Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

N-(5-chloro-2-isopropylbenzyl)-*N*-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1*H*-pyrazole-4-carboxamide; isoflucypram

EC Number: -
CAS Number: 1255734-28-1

CLH-O-0000006854-65-01/F

Adopted

8 October 2020

8 October 2020

CLH-O-0000006854-65-01/F

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

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

Chemical name: ***N*-(5-chloro-2-isopropylbenzyl)-*N*-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1*H*-pyrazole-4-carboxamide; isoflucypram**

EC Number: **-**

CAS Number: **1255734-28-1**

The proposal was submitted by **the United Kingdom** and received by RAC on **4 April 2019.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **27 May 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **26 July 2019**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Nathalie Printemps**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 October 2020** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	*N*-(5-chloro-2-isopropylbenzyl)-*N*-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1*H*-pyrazole-4-carboxamide; isoflucypram	-	1255734-28-1	Acute Tox. 4 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H332 H317 H400 H410	GHS07 GHS09 Wng	H332 H317 H410		inhalation: ATE = 2.2 mg/L M = 10 (acute) M = 1 (chronic)	
RAC opinion	TBD	*N*-(5-chloro-2-isopropylbenzyl)-*N*-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1*H*-pyrazole-4-carboxamide; isoflucypram	-	1255734-28-1	Repr. 2 Acute Tox. 4 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H361f H332 H317 H400 H410	GHS07 GHS09 Wng	H361f H332 H317 H410		inhalation: ATE = 2.2 mg/L (dusts or mists) M = 10 M = 1	
Resulting Annex VI entry if agreed by COM	TBD	*N*-(5-chloro-2-isopropylbenzyl)-*N*-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1*H*-pyrazole-4-carboxamide; isoflucypram	-	1255734-28-1	Repr. 2 Acute Tox. 4 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H361f H332 H317 H400 H410	GHS07 GHS09 Wng	H361f H332 H317 H410		inhalation: ATE = 2.2 mg/L (dusts or mists) M = 10 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Isoflucypram is a pesticide active substance approved under regulation (EC) No 1107/2009. There is no existing entry in Annex VI of the CLP regulation for isoflucypram. Therefore, the proposal of the dossier submitter (DS) addressed all physical, human health and environmental endpoints. Isoflucypram is a succinate dehydrogenase inhibiting (SDHI) fungicide with a broad spectrum and is used against fungal diseases of cereal crops (wheat, triticale, rye, barley and oats). It is a new active substance in the scope of the Regulation (EC) No 1107/2009.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification is proposed by the DS for explosive properties based on negative results obtained in three tests performed according to UN Recommendations of the Transport of Dangerous Goods (UN RTDG) test series 1 and 2 (Dreisch, 2018).

No classification as flammable solid is proposed as the substance failed to ignite in the preliminary UN Test N.1 screening test (Winkler, 2017) and therefore does not meet the criteria for classification (time of burning < 45 s or rate of burning > 2.2 mm/s).

In a Differential Scanning Calorimetry (DSC) measurement of the substance (OECD TG 113), an exothermic effect was observed in the temperature range 360-440°C with a decomposition energy of -1110 J/g. On this basis, the DS proposed no classification for self-reactivity as the Self-accelerating Decomposition Temperature (SADT) of the substance could be considered > 75°C.

For pyrophoric solids and for substances that in contact with water emit flammable gases hazard classes, no data were available. Nevertheless, no classification is proposed based on experience in handling of isoflucypram and its chemical structure.

No classification as self-heating substance was proposed by the DS as no spontaneous combustion was observed in the standard procedure UN RTDG, test N.4 (Winkler, 2017).

No classification as oxidising solid was proposed by the DS. In an UN Test O.3, the substance cellulose mixture exhibits a lower burning rate than the reference substance (Winkler, 2018). Moreover, the substance does not contain oxygen, fluorine or chlorine bonded only to carbon or hydrogen.

No classification as corrosive to metals is proposed. Isoflucypram is a solid and there are no suitable test method for solid substances. According to the DS, the substance may not become liquid as the melting point of the substance is 108.8°C and as the water solubility is low (1.8 mg/L). Moreover, the substance shows no acidic or basic properties between pH 1 and 12.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with DS's proposal that **no classification for physical hazards** is warranted for isoflucypram based on the available data on the substance.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - Oral route

No classification is proposed based on the absence of mortality at 2000 mg/kg observed in an acute oral toxicity study in rats and supported by an acute neurotoxicity study in rats (Anonymous, 2014a & 2017e).

Acute toxicity - Dermal route

No deaths occurred in an acute rat dermal toxicity study. The acute dermal LD_{50} was greater than 2000 mg/kg in the study (Anonymous, 2014b). On this basis, the DS proposed no classification.

Acute toxicity - Inhalation route

The combined male/female LC_{50} calculated in rats exposed to a single dose of isoflucypram (mist aerosol) was 2.518 mg/L/4h (Anonymous, 2014c). On this basis, the DS proposed to classify the substance as Acute Tox. 4 (H332). The DS proposed to set an acute toxicity endpoint estimate (ATE) value at 2.2 mg/l based on female LC_{50} as females were more sensitive than males in this study.

Comments received during consultation

Two member states (MS) agrees with the proposed classification for acute inhalation toxicity in category 4 (H332). One of the MS also supported the ATE proposed by the DS.

Assessment and comparison with the classification criteria

Acute toxicity - Oral route

Isoflucypram was tested in an OECD TG 425 study (GLP compliant) in female rats at 2000 mg/kg. No deaths occurred and the LD_{50} was thus greater than 2000 mg/kg. Isoflucypram was also tested in an acute neurotoxicity study in rats (OECD TG 424, GLP compliant). In this study, no deaths occurred up to 2000 mg/kg in both males and females. According to the CLP regulation criteria, RAC agrees with the DS that **no classification is warranted for acute oral toxicity**.

Acute toxicity: dermal

The LD_{50} of isoflucypram in rats was greater than 2 000 mg/kg in an OECD TG 402 study (GLP compliant) in both sexes. **No classification for acute dermal toxicity is warranted** according to the CLP criteria.

Acute toxicity: inhalation

In an OECD TG 403 study, rats were exposed nose-only to 1.03, 2.04 and 2.87 mg/l/4h of isoflucypram (mist aerosol diluted in 10-20% (w/w) acetone). Mortality was observed from 2.04 mg/l in both males and females as detailed in the table below:

Dose levels (mg/l)	Mortality	
	Males	Females
1.03	0/5	0/5
2.04	1/5	1/5
2.87	2/5	5/5

The calculated combined LC_{50} in rats was 2.518 mg/l (95% CI: 2.01-3.663 mg/l), 3.131 mg/l for males (95% CI not calculated as the range was too wide) and 2.209 mg/l in females (95% CI not calculated as the range was too wide).

RAC agrees with the DS that isoflucypram fulfilled the criteria for **Acute Tox. 4, H332** based on the LC_{50} values obtained in the acute inhalation toxicity study (1 mg/l < LC_{50} < 5 mg/l for dusts or mists). For the ATE, RAC agrees with the DS to use the LC_{50} calculated in females. Thus, RAC agrees with **an ATE value of 2.2 mg/l**.

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

Summary of the Dossier Submitter's proposal

Four acute toxicity studies were considered by the DS to assess STOT SE hazard class: three acute toxicity studies (oral, dermal and inhalation) and the acute neurotoxicity study available in rats.

In these studies, there were no consistent findings in any organ following a single dose. Therefore, no classification STOT SE in category 1 or 2 was proposed by the DS.

No narcotic effects or respiratory tract irritation were noted in the studies, therefore no classification as STOT SE in category 3 was proposed.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS's proposal of **no classification for STOT-SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an OECD TG 404 study (GLP compliant), isoflucypram (moistened with water) was applied to the skin of 3 rabbits (Anonymous, 2014d). Exposure was for 4 hours under semi-occlusive

dressing. As no irritation was observed, the DS proposed no classification for skin irritation/corrosion.

