Committee for Risk Assessment

RAC

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

EC number:

CAS number: 141112-29-0

CLH-O-0000002522-82-03/A1

Adopted

08 March 2013

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Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Isoxaflutole (ISO);** (5-Cyclopropyl-1,2-oxazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
EC number:	**No EC number listed in annex VI**
CAS number:	**141112-29-0**
Annex VI Index number:	**606-054-00-7**
Degree of purity:	**> 950 g/kg**
Impurities:	**Impurity profile has been claimed confidential. However, based on the available data, the impurities present are considered to not change the classification and labelling for isoxaflutole**

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Repr. 2 (H361d***) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)	Repr. Cat. 3; R63 N; R50-53
Current proposal for consideration by RAC	Addition of an acute M-factor of 10 Addition of a chronic M-factor of 100	Addition of SCL: Cn ≥ 2.5%: N; R50-53 0.25% ≤ Cn <2.5%: N; R51-53 0.025% ≤ Cn <0.25%: R52-53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 2 (H361d***) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410) Acute M-factor 10 Chronic M-factor 100	Repr. Cat. 3; R63 N; R50-53 SCL Cn ≥ 2.5%: N, R50-53 0.25% ≤ Cn <2.5%: N, R51-53 0.025% ≤ Cn <0.25%: R52-53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

A review of the available hazard data for isoxaflutole has revealed that the classification listed in Annex VI of Regulation EC no. 1272/2008 is in line with the data. In that respect, there is no need to change the current classification of isoxaflutole. However, a harmonized M-factor according to Regulation EC no. 1272/2008 and SCLs according to Directive 1999/45/EC as amended by Directive 2006/8/EC are not listed in Annex VI of Regulation EC no. 1272/2008. In this dossier, harmonized M-factors and SCLs for isoxaflutole are proposed, taking into account the criteria of the 2nd ATP.

Proposed classification according to the CLP Regulation

It is proposed to add to the existing entry in Annex VI an M-factor of 10 for acute aquatic toxicity and an M-factor of 100 for chronic aquatic toxicity.

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				conclusive but not sufficient for classification
2.2.	Flammable gases				conclusive but not sufficient for classification
2.3.	Flammable aerosols				conclusive but not sufficient for classification
2.4.	Oxidising gases				conclusive but not sufficient for classification
2.5.	Gases under pressure				conclusive but not sufficient for classification
2.6.	Flammable liquids				conclusive but not sufficient for classification
2.7.	Flammable solids				conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				conclusive but not sufficient for classification
2.10.	Pyrophoric solids				conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures				conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with				conclusive but not sufficient for

	water emit flammable gases				classification
2.13.	Oxidising liquids				conclusive but not sufficient for classification
2.14.	Oxidising solids				conclusive but not sufficient for classification
2.15.	Organic peroxides				conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				conclusive but not sufficient for classification
3.1.	Acute toxicity - oral				conclusive but not sufficient for classification
	Acute toxicity - dermal				conclusive but not sufficient for classification
	Acute toxicity - inhalation				conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation				conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation				conclusive but not sufficient for classification
3.4.	Respiratory sensitisation				conclusive but not sufficient for classification
3.4.	Skin sensitisation				conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				conclusive but not sufficient for classification

3.6.	Carcinogenicity				conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Repr.2 (H361d***)		Repr. 2: (H361d***)	
3.8.	Specific target organ toxicity – single exposure				conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure				conclusive but not sufficient for classification
3.10.	Aspiration hazard				conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)	Acute M-factor 10 Chronic M-factor 100	Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)	
5.1.	Hazardous to the ozone layer				conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word:	Warning
Pictogram:	GHS08, GHS09
Hazard statements:	H361d*** (Suspected of damaging the unborn child)
	H410 (Very toxic to aquatic life with long lasting effects)
Precautionary statements:	No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry: A note is not proposed.

Table 4: Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness				conclusive but not sufficient for classification
Oxidising properties				conclusive but not sufficient for classification
Flammability				conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*				conclusive but not sufficient for classification
Thermal stability				conclusive but not sufficient for classification
Acute toxicity				conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure				conclusive but not sufficient for classification
Repeated dose toxicity				conclusive but not sufficient for classification
Irritation / Corrosion				conclusive but not sufficient for classification
Sensitisation				conclusive but not sufficient for classification
Carcinogenicity				conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity				conclusive but not sufficient for classification
Toxicity to reproduction – fertility				conclusive but not sufficient for classification
Toxicity to reproduction – development	Repr. Cat. 3; R63		Repr. Cat. 3;.R63	

Toxicity to reproduction – breastfed babies. Effects on or via lactation				conclusive but not sufficient for classification
Environment	N;R50/53	Cn ≥ 2.5%: N; R50-53 0.25 % ≤ Cn < 2.5 %: N; R51-53 0.025 % ≤ Cn < 0.25 %: R52-53 where Cn is the concentration of isoxaflutole	N;R50/53	

1) Including SCLs
2) Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Xn, N
R-phrases: 50/53 - 63
S-phrases: (-2) 36/37-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Isoxaflutole has been assessed in the Draft Assessment Report, the Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of isoxaflutole in Annex I of Council Directive 91/414/EEC (DAR 1997 + subsequent addenda, RMS The Netherlands) concerning placing isoxaflutole on the market as a plant protection product (PPP). The final examination was finalized in April 2003.

