European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

ISOFLUCYPRAM

Volume 1

Rapporteur Member State : United Kingdom
Co-Rapporteur Member State : France

Version History

When	What
March 2019	Initial DAR

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Level 1

ISOFLUCYPRAM

1. STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1. CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1. Purpose for which the draft assessment report was prepared

This draft assessment report has been prepared to evaluate the dossier for the new active substance BCS-CN88460 (provisional ISO name, isoflucypram) and its formulated product Isoflucypram EC 50. The dossier was submitted for the first active approval under Regulation (EC) No 1107/2009 with the United Kingdom carrying out the assessment as the Rapporteur Member State.

The active substance is a fungicide for the control of stem-base, foliar and ear diseases in wheat (including Durum wheat and spelt), triticale, rye, barley and oats. Isoflucypram belongs to the chemical class of Succinate DeHydrogenase Inhibitor (SDHI) fungicides. This dossier contains data and information to support a limited range of representative uses of the active substance for which it is intended to demonstrate that, for one preparation, the requirements of Regulation (EC) No 1107/2009, Article 4 can be met.

The representative formulation, Isoflucypram EC 50, is an emulsifiable concentrate containing 50 g/L (5.15 % w/w) Isoflucypram. The representative uses for Isoflucypram EC 50 are: cereals. These uses are intended to include the proposed major commercial applications and represent exposure scenarios sufficiently rigorous to allow adequate evaluation of risk to humans and the environment.

This application from Bayer AG is for the first approval of isoflucypram in accordance with Regulation (EC) No. 1107/2009. So far, no provisional or final registrations have been granted in any country, so neither EU nor CODEX MRLs exist for isoflucypram at this point in time. However, alongside this application Bayer AG has submitted an application to set specific maximum residue levels (MRLs) as the new active substance does not satisfy the requirements of Annex II/III/IV of Regulation (EC) No 396/2005.

A Harmonised Classification and Labelling report in line with Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 has also been produced. This has been submitted by the UK CLP Competent Authority to ECHA in October 2018 to follow the aligned evaluation process.

1.1.2. Arrangements between rapporteur Member State and co-rapporteur Member State

The UK acting as the Rapporteur Member State (RMS) evaluated the dossier and produced a DAR (Draft Assessment Report). The DAR was the subject of a peer review by France (co-RMS).

1.1.3. EU Regulatory history for use in Plant Protection Products

Isoflucypram is a new active substance and products containing it have not previously authorized in the EU.

1.1.4. Evaluations carried out under other regulatory contexts

Isoflucypram is a new active substance with fungicidal action, developed by Bayer AG. This dossier is the application of Bayer AG for the first approval of isoflucypram in accordance with Regulation (EC) No. 1107/2009. No registrations or authorizations of isoflucypram containing plant protection

products are existent in EU Member States or elsewhere. Currently there are also no other relevant EU-evaluations of the active substance carried out in the framework of other relevant EU-legislation (e.g. biocides, flavourings, food additives, cosmetics).

1.2. APPLICANT INFORMATION

1.2.1. Name and address of applicant(s) for approval of the active substance

Name: Bayer AG
Address: Alfred-Nobel-Strasse 50
40789 Monheim am Rhein
Germany

Person to contact: [redacted]
Bayer AG
Research & Development, Crop Science
[redacted]
Phone: [redacted]
Email: [redacted]

1.2.2. Producer or producers of the active substance

Name: Bayer AG
Address: Alfred-Nobel-Strasse 50
40789 Monheim am Rhein
Germany

Person to contact: as applicant

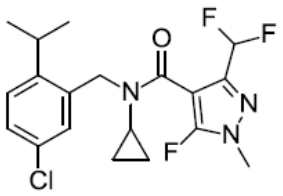
1.2.3. Information relating to the collective provision of dossiers

This application is submitted for the first approval of isoflucypram in accordance with Article 7f of Regulation (EU) No. 1107/2009.

Bayer AG is the only applicant and is the sole owner of the supplementary dossier submitted in support of this applicantion.

1.3. IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1. Common name proposed or ISO-accepted and synonyms	Isoflucypram, ISO provisionally approved, no synonyms
1.3.2. Chemical name (IUPAC and CA nomenclature)	
IUPAC	N-(5-chloro-2-isopropylbenzyl)-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1H-pyrazole-4-carboxamide

CA	*N*-[[5-chloro-2-(1-methylethyl)phenyl]methyl]-*N*-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl-1*H*-pyrazole-4-carboxamide 1H-Pyrazole-4-carboxamide, N-[[5-chloro-2-(1-methylethyl)phenyl]methyl]-N-cyclopropyl-3-(difluoromethyl)-5-fluoro-1-methyl- (CAS index name)
1.3.3. Producer's development code number	*This item should be also included in the LoEP* BCS-CN88460
1.3.4. CAS, EEC and CIPAC numbers	
CAS	1255734-28-1
EEC	not allocated
CIPAC	not allocated
1.3.5. Molecular and structural formula, molecular mass	
Molecular formula	C_{19} H_{21} Cl F_3 N_3 O
Structural formula	
Molecular mass	399.84 g/mol
1.3.6. Method of manufacture (synthesis pathway) of the active substance	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the RAR.
1.3.7. Specification of purity of the active substance in g/kg	Minimum purity: 960 g/kg
1.3.8. Identity and content of additives (such as stabilisers) and impurities *Isomers not covered by the common name should be listed here, as impurities.*	
1.3.8.1. *Additives*	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the RAR.
1.3.8.2. *Significant impurities*	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the RAR.
1.3.8.3. *Relevant impurities*	BCS-CN45153 Max. 1.0 g/kg
1.3.9. Analytical profile of batches	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the RAR.

1.4. INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1. Applicant	Bayer AG Alfred-Nobel-Strasse 50 40789 Monheim am Rhein Germany
1.4.2. Producer of the plant protection product	Bayer AG Alfred-Nobel-Strasse 50 40789 Monheim am Rhein Germany
1.4.3. Trade name or proposed trade name and producer's development code number of the plant protection product	Isoflucypram EC50
1.4.4. Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1. **Composition of the plant protection product**	Refer to Volume 4 Confidential Information
1.4.4.2. **Information on the active substances**	Refer to Volume 4 Confidential Information
1.4.4.3. **Information on safeners, synergists and co-formulants**	Refer to Volume 4 Confidential Information
1.4.5. Type and code of the plant protection product	Emulsifiable Concentrate [Code: EC]
1.4.6. Function	Fungicide
1.4.7. Field of use envisaged	Cereals
1.4.8. Effects on harmful organisms	

1.5. DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1. Details of representative uses

PPP (product name/code)	Isoflucypram EC50	Formulation type:	EC (emulsifiable concentrate)
active substance	Isoflucypram	Conc. of as:	50 g/L
safenern.a.		Conc. of safener:	n.a.
synergist	n.a.	Conc. of synergist:	n.a.
Applicant:	Bayer AG, Monheim, Germany	professional use	☒
Zone(s):	Northern / Central / Southern EU	non-professional use	☐
Verified by MS:	y/n		

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. g/kg (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	Kg a.i./hl min max (g/hl)	Water l/ha min max	Kg a.i./ha min max (*) (g/ha)		
wheat	EU	Isoflucypram EC50	F	*Mycosphaerella graminicola, Puccinia recondita, Puccinia striiformis, Pyrenophora tritici-repentis*	EC50	50g/L	Foliar spray	BBCH 30-69	1	-	-	100-400	0.075	*	
rye	EU	Isoflucypram EC50	F	*Puccinia recondita, Rhynchosporium secalis*	EC50	50g/L	Foliar spray	BBCH 30-69	1	-	-	100-400	0.075	*	
triticale	EU	Isoflucypram EC50	F	*Mycosphaerella graminicola, Puccinia recondita, Puccinia striiformis, Pyrenophora tritici-repentis*	EC50	50g/L	Foliar spray	BBCH 30-69	1	-	-	100-400	0.075	*	
barley	EU	Isoflucypram EC50	F	*Rhynchosporium secalis, Pyrenophora teres, Puccinia hordei,*	EC50	50g/L	Foliar spray	BBCH 30-61	1	-	-	100-400	0.075	*	

				Ramularia collo-cygni											
oats	EU	Isoflucypram EC50	F	*Puccinia coronata, Pyrenophora avenae*	EC50	50g/L	Foliar spray	BBCH 30-61	1	-	-	100-400	0.075	*	

* For uses where the column „Remarks“ in marked in grey further consideration is necessary. Uses should be crossed out when the notifier no longer supports this use(s).

(a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)

(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds

(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

(m) PHI - minimum pre-harvest interval

1.5.2. Further information on representative uses

Not applicable.

1.5.3. Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not applicable.

1.5.4. Overview on authorisations in EU Member States

Not applicable. Isoflucypram is a new active substance not previously approved in the EU.

Level 2

ISOFLUCYPRAM

2. SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

2.1. IDENTITY

Acceptable data have been submitted to support two manufacturing sites of isoflucypram and a joint specification has been proposed to cover both sites. The data are currently from pilot scale production therefore further data will be required once full scale manufracturing has commenced.

2.2. PHYSICAL AND CHEMICAL PROPERTIES

2.2.1. Summary of physical and chemical properties of the active substance

Pure isoflucypram is a white, odourless powder. The technical material is also a white powder but has a weak non-characteristic odour. Pure isoflucypram has a vapour pressure of 1.2 x 10-7 Pa at 20 °C, indicating that it is only very slightly volatile. Its volatility, as determined by Henry's law Constant, is 2.7 x 10-5 Pa m^3 mol^{-1}. It has a melting point of 108.8 °C, but showed no boiling point up to the start of decomposition at 215 °C. It has a relative density of 1.22 and a surface tension of 68.2 mN/m at 20 °C. It is readily soluble in organic solvents but only slightly soluble in water (1.8 mg/L at 20 °C, pH 5.8). No dissociation constant was observed in the pH range 1-12. The log Pow for isoflucypram is 4.0 at pHs 4, 7 and 10, indicating that it has the potential to bioaccumulate. Isoflucypram is not classified as flammable, explosive or oxidising.

2.2.2. Summary of physical and chemical properties of the plant protection product

'Isoflucypram EC 50' is an emulsifiable concentrate formulation containing 50 g/l pure isoflucypram. It is a light brown clear liquid with a weak, paint-like odour. The pH of a 1% aqueous solution of 'Isoflucypram EC 50' is 7.3. 'Isoflucypram EC 50' has dynamic viscosities of 36.6 and 16.5 mPa s at 20 °C and 40 °C respectively. The kinematic viscosities are 37.6 and 17.2 mPa s at 20 oC and 40 °C. relative density of 1.144. The surface tension of the undiluted product is 27 mN/m at 25 °C and the surface tension of a 1 g/L (~0.1% v/v) aqueous dilution of the product is 32 mN/m at 20 °C, indicating that it is surface active. 'Isoflucypram EC 50' has a self-heating temperature of 355 °C and a flash point of 136 °C. It is not classified as flammable, explosive or oxidising but is classified as a Category 1 aspiration hazard and the hazard statement H304 should be included on the label.

Persistent foam data were gemnerated at the highest in-use concentration only. Data are also required at the lowest in-use concentration (0.375 %v/v) – this is a data gap.