Comments received during consultation

No specific comments were received.

Assessment and comparison with the classification criteria

In the absence of any signs of irritation in an OECD GLP compliant study, RAC agrees with the proposal of the DS **not to classify isoflucypram for skin irritation/corrosion**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In an OECD TG 405 study (GLP compliant), 100 mg of solid isoflucypram was instilled in the eyes of 3 male rabbits. No deviation were noted. The substance induced slight to moderate conjunctival redness and chemosis from 1 hour after application. The effects were fully reversible by 72 hours after instillation. The mean individual 24-72h scores were as follow:

- Corneal opacity and iritis: 0 in 3 out of 3 animals;
- Conjunctival redness: 1 in 3 out of 3 animals;
- Conjunctival Chemosis: 0.33 in 3 out of 3 animals.

According to the CLP criteria, no classification is warranted.

Comments received during consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Based on the available eye irritation study in rabbits, RAC agrees with the DS's proposal **not to classify isoflucypram for eye irritation**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No classification is proposed by the DS due to lack of data.

Comments received during consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC is unable to evaluate respiratory sensitisation due to lack of data.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Isoflucypram was tested in a GLP compliant Local Lymph Node assay (LLNA), in mice, conducted according to OECD TG 429 (Anonymous, 2015a). The vehicle used in the study was Acetone/olive oil (4:1 v/v). The following stimulation index values were obtained: 1.2 at 5%, 1.2 at 10%, 2.5 at 25%, and 5.6 at 50%. Based on interpolation, the EC_3 value was calculated to be 29%. On this basis, the DS proposed to classify the substance as Skin Sens. 1B, H317.

Comments received during consultation

One MS agreed to classify the substance as Skin Sens. 1B, H317.

Assessment and comparison with the classification criteria

No human data were available in the dossier.

RAC considers the available LLNA study reliable. As the calculated EC3 value was above 2%, (EC_3 = 29%), RAC agrees to **classify isoflucypram as <u>Skin Sens. 1B, H317</u>**.

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

Summary of the Dossier Submitter's proposal

The evaluation of STOT RE endpoint was based on ten oral repeated-dose toxicity studies performed according to or similar to OECD TG and GLP compliant:

- Three studies in dogs: 28 days, 90 days and one year;
- Three studies in mice: 28 days, 90 days and 18 months (including 1-year time point);
- Four studies in rats: 28 days, 90 days, 2 years (including 1-year time point) and the 2-generation reproductive toxicity study.

The liver was identified as the common target organ in dogs, rats and mice. Moreover, in rats, the kidneys and the thyroid were also identified as target organs.

Effects in the liver comprised treatment-related increases in liver weight and histopathological findings (e.g. hepatocellular hypertrophy). The DS considered the findings related to enzyme induction and adaptive. The liver effects were not consistent across sex and species, often without progress in severity with study duration and not sufficiently severe or significant to warrant classification.

The thyroid effects were only seen in rats. Increased thyroid weight and thyroid follicular cell proliferation were noted in the 28-day and 90-day studies. These findings were considered as a secondary consequence of increased activity of hepatic enzymes (UDPG-transferase and CYPs). Moreover, both liver and thyroid effects were considered likely due to the CAR/PXR mode of action (MoA), which is not relevant to humans, although acknowledging that other MoA have not been ruled out.

In kidney, the histopathological changes were considered as precursor of male rat specific chronic progressive nephropathy related to the accumulation of alpha2u-globulin. The DS considered the MoA not relevant to humans.

Overall, the DS proposed no classification for the STOT RE hazard class.

Comments received during consultation

One MS agreed that the effects were not severe enough to warrant classification under the CLP classification. Nevertheless, the MS considered the proposed CAR/PXR-MoA insufficiently substantiated and the non-relevance to humans not supported by compounds specific comparative mechanistic data (human *vs* rats).

One MS agreed with the DS proposal and considers that CAR/PXR activation was likely the MoA for liver findings.

Assessment and comparison with the classification criteria

RAC considered the same studies as the DS for STOT RE hazard assessment. In addition, liver findings observed in the developmental toxicity studies in rats and rabbits were also taken into account.

Liver effects

Liver was the target organ in all the repeated-dose toxicity studies (including carcinogenicity and reproductive toxicity studies) in rats, mice, dogs and rabbits.

In rats, at dose levels below the guidance value for STOT RE 2 classification, the following findings were noted:

- Increased liver weight *(*above 10%) at ≥ 64 mg/kg and cholesterol levels ≥ 81 mg/kg in the 28-day and 90-day toxicity studies;
- Hepatocellular hypertrophy graded minimal to slight in the 28 and 90-day studies;
- Hepatocellular periportal micro-vacuolation in the 28-day study.

RAC considers the liver findings observed in rats not significant health effects relevant for classification as STOT RE.

In mice, at dose below the guidance value for classification STOT RE 2, the following findings were noted:

- Increased liver weight *(*above 10%) at ≥ 51 mg/kg in the 90-day study;
- Hepatocellular centrilobular vacuolation graded minimal to slight in the 90-day study;
- Hepatocellular single cell necrosis and necrotic foci graded slight to minimal at ≥ 133 mg/kg in the 28-day study.

RAC considers the liver findings observed in mice not significant health effects relevant for classification as STOT RE.

In dogs, the following liver findings were noted at dose below the guidance value for classification:

- Increased liver relative weight (25 -52 % *vs* controls) in the 28-day and in the 90-day studies;
- Increased by 2 to 3-fold of alkaline phosphatase (AP) in the 90-day study;
- Centrilobular hypertrophy graded minimal in males in the 90-day study and in males and females in the 1-year study.

In dogs, the severity of the liver toxicity (weight changes, AP, histopathological changes) increased with dose levels and study duration. Nevertheless, as listed above, at dose below the

guidance values for classification STOT RE, not significant health effects relevant for classification were noted.

In rabbits, based on the developmental toxicity study, no liver findings were observed at dose below the guidance value for classification.

Overall, no classification is warranted for liver toxicity according to the CLP criteria.

Kidney effects

Kidney was a target organ in male rats. Kidney effects, consisting of hyaline droplets in proximal tubules and bilateral basophilic tubules, were observed in several repeated-dose toxicity studies, in male rats. The table below summarised the of relevant kidney findings in rats.

***Table**: kidney effects in repeated dose toxicity in rats*

Effects	Concentration (mg/kg)(sex)	Study duration	Reference
↑ Hyaline droplets in proximal tubular cells	≥ 23 (m) ≥ 64 (m)	28-day 90-day	Anonymous, 2017j Anonymous, 2013a
Alpha-2u-globulin accumulation	≥ 83(m)	28-day	Anonymous, 2017j
Bilateral basophilic tubules/ granular cast	≥ 240 (m) ≥ 64 (m)	28-day 90-day	Anonymous, 2017j Anonymous, 2013a

These effects are not considered severe effects relevant for classification according to the CLP criteria.

Thyroid effects

Thyroid was a target organ in male and female rats. At dose levels below the guidance value for classification, an increased in thyroid relative weight was noted in males in the 28-day study at ≥ 240 mg/kg and in the 90-day study at ≥ 63.5 mg/kg. Moreover, follicular cell hypertrophy (graded minimal to slight) and/or colloid alteration were noted in males and females in the 28-day study at ≥ 240 mg/kg and in the 90-day study at ≥ 63.5 mg/kg. In the reversibility group (4-week recovery), the incidence of colloid alteration, but not the severity, was still increased.

These effects are not considered severe effects relevant for classification according to the CLP criteria.