Isoxaflutole was added to Annex I of Directive 67/548/EEC in the 28th ATP (Directive 2001/59/EC) with the classification Repr.Cat.3;R63, N;R50-53.

Isoxaflutole is currently listed in Annex VI of Regulation EC no. 1272/2008 with the same classification as was listed in the 28th ATP to Directive 67/548/EEC.

2.2 Short summary of the scientific justification for the CLH proposal

Isoaxflutole is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (Regulation EC no. 1272/2008, article 36.2).

Isoxaflutole is classified as Aquatic Acute 1 and Aquatic Chronic 1 under Regulation EC no. 1272/2008 and N; R50/53 under Directive 67/548/EEC. Harmonised M-factors or SCLs are not listed in Annex VI. However, the level of aquatic toxicity observed, the lowest EC50 and EC10 values of 0.0219 mg/L and 0.0004 mg/L, respectively, does give reason for the addition of M-factors and SCLs to the current Annex VI entry.

In the current CLH report, acute and chronic M-factors and SCL for isoxaflutole are proposed.

2.3 Current harmonised classification and labelling

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

Table 5

Classification		Labelling		
Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)
Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H361d*** H400 H410	GHS08 GHS09 Wng	H361d*** H410	

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

Table 6

Classification	Labelling
Repr. Cat. 3; R63 N; R50-53	Xn; N R: 50/53-63 S: (2-)36/37-60-61

2.4 Current self-classification **and labelling**

Not available.

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

Not available.

2.4.2 Current self-classification and labelling based on DSD criteria

Not available.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Isoaxflutole is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (Regulation EC no. 1272/2008, article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

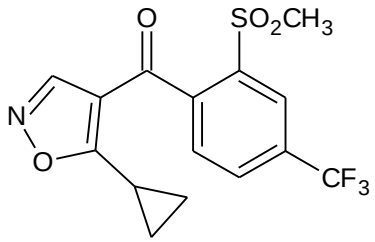
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 7 Substance identity

EC number:	An EC number has not been assigned
EC name:	-
CAS number (EC inventory):	141112-29-0
CAS number:	-
CAS name:	-
IUPAC name:	(5-Cyclopropyl-1,2-oxazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
CLP Annex VI Index number:	606-054-00-7
Molecular formula:	$C_{15}H_{12}F_3NO_4S$
Molecular weight range:	359.5

Structural formula:



1.2 Composition of the substance

Table 8: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Isoxaflutole	> 950 g/kg		

Current Annex VI entry:

Table 3.1: Repr. 2 (H361d***), Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410)
Table 3.2: Repr.Cat.3;R63, N;R50-53

Table 9: Impurities (non-confidential information)

Impurity	**Typical concentration**	**Concentration range**	**Remarks**
			All impurities have been claimed confidential. However, based on the available data, they are not considered to change the classification and labeling

Current Annex VI entry: -

Table 10 Additives (non-confidential information)

Additive	**Function**	**Typical concentration**	**Concentration range**	**Remarks**
				Not applicable

Current Annex VI entry: -

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 11: 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 (pure) or yellow (technical) granular powder	DAR B2.1.7	
Melting/freezing point	Approximately 140 °C Decomposition > 200 °C	DAR B2.1.1	Measured
Boiling point	Decomposition <360 °C	DAR B 2.1.2	Measured
Relative density	1590 g/l at 20 °C	DAR B 2.1.4	Measured
Vapour pressure	$1x10^{-6}$ Pa at 20°C	DAR B 2.1.5	Measured
Surface tension	Data not available		
Water solubility	6.2 mg/L at 20 °C (pH 5.5)	DAR B 2.1.11	Measured
Partition coefficient n-octanol/water	Log Kow = 2.32 (pH independent)	DAR B 2.1.13	Measured
Flash point	Not applicable		Substance is a solid
Henry's law constant	1.87 x 105 Pa mVmol (20 ºC)	DAR B 2.1.6	calculated
Flammability	Not highly flammable	DAR B 2.1.20	
Explosive properties	Not explosive	DAR B 2.1.22	
Self-ignition temperature	Not autoflammable	DAR B 2.1.20	
Oxidising properties	Not oxidizing	DAR B 2.1.23	
Granulometry	Data not available		
Stability in organic solvents and identity of relevant degradation products	Data not available		
Dissociation constant	Not applicable	B 2.1.18	No dissociation anticipated
Viscosity	Not applicable		Substance is a solid

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Isoxaflutole is a plant protection product that is used as an herbicide for crops (maize).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of isoxaflutole were assessed in the Draft Assessment Report, the Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of isoxaflutole in Annex I of Council Directive 91/414/EEC (DAR 1997 + subsequent addenda, RMS The Netherlands) concerning placing isoxaflutole on the market as a plant protection product (PPP).