Accelerated storage stability studies (14 days at 54 °C) in COEX/EVOH and COEX/PA indicated acceptable retention of physical and chemical properties on storage. The content of the isoflucypram deviated by less than 0.5% from the initial concentration over the duration of the studies, and the changes in the observed physical and technical properties were negligible. Limited panelling was observed after storage in the COEX/PA packaging material but this did not affect the integrity of the packaging.

Low temperature storage stability indicated that the active substance content did not decrease by >5% on storage.

Shelf life stability studies were completed after submission of the dossier. They were submitted in an updated dossier but have not been evaluated.

2.3. DATA ON APPLICATION AND EFFICACY

2.3.1. Summary of effectiveness

To evaluate the efficacy of ISY EC 50 in the Maritime EPPO climatic zone, 17 field trials on winter wheat (TRZAW) and 19 field trials on spring/winter barley (HORVS and HORVW) were conducted in the period of 2016-2017. These trials were undertaken by Bayer Crop Science Division country organisations and contract research organisations with 10 trials located in northern France (FRA), 14 trials in Germany (DEU), 2 trials in Ireland (IRL) and 10 trials in the United Kingdom (GBR).

The trials to generate representative efficacy data in spring/winter barley in the North East EPPO climatic zone were undertaken by Bayer Crop Science Division country organisations in Latvia (LVA; 2 trials) and in Poland (POL; 1 trial) in 2017.

All trials have been conducted according to EPPO standards by GEP accredited organisations, either by field development staff of Bayer country subsidiaries or by contract research organisations.
Trials were designed, conducted and reported in accordance with general EPPO standards PP1/225(2), PP1/135(4), PP1/152(4), PP1/026(3), and PP1/181(44) regarding design, analyses and reporting.

No effectiveness data were available from use in the remaining cereal crops of Triticale, Rye, and Oats. However, the uses are identical in terms of dose rate and method of application and therefore within the risk envelope. Further consideration of the scope to extrapolate from Wheat and Barley to these other cereal crops will need to be considered by MS at product autorisation if further crop specific data are not presented at that time .

The results show that generally *Mycospharaella graminicola* (SEPTTR), *Puccinia recondita/P.triticina* (PUCCRE/PUCCRT), *Pyrenophora teres* (PYRNTE), and *Rhynchosporium secalis* (RHYNSE) are controlled, with some variations between zones and application timings. No trials on PYRNTR, PUCCST, PUCCHD, RAMUCC, PUCCCO, and PYRNAV were conducted. For product authorisation, additional results will be required in line with EPPO PP1/226 ‘Number of efficacy trials’ depending on whether species are considered major or minor.

Overall, there is evidence that the proposed dose would be “sufficiently effective” and that the supported GAP is representative.

Refer to Section B.3.13 in Volume 3CP.

2.3.2. Summary of information on the development of resistance

Isoflucypram belongs to the chemical family of Succinate DeHydrogenase Inhibitor (SDHI) which rely on the inhibition of the fungal enzyme succinate dehydrogenase resulting in the inhibition of spore germination and/or a reduction in germ tube elongationat the leaf surface. Although Isoflucypram is a new active it shares the resistance risk typical for all SDHI fungicides.

The active substance Isoflucypram is intended to be used to control various fungal diseases such as *Mycospharaella graminicola* (SEPTTR), *Puccinia recondita/P.triticina* (PUCCRE/PUCCRT), *Pyrenophora teres* (PYRNTE), and *Rhynchosporium secalis* (RHYNSE) in cereals. Resistance is known in various fungal species in field populations and lab mutants. Target site mutations in sdh gene, e.g. H/Y (or H/L) at 257, 267, 272 or P225L, dependent on fungal species. Therefore, the resistance risk is considered medium to high risk and requires active resistance management.

To reduce the risk further it is good agronomic practice to follow the label recommendations and adopt appropriate integrated disease management strategies. The latter could include the use of rotation, cultivation and alternation of fungicides with different modes of action. However the agronomic

resistance risk must be defined and assessed during any zonal evaluation for the authorisation of products at a national specific level in the RAR.

2.3.3. Summary of adverse effects on treated crops

The proposed crops are stated as Wheat, Barley, Rye, Oats and Triticale.

Crop safety was assessed in the 39 effectiveness trials as well as 5 specific field trials on winter wheat (TRZAW) and 5 specific field trials on spring/winter barley (HORVS and HORVW) which were conducted in 2015. These trials were also undertaken by Bayer Crop Science Division country organisations and contract research organisations with 3 trials located in northern France (FRA), 4 trials in Germany (DEU) and 3 trials in the United Kingdom (GBR). The trials were designed and conducted according to approved EPPO standards. ISY EC 50 was applied at 1 N dose rate (1.5 L/ha) in all trials, in some cases including also treatments at lower dose rates. In all trials no phytotoxicity was reported.

No data have been submitted to support use on Rye, Triticale, and Oats. However, the uses are identical in terms of dose rate and method of application. In the absence of any further specific trials further consideration of the scope to extrapolate from Wheat and Barley to Rye, Triticale, and Oats will need to be made by cMS at product authorisation.

The submitted data support crop safety in Wheat and Barley. Further information to support the use of Isoflucypram in terms of crop safety and selectivity will be required and considered at product authorisation.

Refer to Section B.3.15 in Volume 3CP.

2.3.4. Summary of observations on other undesirable or unintended side-effects

Based on results from four multi-plant-back rotational crop trials conducted with a dose rate of isoflucypram of 180 g/ha (covering the plateau concentration of isoflucypram in soil overtime) in three different botanical crop groups (roots, leafy vegetables and small grain cereals) it can be concluded that for isoflucypram-based products applied in cereals at a maximum of 75 g/ha/season according to Good Agricultural Practice neither plant-back-intervals nor maximum residue proposals above 0.01 mg/kg are necessary. No restrictions are on the proposed label in regard to succeeding crops or adjacent crops.

The details of succeeding crops which may be planted following crop failure and subsequent to a normal harvest will be considered at product evaluation stage.

The information submitted for risks to adjacent crops indicates that the risk to adjacent crops is acceptable when Isoflucypram is used at the proposed dose rate of 75 g/ha/season.

Refer to Section B.3.16 in Volume 3 CP.

2.4. FURTHER INFORMATION

2.4.1. Summary of methods and precautions concerning handling, storage, transport or fire

2.4.2. Summary of procedures for destruction or decontamination

2.4.3. Summary of emergency measures in case of an accident

2.5. METHODS OF ANALYSIS

2.5.1. Methods used for the generation of pre-authorisation data

Acceptable methods have been submitted for the determination of the active substance and all significant and relevant impurities in the technical material as manufactured.

Acceptable methods have been submitted for the determination of the active substance and the relevant impurity, BCS-CN45153, in the plant protection product.

Acceptable methods have been submitted for the determination of isoflucypram and selected metabolites in various matrices used in support of all areas of the risk assessment with the following exceptions:

- Study DNM0081 (Renaut, R., 2018; M-612750-02-1) uses methods that have not been evaluated in Section B5. The report references validation data in studies DNM0082 and DNM0085 but these have not been submitted. The company will have to submit these studies for evaluation.

2.5.2. Methods for post control and monitoring purposes

Acceptable methods have been submitted for the determination of isoflucypram and selected metabolites in various matrices for use in post-approval monitoring and control, with the following exceptions:

- The extarction efficiency of method 01520 (Uceda, L., 2017 ; M-588974-01-1) has not been demonstrated for oranges (and thus high acid). This must be addressed.

A summary of the monitoring methods is presented below:

Matrix	**Analytes(s)**	**Method**	**LOQ**	**ILV?**	**Fully validated**
Tomato Orange Wheat grain Coffee bean (green) Rape seed Bean seed	Isoflucypram	LC-MS/MS	0.01 mg/kg	Yes	Yes with the exception of oranges (high acid crops) for which extraction efficiency data is outstanding.
Egg Fat Kidney Liver Milk Muscle (cow) Muscle (hen)	Isoflucypram	LC-MS/MS	0.010 mg/kg (0.005 mg/kg for milk)	Yes	Yes
Silt loam Sandy Loam	Isoflucypram	LC-MS/MS	0.001 mg/kg	n/a	Yes
Surface Water	Isoflucypram	LC-MS/MS	0.0625 µg/L	Yes	Yes
Air	Isoflucypram	LC-MS/MS	4.2 µg/m^3	n/a	Yes
Plasma	Isoflucypram BCS-CX99799	LC-MS/MS	0.05 mg/L	n/a	Yes
Body tissues	Refer to the method for liver				Yes

2.6. EFFECTS ON HUMAN AND ANIMAL HEALTH

Isoflucypram (CAS-No. 1255734-28-1) is a new fungicidal active substance developed by Bayer for application on cereals.

It is a novel broad-spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides and a succinate dehydrogenase inhibitor (SDHI) in fungi. Succinate dehydrogenase is also present in mammals and humans – whether isoflucypram is capable of inhibiting this enzyme in humans is not known; however, it is noted that isoflucypram is extensively metabolised and rapidly eliminated in rats (see section 2.6.1 below).

Isoflucypram representative product (ISY EC 50) is an emulsifiable concentrate (EC) containing 5% isoflucypram.

As this is a new substance, there is no information on its toxicity in publicly available literature (see section B.6.10 of document CA_B6). The available regulatory studies were conducted for the purpose of this approval and have not been previously evaluated in the EU; the studies were GLP-compliant and followed the respective OECD test guidelines.

This document uses the term "isoflucypram" when referring to the active substance. However, other synonyms (development codes) may have been used by the applicant within the individual study reports: BCS-CN88460, '460' and ISY.

The batches of isoflucypram used in the toxicology studies are considered representative of the technical specification (see Vol 4 for more details).

The majority of the methods of analysis for the active substance in different matrices (diet, air, gavage solutions) used in the *in vivo* toxicological studies are either validated or fit for purpose (see document CA_B5 and individual study summaries within document CA_B6 for further details).

The key outcome of the evaluation are:

The human health classification of isoflucypram has been addressed in an aligned CLH dossier submitted to ECHA; classification for acute inhalation toxicity 4 (H332) and skin sensitisation 1B (H317) is proposed. Isoflucypram is not mutagenic, carcinogenic or reprotoxic and does not show any ED (endocrine disruption) potential when compared with the criteria given in Regulation (EC) 605/2018 and against the EFSA/ECHA guidance document on the identification of endocrine disruptors.

The reference values are:

ADI 0.04 mg/kg bw/d

ARfD 0.7 mg/kg bw

AOEL 0.04 mg/kg bw/d

AAOEL – not set

The data requirements of regulation (EC) 1107/2009 and Reg 213/2013 have been met and the RMS concludes that there are no data gaps.

2.6.1. Summary of absorption, distribution and excretion in mammals

The toxicokinetics of isoflucypram have been investigated in rats via the oral route only, following gavage dosing. The available studies comprise two ADME investigations including bile duct-cannulation and two whole body autoradiography experiments using isoflucypram, separately radiolabelled at the pyrazole and phenyl moieties and a comparative *in vitro* metabolism study (including human liver microsomes). None of these studies were conducted to OECD TG, but were GLP compliant and are considered sufficiently robust to provide information on the toxicokinetics in the rat following oral dosing by gavage Limited additional information is also available on blood levels of isoflucypram, and a number of metabolites from repeated dose studies in rats, mice and dogs and a developmental toxicity study in rats dosed via the oral route only. No toxicokinetic studies are available by other routes of exposure. Limited additional toxicokinetic information on a metabolite, isoflucypram carboxylate (M12) is also available.