Overall conclusion

RAC agrees with the DS proposal **not to classify isoflucypram for STOT RE** as the relevant effects observed in liver, thyroid and kidney were not sufficient, at dose levels below the guidance value for STOT RE 2, to warrant classification via the oral route of exposure. RAC notes that no other route of exposure has been investigated. With regards to the hypothesised non-relevant MoA to human for the effects in liver, kidney and thyroid, RAC considers that uncertainties remained and notes that the non-relevance to humans has not been investigated (see in-depth analyses by RAC in the Background Document).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

No classification for germ cell mutagenicity was proposed by the DS. A positive result was obtained in an *in vitro* mammalian chromosome aberration assay in presence and absence of metabolic activation (S9 mix). Nevertheless, negative results were obtained in an Ames assay, an *in vitro* mammalian cell gene mutation assay and an *in vivo* micronucleus test. Based on the negative *in vivo* assay, the DS concluded that isoflucypram does not fulfilled the classification criteria for germ cell mutagenicity.

Comments received during consultation

One MS requested clarification on the *in vitro* mammalian cell gene mutation assay to conclude whether the test is equivocal or negative. The MS requested more information on the cytotoxicity at the dose levels where increased mutation frequency was observed, statistical analysis and historical control data. Indeed, the MS noted that based on the results of cloning efficiency measured after the expression period, no cytotoxicity was observed. The DS agreed with the MS, but noted that based on relative cloning efficiency immediately after treatment, cytotoxicity was evident. Moreover, the DS noted that clear precipitation was observed at the top concentrations. No more information on statistical analysis was provided by the DS. Overall, the DS concluded that the study is negative as the increase in mutation frequency was not reproducible or was observed in presence of clear precipitation.

Assessment and comparison with the classification criteria

In vitro data

One negative bacterial gene mutation assay is available. The study was performed according to OECD TG 471 and was GLP compliant.

An *in vitro* gene mutation assay in mammalian cells was performed according to OECD TG 476 in V79 cells (GLP compliant). The main limitation if the study was that, excluding precipitation dose levels, only 2 or 3 concentrations were analysable in some experiments instead of four recommended. A three-fold increase in mutant frequency was seen at 4 µg/mL, in absence of metabolic activation, in culture I of the first experiment. The increase was around the mean of the historical control range value. Thus, the increase was not considered of toxicological significance. Other 3-fold increases in mutation frequency were only noted at precipitating levels and in presence of cytotoxicity (relative survival < 10%). Observed precipitation may have interfere with the conduct of the test. Therefore, RAC agrees with the DS conclusion that the study is negative noting the limitation of the study.

An *in vitro* mammalian chromosome aberration assay was available with isoflucypram (performed according to OECD TG 473, GLP compliant). Although some limitations were noted (only 50 metaphases evaluated in experiment II without S9mix instead of 100 metaphases), the study is considered acceptable. Statistically significant increases in chromosomal aberration were noted in the study:

- *Without metabolic activation*: a statistically significant increase in chromosomal aberration above historical control range at 13.6 µg/ml was noted following 4h-exposure in the first experiment (3.3% vs 3% maximum in HC). RAC is unable to conclude on dose-response as at 23.9 µg/ml (next concentration), cytotoxicity was noted (mitotic index = 43%). A statistically significant dose-related increase in chromosomal aberration above historical control range at 6.1 µg/ml without S9mix, was also noted following 22-h exposure in the

second experiment (7% compare to 2.5% maximum value in HC). The increase is observed in presence of cytotoxicity (45% mitotic index);
- *With metabolic activation:* a statistically significant dose-related increase in chromosomal aberration with S9mix at 30 µg/ml, was observed following 4-h exposure at the top dose, above historical controls (4.5% vs 3.8% maximum in HC). The dose-related increase was observed only in the second experiment but not in the first experiment. The increase was observed at a cytotoxic concentration (mitotic index =35.6%);

Overall, RAC agrees with the DS that isoflucypram in this study, was clastogenic *in vitro* without S9mix and that equivocal results were observed with S9mix.

In vivo data

In vivo, a negative result was obtained in a micronucleus test performed in mice up to 2000 mg/kg. The study was performed according to OECD TG 476 (GLP compliant) by oral gavage. With regards to bone marrow exposure, there was no direct evidence of bone marrow exposure in the study:

- No alteration of PCE:NCE ratio was observed;
- Although some clinical signs were noted at all dose tested in the study, only ruffled fur was treatment related and such effect does not provide sufficient evidence of systemic bioavailability.

Nevertheless, there are some indirect evidence of systemic toxicity:

- In the 28-day toxicity study in mice, liver toxicity was observed (weight changes and histopathology at ≥ 133 mg/kg);
- In the ADME studies; low level of radioactivity was detected in plasma and bone marrow. Nevertheless, RAC ADME studies were only performed in rats;
- In the 2-year mouse chronic study, plasma sample indicated low concentration of the test item in plasma and high concentration of its two main metabolites in plasma. As bone marrow is well perfused, this is supportive of exposure.

Overall, RAC considers that there are limited evidence of bone marrow exposure.

It may be noted that in relation to germ cell mutagenicity:

- Isoflucypram does not appear to reach or accumulate in the male or female gonads from toxicokinetic studies
- No functional, macroscopic or histopathological changes were observed from reproductive toxicity studies at however, low exposure levels.

Comparison with the classification criteria

RAC agrees with the DS that **no classification is warranted for germ cell mutagenicity**, according to the CLP criteria, based on the negative results observed in the *in vivo* micronucleus assay. RAC notes that *in vivo* study has limited evidence of bone marrow exposure.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenic potential of isoflucypram was investigated in a 2-year rat study and an 18-month mouse study. No neoplastic effects were seen in rats and mice.

The DS considered that the highest dose used in the study was appropriate on the following basis:

- Precursor events of alpha2u-globulin nephropathy were observed in the repeated dose toxicity. Chronic progressive nephropathy may have impacted the survival of males in the study.
- Liver effects likely related to CAR/PXR activation were noted in the repeated dose toxicity studies. The top dose was chosen in order to have liver weight effects at the end of the study without excessive liver toxicity.

The DS considered that the highest dose used in the mice study was appropriate on the following basis:

- Body weight gain was reduced by more than 10 % in both males and females in the high dose groups.
- Relative liver weight was increased in the study by 27% in males and 23% in females;

Therefore, the DS considered that the doses of isoflucypram chosen for long-term studies were sufficiently high to cover the carcinogenic potential of isoflucypram and its metabolites and that no classification is warranted.

Comments received during consultation

One industry representative explained the rationale behind dose selection for the 2-year rat study. The dose selection was based on results of the short-term standard and mechanistic studies. An increase weight, histopathological changes and possibly tumours in the liver and thyroid were expected. Moreover, for male rats, an increased incidence and severity of histopathological findings associated with chronic progressive nephropathy and potentially an increase in mortality late in the long-term study were expected.
The industry representative acknowledged that although toxicity was expected, none was observed (except some adaptive changes in liver and thyroid). In order to understand the differences in response, and the absence of any appreciable amounts of isoflucypram in plasma samples taken at various time points during the cancer bioassay, two studies were conducted and provided during the consultation:

- An *in vitro* mechanistic screen of isoflucypram and its metabolites;
- A physiologically-based toxicokinetic (PBTK) modelling approach.

The *in vitro* screens confirmed that isoflucypram was able to induce CAR/PXR and indicates that the identified three main plasma metabolites do not have this potential. The PBTK modelling approach indicates that an internal exposure plateau for isoflucypram could be identified at dose slightly above the male top dose used in the carcinogenicity study (at least 2-fold) and slightly below in females, thus indicated that higher dose levels would not have led to significant increase in internal exposure to isoflucypram.

One consulting company working for the industry representative concluded that body weight gain reduction was a toxicological indicator of toxicity. The consulting company retrospectively calculated BMD_{10} on body weight gain data observed in sub-chronic and chronic studies with

isoflucypram and with five pyrazole carboxamide being SDHI fungicides. For isoflucypram, in rats, only the BMD10 for male rats after 13 weeks (38.4 mg/kg) and 52 weeks in females (51.6 mg/kg) were calculated. Other BMD (carcinogenicity study, 2-generation study) were not calculated as the data could not be fitted by the hill model. In conclusion, of the BMD analysis, the consulting company concluded that the high dose utilised in the chronic rat study was near to the maximum tolerable dose (MTD).