No changes in the classification for the physico-chemical properties are proposed in this dossier. For this reason, it is considered not warranted to present detailed data relating on physical hazards in this dossier.

Isoxaflutole is not classified or labeled for physico-chemical properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazards of isoxaflutole were assessed in the Draft Assessment Report, the Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of isoxaflutole in Annex I of Council Directive 91/414/EEC (DAR 1997 + subsequent addenda, RMS The Netherlands) concerning placing isoxaflutole on the market as a plant protection product (PPP).

Based on a recent review of the available data on human health hazards, a change in classification for these hazard properties is not needed. For this reason, it is considered not warranted to present detailed data relating to the human health hazards in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate and ecotoxicological properties of isoxaflutole were assessed in the Draft Assessment Report, the Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of isoxaflutole in Annex I of Council Directive 91/414/EEC (DAR 1997 + subsequent addenda, RMS The Netherlands) concerning placing isoxaflutole on the market as a plant protection product (PPP).

Based on a review of the available data on environmental fate and aquatic toxicity, a change in the environmental classification is not needed. However, the level of toxicity does give reason for the addition of M- factors and SCLs to the current Annex VI entry

5.1 Degradation

Table 12: Summary of relevant information on degradation

Method	Results	Remarks	Reference
EPA 161-1 (compatible with EEC-C7)	DT50 for Hydrolysis at 25 ºC pH 5: 11.1 days pH 7: 20.1 hours pH 9: 3.2 hours	Hydrolysis product RPA 202248 was formed.	DAR: Corgier et al., 1994
EPA 162-2	Photochemical DT 50 : 40.0 hours under Xenon lamp in pH 5 buffer, at 25 °C	14C-phenyl labeled, > 98% purity	DAR: Corgier and Plewa, 1995
OECD 302B	11% degradation		DAR: Desmares-Koopmans, 1996
No guideline mentioned; GLP study	Water/sediment aerobic. Loam system (DT50system) Isoxaflutole: 0.53 days Metabolite RPA 202248: 700days Metabolite RPA 205834 97 days Clay loam system (DT50 system): Isoxaflutole: 0.34 days Metabolite RPA 202248: 255 days Metabolite RPA 205834: 52 days.		DAR: Ayliffe and Newby, 1995

5.1.1 Stability

In a study was performed with 14C-labelled isoxaflutol in the phenyl ring (purity 98.3%) , isoxaflutole hydrolysed at all pH levels. The hydrolysis rate increased with increasing pH; DT 50 of 11.1 days, 20.1 hours and 3.2 hours at pH 4, 7 and 9, respectively. The hydrolysis product RPA 202248 was detected.

In a photolysis study carried out according to EPA guidelines, isoxaflutole quickly photodegadated in an aqueous medium by photoreduction by opening of the isoxazol and cyclopropyl rings. The photochemical DT50 was 40.0 hours under Xenon lamp in pH 5 buffer, at 25 °C**.**

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

The biodegradability of isoxaflutole (99% pure) was determined in an OECD 302B (enhanced ready biodegradability study) with an inoculum derived from non-adapted activated sludge and a nominal concentration of 3 mg/L. After 28 days 11% degradation was observed.

5.1.2.3 Simulation tests

Water/sediment studies.

An aerobic water/sediment study with 14C – isoxaflutole (purity 98.7%, label in phenyl-ring), was performed under GLP conditions. The study was found to be acceptable. Test duration was 100 days. Sediment and water were taken from a stream. Loam and clay loam sediment was used, redox potential of sediment- and water-layers were determined. Isoxaflutole was passed through the water layer and incubation in the dark at 20 ºC. Samples were taken from water and sediment layer at different time points. Analysis of concentration of isoxaflutole and its metabolites was carried out by LSC (water) and LSC, TLC and HPLC (sediment).

Results: The total recovery radioactivity was 94 – 99% in both systems.

Loam system: Amount of isoxaflutole in the water was not reported No isoxaflutole was found in the sediment at any time point. Two major metabolites were determined: RPA 202248 with a maximum of 69% after 7 days for the whole system (56% at the end of the study) and RPA 205834 with a maximum of 24% after 2 days for the whole system (12% at the end). The metabolites reached a maximum and subsequently degrade thereafter. The DT50 of the system was 0.53 days for isoxaflutole, 700 days for RPA 202248, and 97 days for RPA 205834.

