curves based on (a) untransformed data, (b) untransformed response versus logarithm-transformed concentration, (c) probit-transformed response versus untransformed concentration, and (d) probit-transformed response versus logarithm-transformed concentration. The regression line which provided the best fit of the untransformed or transformed data was selected based on the highest coefficient of determination, f. This regression equation was then applied to calculate each EC value and its 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981). A computer program was used to assist in these computations.

Results and Discussion

At the beginning and end of one renewal period (day 6 and day 9), one sample was removed from each treatment, control and solvent control solution to be analyzed for isoproturon concentration.

Samples analyzed on day 6 were removed from the newly prepared test solutions subsequent to division into replicate test vessels. Samples analyzed at the end of the renewal period (day 9) were removed from the individual composite solutions after the three replicates of each test concentration, the control and solvent control had been respectively combined.
The concentrations of isoproturon were analysed using HPLC-UV. The measured concentrations were generally consistent between sampling intervals and maintained the expected concentration gradient. Based on mean measured concentrations, the treatment levels were defined as 0.0019, 0.0052, 0.014, 0.034, 0.076, 0.20 and 0.44 mg a.i./L, which ranged from 89 to 110 % of nominal concentrations.
Environmental parameters light, pH and temperature were within the acceptable limits. The validity criteria of the test guideline were fulfilled.

The effects of isoproturon, based on mean measured test concentrations are summarized below:

Table 54: NOEC, LOEC, EC-values of isoproturon related to biomass inhibition

Isoproturon Mean measured concentration [mg/L]	**Frond Biomass (dry weight) Percentage inhibition (14 d)**
Control	0
Solvent control	0
0.0019	8.9
0.0052	14
0.014	22
0.034	42
0.076	73
0.2	96
0.44	98
EbC50 mg a.s. /L	0.037 mg/L
(95% confidence limits)	0.0019 - 0.071 mg/L
NOEC	0.0052 mg/L
LOEC	0.014 mg/L

Table 55: NOEC, LOEC, EC-values of isoproturon related to growth inhibition

Isoproturon Mean measured concentration [mg/L]	**Frond production (growth) Percentage inhibition (14 d)**
Control	0
Solvent control	0
0.0019	1.4
0.0052	8.4
0.014	20
0.034	22
0.076	64
0.2	92
0.44	95
ErC50 mg a.s. /L	0.045 mg/L
(95% confidence limits)	0.015 – 0.13 mg/L
NOEC	0.0019 mg/L
LOEC	0.0052 mg/L

Conclusions

Isoproturon was found to inhibit the growth of the monocotyledon *Lemna gibba* after 14 d. The EC_{50}-values for E_rC_{50} and E_bC_{50} were 0.045 (0.015-0.13) mg/L and 0.037 (0.019-0.071) mg/L, respectively. The related NOEC values were 0.0019 mg/L for growth and 0.0052 mg/L for biomass.

5.4.4 Other aquatic organisms (including sediment)

Author:	Suteau, P.
Title:	Isoproturon toxicity to the sediment dwelling chironomid larvae (Chironomus riparius)
Date:	1997
Doc ID:	SA96316
Guidelines:	According to proposal for BBA guideline (1995)
GLP:	Yes
Validity:	Yes

Comment: Although in the RAR under KIIA 8.5.2 the study was reported without GLP by a mistake, the study was carried out with GLP (certificate available).