Isoflucypram

<u>Absorption</u>

From the available information, isoflucypram is rapidly and well absorbed from the gastrointestinal tract (84 and 88% in males and females respectively). Although uptake from the GIT is extensive, it appears that post-hepatic systemic exposure to unchanged isoflucypram and/or its metabolites accounts for around 15% of the administered dose, as no more that 15% was detected in the urine and a significant amount is excreted in bile within 24 hours. Time to maximal plasma concentration (Tmax) was 1 hour with a maximum concentration of around 0.5µg/ml. There is no evidence of any significant sex differences in absorption. However, absorption of isoflucypram from the GI tract does appear to be saturable. Overall**, an oral absorption** value of **100%** is proposed. For **post-hepatic systemic availability,** the RMS proposes a value of **15%**.

Dermal absorption of isoflucypram from its representative product is 2% and 5% for the concentrate and dilution respectively (further details are available in document CP_B6). There are no data to determine the inhalation absorption of the substance. However, based on the extensive oral absorption, a default inhalation absorption value of 100% can be assumed.

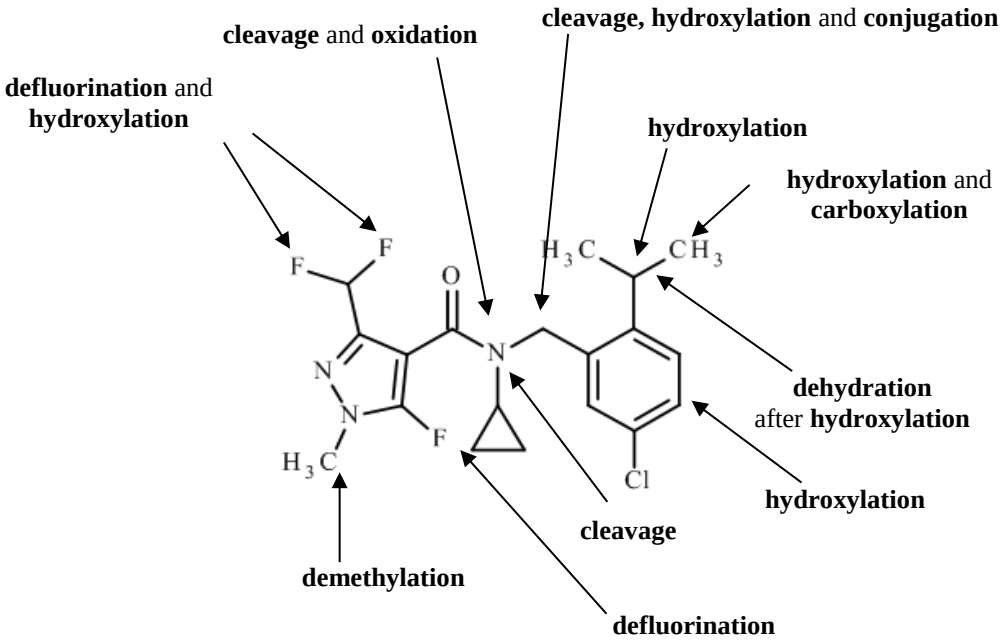
Distribution

Distribution of radiolabelled isoflucypram and/or its metabolites was predominately to the organs of metabolism and excretion, the liver and kidneys. There was no evidence of retention in any organs/tissues. Although blood levels of individual metabolites were not measured, very low levels of total radioactivity were found in terminal blood samples (below 0.1% of the administered dose) 72 hours after dosing.

Limited information on plasma levels of isoflucypram and a number of metabolites, M01, M02, M06, M11, M12 and M58 from repeat dose studies in rats, mice and dogs and from the developmental toxicity study in rats confirmed that there is little or no detectable unchanged isoflucypram in blood plasma following repeated exposure. The levels of the metabolites in plasma were significantly higher than those of the parent.

Metabolism

In vivo toxicokinetic investigations of metabolism found that isoflucypram was rapidly and extensively metabolised such that there was no significant, post-hepatic exposure to unchanged isoflucypram. Studies with ^{14}C pyrazole labelled isoflucypram demonstrated that, in the rat, this moiety was metabolized in the liver principally via N-demethylation of the pyrazole methyl and/or oxidation of the isopropyl group to desmethyl carboxylates or lactate. Investigation of plasma metabolites found desmethyl carboxylic acid (M11) to be the most abundant metabolite. The Phase 1 metabolites act as substrates for a number of Phase II reactions, principally, glucuronidation. There were no significant sex differences in metabolism. Studies with ^{14}C-phenyl labelled isoflucypram found metabolism to be largely similar to that of the ^{14}C pyrazole labelled isoflucypram. However, some benzyl alcohol-related compounds were identified, indicating cleavage of the parent isoflucypram, although this only represents a minor metabolic pathway. No individual metabolite nor the parent compound was found in urine or plasma at levels greater than 10% of the administered dose. Analysis of plasma levels of isoflucypram and a number of metabolites occurring in plants (M01, M02, M06, M11, M12 and M58) from the rat chronic study showed that the levels of these metabolites were significantly higher than those of the parent compound. The principal metabolic reactions and sites for isoflucypram are detailed in the diagram below:



A comparative *in vitro* metabolism study indicated that although pooled human microsomes metabolised isoflucypram less rapidly than other species (rats, mice, rabbits and dog) they did not produce any metabolites that were not observed in the rat or dog, the main species for which most toxicological information is available.

Excretion

Excretion of most metabolites was via the bile, accounting for around 77.6-85.5 % of the administered dose, in males and females respectively. Investigations in bile-duct cannulated rats found no unchanged parent in the bile; therefore, any unchanged parent in the faeces is considered to represent unabsorbed material (20.7% and 15.5% in males and females respectively). Urinary excretion accounted for 8 – 13 % of the total administered dose, with no individual metabolite present above 10%. No radiolabel was detected in the expired air. The radioactivity retained in the carcass accounted for no more than 0.5% of the administered dose.

Although there is no toxicokinetic information from other relevant routes of exposure, given the significant first-pass effect, quantitative differences in the degree of systemic exposure to unchanged isoflucypram and metabolites would be anticipated following inhalation or dermal exposure.

Isoflucypram carboxylic acid (M12)

A limited toxicokinetic study was also conducted in rats by gavage with pyrazolyl-4-^{14}C labelled isoflucypram carboxylic acid (M12), a prominent isoflucypram metabolite. From the information available, it is unclear whether absorption of the isoflucypram carboxylic acid was complete, as nearly 60% of the administered dose was recovered as unchanged parent in the faeces. This is not unexpected as the presence of the carboxylate group will increase the polarity of the molecule, making it less likely to cross plasma membranes unless facilitated by an active transport process.

The most prominent metabolite of M12 was desmethyl carboxylic acid (M11) with 31.4% of the administered dose found in the faeces and 0.07% found in the urine. As around 1.5% of the total administered radioactivity was detected in the urine, it is uncertain whether there is any significant post-hepatic systemic exposure to unchanged M12. Elimination of most of the absorbed dose was via the faeces.

2.6.2. Summary of acute toxicity

The acute toxicity of isoflucypram technical has been investigated *via* the oral, dermal and inhalation routes. An *in vivo* study has been performed to investigate skin irritation while eye irritation has been investigated sequentially *in vitro* and then *in vivo*. Skin sensitisation has been investigated in a murine local lymph node assay. In addition, phototoxicity was investigated *in vitro*. All of these studies were conducted according to standard OECD Test Guidelines and are GLP compliant. Isoflucypram was of low oral and dermal acute toxicity, but was harmful by the inhalation route (4hr LC_{50} = 2.5 mg/L), thus meeting the criteria for classification with **Acute Tox 4 (H332)** under Reg. (EC) No 1272/2008. It was not a skin or eye irritant, but gave a positive response in the LLNA,

triggering classification with **Skin Sens 1B (H317)** under Reg. (EC) No 1272/2008. Isoflucypram was not phototoxic *in vitro*, even though a study was not strictly required. The table below provides an overview of the available acute toxicity studies.

Table 2.2.2-1 Summary of isoflucypram acute toxicity data, with classification according to Regulation (EC) No 1272/2008

Study	Result	Reference	Classification according to Reg. (EC) No 1272/2008
Acute oral rat	Oral LD_{50} > 2000 mg/kg bw	[redacted] 2014a	No classification
Acute dermal rat	Dermal LD_{50} > 2000 mg/kg bw	[redacted] 2014b	No classification
Acute inhalation rat	Inhalation 4hr LC_{50} = 2.518 mg/L (both sexes combined) 3.131 mg/L (males) 2.209 mg/L (females)	[redacted] 2014	**Acute Tox 4, H332** Harmful if inhaled
Skin irritation, rabbit	Negative	[redacted] 2014c	No classification
Eye irritation *in vitro*, isolated chicken eyes	Neither severe irritant nor non-irritant	[redacted], 2014	Inconclusive
Eye irritation, rabbit	Negative	[redacted] 2014d	No classification
Skin sensitization (LLNA)	Sensitizing EC3 = 29.0%	[redacted]2015	**Skin Sens 1B, H317** May cause an allergic skin reaction
Phototoxicity	Negative	Spohr, 2018	Not applicable

In conclusion, isoflucypram is classified for acute toxicity 4 via inhalation (but not by the oral or dermal route) and skin sensitisation 1B. It is most likely that the higher acute inhalation toxicity of isoflucypram compared to the oral route is due to the lack of a first-pass effect by the liver. The low acute dermal toxicity is most likely the consequence of the limited dermal absorption of the substance.

2.6.3. Summary of short-term toxicity

The short-term toxicity of isoflucypram has been investigated in rats, mice and dogs after 28- and 90-days' oral dietary exposure; a one-year dietary study in dogs is also available. Although not conducted to OECD guidelines, the 28-day studies were broadly similar to these and the 90-day and one-year studies complied with the relevant guidelines. No other routes of exposure have been investigated. The main findings are summarised in table B.6.3.4.1 below. Further information on the repeated dose toxicity of isoflucypram is also available from the 2-generation study (see section B.6.6.1.) and in the chronic studies (see section B.6.5.).

The main target organ of toxicity in all species is the liver. The toxicological significance of the effects on the liver has been assessed by the RMS using a weight-of-evidence approach, with a clear distinction being made between effects that are clearly adverse and those which are potentially adaptive. This has been carried out in line with the TAB (Technical Agreements for Biocides) entry[1], agreed at the Biocide WG-IV-2018 meeting (WGIV2018_TOX_6-2); a paper which is based on several international reviews of liver effects (JMPR 2006 and 2015) and which describes a weight-of-evidence approach for the evaluation of liver effects in repeated-dose toxicity studies. Hepatocellular hypertrophy is typically related to increased functional capacity of the liver which allows the maintenance of homeostasis in the organism after xenobiotic exposure. A general increase in the size of the liver is observed (owing to cell enlargement and fluid accumulation); this is considered a potentially beneficial, adaptive response. However, there is the potential that the capacity of the homeostatic mechanisms may be exceeded and in these cases the organism would be unable to return to its previous state once exposure has ended (thus constituting an adverse response). Hypertrophy as an adaptive response should not be accompanied by adverse histopathology (necrosis, apoptosis, pigment deposition or hyperplasia), or by substantial changes in clinical chemistry indicative of liver toxicity (decreased albumin or increased activities of ALT, AST, ALP, GGT, bilirubin or cholesterol). In line with the TAB entry, relative liver weight increases up to 15%, that are not

[1] https://webgate.ec.europa.eu/s-circabc/d/a/workspace/SpacesStore/83e2fb72-5d1f-4ada-be15-2246109e65d4/Interpretation%20of%20liver%20effects.pdf

accompanied by other signs of liver dysfunction have been considered by the RMS to be an adaptive rather than an adverse response.