One MS considers that the MTD was not reached in the rat 2-year study and highlighted that the survival in the study was below 50% in all groups. The MS also pointed out that other SDHI induced tumours when tested at higher dose levels. The DS responded that also high mortality rate was observed in the study, the power of the study to detect a carcinogenic effect was not compromised. Indeed, the increased mortality (> 50%) was only seen during the last three months of the study. Concerning the tested doses, the DS considered that higher dose may have cause chronic nephropathy (leading to death).

One MS agreed with the DS' proposal for no classification regarding carcinogenicity.

Assessment and comparison with the classification criteria

Rats

No tumours were observed following 2-year administration in male and female rats up to an average dose of 18.8 mg/kg bw/day in males and 46.6 mg/kg bw/day in females. Nevertheless, RAC considers that the dose selection in the carcinogenicity study is an issue.

With regards to general toxicity, no treatment related effect on survival was noted in the study. Body weight in males was not affected at any dose levels during any phase of the study. In females, at the top dose, body weight was slightly reduced during the second half of the study. Body weight gain was reduced from study day 50 and was occasionally statistically significant (↓ 4% *vs* control at the end of the study).

No severe systemic toxicity was observed. Indeed, liver findings consisted of an increased in liver weight at the top dose in females (~10%) which is not considered adverse in absence of other concomitant findings. Thyroid effects consisted of an increased in the incidence of colloid alteration and pigmentation in the follicular cells were noted in both males and females (graded minimal to moderate). No kidney findings were noted.

In males, the top dose selection was based on the observed kidney toxicity in the short-term repeated-dose toxicity studies. The kidney toxicity was considered likely to be related to αu-globulin MoA, and precursor effects of chronic progressive nephropathy and potentially tumours. RAC agrees that survival at the late stage of the study may have been decreased secondary to severe chronic progressive nephrotoxicity. Nevertheless, minimal to moderate chronic progressive nephropathy may not have affected the survival and the interpretation of the results.

In females, dose selection was based on the observed liver toxicity in the 13-week sub-chronic toxicity study and a 28-day mechanistic study investigating liver and thyroid effects. Although liver toxicity was expected by industry due to the likely mediated CAR/PXR MoA, none was observed.

Based on the above consideration, RAC considers that the long-term rat study does not satisfy the MTD requirement.

In order to assess whether the dose levels used in the carcinogenicity study were sufficient to investigate potential carcinogenicity hazard, RAC analysed the available toxicokinetics data

performed during the 2-year study and the 2-generation study and the provided PBTK modelling approach (summarised in the additional key elements above).

Plasma measurements performed in the carcinogenicity study and in the 2-generation reproductive toxicity study showed that the mean plasma concentration of the substance was very low throughout the studies and non-linear. On the contrary, mean plasma concentrations of two metabolites were higher than the parent compound throughout the studies. For the metabolites, the plasma concentrations increased with increasing dose-levels. Therefore, while higher dietary concentrations of isoflucypram may not have significantly increased systemic exposure to the parent, it can be considered that systemic exposure to its main metabolites may have significantly raised.

	Mean plasma concentration_mean values (µg/L)					
	Males			**Females**		
Dose (mg/kg)	**1.24**	**6.27**	**18.6**	**1.746**	**8.54**	**46.6**
3-4 month time point_carcinogenicity study (µg/L)						
Isoflucypram	< LOQ	<10	<19	< LOQ	< 12	15
Metabolite I	68	282	526	135	339	610
Metabolite II	29	162	510	14	146	1113
1-yr time point_carcinogenicity study (µg/l)						
Isoflucypram	<10	<15	<15	< 10	14	<68
Metabolite I	74	320	523	155	423	813
Metabolite II	20	123	377	15	143	1278
2-yr time point_carcinogenicity study (µg/l)						
Isoflucypram	<10	<10	32	<10	<17	27
Metabolite I	111	540	895	121	682	1470
Metabolite II	<15	69	310	<12	83	1230

	F0 males			**F0 females**		
Dose (mg/kg)	**11**	**34**	**94**	**13**	**40**	**104**
Plasma levels, _mean values (µg/L), 2-generation study						
Isoflucypram	4.6	8.9	23.8	15.3	19.4	24.3
Metabolite I	130	509	1343	94	541	2073
Metabolite II	95	281	557	86	358	1291

According to the PBTK modelling, a point of non-linearity was expected for the substance at 40 mg/kg in males and 30 mg/kg in females for isoflucypram. Nevertheless, RAC considers that even if a point of linearity was identified at such low dose, higher doses would still have led to higher internal exposure to the metabolites. This statement is also supported by data available in the repeated dose toxicity studies as increased liver toxicity and dose-related decrease in bilirubin (which is a good indicator of isoflucypram exposure) was observed with increasing dose levels.

Mice

No tumours were observed in the carcinogenicity study in mice. The top dose in males (300 mg/kg/d) resulted in a reduction of body weight gain of more than 10%). Moreover, a statistically significant increase in liver weight (12-18%) and histopathological findings were found in the liver. Therefore, RAC agrees with the DS that isoflucypram was not carcinogenic in mice.

Conclusion

Overall, based on the absence of tumours in the carcinogenicity studies, **no classification is warranted for isoflucypram due to inconclusive data**. The dosing chosen in the study aimed

at producing some toxicity as recommended in the test guideline, but the results unfortunately show insufficient dosing of rats to fulfil the requirement of the guideline. RAC thus notes that the data from rat studies is not adequate to inform on liver, thyroid and kidney or other organ carcinogenicity due to insufficient dosing.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Isoflucypram was evaluated for reproductive and developmental toxicity in a 2-generation reproductive toxicity study in rats (Anonymous, 2018) and in prenatal developmental toxicity studies in rats (Anonymous, 2017h) and rabbits (Anonymous, 2017i). All studies were conducted according to OECD TG (OECD 416 and OECD 414) and were GLP compliant. Additionally, a modified *in vivo* rat uterotrophic assay was available in the dossier (Anonymous, 2011).

Sexual function and fertility

In the 2-generation reproductive performance study, rats were administered isoflucypram orally *via* the diet at 0, 11.27, 34.1 and 94.4 mg/kg/day for males and 13.03, 40.8 and 104.4 mg/kg/day for females (1-10 weeks), respectively.
The DS provided the dose selection rational. Based on the absence of adverse effects related to treatment in a preliminary one-generation study up to 65.2 mg/kg in males and 76.7 mg/kg in females, higher dietary concentration were used in the main study. The top dose was expected to produce significant systemic toxicity.

In the main study, a 5-day delay in the mean age of vaginal opening was noted at the top dose in females F1 and was discussed by the DS. Although the delay was not a reflection of a delayed physical development (body weight was not lower at the age of completion), the finding was not considered adverse as:

- Subsequent oestrous cycle, offspring survival, mating performance, fertility were not affected;
- Other developmental landmarks were not affected (e.g. anogenital distance);
- Historical controls showed an intrinsic variability of this parameter;
- No effects on vaginal opening was observed in the modified rat uterotrophic assay.

In F1 adult female, at the top dose, a lower number of females with a 23-day gestation period was noted as more females had a gestation period of 22/22.5 days compared to control. The DS did not consider the effect as a specific reproductive effect as the shortest gestation length of 22 days was similar to that observed in F0 controls. The DS also suggested that the finding could be a consequence of liver toxicity.
No other effects related to sexual function and fertility were noted in the study.

Overall, the DS proposed no classification for isoflucypram for sexual function and fertility.

Developmental toxicity

In the 2-generation study, at the top dose, an increase in the litter incidence of bilateral or unilateral renal pelvis was observed in F1 offspring's sacrificed at PND21. As no pathology in kidneys was seen in F1 parental animals, the DS considered that the observation was of no toxicological significance.