Clay loam system: Amount of isoxaflutole in the water was not reported. No isoxaflutole was found in the sediment at any time point. Two major degradation products were determined. degradation product RPA 202248 reached a maximum after 1 day of 70% (51% at the end of study) and degradation product RPA 205834 reached a maximum of 26% after 7 days (7% at the end of study). A minor degradation product (RPA 203328) was found with a maximum of 11% at the end of the study (100 days). The DT50 of the system was 0.34 days for isoxaflutole, 255 days for RPA 202248, and 52 days for RPA 205834. DT50 for RPA 203328 could not be determined.

CO_2 was hardly formed in both systems, only 0.1% was determined after 100 days.

Degradation products found in the aerobic water/sediment study:

RPA 202248: 2-cyano-3-cyclopropyl-1-(2-methylsulfonyl-4-trifluoromethylphenyl)propan-1,3-dione.
RPA 205834: 2-aminomethylene-1 -cyclopropyl-3-(2-methylsulfonyl-4-trifluoromethylphenyl)propan-1,3-dione.
RPA 203328: 2-methanesulfonyl-4-trifluoromethylbenzoic acid.

5.1.3 Summary and discussion of degradation

Isoxaflutole hydrolysed at all pH levels. The photolysis of isoxaflutole was 40 hours in a pH 5 buffer under artificial light conditions. In an enhanced ready biodegradability study (OECD 302B), 11% degradation of isoxaflutole was found. In an aerobic water/sediment system with two sediments, DT50 (system) values of 0.34 – 0.54 days for isoxaflutole were established. Isoxaflutole was not found in the sediment. In the water/sediment studies the DT50(system) values for the major degradation products RPA 202248 and RPA 205834 were 255-700 days and 52-97 days, respectively. No DT50 could be determined for degradation product RPA203328. Negligible CO2 was formed in both systems. It can be concluded that isoxaflutole undergo rapid primary degradation in the environment. However, the formed degradation products do not degrade rapidly. Negligible mineralisation occurs.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

An adsorption/desorption study was performed with 14C-isoxaflutole (purity 98.5%, label in the phenyl ring) with four different soils. The study was found to be reliable. Four concentrations were used and the shaking time was 54 hours. Soils and supernatants were analysed water directly with LSC, HPLC, and GC-MS, soil after extraction with acetonitrile:water 1:1 (twice). A desorption step was performed. Total recovery was 98 - 104%. Some transformation of isoxaflutole to metabolite RPA 202248 occurred (3.8 % - 14%). Degradation was highest for soils with higher pH. The resulting Kom values ranged between 54 l/kg and 79 l/kg (Koc 92 l/kg - 134 l/kg). The desorption showed that the adsorption is a reversible process.

5.2.2 Volatilisation

A Henry coefficient of 1.87 x 10^5 Pa m^3/mol at 20 º C was calculated.

5.2.3 Distribution modelling

From the adsorption/desorption study can be concluded that isoxaflutole is slightly mobile in soil and adsorption is reversible. The sorption appears to be correlated with the organic matter content of the soil.

5.3 Aquatic Bioaccumulation

Table 13 Summary of relevant information on aquatic bioaccumulation

Method	Results	Remark	Reference
Log Kow	2.32	Measured	DAR B. 8.2.20

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log Kow of isoxaflutoel is 2.32 (Kow 220). Based on this result, it can be concluded that the potential to bioaccumulate is low.

5.3.1.2 Measured bioaccumulation data

Bioaccumulation studies in aquatic environment are not available.

5.3.2 Summary and discussion of aquatic bioaccumulation

The log Kow of 2.32 shows that isoxaflutole does not have a potential to bioaccumulate.

5.4 Aquatic toxicity

A brief summary of the aquatic toxicity studies listed in the DAR for the three trophic levels fish, aquatic invertebrates and algae/aquatic plants are reported below.

Table 14: Summary of relevant information on aquatic toxicity for isoxaflutole.

Method	Results	Remarks	Reference
EPA	96-h LC50: > 2.7 mg/L	Flow-through, *Lepomis macrochirus.*	DAR: Bettencourt, 1993
EPA	96-h LC50: > 1.7 mg/L	Flow-through, *Oncorhynchus mykiss.*	DAR: Bettencourt, 1993
OECD draft	28-d NOEC: 0.08 mg/L	Flow-through, *Oncorhynchus mykiss*	DAR: Sewell and Bartlett, 1995
EPA	48-h EC50: > 1.5 mg/L	Flow-through, *Daphnia magna*	DAR: Putt,1993
OECD 202	21-d NOEC: 0.35 mg/L	Flow-through, *Daphnia magna*	DAR: Mc Elligott, 1995
EPA	120-h E_bC50: 0.12 mg/L 120-h NOEbC: 0.016 mg/L	Static, *Selenastrum capricornutum*	DAR: Hoberg, 1993
EPA	6-d E_rC50: 0.0219 mg/L 6-d E_rC10: 0.0004 mg/L	Semi-static, *Lemna gibba.* 6-day EC50 and EC10 values were calculated by the dossier submitter.	DAR: Hoberg, 1994
EPA	14-d NOEC: 0.61 mg/L 14-d NOEC: < 0.0080 mg/L (conservative value)	*Lemna gibba*, exposure only the first three days. Reduction in frond density was observed at 0.61 mg/L (41%), therefore a conservative value was estimated)	DAR: Hoberg, 1999

Table 15: Summary of relevant information on aquatic toxicity of the degradation product RPA 202248.