Rat

In rats, the main target organs of toxicity were the liver, thyroid and kidney.

Adverse (> 15%) increased liver weights (with or without hypertrophy) were seen from 86 mg/kg bw/d in the 28-day study and at the top dose of 63 mg/kg bw/d in the 90-day study. In addition, similar effects were seen from 34 mg/kg bw/d in the 2-generation study and at the top dose of 46 mg/kg bw/d in the 2-year carcinogenicity study. These effects were associated with alterations of some clinical-chemistry parameters indicative of liver toxicity (e.g. Increased cholesterol) at the top dose of 240 mg/kg bw/d in the 28-day study, at the top dose of 63 mg/kg bw/d in the 90-day study and from 34 mg/kg bw/d in the 2-generation study.

Increased thyroid weights with or without associated histopathology (follicular cell hypertrophy, colloid alterations and pigmentation) were seen at the top dose of 240 mg/kg bw/d in the 28-day study and at the top dose of 63 mg/kg bw/d in the 90-day study. In addition, similar effects were seen from 34 mg/kg bw/d in the 2-generation study and from 19 mg/kg bw/d in the 2-year carcinogenicity study. Mechanistic investigations (see section B.6.8.2) have shown that these changes were associated with increases in TSH and that the most likely MoA (mode of action) underpinning the induction of these effects is activation of CAR/PXR in the liver followed by enzyme induction, including T4-UDPGT, increased clearance of T4, stimulation of the pituitary with increased secretion of TSH leading to stimulation of the thyroid and follicular cell hypertrophy. It is well established that there are large quantitative differences between rats and humans in the regulation of thyroid homeostasis (Colnot & Dekant, 2017). Therefore, these thyroid effects seen in the rat as a secondary consequence of liver enzyme induction are considered to be not relevant to humans.

Kidney histopathology, related to α2u globulin accumulation, was seen in males only from 23 mg/kg bw/d in the 28-day study and at the top dose of 63 mg/kg bw/d in the 90-day study. It was not seen in the 2-generation study up to the top dose of 93 mg/kg bw/d or in the 2-year carcinogenicity study up to the top dose of 19 mg/kg bw/d. The effects in the kidney are male rat-specific and not relevant to humans.

In addition to toxic effects in these organs, decreases in body weight and/or body weight gain were observed at the top dose of 240 mg/kg bw/d in the 28-day study, at the top dose of 63 mg/kg bw/d in the 90-day study and at the top dose of 47 mg/kg bw/d in the 2-year carcinogenicity study.

Mouse

In mice, the main target organs of toxicity were the liver and kidney. There were no effects on the thyroid.

Increased liver weights with associated histopathology (necrosis and/or vacuolation and/or bile-duct hyperplasia) were seen from 133 mg/kg bw/d in the 28-day study, from 168 mg/kg bw/d in the 90-day study and at the top dose of 147 mg/kg bw/d in the 18-month carcinogenicity study. These effects were associated with alterations of some clinical-chemistry parameters indicative of liver toxicity (e.g. Increased cholesterol, AST, ALT, etc) at the top dose of 330 mg/kg bw/d in the 28-day study. The effects on the liver in the mouse are more severe than those seen in the rat, but they occur at higher dose levels than the adverse effects observed in the rat.

Increased kidney weights with associated histopathology (hyaline casts, tubule dilatation and basophilia) were seen in male mice and only in the carcinogenicity study only at the top dose of 147 mg/kg bw/d. These kidney effects in the mouse were considered relevant to humans.

In addition to toxic effects in these organs, decreases in body weight and/or body weight gain and increased mortality rate (in females) were observed in the carcinogenicity study at the top dose of 147 mg/kg bw/d.

Overall, the mouse appears less sensitive to the toxic effects of isoflucypram than the rat.

Dog

In dogs, the main target organ of toxicity is the liver. There were no effects on the thyroid or kidney, providing further evidence supporting the lack of human relevance of the thyroid effects that were seen in the rat (see section B.6.8.2 in document CA_B6).

Adverse increased liver weights with associated hypertrophy were seen at the top dose of 50 mg/kg bw/d in the 90-day study and from 17 mg/kg bw/d in the 12-month study. More severe histopathological findings (Kupffer cell pigmentation, necrosis and cytoplasmic changes) occurred in the 12-month study at the higher dose of 50 mg/kg bw/d. These effects were associated with increased levels of ALP seen at the top dose of 50 mg/kg bw/d in the 90-day study and from 17 mg/kg bw/d in the 12-month study. The liver hypertrophy seen in the dog after 90-days' exposure is similar to that observed in the rat and it occurred at similar dose levels; however, in the 12-month study, more severe histopathological findings were noted. In conclusion, adverse effects were seen in the liver after both 90-days and 12-months' exposure in dogs.

In addition to toxic effects in the liver, decreases in body weight and/or body weight gain were seen at the top dose of 50 mg/kg bw/d in both the 90-day and 12-month study.

Overall, the dog appears to be as sensitive as the rat to the toxic effects of isoflucypram.

When compared with the classification criteria for STOT-RE, the liver was the only target organ at doses below the cut-off values for classification into category 2. However, the RMS concluded that the findings were neither consistent nor severe enough to warrant classification for STOT-RE 2 (see CLH report for further details). The following NOAELs were identified from the repeated-dose toxicity studies:

Table 2.6.3-1: Summary of NOAEL values for the repeated-dose toxicity of isoflucypram

Study, guideline, reference	**Species, doses tested**	**NOAEL mg/kg bw/d**	**LOAEL mg/kg bw/d**	***Adverse* effects at LOAEL**
Dietary 28-day No guideline [redacted] 2017)	Rat (Wistar) 0, 300, 100, 3000 ppm Equivalent to 0, 22.8/25.6, 83.3/86.5, 240/285 mg/kg bw/d in M/F	83.3 (M) – 1000 ppm 25.6 (F) – 300 ppm	240 (M) – 3000 ppm 86.5 (F) – 1000 ppm	M (240 mkd):↓ body-weight gain & final body weight; ↑ abs & rel liver weight with associated hypertrophy & ↑ rel thyroid weight with associated follicular cell hypertrophy; effects on clinical-chemistry parameters indicative of liver toxicity; kidney effects (α2u accumulation) – not relevant to humans F (86.5 mkd): ↑ abs & rel liver weight with associated hypertrophy
Dietary 90-day OECD 408 [redacted] 2014)	Rat (Wistar) 0, 100, 300, 1000 ppm Equivalent to 0, 6.3/7.9, 18.4/21.9, 63.5/80.9 mg/kg bw/d in M/F	18.4 (M) – 300 ppm 21.9 (F) – 300 ppm	63.5 (M) – 1000 ppm 80.9 (F) – 1000 ppm	M&F:↓ body-weight gain; ↑ abs & rel liver weight with associated hypertrophy; ↑ abs & rel thyroid weight with associated follicular cell hypertrophy/colloid alterations; increased cholesterol; kidney histopathology and effects on urinalysis parameters (α2u accumulation) in males only – not relevant to humans
Dietary 28-day No guideline [redacted] 2012)	Mouse (C57BL/6J) 0, 200, 800, 2000 ppm Equivalent to 0, 32/41, 133/149, 330/374 mg/kg bw/d in M/F	32 (M) – 200 ppm 149 (F) – 800 ppm	133 (M) – 800 ppm 374 (F) – 2000 ppm	M (133 mkd): Hepatocellular necrotic foci, single cell necrosis; ↑ rel liver weight; F (374 mkd): Hepatocellular necrotic foci, single cell necrosis; ↑ rel liver weight; effects on clinical-chemistry indicative of liver toxicity

Study, guideline, reference	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	*Adverse* effects at LOAEL
Dietary 90-day OECD 408 [redacted] 2013)	Mouse (C57BL/6J) 0, 100, 300, 1000 ppm Equivalent to 0, 17/19.5, 51/59.8, 168/207 mg/kg bw/d in M/F	51 (M) – 300 ppm 59.8 (F) – 300 ppm	168 (M) – 1000 ppm 207 (F) – 1000 ppm	M&F: ↑ rel liver weight and associated histopathology (vacuolation)
Dietary 90-day OECD 409 [redacted] 2015)	Dog (Beagle) 0, 170, 500, 1500 ppm Equivalent to 0, 5.5/5.5, 15.9/16.2, 50.4/54 mg/kg bw/d in M/F	15.9 (M) – 500 ppm 16.2 (F) – 500 ppm	50.4 (M) – 1500 ppm 54 (F) – 1500 ppm	↑ rel liver weight with associated hypertrophy; ↑ ALP; ↓ body-weight gain
Dietary 12-month OECD 452 [redacted] 2017a)	Dog (Beagle) 0, 150, 600, 1800 ppm Equivalent to 0, 4.2/4.2, 18.8/17.6, 60.2/49.8 mg/kg bw/d in M/F	**4.2 (M & F) – 150 ppm**	18.8 (M) – 600 ppm 17.6 (F) – 600 ppm	↑ rel liver weight with associated hypertrophy, ↑ ALP, enlarged liver

The overall/most sensitive **NOAEL for short-term toxicity** was **4.2 mg/kg bw/d** for males and females (with a respective LOAEL of 18.8/17.6 mg/kg bw/d in males/females) taken from the 12-month dog study. At the LOAEL, there were adverse (>15%) increases in liver weights and in ALP in both sexes.

Overall, therefore, repeated-dose toxicity has been adequately investigated in studies in rats, mice and dogs; the critical target organ was the liver and adverse effects observed in this organ are relevant to humans. Classification for repeated-dose toxicity is not required.

2.6.4. Summary of genotoxicity

The genotoxicity of isoflucypram was tested in a S. typhimurium reverse mutagenesis assay (Ames test), an in vitro V79/HPRT gene mutation assay in Chinese hamster V79 cells, an in vitro chromosome aberration study using human lymphocytes and an *in vivo* mouse micronucleus study. The studies were all conducted according to the relevant OECD TGs and were GLP compliant.

Isoflucypram did not induce gene mutations in bacteria or mammalian cells *in vitro* but was clastogenic *in vitro* with and without metabolic activation. However, when tested *in vivo* in a valid mouse bone marrow micronucleus study up to the limit dose of 2000 mg/kg bw, at which clinical signs of toxicity occurred, the clastogenic activity seen *in vitro* was not expressed *in vivo*. Although there was no direct evidence of bone marrow exposure in the study, in the chronic mouse study, isoflucypram and two major metabolites were detected in plasma, demonstrating systemic availability in the mouse. As the bone marrow is a highly-perfused tissue, it must be inferred that exposure of the bone marrow in the mouse occurred. In addition, the occurrence of clinical signs of toxicity in the

micronuclues study itself may indicate that the test substance (and/or its metabolites) was systemically available in this test. Furthermore, there were systemic effects (decreases in body weight) in mouse repeated dose toxicity studies, indicating again potential systemic availability of isoflucypram and/or its metabolites in the mouse. It is also noted that kinetic studies in the rat showed that the substance and/or its metabolites were systemically available and reached the bone marrow in the rat. Thus, it can be concluded that isoflucypram is not clastogenic or aneugenic in a valid *in vivo* mouse micronucleus assay.