In the rat developmental toxicity study, no treatment-related malformations (external, skeletal or visceral) or external variations were observed at any dose level. An increase in the incidence of distended bladder, dilated renal pelvis (above HCD range) and thymic remnant (within HCD range) were noted at the top dose. These increases were considered as variations by the DS and as the unspecific, secondary consequence of the maternal toxicity observed at the top dose of 625 mg/kg bw/d by the DS.

In the rabbit developmental toxicity study, no developmental toxicity was noted up to the highest dose level of 500 mg/kg at which dose maternal toxicity was observed.

Overall, the DS proposed no classification for developmental toxicity.

Comments received during consultation

Sexual function and fertility

One MS commented that the MTD was not reached in the 2-generation study. The MS also pointed out that the increase of the mean age of the vaginal opening was outside the HCD mean. Moreover, the MS highlighted that although females went to mate successfully and produce F2 generation, a shift in gestation length was noted at the top dose. In addition, the MS considered that the non-guideline modified rat uterotrophic assay should not be given much weight as only 6 animals were used per group instead of 15 recommended in the OECD TG. Moreover, the assay did not cover *in utero* exposure and from individual data, vagina was not opened at PND 39 for 1/6 females and 2/4 females at 400 mg/kg and 800 mg/kg, respectively. Therefore, based on these considerations, the MS concluded that a classification for reproductive toxicity may be warranted. The DS responded that the 2-generation study was enough sensitive as the top dose caused reasonable level of toxicity in parental animals (food consumption, liver, thyroid and kidney weight changes and effects on clinical chemistry indicative of liver toxicity. Moreover, the DS considered the delay in vaginal opening as a chance finding as these females went to mate successfully and produce F2 generation and as no other effects on other developmental landmarks were noted in the study. The small decrease in gestation length in the F1 generation at the top dose only was considered likely of a consequence of the liver toxicity. The limitations of the rat uterotrophic assays were acknowledge but, still, the study was considered supportive of an absence of effect.

One MS agreed with the DS's proposal for no classification. The MS agreed that the delay in the age at vaginal opening was not an adverse effect based on the justification provided by the DS.

One research and consulting company commented that based on retrospective BMD calculation, the BMD was 38.4 mg/kg /day in males after 13 weeks and 51.6 mg/kg/day in females after 52 weeks. Thus, they concluded that the high doses of isoflucypram used in the 2-generation study approximated the maximum tolerable dose.

Developmental toxicity

One MS agreed with the DS that no classification is warranted for developmental toxicity.

Assessment and comparison with the classification criteria

Sexual function and fertility

Reproductive effects

No treatment-related effects on fertility parameters were observed in the 2-generation study in rats.
In F1 female pups, the mean age at which vaginal opening occurred was statistically significantly higher in the high dose group than in control. Indeed, at the top dose, a 5-day delay was noted in the mean age and 11 females had vaginal opening at days outside the maximum age observed in controls (≥ 39 days). Moreover, the lowest value in the top dose group was at the mean of the concurrent controls. The mean age at which vaginal opening occurred was outside the HCD. At the day of measurement, the body weight of F1 female pups was statistically significantly increased. Thus, the increase was not related to a delayed development. Nevertheless, these F1 females went to mate on successfully and produce F2 generation. Moreover, in some of the historical controls, a vaginal opening was noted at 43 days (but mean age in this study was higher than in this study). Thus, the biological relevance of the delay is unknown.

Parameter	Delay of the onset of puberty				HCD[1]
Dose (mg/kg)	0	13	40	104	
Vaginal patency					
Days (mean) Days (Range)	33 29-38	33 28-36	33 29-38	38** 33-43	31.7-36.9 28-43
Body weight at criterion (g)	105	103	104	124**	

** p≤0.01; [1] 13 studies, 2013-2017, same strain, same laboratory (detailed in page 35 of the CLH report)

In the available modified *in vivo* uterotrophic assay, no effects on vaginal opening was seen up to 400 mg/kg. Nevertheless, RAC considers the data of limited relevance since as only 6 animals per group were used instead of 15 recommended in the OECD TG and as *in utero* exposure was not covered by the assay.

No effects were either seen in the preliminary study for reproductive performance. In this study dose up to 76.7 mg/kg were achieved (pre-pairing dose). The top dose is slightly below the dose where effects were seen in the main study. Moreover, only eight dams per groups were used in this preliminary study. Thus, RAC considers also the data from this study of lower weight than in the two-generation study.

A small decrease in gestation length in the F1 females was also noted at the top dose. Some historical controls from the laboratory were provided during the consultation. HCD comes from studies performed in 2011, 2014 and 2015. RAC notes that the laboratory data from 2011 are outside of the preferred 5-year period (reducing their relevance). In these HCD, gestation length was between 22 and 23 days and in some case up to 24 days. No trend was identified in the proportion of animals that has a gestation length of 22, 22.5 or 23 days. Therefore, although from the available data a shift was observed at the top dose, there are no indication that the effects would be adverse as still in the expected range of 22-23 days for all animals.

Parameter	F0				F1			
Dose (mg/kg)	0	13	40	104	0	13	40	104
Gestation length (days)								
22	25%	32%	25%	14%	17%	9%	33%	29%
22.5	25%	18%	38%	39%	48%	65%	42%	58%
23	50%	50%	38%	46%	35%	26%	25%	13%

In the available repeated-dose toxicity studies, no effects on reproductive organs were seen in rats and mice. In the dog one-year study, in males, a decrease in the absolute weight of the epididymides and a decrease in the weight (both absolute and relative to brain weight) of the prostate were noted. These organ weight changes were not accompanied by any macroscopic or microscopic findings, and thus are not considered indicative of adverse effect.

Parental toxicity and dose selection

There were no mortality or clinical signs considered related to treatment in the study. Body weight was not affected by treatment during the pre-mating period in males and females and during gestation. Although some isolated changes in body weight gain were noted in females F0 during lactation, this was considered unrelated to treatment by the study authors as no similar findings were noting in F1 females during lactation. Cholesterol levels were higher in females of the F0 generation at the top dose and were also higher in males and females of the F1 generation at the mid and high dose levels. Higher relative liver weight was apparent in both generations (11 to 20% increased). For females and offspring in both generations these effects were seen at the mid and high dose levels. No macroscopic or histopathological changes were considered relative to treatment.

Plasma concentration and its metabolites were measured in the study and plasma levels were as follow (For F0, similar results obtained for F1 and F2):

	F0 males			F0 females		
Dose (mg/kg)	11	34	94	13	40	104
Plasma levels_mean values (µg/L)						
Isoflucypram	4.6	8.9	23.8	15.3	19.4	24.3
Metabolite I	130	509	1343	94	541	2073
Metabolite II	95	281	557	86	358	1291

The measurements showed low levels of the substance in plasma while higher levels of metabolites were found.

Overall, RAC notes that the top dose selection was not appropriate as no adverse findings were noted in parental animals. In line with one MS comment received during consultation, this raises the issue of whether the endpoints were fully investigated or indeed if the study was truly OECD 416 guideline compliant with regard to the selection criteria for determining the highest dose.

Conclusion

Exposure to isoflucypram delayed the mean age of onset of vaginal opening at the top dose in females by 5 days. This delay was statistically significant compared to control values ($p < 0.01$). The mean 5 day delay was above the available mean historical control range and is thus considered treatment-related and biologically relevant. There is no evidence to conclude that the delay was secondary to a delayed development and not a direct effect of isoflucypram. A shift in the gestational length was also noted in the study in F1 but was still within the expected range in rats. RAC considers that the top dose use was not appropriate. Thus, the 2-generation study does not fully inform on potential reproductive outcome. RAC considers the adverse effect on the onset of female puberty relevant for classification. In a modified uterotrophic assay, no effect on the day at vaginal opening was observed but RAC notes several limitations in the study (low number of animals, no *in utero* exposure). Overall, considering the available data and the limitation of the studies, a classification in category 1B is not warranted. However, RAC concludes that **classification in Category 2 for sexual function and fertility is warranted** for the adverse effect on the onset of puberty.