Method	Results	Remarks	Reference
EPA	96-h LC50 : > 15 mg/L	Semi-static, *Oncorhynchus mykiss.* (Undissolved particles at the two highest concentrations of 30 and 60 mg/L)	DAR 8.2.1.2/01
OECD 202	48-h EC50: > 60 mg/L	Semi-static, *Daphnia magna* 60 mg/L was the highest concentration tested.	DAR 8.2.4.2/01
OECD 201	72-h Eb,rC50: > 20 mg/L 72-h NOEC: > 20 mg/L	Static, *Scenedesmus subspicatus*	DAR 8.2.6.2/01
FIFRA 122-2 and 123-2	14-d EC50 (frond density): 0.083 mg/L* 14-d NOEC (fond density): 0.022 mg/L 14-d EbC50: 0.055 mg/L 14-d NOEbC: 0.022 mg/L	Semi-static, *Lemna gibba*, tested concentrations 0-30 mg/L. Analytical monitoring.	DAR 8.2.8/03

* As these findings will only be used to determine whether the degradation product will be classifiable, the data were not recalculated to derive the EC_{50} or NOEC for growth rate at the exponential growth period.

Table 16: Summary of relevant information on aquatic toxicity of the degradation product RPA 205834.

Method	**Results**	**Remarks**	**Reference**
EPA	96-h LC50: > 35 mg/L	Semi-static, *Oncorhynchus mykiss*	DAR 8.2.1.2/03
OECD 202	48-h EC50: > 100 mg/L	Semi-static, *Daphnia magna*	DAR 8.2.4.2/03
OECD 201	72-h Eb,rC50: > 15 mg/L 72-h NOEC: > 15 mg/L	Static, *Scenedesmus subspicatus*	DAR 8.2.6.2/03

Table 17: Summary of relevant information on aquatic toxicity of the degradation product RPA 203328.

Method	Results	Remarks	Reference
EPA	96-h LC50: 160 mg/L	Flow-through, *Oncorhynchus mykiss.* (pH at the highest test concentration was too low(pH < 4)	DAR 8.2.1.2/02
EPA	48-h EC50: > 150 mg/L	Flow-through, *Daphnia magna*	DAR 8.2.4.2/02
EPA	120-h EbC50: > 9.4 mg/L 120-h NOEbC: 2.4 mg/L	Static, *Selenastrum capricornutum* 10 mg/L, nominal, was the highest concentration tested	DAR 8.2.6.2/02

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1: The acute aquatic toxicity of isoxaflutole (purity 98.7%) was tested at five concentrations (0.65-5.0 mg/L nominal with undissolved material observed at the highest concentration, mean measured concentrations were 92% of nominal) in *Lepomis macrochirus* (bluegill) in a 96-h flow-through study following EPA guidelines with analytical monitoring of the test concentrations. An LC50 of >2.7 mg/L was determined in this study.

Study 2: The acute aquatic toxicity of isoxaflutole (purity 98.7%) was tested at five concentrations (0.32-2.5 mg/L nominal, mean measured concentrations 68-113%) in *Onchorhynchus mykiss* (rainbow trout) in a 96-h flow-through study following EPA guidelines with analytical monitoring of the test concentrations. An LC50 of >1.7 mg/L was determined in this study.

5.4.1.2 Long-term toxicity to fish

Study 1: The chronic aquatic toxicity of isoxaflutole (purity 99.2%) was tested at five concentrations (0.10-0.80 mg/L nominal, 0.08-0.73 mg/L measured) in *Onchorhynchus mykiss* (rainbow trout) in a 28-d flow-through study following draft OECD Guideline (Fish, juvenile growth test-28 days, 1992) with analytical monitoring of the test concentrations. First mortalities were observed on day 9. A NOEC of 0.08 mg/L was derived in this study

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1: The acute aquatic toxicity of isoxaflutole (purity 98.7%) was tested at five concentrations (0.32-2.5 mg/L nominal, 0.2-1.5 mg/l initial measured with concentrations increasing during the test) in *Daphnia magna* in a 48-h flow-through study following EPA guidelines with analytical monitoring of the test concentrations. An EC50 of > 1.5 mg/L was determined in this study.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1: The chronic aquatic toxicity of isoxaflutole (purity 99.6%) was tested at five concentrations (0.09-1.5 mg/L) in *Daphnia magna* in a 21-d flow-through study following OECD Guideline 202 with analytical monitoring of the test concentrations. At the two highest concentrations tested, 11 and 14 daphnids died. No effects on length and reproduction were observed in the other groups. A NOEC of 0.35 mg/L was derived from this study

5.4.3 Algae and aquatic plants

Algae

Study 1: Isoxaflutole (purity 98.7%) was tested at five concentrations (0.016-0.50 mg/L nominal, measured concentrations were 76-100% at t=0 but 22-50% at termination) in *Selenastrum capricornutum* in a 120-h static study following EPA guidelines with analytical monitoring of the test concentrations. An EbC50 of 0.12 mg/L and a NOEbC of 0.016 mg/L were determined in this study.