Overall, it can be concluded that isoflucypram is not genotoxic *in vivo* and the data requirements of Regulation 283/2013 have been met. Classification of isoflucypram for mutagenicity is not warranted (see also aligned CLH report). A summary of all the available genotoxicity studies is shown in the table below.

Table 2.6.4-1: Summary of genotoxicity studies with isoflucypram

Study	Concentrations of Substance tested	Result	Reference
***In vitro* assays**			
Ames test	0, 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate	negative	Sokolowski, 2014
Chromosomal aberrations study in human lymphocytes *in vitro*	Concentrations evaluated: Without S9, exp.1: 0, 7.8, 13., 23.9 µg/mL; exp.2: 0, 2.0, 3.5, 6.1 µg/mL With S9, exp.1: 0, 13.6, 23.9, 41.8 µg/mL; exp.2: 0, 10.0, 20.0, 30.0, 40.0 µg/mL	Clastogenic with and without S9	Bohnenberger, 2014
V79 / HPRT mammalian mutagenicity study	Without S9, exp.1 (4 hours): 0, 4.0, 8.0, 16.0, 24.0, 32.0 µg/mL; exp. 2 (24 hours): 0, 8.0, 16.0, 32.0, 48.0, 64.0 µg/mL. With S9, exp.1 (4 hours): 0, 8.0, 16.0, 32.0, 48.0, 64.0 µg/mL; exp.2 (4 hours): 0, 8.0, 16.0, 32.0, 48.0, 64.0, and 128.0 µg/mL	Negative	Wollny, 2014
***In vivo* assay**			
Mouse micronucleus assay *in vivo*	0, 500, 1000, and 2000 mg/kg bw	Negative	[redacted]2014

2.6.5. Summary of long-term toxicity and carcinogenicity

The long-term toxicity and carcinogenic potential of isoflucypram have been investigated in rats and mice in dietary studies of 2-years and 18-months respectively; both were conducted in compliance with OECD test guidelines (453 for rats and 451 in mice). There were no neoplastic findings in either species.

In rats, no increase in the incidence, severity or onset of tumours was observed up to and including the highest-dose tested (450 ppm/18.6 mg/kg bw/d in males and 800 ppm/46.6 mg/kg bw/d in females) at which some limited generalised toxicity (hair loss, decreased body weight gain and increased absolute liver weight in females and minimal thyroid histopathological findings- colloid alteration and diffuse pigmentation of the follicular cells- in both sexes) occurred. No other systemic effects occurred at lower doses. Therefore, isoflucypram was not carcinogenic in rats at the highest dose tested of 450/800 ppm (18.6/46.6 mg/kg bw/d in M/F). The NOAEL for systemic chronic toxicity is 150 ppm (6.27/8.54 mg/kg bw/d in M/F).

In mice, there were no treatment-related increases in the incidence, severity or onset of tumours in any tissue up to the top dose of 1250 ppm (147/190 mg/kg bw/d in M/F) at which systemic toxicity occurred (higher mortality rate in females, adverse effects on body weight and body weight gain in both sexes, increased liver weight with associated histopathology, including necrosis and bile-duct hyperplasia in both sexes and increased kidney weight with associated histopathology, including hyaline casts, tubule dilation and tubule basophilia in males) occurred. No other systemic effects occurred at lower doses. Overall, isoflucypram was not carcinogenic in mice at the highest dose tested of 1250 ppm (147/190 mg/kg bw/d in M/F). The NOAEL for systemic chronic toxicity is 250 ppm (29/38.1 mg/kg bw/d in M/F).

Overall, therefore, isoflucypram is not carcinogenic in rats or mice. No classification for carcinogenicity is warranted (see aligned CLH report).

The following NOAELs have been identified for the chronic toxicity and carcinogenicity of isoflucypram.

Table 2.6.5-1: Summary of NOAEL values for the chronic toxicity/carcinogenicity of isoflucypram

Study, guideline, reference	**Species, Doses tested**	**NOAEL mg/kg bw/d**	**LOAEL mg/kg bw/d**	**Adverse effects at the LOAEL**
Dietary 24-month OECD 453 [redacted] 2018)	Rat (Wistar) 0, 30, 150, 450/800 ppm Equivalent to 0, 1.2/1.7, 6.27/8.54, 18.6/46.6 mg/kg bw/d in M/F	*Carcinogenicity* 18.6 (M) – 450 ppm 46.6 (F) – 800 ppm	*Carcinogenicity* >18.6 (M) – 450 ppm >46.6 (F) – 800 ppm	*Carcinogenicity* No tumours observed up to the top-dose
		Chronic toxicity **6.27 (M) – 150 ppm** 8.54 (F) – 150 ppm	*Chronic toxicity* 18.6 (M) – 450 ppm 46.6 (F) – 800 ppm	*Chronic toxicity* Hair loss (F) ↓ body weight gain (F) ↑ abs liver weight (F) Minimal thyroid histopath changes (M & F)
Dietary 18-month OECD 451 [redacted], 2017)	Mouse (C57BL/6J) 0, 50, 250, 1250 ppm Equivalent to 0, 5.9/7.8, 29/38.1, 147/190 mg/kg bw/d in M/F	*Carcinogenicity* 147 (M) – 1250 ppm 190 (F) – 1250 ppm	*Carcinogenicity* >147 (M) – 1250 ppm >190 (F) – 1250 ppm	*Carcinogenicity* No tumours observed up to the top dose
		Chronic toxicity 29 (M) – 250 ppm 38.1 (F) – 250 ppm	*Chronic toxicity* 147 (M) – 250 ppm 190 (F) – 250 ppm	*Chronic toxicity* ↑ mortality rate (F) ↓ body weight and body weight gain (M & F) ↑ liver weight and histopathological correlates (M & F) ↑ kidney weight and histopathological correlates (M)

The overall/most sensitive **NOAEL for chronic toxicity** is **6.7 mg/kg bw/d** identified in the rat 2-year study. Effects at the LOAEL of 18.6/46.6 mg/kg bw/d (M/F) included hair loss, decreased body weight gain and increased absolute liver weight in females and minimal thyroid histopathological changes in both sexes.

2.6.6. Summary of reproductive toxicity

The reproductive toxicity of isoflucypram has been investigated in a standard guideline dietary 2-generation study in rats and in guideline gavage pre-natal developmental toxicity studies, one in rats and one in rabbits.

Effects on Sexual Function and Fertility

The potential of isoflucypram to adversely affect sexual function and fertility has been well investigated in a standard 2-generation dietary study, conducted in rats.

There were no effects of treatment on mating behaviour, fertility, litter size, oestrus cycle, ovarian primordial follicle counts and gestation up to the top dose of 1200/600 ppm (93 – 104 mg/kg bw/d) at which parental and offspring toxicity occurred. In addition, examination of the reproductive organs did not reveal any treatment-related changes. Specific investigations of the spermatogenic cycle did not find any cell or stage-specific abnormalities at any dose level. Therefore a **NOAEL for reproductive toxicity** of **93-104 mg/kg bw/d** (highest dose tested) can be identified from this study.

In relation to general toxicity in parental animals and offspring, liver and thyroid weights (with no associated histopathology) were adversely increased from the mid dose of 450/225 ppm (equivalent to 34.1-40.8 mg/kg/day) in adults and pups in both generations. Clinical-chemistry parameters associated with liver toxicity were also affected from the mid dose in parental animals of both generations. In addition, food consumption was decreased in F1 adult females at the top dose. There were no adverse effects in parental animals and offspring at the lowest dose tested of 150/75 ppm (11-14 and mg/kg/d). Therefore a **NOAEL** of **11-14 mg/kg bw/d** can be identified for **parental and offspring toxicity** from this study.

Overall, classification of isoflucypram for effects on fertility is not warranted (see also aligned CLH report).

Developmental toxicity

The developmental toxicity of isoflucypram has been investivated in guideline gavage pre-natal developmental toxicity studies, conducted in rats and rabbits. Additional information on the developmental toxicity potential of isoflucypram is also available from the rat 2-generation study.

In the rat developmental study, no malformations (skeletal or visceral) were observed at any dose. Foetal weight was reduced in females at the top dose of 625 mg/kg bw/d. Some skeletal and visceral variations were also observed at the top dose only. These common variations are considered to be of minimal toxicological significance and the unspecifc, secondary consequence of the maternal toxicity (reduced BWG and food consumption and increased liver weight with associated hypertrophy) observed at the top dose of 625 mg/kg bw/d. No maternal toxicity was observed at lower dose levels. Overall, it can be concluded that isoflucypram is not a specific developmental toxicant in rats. Based on these findings, the **NOAELs** proposed by the RMS for **developmental and maternal toxicity** in the rat are **125 mg/kg bw/d**, based on the lack of relevant effects at these dose levels.

In the rabbit developmental study, there was no evidence that isoflucypram was a developmental toxicant up to the top dose of 500 mg/kg bw/d at which severe maternal toxicity (2 abortions, initial body weight loss and initial reduction in food consumption, overall decrease in body weight gain during the dosing period and increased liver weight) occurred. Based on these data, the **NOAEL**s proposed by the RMS for **developmental and maternal toxicity** in rabbits are **500 and 70 mg/kg bw/d**, respectively based on the lack of relevant effects at these dose levels.

In addition, in the rat 2-generation study, there were no effects of treatment on pup survival, sex ratio, pup bodyweight, preputial separation or ano-genital distance (AGD) up to the top dose of 1200/600 ppm (93 – 104 mg/kg bw/d) at which parental and offspring toxicity occurred.

The mean age at vaginal opening for F1 females in the 1200/600 ppm group occurred five days later than in controls. The body weight of these F1 females was also significantly increased. In the presence of an increase in body weight (usually delayed vaginal opening is associated with reduced body weight), it is difficult to establish the toxicological significance of the observed delay in vaginal opening. However, in the absence of any effects on other developmental landmarks and on AGD and considering that these females went on to mate successfully and produce the F2 generation, the RMS concludes that it is most likely this observation was a chance finding. In addition, no effects on vaginal opening were seen up to the high gavage dose of 400 mg/kg bw/d for 20 days in a modified rat uterotrophic assay in immature animals (Kennel, 2011; see section B.6.8.3) which included specific investigations of vaginal opening.

Overall, classification of isoflucypram for developmental toxicity is not warranted (see also aligned CLH report).

The table below summarises the relevant NOAEL and LOAEL values identified in the reproductive toxicity studies.