Developmental toxicity

In the rat developmental toxicity study performed in 2014, the following developmental findings were observed (statistically-significant and/or above HCD):

- An increase in delayed ossification in the zygomatic arch, hyoid centrum, femur and humerus;
- An increased in the incidence of visceral variations: distended bladder, dilated renal pelvis and thymic remnant;

	% foetuses affected / % litters affected				**HCD (% Foetal affected/%litter affected)**
Dose (mg/kg)	0	25	125	625	
Visceral variations					
Thymic remnant presence (uni/bi)	2 / 14.3	4.1 / 19	4.4/ 26.1	**7.2*** / 34.8	2-7.1/ 8.7-39.1
Bladder, distended	0 / 0	0 / 0	0 / 0	**1.8 / 4.3**	0/0
Dilated renal pelvis (uni/bi) (less than severe)	0 / 0	2 / 4.8	1.3 / 8.7	**4.2* /26.1***	0 – 2.9/0-13.6
Skeletal variations- incomplete ossification					
At least one bone of zygomatic arch (uni/bi)	2.5 / 14.3	3.8 / 14.3	5.7 / 30.4	**8.5* /52.2***	0 – 8.8/0-34.8
Squamosal (uni/bi)	0.6 / 4.8	1.3 / 4.8	1.7 / **13.0**	**2.3 / 17.4**	0 – 2/0-9.1
Hyoid centrum	2.5 / 19.0	1.3 / 9.5	6.3/ 34.8	**8.0* / 39.1**	0 – 7.9/0-34.8
Femur (uni/bi)	1.3 / 9.5	2.5 / 19.0	4.6 / 21.7	**8.5 **/** 21.7	0.6 – 6.4/ 4.3-26.1
Humerus (uni/bi)	0 / 0	0 / 0	**2.3 / 13.0**	**1.7 / 8.7**	0 – 1.3/0-4.5

In bold: statistically significant or outside HCD range. [1]HCD: 9 studies from same strain and same laboratory (2012-2017)

Based on an expert opinion provided by industry and available in the dossier, the DS concluded that the observed visceral findings (dilated renal pelvis, distended urinary bladder and thymic remnant) should be considered as variations rather than malformations.

Concerning thymic remnants, the dose-related statistically significant increase can be considered treatment-related. The increase was only slightly above HCD range in a fetal basis and inside HCD range for the affected litters. Embryonic thymic remnants can give rise to ectopic thymic tissue in the neck and from birth to three month of age, small remnants of thymus tissue can still be observed in the neck. According to the expert opinion, these thymic remnant results from a normal embryological process and the bulk of the thymic tissue has already reached the correct final position and thus is considered to reflect a delay in the migration process. For these reasons, thymic remnants should be considered as a variation. RAC agrees that thymic remnant should be considered as a variation on the basis of the above considerations.

Dilated renal pelvis were considered as variation as the finding was less than severe and is generally reversible. RAC agrees with the proposed classification and agrees that the reversibility was indeed observed in the 2-generation study available with isoflucypram. Although dilated renal pelvis was observed in 9/28 F1 litters, no pathological changes were noted in the kidney in parental animals.

With regards to distended bladder, this is a rare finding as it was not observed in the studies before. According to the industry expert opinion, the output of the foetal kidney must be sufficient to maintain amniotic fluid volume, and the urine, by means of excretion into the amniotic fluid (via the bladder) is ultimately recycled back to the foetus by swallowing. The major result of failure to pass urine would thus be a significant reduction in amniotic fluid. In the absence of evidence suggesting the presence of a structural malformation of the bladder or marked reduction

of amniotic fluid volume, the industry expert opinion concluded that the distension should be a transient effect and classified as a variation. The severity of the effect was not provided in the dossier. RAC agrees with the proposed classification of the finding as variation. Moreover, RAC noted that based on the provided percentage, the incidence was clustered to 3 foetuses from the same litter that may reduce the concern.

Concerning the maternal toxicity observed in the study at the top dose, no statistically significant changes in body weight and body weight gain was noted. A sporadic decrease in body weight gain was noted on GD 6-8 (by 43%, not statistically significant). Reduced food consumption was also noted during GD 6-8 (by 12%, statistically significant). No mortality or clinical signs were seen in the study. Minimal to slight liver toxicity was observed at the top dose in dams as an increase in the incidence of enlarged liver and minimal to slight hepatocyte hypertrophy was noted in 10 out of 23 dams of the study. Therefore, RAC considers, in contrast to the DS that only mild maternal toxicity was noted in the study at the top dose.

Overall, the observed developmental effects (visceral variations and skeletal developmental delays) in rats are considered treatment-related and were observed in presence of mild maternal toxicity that may not explain the observed effects. Nevertheless, as the observed findings were mainly the reflection of delayed post-natal development, the findings are not considered sufficient for classification.

In the rabbit developmental toxicity study, no developmental toxicity was noted up to 500 mg/kg.

Overall, in agreement with the DS, RAC concludes that **no classification for developmental toxicity of isoflucypram is warranted**.

Effects on or via lactation

In the 2-generation rat study, there was no effect on survival, body weight or body weight gain of offspring in either F1 or the F2 generation. Therefore, **no classification for effects on or via lactation is warranted**.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

No classification is proposed, as the endpoint is not relevant for a solid.

Comments received during consultation

No comments received during the consultation.

Assessment and comparison with the classification criteria

RAC agrees with DS's proposal.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The present evaluation exclusively relies on data submitted in the context of the application for approval as an active substance under Regulation (EC) No 1107/2009.

Overall, the dossier submitter (DS) concluded that isoflucypram is 'not rapidly degradable', has a low potential for bioaccumulation and proposed classification based on aquatic and chronic toxicity to fish:

Aquatic Acute 1 with an M-factor of 10, based on the lowest 96-hour nominal LC_{50} value of 0.081 mg/L for *Pimephales promelas*; and

Aquatic Chronic 1 with an M-factor of 1, based on the lowest 33-d NOEC of 0.01328 mg/L for *Pimephales promelas*.

Degradation

The results of a hydrolysis study (OECD TG 111, GLP) showed that isoflucypram is hydrolytically stable in the laboratory in the dark for 7 days at all three pH values (pH 4, 7, 9) at 50°C temperatures (Heinemann; Kasel; 2015;).

No significant degradation was observed in aerobic mineralisation study (OECD TG 309, GLP). <10% degraded after 61 days at 20°C (Gabbert; Smith; 2017).

Aerobic water/sediment degradation study (OECD TG 308, GLP) was carried out using two natural water/sediment systems (i.e. Anglersee and Wiehltalsperre). In the total water/sediment system, isoflucypram was degraded slowly. The calculated values for the total system at 20°C based on whole system were DT_{50} 222 (Anglersee) and 681 (Wiehltalsperre) days, mineralisation <1% applied radioactivity (Hein, E. M.; Kasel, D.; 2017).

Three aerobic soil degradation studies (OECD TG 307, GLP) have been performed in a number of soils in the dark in the laboratory (Hellpointner, E.; Junge, T.; 2014. Gabbert, D.; McConnell, L. L.; Arthur, E. L.; 2017. Heinemann, O.; Kasel, D.; 2017). In all of them isoflucypram was slowly degraded in soil under aerobic conditions. The degradation rates derived from these studies were kinetically evaluated according to FOCUS Kinetics (2006, 2014). The calculated DT_{50} values of isoflucypram were in the range of 222 to 709 days in the tested soils (Reinken; Kallweit; 2017).

Aerobic soil degradation in the field (GLP) was between DT_{50} 16.5 and 177 days in all tested soil (Heinemann, O.; Junge, T.; 2017).

Anaerobic soil degradation (OECD TG 307, GLP) was >1000 days in the dark in the laboratory at 20.4°C (Heinemann, O.; Kasel, D.; 2015).