Aquatic plants

Study 1: The effects of isoxaflutole on the aquatic plant *Lemna gibba* have been investigated in a 14-day study following EPA guidelines using semi-static test conditions with test solution renewals at days 3, 6, 9, 12 and 14. Isoxaflutole (purity > 97% was tested in triplicate at six concentrations ranging from 0.00063 to 0.02 mg/L (nominal), with concurrent control and solvent control (acetone ≤ 1 ml/l). The concentrations of isoxaflutole in the test solutions were analytically monitored using HPLC before and after test solution renewal. Fronds were counted and observed on day 3, 6, 9, 12, and 14. Actual concentrations were 87-98% of nominal. Results are based on mean measured concentrations. At test termination fronds exposed to the two highest treatment levels were observed to be chlorotic, with smaller fronds and less root formation compared to control. Effects declined with lower test concentrations. Fronds exposed to 0.011 mg/L were observed to be smaller than the control fronds at test termination. Fronds in the lowest test concentration were normal compared to control fronds at test termination. No effects were observed in the control and solvent control during the test. At the end of the test, a significant reduction in frond production was determined at the four highest test concentrations (inhibition ranged from 26% to 74% at the highest concentration).

This study was carried out for 14 days whereas OECD guideline 221 requires test duration of 7 days. Examination of the growth rate over time obtained in this study showed that control cultures were no longer in exponential growth on days 9, 12 and 14. OECD guideline 221 states that one of the principles of this test is exponential growth in the control cultures. Any deviations from exponential growth in the controls skew the results. Based on this information, it was considered most appropriate to recalculate the ErC50 and ErC10 using measurements for days 0 through 6 as the control cultures were shown to be in exponential growth during this period. This 6-day exposure time is in good agreement with the 7-day exposure recommended in OECD guideline 221.

Using the calculation methods recommended by OECD guideline 221, a 6-day ErC50 value of 0.0219 mg/L and a 6-day ErC10 value of 0.0004 mg/L were calculated

Study 2: Another study examined the effects of isoxaflutole on *L. gibba* during a pulse-dose exposure in accordance with EPA guidelines. The plants were exposed to isoxaflutole (purity 99.7%) during the initial three days of the exposure and then transferred to fresh untreated medium on days 3, 6, 9 and 12. The test was terminated on day 14. Isoxaflutole was tested at 7 concentrations, ranging from 0.016 to 4.0 mg/L (nominal) with mean measured concentration ranging from 0.0080 to 3.9 mg/L. Results are based on mean measured concentrations. The 3, 6, 9, 12 and 14-day EC50 values were >3.9, 0.56, 1.0, 0.72 and >3.9 mg/L, respectively. 14-day NOEC was 0.61 mg/L. However, 18%-41% reductions in frond

densities were observed at concentrations between 0.0080 and 0.61 mg/L. Therefore, a conservative NOEC for frond density was empirically estimated to be < 0.0080 mg/L.

5.4.4 Other aquatic organisms (including sediment).

No data available.

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

No change in the environmental classification of isoxaflutole is proposed in this report. Data on degradation and bioaccumulation are presented for information only.

Isoxaflutole undergoes rapid primary degradation in water through hydrolysis. In an enhanced ready biodegradability study, only 11% degradation was observed. In a water/sediment study, isoxaflutole disappears rapidly from the system with a DT50system of < 1 day. However, three degradation products are formed which do not degrade rapidly. For one degradation product, a DT50 could not be determined. The DT50 system for the other two degradation products was 52-97 days and 255-700 days. Negligible mineralisation was observed throughout the study period (100 days).
The available data show that the aquatic plant *Lemna gibba* is the most sensitive aquatic species for isoxaflutole. For degradation product RPA202248, *L gibba* was also the most sensitive species with an EC_{50} and NOEC value <1 and 0.1 mg/L, respectively, For the other degradation products no information on the toxicity for *L. gibba* is available.. Based on this information degradation product RPA202248 would be classifiable. For the other degradation products it can not be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment. It is therefore concluded that isoxaflutole is not rapidly degradable.

The data on aquatic plants are considered the most appropriate for the derivation of M-factors and SCLs. Study 1 on *L. gibba* (section 5.4.3) will be used as the key study for deriving M-factors and SCL.