Table 2.6.6-1: Summary of reproductive toxicity studies with isoflucypram

Study, guideline and reference	Species and dose tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at the LOAEL
2-gen study (dietary) OECD 416 [redacted] 2018)	Rat (Wistar) 0, 150/75, 450/225, 1200/600 ppm Equivalent to 0, 11-14, 34-44, 93-140 mg/kg bw/d	*Reproductive* 93-140 (1200/600 ppm) *Parental* **11-14 (150/75 ppm)** *Offspring* 11-14 (150/75 ppm)	*Reproductive* >93-140 (>1200/600 ppm) *Parental* 34-44 (450/225 ppm) *Offspring* 34-44 (450/225 ppm)	*Reproductive* No adverse effects up to the top dose *Parental* ↑ liver and thyroid wt; effects on some clinical-chemistry parameters indicative of liver toxicity *Offspring* ↑ liver and thyroid wt
Pre-natal dev tox study (gavage) OECD 414 [redacted] 2017b)	Rat (SD) 0, 25, 125, 625 mg/kg bw/d	*Developmental* 125 *Maternal* 125	*Developmental* 625 *Maternal* 625	*Developmental* ↓ foetal wt, ↑ incidence of skeletal and visceral common variations *Maternal* ↓ bwg, ↓ food consumption, ↑ liver wt with associated hypertrophy
Pre-natal dev tox study (gavage) OECD 414 [redacted] 2017)	Rabbit (NZW) 0, 10, 70, 500 mg/kg bw/d	*Developmental* 500 *Maternal* 70	*Developmental* >500 *Maternal* 500	*Developmental* No adverse effects up to top dose *Maternal* 2 abortion, initial body weight loss, ↓ overall bwg, ↓ initial food consumption, ↑ liver wt

Overall classification of isoflucypram for reproductive toxicity is not warranted. Reproductive toxicity has been adequately investigated in studies that comply with OECD guidelines 414 (2001) and 416 (2001).

2.6.7. Summary of neurotoxicity

The neurotoxicity potential of isoflucypram has been investigated in a guideline oral acute neurotoxicity study in the rat. In this study, isoflucpyram did not show any potential for neurotoxicity up to the limit dose of 2000 mg/kg bw. No generalised toxicity was evident up to the top dose.

2.6.8. Summary of further toxicological studies on the active substance

Mechanistic studies investigating liver and thyroid effects

A series of dietary studies have been conducted to investigate the potential mechanism for the effects seen in the liver of rats and mice and the thyroid of rats. These comprised one 7-day and two 28-day studies in rats and a 7-day study in mice. All studies were conducted in females and the applicant has reasoned that this is the most appropriate sex, because the liver effects observed in this sex were more severe; however, although this is true, the

RMS considers that testing in males (or both sexes) would have been more appropriate, owing to the thyroid effects being observed in males or both sexes. The applicant has proposed that isoflucypram is an activator of CAR and/or PXR and the results of this set of studies appear to support this finding, based upon investigations conducted on several parameters including liver/thyroid weights, thyroid hormones, liver/thyroid histopathology, liver/thyroid cell proliferation, liver enzyme induction and gene transcription.

Consistent with the findings of the previously conducted repeated-dose studies, relative and/or absolute liver weights were increased after 7-days in rats and mice and to a greater extent after 28-days' administration in rats. Isoflucypram did not have any effect on thyroid weights (measured in rats only). The increase in liver weight was accompanied by an enlarged liver in rats at 28-days and in mice at 7 days. Where the tissues were examined microscopically, hepatocellular and thyroid follicular cell hypertrophy was found to be present in rats after 28-days (but not 7 days); no hypertrophy was observed in mice. Cell proliferation was measured after 7- and 28-days' exposure in rats and was found to be increased in the liver and thyroid (only the proliferation in the liver persisted into recovery). No conclusion could be drawn on the extent of cell proliferation in mice owing to the lack of a clear dose-response.

Analysis of liver enzyme induction and associated gene transcription indicated that a PXR and/or a CAR MoA is the most likely MoA. In both 28-day rat studies, BROD (associated with PXR activation) was increased as well as the genes associated with both BROD and PROD (associated with CAR actication). In the 7-day studies (in rats and mice) an increase in PROD and associated genes was more prominent.

Therefore it is likely that the effects observed in the liver and thyroid are a result of liver enzyme induction, arising from the activation of CAR and/or PXR.

Endocrine Disruption (ED)

An assessment for potential endocrine disrupting properties of isoflucypram in line with the new EFSA/ECHA guidance (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311) and the recently published scientific criteria (Regulation 605/2018) has been provided by the applicant. The following summary conclusions are reached by the RMS.

Estrogenity (E), androgencity (A) and steroidogenesis (S) modalities

No effects on reproduction or reproductive organs were observed in a modern 2-generation study in rats, pre-natal developmental toxicity studies in rats and rabbits and repeated dose toxicity studies in rats, mice and dogs. The 2-generation study included investigations of all the required ED-sensitive parameters (with the exception of nipple retention). In addition, no (anti-)androgenic or oestrogenic activity was seen in a modified Hershberger assay and uterotrophic test in immature animals. Overall, it can be concluded that isoflucypram is not an ED in relation to these modalities.

Thyroid (T) modality

Endocrine-mediated thyroid toxicity was seen in the rat. Based upon the large quantitative differences between rats and humans in the regulation of thyroid homeostasis (Boobis et al., 2006; Lewandowski et al., 2004; Capen, 1997; Capen, 1998; Colnot and Dekant, 2017), these effects are considered to be not relevant to humans, especially when taking into account the mild nature and low incidence of the observed changes. Overall, it can be concluded that isoflucypram is not a thyroid ED in humans.

Overall

Isoflucypram is not an ED of potential relevance to human health in accordance with the recently published scientific criteria (Reg 605/2018).

Immunotoxicity

No specific immunotoxicity study with isoflucypram is available. However, an assessment of the immunotoxicity potential of isoflucypram can be performed by considering the available repeat dose toxicity, carcinogencicity and reproductive toxicity studies.

Various repeat dose toxicity studies conducted with isoflucypram in the rat, mouse, and dog show that there is no indication of an immunotoxic effect in adult animals. The weight of the spleen, thymus and lymph nodes were unaffected in all three species, and there was no alteration in morphology in these organs which would suggest an effect of isoflucypram on the immune system. In addition, there were no relevant effects on any haemathological parameters.

There were also no relevant or specifc effects on spleen and thymus in rat and rabbit foetuses and in rat pups in the available reproductive toxicity studies.

It can therefore be concluded that isoflucypram does not affect the immune system, and an *in vivo* immunotoxicity study is not required.

2.6.9. Summary of toxicological data on relevant impurities and metabolites

Relevant impurities

The impurity BCS-CN45143 was initially under investigation as a potential active substance; during this time it was tested in two 28-day studies (one in rats and one in mice) and in a uterotrophic assay in rats. Liver effects were seen in rats (at top dose of 150 mg/kg bw/d) and mice (at ≥ 377 mg/kg bw/d) and histopathological findings of the uterus were noted in rats (at 150 mg/kg bw/d). In the uterotrophic assay, an acceleration of vaginal opening was noted at 450 mg/kg bw/d. Similar effects on vaginal opening were not seen with isoflucypram. Therefore, on the basis of these effects, impurity BCS-CN45153 is considered to be a relevant impurity. The development of this compound as a potential active substance was halted early as a result of these adverse effects.

Metabolites

The following metabolites were selected for potential inclusion in the residue definitions based on their significant occurrence in the plant and livestock metabolism studies:

- Isoflucypram-propanol (M01) and its conjugates (M18, M19 & M21)
- Isoflucypram-2-propanol (M02) and its conjugates (M20 & M22)
- Isoflucypram-desmethyl-propanol (M06) and its conjugates (M37 & M41)
- Isoflucypram-desmethyl-1,2-propandiol (M07) and its conjugate (M36)
- Isoflucypram-desmethyl-carboxylic acid (M11)
- Isoflucypram-carboxylic acid (M12)

To assess the toxicological properties of these metabolites, all the available data (including data relating to isoflucypram) were considered. These included presence of these metabolites in rat ADME and toxicity studies performed with the parent, structural similarity to the parent and in silico genotoxicity assessment. No specific toxicity studies are available on these metabolites.

For **M01**, **M02**, **M06**, **M11** and **M12**, there are close structural similarities to the parent compound and negative genotoxicity QSAR predictions. In addition, plasma levels of these metabolites in the 2-year study in rats were similar or higher than those of the parent. Therefore the toxicity of these metabolites is covered by the toxicity data of the parent and if a risk assessment were to be required, the reference values of isoflucypram could be used.

For **M07**, there is close structural similarity to the parent compound and negative genotoxicity QSAR predictions, but no information on relative levels in rat plasma compared to the parent. Therefore, the toxicity of M07 is not sufficiently covered by the toxicity data of the parent and if a risk assessment were to be required, the Cramer class III TTC value could be used.

2.6.10. Summary of medical data and information

No medical problems related to handling of isoflucypram during the piloting phase have been reported to the plant Medical Department and HSE Management. There is no production currently ongoing. All plant employees undergo annual medical examinations, including clinical examinations, clinical-pathology, spirometry, audiogram and ECG (Steffens, 2017).

2.6.11. Overview of all available studies relevant to reference value setting

The following table gives an overview of the all the available studies relevant to reference values setting.

Table 2.6.11-1 Summary of all studies relevant to setting of reference values

Study, guideline, reference	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	*Adverse* effects at LOAEL
Dietary 28-day No guideline [redacted] 2017)	Rat (Wistar) 0, 300, 1000, 3000 ppm Equivalent to 0, 22.8/25.6, 83.3/86.5, 240/285 mg/kg bw/d in M/F	83.3 (M) – 1000 ppm 25.6 (F) – 300 ppm	240 (M) – 3000 ppm 86.5 (F) – 1000 ppm	M (240 mkd):↓ body-weight gain & final body weight; ↑ abs & rel liver weight with associated hypertrophy & ↑ rel thyroid weight with associated follicular cell hypertrophy; effects on clinical-chemistry parameters indicative of liver toxicity; kidney effects (α2u accumulation) – not relevant to humans F (86.5 mkd): ↑ abs & rel liver weight with associated hypertrophy
Dietary 90-day OECD 408 [redacted] 2014)	Rat (Wistar) 0, 100, 300, 1000 ppm Equivalent to 0, 6.3/7.9, 18.4/21.9, 63.5/80.9 mg/kg bw/d in M/F	18.4 (M) – 300 ppm 21.9 (F) – 300 ppm	63.5 (M) – 1000 ppm 80.9 (F) – 1000 ppm	M&F:↓ body-weight gain; ↑ abs & rel liver weight with associated hypertrophy; ↑ abs & rel thyroid weight with associated follicular cell hypertrophy/colloid alterations; increased cholesterol; kidney histopathology and effects on urinalysis parameters (α2u accumulation) in males only – not relevant to humans
Dietary 28-day No guideline [redacted], 2012)	Mouse (C57BL/6J) 0, 200, 800, 2000 ppm Equivalent to 0, 32/41, 133/149, 330/374 mg/kg bw/d in M/F	32 (M) – 200 ppm 149 (F) – 800 ppm	133 (M) – 800 ppm 374 (F) – 2000 ppm	M (133 mkd): Hepatocellular necrotic foci, single cell necrosis; ↑ rel liver weight; F (374 mkd): Hepatocellular necrotic foci, single cell necrosis; ↑ rel liver weight; effects on clinical-chemistry indicative of liver toxicity
Dietary 90-day OECD 408 [redacted] 2013)	Mouse (C57BL/6J) 0, 100, 300, 1000 ppm Equivalent to 0, 17/19.5, 51/59.8, 168/207 mg/kg bw/d in M/F	51 (M) – 300 ppm 59.8 (F) – 300 ppm	168 (M) – 1000 ppm 207 (F) – 1000 ppm	M&F: ↑ rel liver weight and associated histopathology (vacuolation)
Dietary 90-day OECD 409	Dog (Beagle) 0, 170, 500, 1500 ppm	15.9 (M) – 500 ppm 16.2 (F) – 500 ppm	50.4 (M) – 1500 ppm 54 (F) – 1500 ppm	↑ rel liver weight with associated hypertrophy; ↑ ALP; ↓ body-weight gain