The photolytic route and rate of degradation (OECD TG 316, GLP) were studied in sterile aqueous buffer solution at pH 7 under exposure to simulated sunlight and aerobic conditions in the laboratory at 24.5°C for 10 days. Based on the experimental half-life for isoflucypram of 150 days for irradiated samples, the DT_{50} value of isoflucypram under environmental conditions was calculated to be e.g. 484 solar summer days at Phoenix (Arizona, USA) or 750 solar summer days at Athens (Greece) (Heinemann; Kasel; 2015;).

Direct photolysis in water (OECD TG 101, 316, GLP) half-lives were estimated of 8 to 22 days at pH 7 for the periods of main use during spring and fall (Heinemann, O.; 2013).

Soil photolysis (OECD Draft Test Guideline: Phototransformation of Chemicals on Soil Surfaces, GLP) was studied on one soil under exposure to simulated sunlight and aerobic conditions in the laboratory for 10 days at 20.0°C (Heinemann, O.; 2013). Photolytic degradation did not occur (<10% degradation after 10 days).

A study on the ready biodegradability of isoflucypram was not performed.

Overall, due to the results summarized above DS concluded that isoflucypram is not ultimately degraded to >70 % within 28 days (equivalent to a half-life < 16 days), or rapidly transformed to non-classifiable products. As a consequence, isoflucypram was considered as not rapidly degradable, according to CLP criteria.

Aquatic Bioaccumulation

An experimental aquatic study (OECD TG 305, GLP) to determine the bioconcentration potential (BCF) of isoflucypram is available. It was conducted in a flow-through system with Bluegill sunfish (*Lepomis macrochirus*) and exposure to two different treatment levels (i.e. 0.500 and 5.00 µg/L). Since the lipid content was not determined in all sampled fish a mean lipid value of 5.13 % which is the mean value from day 0 to 28 considering all treatment groups was used to calculate the lipid normalization factor. For the whole fish, the lipid normalized steady-state bioconcentration factor (BCFSSL) was calculated to be 308 L/kg and 383 L/kg for the treatment level of 0.500 and 5.00 µg/L, respectively. For the whole fish, the lipid normalized and growth corrected kinetic bioconcentration factor (BCFKLG) was calculated to be 370 L/kg and 361 L/kg for the treatment level of 0.500 and 5.00 µg/L, respectively (Anonymous, 2017l).

The determined log P_{ow} of 4.0 at pH 4, pH 7 and pH 9 (OECD TG 117, GLP) meet the CLP trigger value of ≥4 indicating a potential for bioaccumulation and that log P_{ow} is not pH dependent (Ziemer, F.; Peschke, C.; 2014).

Consequently, as preference is given to the fish BCF of 370 L/kg, which is below the CLP criterion of ≥ 500, the DS concluded that isoflucypram can be considered as having low potential for bioaccumulation.

Aquatic Toxicity

The aquatic toxicity test results from available acute and chronic studies for all trophic levels of isoflucypram are summarised in the following table and sections. All provided studies were considered as acceptable and reliable by the DS. As well all effect concentrations were corrected for purity.

Acute aquatic toxicity

Test organism	**Guideline, test method**	**Short-term result (endpoint)**	**Reference / Test item Study No**
Fish			
Pimephales promelas	OECD TG 203, EC No. 440/2008, C.1, EPA OCSPP 850.1075, MAFF Guideline 12 Nousan No. 8147 / GLP	**96h LC_{50} 0.0861 mg/L (nom)**	Anonymous (2018c) / Isoflucypram, **M-542897-02-1**
Oncorhynchus mykiss	OECD TG 203, EC No. 440/2008, C.1, EPA OCSPP 850.1075, MAFF Guideline 12 Nousan No. 8147 / GLP	96h LC_{50} 0.098 mg/L (nom)	Anonymous (2015c) / Isoflucypram **M-543443-01-1**
Cyprinodon variegatus	OECD TG 203, EPA OCSPP 850.1075 / GLP	96h LC_{50} 0.544 mg/L (mm)	Anonymous (2015d) / Isoflucypram **M-537137-01-1**

Aquatic invertebrates			
Daphnia magna	OECD TG 202, EC No. 440/2008, C.2, EPA OCSPP 850.1010, MAFF Guideline 12 Nousan No. 8147, GLP	48h EC_{50} = 0.201 mg/L (gm)	Kuhl, K. (2016) / Isoflucypram **M-574184-01-1**
Americamysis bahia	EPA OCSPP 850.1035 / GLP	48h EC_{50} = 0.270 mg/L (mm)	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P. (2016) / Isoflucypram **M-547041-01-1**
Algae			
Pseudokirchneriella subcapitata	OECD TG 201, EPA OCSPP 850.4500 / GLP	72h E_rC_{50} >2.02 mg/L (gm) 72h E_yC_{50} >2.02 mg/L (gm)	Kuhl, K. (2017) / Isoflucypram **M-586715-01-1**
Anabaena flos-aquae	OECD TG 201, EPA OCSPP 850.4550 / GLP	72h E_rC_{50} 4.8 mg/L (gm) 72h E_yC_{50} 3.4 mg/L (gm) 72h E_bC_{50} 3.5 mg/L (gm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H. (2017) / Isoflucypram **M-605074-01-1**
Skeletonema costatum	OECD TG 201, EPA OCSPP 850.4500 / GLP	96h E_rC_{50} >2.538 mg/L (gm) 96h E_yC_{50} 1.5 mg/L (gm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H. (2017) / Isoflucypram **M-604811-01-1**
Navicula pelliculosa	OECD TG 201, EPA OCSPP 850.4500 / GLP	72h E_rC_{50} >2 mg/L (gm) 72h E_yC_{50} >2 mg/L (gm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H. (2017) / Isoflucypram **M-604809-01-1**
Aquatic plants			
Lemna gibba	OECD TG 221, EPA OCSPP 850.4400 / GLP	7d E_rC_{50} >2.48 mg/L (gm)	Kuhl, K. (2017) / Isoflucypram **M-593965-01-1**

mm: mean measured concentration, gm: geometric mean measured concentration, nom: nominal concentration

Acute aquatic toxicity data on isoflucypram are available for fish, invertebrates, algae and aquatic plants. Fish are the most acutely sensitive trophic group. The lowest reliable acute endpoint is the 96-hour nominal LC_{50} of 0.081 mg isoflucypram/L for *Pimephales promelas*. This value is <1 mg/L and thus, the DS proposed that isoflucypram should be classified as Aquatic Acute 1. As the LC_{50} is >0.01 mg/L but ≤0.1 mg/L an Acute M-factor of 10 should also be applied.

Chronic aquatic toxicity

Test organism	**Guideline, test method**	**Short-term result (endpoint)**	**Reference / Test item Study No.**
Fish			
Pimephales promelas	OECD TG 210, EPA OCSPP 850.1400, ASTM E 1241-92 / GLP	**33d NOEC 0.0133 mg/L (mm)**	Anonymous (2017) / Isoflucypram **M-580247-01-1**
Cyprinodon variegatus	OECD TG 210, EPA OCSPP 850.1400 / GLP	35d NOEC 0.025 mg/L (mm)	Anonymous (2016a) / Isoflucypram **M-575119-01-1**
Aquatic invertebrates			
Daphnia magna	OECD TG 211, EPA OCSPP 850.1300 / GLP	21d EC_{10} 0.0584 mg/L (nom) 21d NOEC 0.072 mg/L (nom)	Bruns, E (2017) / Isoflucypram **M-593961-01-1**
Americamysis bahia	EPA OCSPP 850.1350 / GLP	28d NOEC 0.020 mg/L (mm)	Milligan, A. L.; Siddiqui, A. I.; Gallagher, S. P.; Krueger, H. O. (2016) / Isoflucypram **M-567966-01-1**