Acute M-factor (CLP)
The lowest EC50 value of 0.0219 mg/L obtained in *L. gibba* lies between 0.01 and 0.1 mg/L. Isoxaflutole fulfils criteria for classification as Aquatic Acute Cat. 1 with an acute M-factor of 10.

Chronic M-factor (CLP)
Isoxaflutole is not rapidly degradable. The lowest EC10 value of 0.0004 mg/L obtained in *L. gibba* lies between 0.0001 and 0.001 mg/L. Isoxaflutole fulfils criteria for classification as Aquatic Chronic Cat. 1 with a chronic M-factor of 100.

SCL (DSD)
The lowest L(E)C50 value of 0.0219 mg/L obtained in *Lemna gibba* lies between 0.01 and 0.1 mg/L. Isoxaflutole fulfils criteria for classification with N;R50/53, with an SCL of Cn ≥ 2.5% N; R50-53, 0.25% ≤ Cn <2.5% N; R51-53 and 0.025% ≤ Cn < 0.25%; R52-53.

Table 18: comparison toxicity of isoxaflutole with the CLP criteria

Lowest toxicity values: *Lemna gibba*	Criteria CLP	Toxicity Category	Criteria M factor CLP	M factor
6-d E_rC_{50}: 0.0219 mg/L	≤ 1 mg/L	Aquatic Acute category 1	0.01 < EC_{50} ≤ 0.1	10
6-d E_rC_{10}: 0.0004 mg/L	≤ 0.1 mg/L*	Aquatic Chronic category 1	0.0001 < NOEC ≤ 0.001 *	100

*: not rapidly degradable

Table 19: comparison toxicity of isoxaflutole with the DSD criteria

Lowest toxicity values: *Lemna gibba*	Classification according to DSD	SCLs
6-E_rC_{50}: 0.0219 mg/L	N; R50-53 (0.01 < EC_{50} ≤ 0.1 mg/L)	Cn * ≥ 2.5%, N; R50-53 0.25% ≤ Cn < 2.5%, N; R51-53 0.025% ≤ Cn < 0.25%, R52-53

*: Cn is the concentration of isoxaflutole in the mixture.

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

The available data show that the current CLP classification Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) and DSD classification N;R50-53 are in line with the available data. No change in the classification is needed.

CLP
However, the available data show that based on the 6-day E_rC_{50} in *Lemna gibba* of 0.0219 mg/L the acute M-factor is 10.
Based on the 6-day E_rC_{10} in *Lemna gibba* of 0.0004 mg/L the chronic M-factor is 100.

DSD
Based on the 6-day E_rC_{50} in *Lemna gibba* of 0.0219 mg/L the concentration limits are
Cn ≥ 2.5%: N; R50-53;
0.25% ≤ Cn < 0.25%: N; R51-53;
0.025% ≤ Cn < 0.25%: R52-53,
where Cn is the concentration of isoxaflutole in the mixture.

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Isoxaflutole has an existing environmental classification in Annex VI to the CLP Regulation: Aquatic Acute 1 and Aquatic Chronic 1 (CLP Regulation) and N; R50-53 (DSD). The dossier submitter (DS) has reviewed the environmental hazard data and concluded that no change to the environmental classification is necessary but proposed to update the Annex VI entry by including an acute M-factor of 10 and a separate chronic M-factor of 100 based on available chronic toxicity data.
Similarly, the following Specific Concentration Limits (SCL) according to DSD are proposed:
N; R50-53: C ≥ 2,5%
N; R51-53: 0,25% ≤ C < 2,5%
R52-53: 0,025% ≤ C < 0,25%

No other changes to the existing harmonised environmental classification are proposed.

The proposal for setting an acute M-factor is based on the acute toxicity data from test results for all three trophic levels, i.e. two fish species (*Lepomis macrochirus* and *Onchorhynchus mykiss*), one species of crustacean (*Daphnia magna*), one species of algae (*Selenastrum capricornutum*) and two tests with the duckweed *Lemna gibba*. In addition, chronic test results are available for fish (*Onchorhynchus mykiss*) and *Daphnia magna* which enable a separate chronic M-factor to be set.

The available data show that the aquatic plant *Lemna gibba* is the most sensitive aquatic species with an **EC_{50}** and an **EC_{10}** of **0.0219 mg/l** and **0.0004 mg/l**, respectively. Data on *Lemna gibba* were, therefore, considered the most appropriate for the derivation of both acute and chronic M-factors, and for SCLs according to DSD criteria.

One particular study performed with *Lemna gibba* was selected as the key study for deriving M-factors and SCLs. This study was in conformity with the relevant EPA test guidelines (U.S. EPA FIFRA Test Guidelines 122-2 and 123-2) and the exposure lasted for 14 days. The examination of the growth rate over time in this study showed that the control cultures were no longer in an exponential growth phase on days 9, 12 and 14. Any deviations from exponential growth in the controls can skew the results. For this reason, the ErC_{50} and ErC_{10} were re-calculated using measurements for days 0 to 6. This 6-day exposure time is considered to be sufficiently close to the 7-day exposure recommended in OECD guideline 221.