Study, guideline, reference	**Species, doses tested**	**NOAEL mg/kg bw/d**	**LOAEL mg/kg bw/d**	***Adverse* effects at LOAEL**
([redacted] 2015)	Equivalent to 0, 5.5/5.5, 15.9/16.2, 50.4/54 mg/kg bw/d in M/F			
Dietary 12-month OECD 452 [redacted] 2017a)	Dog (Beagle) 0, 150, 600, 1800 ppm Equivalent to 0, 4.2/4.2, 18.8/17.6, 60.2/49.8 mg/kg bw/d in M/F	**4.2 (M & F) – 150 ppm**	18.8 (M) – 600 ppm 17.6 (F) – 600 ppm	↑ rel liver weight with associated hypertrophy, ↑ ALP, enlarged liver
Dietary 24-month OECD 453 [redacted] 2018)	Rat (Wistar) 0, 30, 150, 450/800 ppm Equivalent to 0, 1.2/1.7, 6.27/8.54, 18.6/46.6 mg/kg bw/d in M/F	*Carcinogenicity* 18.6 (M) – 450 ppm 46.6 (F) – 800 ppm	*Carcinogenicity* >18.6 (M) – 450 ppm >46.6 (F) – 800 ppm	*Carcinogenicity* No tumours observed up to the top-dose
		Chronic toxicity **6.27 (M) – 150 ppm** 8.54 (F) – 150 ppm	*Chronic toxicity* 18.6 (M) – 450 ppm 46.6 (F) – 800 ppm	*Chronic toxicity* Hair loss (F) ↓ body weight gain (F) ↑ abs liver weight (F) Minimal thyroid histopath changes (M & F)
Dietary 18-month OECD 451 [redacted] 2017)	Mouse (C57BL/6J) 0, 50, 250, 1250 ppm Equivalent to 0, 5.9/7.8, 29/38.1, 147/190 mg/kg bw/d in M/F	*Carcinogenicity* 147 (M) – 1250 ppm 190 (F) – 1250 ppm	*Carcinogenicity* >147 (M) – 1250 ppm >190 (F) – 1250 ppm	*Carcinogenicity* No tumours observed up to the top dose
		Chronic toxicity 29 (M) – 250 ppm 38.1 (F) – 250 ppm	*Chronic toxicity* 147 (M) – 250 ppm 190 (F) – 250 ppm	*Chronic toxicity* ↑ mortality rate (F) ↓ body weight and body weight gain (M & F) ↑ liver weight and histopathological correlates (M & F) ↑ kidney weight and histopathological correlates (M)
2-gen study (dietary) OECD 416 [redacted], 2018)	Rat (Wistar) 0, 150/75, 450/225, 1200/600 ppm	*Reproductive* 93-140 (1200/600 ppm) *Parental*	*Reproductive* >93-140 (>1200/600 ppm) *Parental*	*Reproductive* No adverse effects up to the top dose *Parental*

Study, guideline, reference	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	*Adverse* effects at LOAEL
	Equivalent to 0, 11-14, 34-44, 93-140 mg/kg bw/d	**11-14 (150/75 ppm)** *Offspring* 11-14 (150/75 ppm)	34-44 (450/225 ppm) *Offspring* 34-44 (450/225 ppm)	↑ liver and thyroid wt; effects on some clinical-chemistry parameters indicative of liver toxicity *Offspring* ↑ liver wt
Pre-natal dev tox study (gavage) OECD 414 [redacted] 2017b)	Rat (SD) 0, 25, 125, 625 mg/kg bw/d	*Developmental* 125 *Maternal* 125	*Developmental* 625 *Maternal* 625	*Developmental* ↓ foetal wt, ↑ incidence of skeletal and visceral common variations *Maternal* ↓ bwg, ↓ food consumption, ↑ liver wt with associated hypertrophy
Pre-natal dev tox study (gavage) OECD 414 [redacted] 2017)	Rabbit (NZW) 0, 10, 70, 500 mg/kg bw/d	*Developmental* 500 *Maternal* **70**	*Developmental* >500 *Maternal* 500	*Developmental* No adverse effects up to top dose *Maternal* 2 abortion, initial body weight loss, ↓ overall bwg, ↓ initial food consumption, ↑ liver wt

2.6.12. Toxicological end point for assessment of risk following long-term dietary exposure – ADI

The most suitable studies for the derivation of the ADI (Acceptable Daily Intake) are chronic studies. For isoflucypram, the lowest NOAEL from these studies is 6.27 mg/kg bw/d identified from the rat chronic/carcinogenicity study, with a LOAEL of 18.6 mg/kg bw/d for effects on the liver, thyroid and body weight gain. However, a slightly lower NOAEL of 4.2 mg/kg bw/d was identified in the dog 12-month study with a LOAEL of 18.8 mg/kg bw/d for effects on the liver and associated increase in ALP. Overall, the RMS considers that the **NOAEL of 4.2 mg/kg bw/d** from the 12-month dog study is the most appropriate starting point for the derivation of the ADI. This is supported by the NOAEL of 6.27 mg/kg bw/d from the rat 2-yeat study and the NOAEL of 11 mg/kg bw/d for parental and offspring toxicity from the rat 2-generation study. The liver effects seen in the dog are considered to be relevant to humans.

By applying a standard assessment factor of 100 (there is no evidence to suggest that it is possible to deviate from this default), an **ADI value of 0.04 mg/kg bw/d** is derived.

2.6.13. Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Isoflucypram is not acutely toxic by the oral route, is not a neurotoxicant and is not a developmental toxicant. Therefore, in principle an ARfD (Acute Reference Dose) should not be derived. However, in the rabbit gavage developmental study, maternal body weight loss and reduction in food consumption were seen at the beginning of the dosing period at the top dose of 500 mg/kg bw/d. These effects in the rabbit are considered to be potentially relevant to humans. A **NOAEL of 70 mg/kg bw/d** (for maternal toxicity) was identified from this study.

By applying a standard assessment factor of 100 (there is no evidence to suggest that it is possible to deviate from this default), an **ARfD value of 0.7 mg/kg bw** is derived.

2.6.14. Toxicological end point for assessment of occupational, bystander and residents risks – AOEL

The most suitable studies for the the derivation of the systemic AOEL (Acceptable Operator Exposure Level) are medium-term studies. For isoflucypram, the lowest NOAEL from such studies and the whole dataset is 4.2 mg/kg bw/d identified from the 12-month dog study, with a LOAEL of 18.8 mg/kg bw/d for effects on the liver and associated increase in ALP. Overall, the RMS considers that the **NOAEL of 4.2 mg/kg bw/d** from the 12-month dog study is the most appropriate starting point for the derivation of the AOEL. The liver effects seen in the dog are considered to be relevant to humans.

An oral absorption value of 100% and a value for post-hepatic systemic availaibility of 15% (see section 2.6.1 above) have been established. The value of 15% should in principle be used to derive the systemic AOEL; however, as the most sensitive effects driving the NOAEL of 4.2 mg/kg bw/d are liver effects, the post-hepatic systemic availaibility value of 15% is not appropriate. Therefore, the oral absorption value of 100% is the most suitable value to use in adjusting the NOAEL in this case.

By applying an oral absorption value of 100% and a standard assessment factor of 100 (there is no evidence to suggest that it is possible to deviate from this default), an **AOEL of 0.04 mg/kg bw/d** is derived.

2.6.15. Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL

The RMS is of the view that an AAOEL (Acute Acceptable Operator Exposure Level) should not be derived from the ARfD as the effects driving its NOAEL (maternal body weight loss and reduction in food consumption seen at the beginning of the dosing period at the top dose of 500 mg/kg bw/d in the rabbit gavage developmental study) are regarded to be specific to the oral gavage administration of the test substance and should not be extrapolated to the inhalation and dermal route. If an AAOEL is still required, additional data may have to be generated.

2.6.16. Summary of product exposure and risk assessment

'ISY EC 50' is an emulsifiable concentrate containing 50 g isoflucypram/L. It is used as a professional fungicide on cereal crops and it is applied via tractor mounted/trailed boom sprayer. The product is applied at 1 x 0.075 kg a.s./ha, using a water rate from 100 to 400 L/ha and an application timing ranging from BBCH 30 to BBCH 69. Isoflucypram is a non-volatile active substance with a vapour pressure < 5 x 10^{-5} Pa at 25 °C. For the purpose of the non-dietary assessment an AOEL of 0.04 mg/kg bw/d, a dermal absorption of 2% for the neat formulation (50 g a.s. /L) and 5% for the in-use dilutions (0.1875 g a.s./L) was applied for isoflucypram.
Estimates of operator, worker, bystander and resident exposure were conducted in line with the EFSA guidance (EFSA Journal 2014;12(10):3874, 55 pp.,) using the default values in accordance with the guidance and the respective calculator (Version: 30 March 2016). It was assumed that the product is applied using standard spray nozzles. It is noted that the product 'ISY EC 50' contains isoflucypram that has no significant acute toxicity and/or the potential to exert effects after a single dose and hence in this instance an acute exposure risk assessment is not required and only a long term exposure risk assessment was conducted. Exposure in this case will be determined by average exposure over a longer duration, and higher exposures on one day will tend to be offset by lower exposures on other days. Hence, exposure assessment for residents also covers bystander exposure.

2.6.16.1. Operator exposure risk assessment

Systemic exposure risk assessment

The operator exposure assessment undertaken indicates that the proposed uses of 'ISY EC 50' through field crop boom sprayers will result in acceptable systemic operator exposure of 0.0052 mg a.s./kg bw/day equal to 13% of the AOEL for isoflucypram, for an operator that applies the product 'ISY EC 50' without wearing PPE. Detailed exposure calculations can be found in Vol 3- B6(PPP)- Isoflucypram EC 50.

Local effects risk assessment

The product 'ISY EC 50' is classified for human health effects as eye irritant Cat 2, skin irritant Cat 2, skin sensitizer Cat 1, STOT SE 3 and Asp. Tox 1. Based on the classification the following PPE is required: suitable protective clothing (coveralls), suitable protective gloves and face protection (face shield) when handling the concentrate.

2.6.16.2. Bystander and resident exposure risk assessment

An exposure risk assessment for resident was conducted, which covers also the bystanders. For the proposed uses of the product 'ISY EC50' an acceptable systemic resident (and bystander) exposure to isoflucypram is predicted for an unprotected child and adult for individual pathways and the mean of all pathways for which exposure is

5.6% (child) and 1.6% (adult) of the AOEL respectively. A summary of the resultant exposure is presented in the table below. Detailed exposure calculations can be found in Vol 3- B6(PPP)- Isoflucypram EC 50. It is noted that the spray dilution is not classified for human health effects (Vol 3- B6(PPP)- Isoflucypram EC 50), thus no risk assessment for local effects is required for bystanders and residents.