Crassostrea virginica	EPA OPPTS 850.1025 / GLP	96h NOEC 0.049 mg/L (nom)	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P. (2016) / Isoflucypram **M-547035-01-1**
Algae			
Pseudokirchneriella subcapitata	OECD TG 201, EPA OCSPP 850.4500 / GLP	72h E_rC_{10} >2.02 mg/L (gm) 72h E_yC_{10} 0.42 mg/L (gm) 72h NOErC 0.598 mg/L (gm)	Kuhl, K. (2017) / Isoflucypram **M-586715-01-1**
Anabaena flos-aquae	OECD TG 201, EPA OCSPP 850.4550 (2012) / GLP	72h E_rC_{10} 4.3 mg/L (gm) 72h E_yC_{10} 2.6 mg/L (gm) 72h NOE_rC 2.2 mg/L (gm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H. (2017) / Isoflucypram **M-605074-01-1**
Skeletonema costatum	OECD TG 201, EPA OCSPP 850.4500 / GLP	96h E_rC_{10} 0.91 mg/L (gm) 96h NOE_rC 1.478 mg/L (gm) 96h E_yC_{10} 0.27 mg/L (gm) 96h NOE_yC 0.186 mg/L (gm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H. (2017) / Isoflucypram **M-604811-01-1**
Navicula pelliculosa	OECD TG 201, EPA OCSPP 850.4500 / GLP	72h E_rC_{10} >2 mg/L (gm) 72h E_yC_{10} 1.7 mg/L (gm) 72h NOE_rC 0.67 mg/L (gm)	Arnie, J. R.; Siddiqui, A. I.; Porch, J. R.; Martin, K. H. (2017) / Isoflucypram **M-604809-01-1**
Aquatic plants			
Lemna gibba	OECD TG 221, EPA OCSPP 850.4400 / GLP	7d E_rC_{10} >2.48 mg/L (gm)	Kuhl, K. (2017) / Isoflucypram **M-593965-01-1**

Chronic/long-term aquatic toxicity data on isoflucypram are available for fish, aquatic invertebrates, algae and aquatic plants. The lowest reliable chronic value considered by the DS was the 33-day (geometric mean measured) NOEC of 0.0133 mg isoflucypram/L for *Pimephales promelas*. As this is ≤0.1 mg/L and isoflucypram is 'not rapidly degradable' it should be classified as Aquatic Chronic 1. The NOEC value falls within the range >0.01 to < 0.1 and thus, a Chronic M-factor of 1 should be applied.

Comments received during consultation

Two MSs submitted comments on the environmental part of the DS's proposals. One MS agreed with the proposed classification by the DS with only editorial comment. The other MS only asked for correct DT_{50} values for the water/sediment studies and DT_{50} values in aerobic soil degradation. The DS confirmed these DT_{50} corrections and that they can be found in the revised LoEP in the DAR.

Assessment and comparison with the classification criteria

Degradation

No hydrolysis of isoflucypram was observed and the substance was stable at pH 4, 7 and 9 at 50 °C.

In an aerobic mineralisation study, <10 % degraded after 61 days at 20 °C.

An aerobic water/sediment study showed that the whole system DT_{50} was 222 – 681 days at 20 °C, while mineralisation was <1% of applied radioactivity.

Phototransformation in water shows that isoflucypram was slowly degraded in aqueous buffer solution at pH 7 under exposure to simulated sunlight and aerobic conditions in the laboratory. No degradation products > 10% AR were observed. The experimental DT_{50} for isoflucypram was 150 days in irradiated samples. Kinetic evaluation could not be performed for dark samples due

to the stability of isoflucypram in the dark. Based on experimental half-life of 150 for irradiated samples, the DT50 value of isoflucypram under environmental conditions was calculated to be e.g. 484 solar summer days at Phoenix (Arizona, USA) or 750 solar summer days at Athens (Greece).

Based on direct photo-degradation in water the environmental half-lives of sunlight exposed top surface water layers were estimated to 8 to 22 days for a direct phototransformation of isoflucypram during periods of main use in spring to fall.

Although a study on the ready biodegradability of isoflucypram is not available, RAC agrees with the assessment of the DS that isoflucypram is not ultimately degraded to >70 % within 28 days (equivalent to a half-life < 16 days), or rapidly transformed to non-classifiable products. Consequently, RAC agrees that isoflucypram should be considered as not rapidly degradable under the CLP regulation.

Bioaccumulation

In the available experimental study to determine the bioconcentration potential, the determined whole fish BCF value of 370 L/kg for isoflucypram (kinetic BCF lipid normalised and growth corrected) is below the CLP trigger value of ≥500. However, the derived Log P_{ow} value of 4 (the same in different buffered media (pH 4, 7 and 9) at 25 °C) meets the CLP trigger value for indication of bioaccumulation (Log K_{ow} ≥4). Following the CLP regulation (section 4.1.2.8.1), the available, reliable experimental BCF determined in fish is taken in preference to the Log K_{ow}. Therefore, based on the BCF_{fish} below 500, RAC agrees with the DS that isoflucypram is not bioaccumulative according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels and agrees that the provided studies are acceptable and reliable. The most acutely sensitive trophic group is fish. The most chronically sensitive trophic groups are fish and invertebrates, both in same order of magnitude. The most sensitive species were fish *Pimephales promelas* at both testing (acute and chronic).

RAC also notes that new information was provided from the approval process under Regulation (EC) No 1107/2009. The information relevant for CLH has been included in the opinion and taken into account for hazard classification. The new study did not provide data that would affect the classification outcome. RAC acknowledges that while the reassessments of studies included in the CLH dossier do not change the conclusions regarding reliability and the acute endpoint values have not changed, the reassessed chronic endpoints are accepted and should be taken into account, although the recalculated endpoints do not change the classification outcome.

Consequently, RAC agrees that the lowest acute endpoint for aquatic acute classification is the 96-hour LC_{50} value for *Pimephales promelas* of 0.0861 mg/l (Anonymous 2018c, M-542897-02-1) based on nominal concentration.

The lowest chronic endpoint used for aquatic chronic classification purpose was initially recalculated based on morphological and behavioural observations and the statistical analysis of hatching success, larval survival and larval growth (expressed as dry weight and total length) (Bayer, 2017, M-580247-01-1). It was then further reassessed due to an initially wrongly assumed LOQ with the new value based on the concept of time weighted average (TWA) calculated concentrations (Bayer, 2019, M-648245-01-1). RAC accepts the reassessment of this study and the recalculated endpoint value. Therefore, the lowest chronic endpoint for aquatic chronic classification purpose is the 33d NOEC value for *Pimephales promelas* of 0.0147 mg/l. This result is in the same range as the value previously provided for this study (33d NOEC 0.0133 mg/L) and does not alter the classification outcome.

Conclusion on classification

Isoflucypram is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and reliable information, RAC agrees with the DS that isoflucypram warrants classification as:

Aquatic Acute 1 based on LC_{50} = 0.0861 mg/L for *Pimephales promelas.* As this acute toxicity value falls within the 0.01 < $L(E)C_{50}$ ≤ 0.1 mg/L range, the **acute M-factor is 10**.

Aquatic Chronic 1 based on NOEC =0.0147 mg/L for *Pimephales promelas*. As this chronic toxicity value falls within the 0.01 < NOEC ≤ 0.1 mg/L range, the **chronic M-factor is 1.**

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The DS indicated that due low volatility (vapour pressure = 1.2 × 10-7 Pa at 20°C) is expected that isoflucypram would be highly unlikely to deplete the stratospheric ozone layer.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

A substance shall be classified as hazardous to the ozone layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

Isoflucypram is a solid at room temperature and has a very low vapour pressure of 1.2×10^{-7} Pa at 20°C. Therefore, significant volatilisation of isoflucypram is not expected. In addition, estimates of the chemical lifetime in the troposphere resulted in half-lives <2 days for isoflucypram. Based on this, RAC concludes that it is highly unlikely that isoflucypram would be available in the stratosphere.

Consequently, RAC agrees that isoflucypram is not expected to be hazardous to stratospheric ozone and **does not warrant classification for hazards to the ozone layer**.

ANNEXES:

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

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

Annex 3 Records of the targeted consultation following the submission of studies and expert statements containing additional information on the hazard to human health