Executive Summary

The purpose of this study was to estimate the toxicity of technical isoproturon on the sediment dwelling life stage of *Chironomus riparius* in a sediment-water system. A total of 500 organisms (25 per replicate 4 replicates per concentration) were exposed to 4 concentrations of isoproturon (lot nO 9004302), and a dilution water-sediment control for an exposure period of 28 days. The test substance isoproturon had a measured purity of 1004 g/kg. The definitive test was carried out using the following nominal concentrations: 0.063, 0.125, 0.25 and 0.5 mg/L. The concentrations of isoproturon were verified by chemical analysis in test solutions one hour after the test initiation, on Day 7 and at the test termination (Day 28). One hour after test initiation analytical verification of the test concentrations in the overlying dilution water demonstrated the measured values to be slightly lower than nominal concentration values (70 – 82 % recovery). Analytical verification of these test concentrations on Day 7 of the test showed a little decrease, compared to initial measured values (64 – 83 % recovery). At test termination (Day 28), analytical measurements confirmed this decrease (41 – 69 % recovery from initial values).
Following 28 days of exposure to isoproturon, there was no statistical significant difference between the emergence rate of adult chironomid midges at any of the test concentrations, compared to the dilution water-sediment control group.
No statistical significant difference in development rate of midges during the test period was observed between the control group and any of the exposed groups.
The No Observed Effect Concentration (NOEC) is reported to be 0.5 mg/L (nominal) and 0.344 mg/L (mean measured).

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

Isoproturon produces acute $L(E)C_{50}$ values in concentrations > 0.01 ≤ 0.1 mg/L for algae and aquatic plants, > 0.1 ≤ 1 mg/L for crustaceans and > 1 mg/L for fish. Chronic NOEC values in concentrations > 0.001 ≤ 0.01 mg/L for aquatic plants and algae and > 0.1 ≤1 mg/L for invertebrates and fish were determined.

The results of the test on the biodegradation of isoproturon in the water/sediment system and abiotic degradation show that isoproturon is considered not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

Isoproturon has a log $K_{o/w}$ of 2.6 (25°C). The experimentally derived steady state BCF of 2.6 -3.6 for isoproturon related to parent and whole fish is below the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008) for not rapidly degradable substances.

CLP- Acute aquatic hazards

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

The lowest $L(E)C_{50}$ obtained for isoproturon are 0.046, 0.58 and 23.83 mg/L in algae, invertebrates and fish, respectively. Because EC_{50} for aquatic plants was only determined over 14 days and not after 7 days, the E_rC_{50} of 0.045 mg/L (lowest value of three trophic levels) was used only as supplementary information for acute classification. Isoproturon therefore fulfils the criteria for classification as Aquatic Acute Cat. 1.

An M-factor of 10 for acute toxicity is proposed based on $L(E)C_{50}$ value of 0.046 mg/L in algae. ($0.01 < L(E)C_{50} \leq 0.1$ mg/L)

CLP - Aquatic chronic hazards

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 1 mg/L is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

Isoproturon is considered not rapidly degradable (see section 5.1.3). NOEC values for isoproturon are available for all trophic levels. The lowest NOE_rC is 0.0019 mg/L obtained for aquatic plants and 0.0064 mg/L for algae. Isoproturon therefore fulfils criteria for classification as Aquatic Chronic Cat.1.

An M-factor of 10 for chronic toxicity is proposed based on the NOE_rC value of 0.0019 mg/L for aquatic plants and 0.0064 mg/L algae, respectively. ($0.001 < NOEC \leq 0.01$ mg/L).

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

Isoproturon fulfils the criteria for classification as Aquatic Acute 1 with an M-factor of 10.

Isoproturon fulfils the criteria for classification as Aquatic Chronic 1 with an M-factor of 10.

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 25ºC 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 K_{ow} 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 K_{ow} 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

<u>Acute (short-term) aquatic toxicity</u>

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 inmobilisation 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 K_{ow} 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).

Conclusion on Classification

Based on the above information, RAC agrees with the DS's proposal that isoproturon fulfills 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**.

6 OTHER INFORMATION

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II A 5	Anonym	2002	Review report for the active substance isoproturon *SANCO/3045/99-final* ASB2013-4639	N	---
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Codes of owner

ACI: ACI International
BBA: Biologische Bundesanstalt für Land-und Forstwirtschaft
BCL: Barclay Chemicals Manufacturing Ltd.
GHA: Gharda Chemicals Ltd.
ITF: Isoproturon Task Force
MAK: Makhtheshim-AGAN
PUS: Phytorus SA
ROP: Rhone-Poulenc Agro
RPA: RHONE-POULENC AGRO GmbH
SAC: Sanachem GmbH
SCC: SCC GmbH Chemisch-Wissenschaftliche

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8 ANNEXES

Confidential Annex