Regarding degradation, isoxaflutole undergoes rapid primary degradation in water through hydrolysis. However, data on primary degradation may be used for classification purposes only when it can be satisfactorily demonstrated that the degradation products formed do not meet the criteria for environmental classification. For one of the degradation products (RPA202248) an EC_{50} < 1 mg/l and a chronic NOEC of < 0.1 mg/l (*Lemna gibba*) was determined which would result in a classification as hazardous to the aquatic environment. Biodegradability of isoxaflutole was tested in an enhanced ready biodegradability study and in a water/sediment simulation test. In the enhanced ready biodegradability study, only 11% degradation was observed. In the water/sediment study, isoxaflutole disappears rapidly from the system with a DT_{50} of < 1 day. However, in the water/sediment study three degradation products were formed. For one degradation product, the DT_{50} could not be determined, while for the other two degradation products DT_{50} values of 52-97 days and 255-700 days were established.

In conclusion, while isoxaflutole hydrolysed at all pH levels tested, the degradation products do not degrade rapidly. Furthermore, tests also show that negligible mineralisation occurs. For this reason isoxaflutole is considered **not rapidly degradable**.

The above findings allow an acute M-factor of 10 ($0.01 < EC_{50} \leq 0.1$) and a chronic M-factor of 100 (non-rapidly degradable and $0.0001 < EC_{10} \leq 0.001$) to be determine according to CLP and SCLs according to DSD of:
N; R50-53: C ≥ 2,5%
N; R51-53: 0,25% ≤ C <2,5%
R52-53: 0,025% ≤ C < 0,25%

Comments received during public consultation

Two comments on the environmental classification were submitted during the public consultation by MSCAs. One MS agreed with the proposed M-Factors and SCLs. The other MS requested further clarification on the relevance of re-calculating the toxicity for *Lemna gibba* after 6 days of test duration in the context of the 14-days EPA test guidelines used i.e. U.S. EPA FIFRA Test Guidelines 122-2 and 123-2 (compared to the OECD test guideline) as this would influence the value of the proposed chronic M-factor.

Additional key elements
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Assessment and comparison with the classification criteria

Degradation

The RAC confirms the DS's conclusion that isoxaflutole is non-rapidly degradable. In spite of rapid primary hydrolytic degradation, mineralisation rates were far below the cut-off criterion of 70% within 28 days, both in an enhanced ready biodegradability test and in a water/sediment simulation study. While the RAC notes that results from tests on inherent biodegradability (e.g. OECD 302) would not be appropriate to confirm rapid degradability for classification purposes, the Committee considers the low mineralisation rate (11%) as supporting evidence for the non-rapid degradation of isoxaflutole. Moreover, in the water/sediment study, one degradation product of isoxaflutole is classifiable and two others showed high DT50 values.

Aquatic Toxicity

The particularly high sensitivity of duckweed to isoxaflutole is consistent with the substance's herbicidal mode of action. As micro-algae are about two orders of magnitude less sensitive according to the available test, it is appropriate to use the key study with *Lemna* for deriving both the acute and chronic M factors. The Committee supported the DS's argumentation for the recalculation of the study data to derive 6d ErC_{50} and ErC_{10} values, i.e. sufficiently in line with the 7d requirement of the OECD 221 guideline. .

The recalculated 6-day EC_{50} of 0.0219 mg/L is well below the 1 mg/L criterion for CLP Category Aquatic Acute 1, and the corresponding acute M-factor for $0.01 < 0.0219 \leq 0.1$ mg/L is 10.

The recalculated 6-day EC_{10} of 0.0004 mg/L is well below the ≤ 0.1 mg/L criterion for CLP Category Aquatic Chronic 1 (non-rapidly degradable substances), and the corresponding chronic M-factor for $0.0001 < 0.0004 \leq 0.001$ mg/L is 100.

Conclusion on classification

Isoxaflutole is **non-rapidly degradable**. Based on a measured log Kow of 2.32, its bioaccumulation potential is low.

The RAC agrees with the DS that adequate M-factors for the existing classification of isoxaflutole's aquatic toxicity should be based on recalculated 6-day EC-values from the key study with duckweed, and that the appropriate classification are CLP Categories **Aquatic Acute 1 (H400) with M = 10** and **Aquatic Chronic 1 (H410) with M = 100.**

RAC also agrees with the proposed SCLs according to DSD:

N; R50-53: C ≥ 2,5%
N; R51-53: 0,25% ≤ C <2,5%
R52-53: 0,025% ≤ C < 0,25%

Supplemental information - In depth analyses by the RAC

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6 OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of the active substance isoxaflutole according to Directive 91/414/EEC. The summaries included in this proposal are partly copied from the DAR. Some details of the

summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the DAR

7 REFERENCES

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8 ANNEXES