Table 2.3.16.2-1: Estimated resident exposure (longer term exposure)

		Isoflucypram	
Exposed group		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0010	2.56%
	Vapour (75th perc.)	0.0011	2.68%
	Deposits (75th perc.)	0.0001	0.29%
	Re-entry (75th perc.)	0.0006	1.58%
	Sum (mean)	0.0022	5.56%
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0002	0.61%
	Vapour (75th perc.)	0.0002	0.58%
	Deposits (75th perc.)	0.0000	0.06%
	Re-entry (75th perc.)	0.0004	0.88%
	Sum (mean)	0.0006	1.61%

2.6.16.3. Worker exposure risk assessment

The worker exposure risk assessment undertaken indicates that the proposed uses of 'ISY EC 50' on cereal crops will result in an acceptable systemic worker exposure of 0.0005 mg a.s./kg bw/day equal to 1.31% of the AOEL for isoflucypram. for a worker performing crop inspection/ irrigation activities wearing normal workwear (arms, legs and body coverd) and no PPE. As worker re-entry activities will take place outdoors, it is assumed that workers will be wearing normal work wear (arms, body and legs covered). Detailed calculations can be found in Vol 3- B6(PPP)- Isoflucypram EC 50.

2.7. RESIDUE

2.7.1. Summary of storage stability of residues

Plant matrices

Storage stability results for **isoflucypram** and metabolite **M49** are summarised in the Table below:

Table 2.7.1-1 : Stability of isoflucypram and metabolite M49 in frozen plant matrices

Plant products (Category)	**Commodity**	**T** (°C)	**Stability** (Month/Year)			
			Isoflucypram	**M49**		
High water content	Tomato	-18	25 months	25 months		
High oil content	Rape seed	-18	25 months	25 months		
High protein content	Bean (dry seed)	-18	25 months	25 months		
High starch content	Wheat grain	-18	25 months	25 months		
High acid content	Orange	-18	25 months	25 months		

Based on the above results, it can be concluded that residues of **isoflucypram** and its metabolite **M49** are stable for at least 25 months in frozen storage at around -18°C in orange fruit (high acid), tomato fruit (high water), wheat grain (high starch), bean dry seed (high protein) and rape seed (high oil). An

additional period of 6 days at -1 ± 2°C also did not result in significant degradation of residues of either compound. Since stability has been demonstrated in one commodity from each of the crop categories as specified in OECD 506, it can be assumed that residues of **isoflucypram** and **M49** are stable for 25 months at ≤ -18 °C in all raw agricultural and processed commodities.

These results validate the storage periods used in the supervised field trials and processing studies with respect to storage stability of samples frozen prior to analysis.

Animal matrices

In the ruminant and poultry feeding studies, the analyses were completed within 30 days of sample collection. Therefore, further storage stability data in animal matrices are not necessary.

2.7.2. Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Plants

The metabolism of **isoflucypram** (BCS-CN88460) in **tomato fruits** was investigated after two post-emergence spray applications, at a targeted single application rate of 75 g **isoflucypram**/ha. After foliar application of [pyrazole-4-^{14}C]**isoflucypram** or [phenyl-UL-^{14}C]**isoflucypram** to tomatoes the TRR in tomato fruits was calculated based on the radioactivity in the surface wash solution and the fruit sample and amounted up to 0.170 mg a.s. equivalents/kg in total. Most of the radioactive residues (up to 75% of the TRR) were recovered in the surface wash of tomatoes. The only substance identified in rinse and fruit was unchanged **isoflucypram** amounting up to 98% of the TRR. Consequently no relevant metabolisation of **isoflucypram** occurs in tomato fruits after foliar treatment.

The metabolism of **isoflucypram** (BCS-CN88460) was investigated in **wheat plants** after two spray applications using a nominal application rate of 65 g **isoflucypram**/ha each. The applications were performed at the growth stage of BBCH 30 (beginning of stem elongation) and BBCH 69 (end of flowering). After post-emergence spray application of [pyrazole-4-^{14}C]**isoflucypram** or [phenyl-UL-^{14}C] **isoflucypram** to wheat plants, TRR values in wheat straw and wheat hay were high (up to 16.031 mg/kg and 4.032 mg/kg, respectively) and the TRR values in grains were moderate and amounted up to 0.385 mg/kg. Parent compound was the main component (up to 64% of the TRR) in wheat hay and straw samples and the only component in wheat grain samples (up to 92% of the TRR). Metabolites in wheat hay and straw were detected in lower amounts (equal or below 10% of the TRR) and were formed predominantly by hydroxylation at the benzyl moiety followed by hexose and malonic acid conjugation and to a lesser extent by additional demethylation. No label specific metabolism was observed in wheat.

The metabolism of **isoflucypram** (BCS-CN88460) was investigated in **oilseed rape plants** after two foliar applications using a nominal application rate of 60 g **isoflucypram**/ha each. The applications were performed at the growth stage of BBCH 14 (trifoliolate on the 3rd up to 5th node unfolded) and BBCH 77 (70% of pods have reached final size). After post-emergence spray application of [pyrazole-4-^{14}C]**isoflucypram** or [phenyl-UL-^{14}C] **isoflucypram** to oilseed rape, TRR values in oilseed rape intermediate harvest and mature plants were high and amounted up to 4.751 mg/kg and 4.076 mg/kg, respectively. The TRR values in oilseed rape forage and seeds were low and amounted up to 0.012 mg/kg and to 0.126 mg/kg, respectively. Parent compound **isoflucypram** was the main component in intermediate harvest and mature plants (up to 88% of the TRR) and the only component in oilseed rape seeds (up to 74% of the TRR). The forage extract contained no residue above the limit of detection. Hexose and malonic acid conjugated metabolites after hydroxylation at the benzyl moiety were formed in oilseed rape intermediate harvest and mature plants but were detected in low amounts (equal or below 5% of the TRR). No label specific metabolism was observed in oilseed rape.

The metabolism of **isoflucypram** (BCS-CN88460) was investigated in **soybean plants** after three post-emergent plant applications using a nominal application rate of 60 g **isoflucypram** /ha each. The

plants were applied at three different growth stages (BBCH 14, 51 and 84). After post-emergence spray application of [pyrazole-4-^{14}C]**isoflucypram** or [phenyl-UL-^{14}C]**isoflucypram** to soybean, TRR values in soybean straw were highest and amounted up to 17.715 mg/kg. The TRR in soybean forage and soybean hay were high and amounted up to 4.371 mg/kg and 4.679 mg/kg, respectively. The TRR values in seeds were low and amounted up to 0.035 mg/kg. Parent compound **isoflucypram** was the main residue component in soybean straw (up to 70% of the TRR), and a major residue in soybean forage and hay (up to 20% of the TRR). In soybean seeds parent compound was the only component (up to 77% of the TRR). **Isoflucypram** was moderately metabolised in soybean forage, hay and straw samples after three post-emergence applications. Besides parent compound, the following metabolites were identified: BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (**M48**), BCS-CN88460-desfluoro-homoGSH (**M44**), BCS-CN88460-desfluoro-mercapto-lactic acid-OH (**M46**), BCS-CN88460-desfluoro-mercapto-lactic acid-Glyc (**M47**) and BCS-CN88460-desfluoro-Cys-MA (**M45**), accounting for up to 23% of the TRR. The main metabolic reaction observed was de-fluorination at position 5 of the pyrazole ring. Subsequently, conjugation with homoglutathione and degradation of the homoglutathione moiety followed by conjugation with malonic acid or degradation and desamination to mercapto lactic acid group and hydroxylation of the benzyl moiety or of the propyl group. No label specific metabolism was observed in soybean.

Overall **isoflucypram** is moderately metabolized in primary crops after foliar application and unchanged **isoflucypram** was the main or a major residue in all plants investigated. In human edible commodities (tomato fruit, soybean grain, rape seed and wheat grain) no metabolites were identified and parent represented the only residue. In general, no label specific metabolisation of the active substance was observed for all primary crops after foliar application. Metabolites in soybean feed items (straw, forage, hay) were different from the metabolites found in feed items from rape (intermediate harvest, mature plants) and wheat (hay, straw), involving metabolism in soybean through GST (glutathione S-transferase).

Most important metabolisation in soybean occurred at the pyrazole ring: De-fluorination at position 5 of the pyrazole ring followed by conjugation with homoglutathione resulted in metabolite BCS-CN88460-desfluoro homoGSH (**M44**). Degradation of the homoglutathione moiety followed by conjugation with malonic acid or desamination to mercaptic acid group and hydroxylation at the benzyl moiety resulted in metabolites BCS-CN88460-desfluoro-Cys-MA (**M45**), BCS-CN88460-desfluoro-mercapto-lactic acid-OH (**M46**) and BCS-CN88460-desfluoro-mercapto-lactic acid-propyl-OH-Glyc (**M48**). Furthermore, glycosilation was clearly observed at the mercapto lactic acid group and formed metabolite BCS-CN88460-desfluoro-mercapto lactic acid-propyl-OH-Glyc (**M48**).

In feed items from rape (intermediate harvest, mature plants) and wheat (hay, straw) parent compound represented the most prominent residue. In contrast to soybean - where the main metabolic route involved metabolism through GST at the pyrazole moiety - the most important metabolisation observed in rape and wheat was hydroxylation at the benzyl moiety followed by conjugation with hexose and malonic acid. In wheat, hydroxylation at position 1 of the propyl group followed by hexose and malonic acid conjugation and to a minor extent demethylation of the pyrazole moiety formed the metabolites BCS-CN88460-propanol (**M01**), BCS-CN88460-propanol-Glyc (**M18**), BCS-CN88460-propanol-Glyc-MA (**M21**), BCS-CN88460-desmethyl-propanol (**M06**) and BCS-CN88460-desmethyl-propanol-Glyc-MA (**M41**). In rape (intermediate harvest, mature plants) metabolites were generally minor (<5% of TRR). Besides formation of BCS-CN88460-propanol-Glyc-MA (**M21**) also hydroxylation and conjugation at position 2 of the propyl group and of the phenyl moiety was observed forming the metabolites BCS-CN88460-2-propanol-Glyc-MA (**M22**), BCS-CN88460-hydroxyphenyl-Gluc-MA (**M23**) and BCS-CN88460-hydroxyphenyl-Glyc-MA (**M24**).

The metabolism of **isoflucypram** (BCS-CN88460) was further investigated in potato after seed treatment at a nominal application rate of 25 g a.s./ha, representing the envisaged use rate (1x). Additionally, a 10x overdose experiment was conducted at a nominal application rate of 250 g a.s./ha. After seed treatment with [pyrazole-4-^{14}C]isoflucypram or [phenyl-UL-^{14}C]isoflucypram, TRR values in potato tubers of the normal dose experiment were very low and amounted to 0.009 mg/kg and 0.002 mg/kg, respectively and no identification and quantitation of individual components of the TRR was performed due to the very low TRRs. The TRR in potatoes of the 10x overdose experiment amounted to 0.064 mg/kg and 0.042 mg/kg, respectively. Parent compound represented by far the major component of the residue in tubers of the overdose experiment. As additional matrix, leaves were investigated in both 1x and 10x experiment in order to facilitate the identification of formed metabolites and investigation of the metabolic pathway. Hydroxylation at the benzyl moiety followed by hexose and malonic acid conjugation was the major metabolic process of isoflucypram identified in potato leaves. One metabolite specific for the pyrazole label was identified in leaves and in low amounts (<0.010 mg eq/kg) in tubers of the 10x overdose experiment. Parent compound and/or metabolites were taken up and translocated into leaves and tubers only to a very small extent (calculated rate of uptake into tubers and leaves based on the applied radioactivity (RA) amounts to ≤0.3% and ≤1.0% of the applied RA for tubers and leaves, respectively).

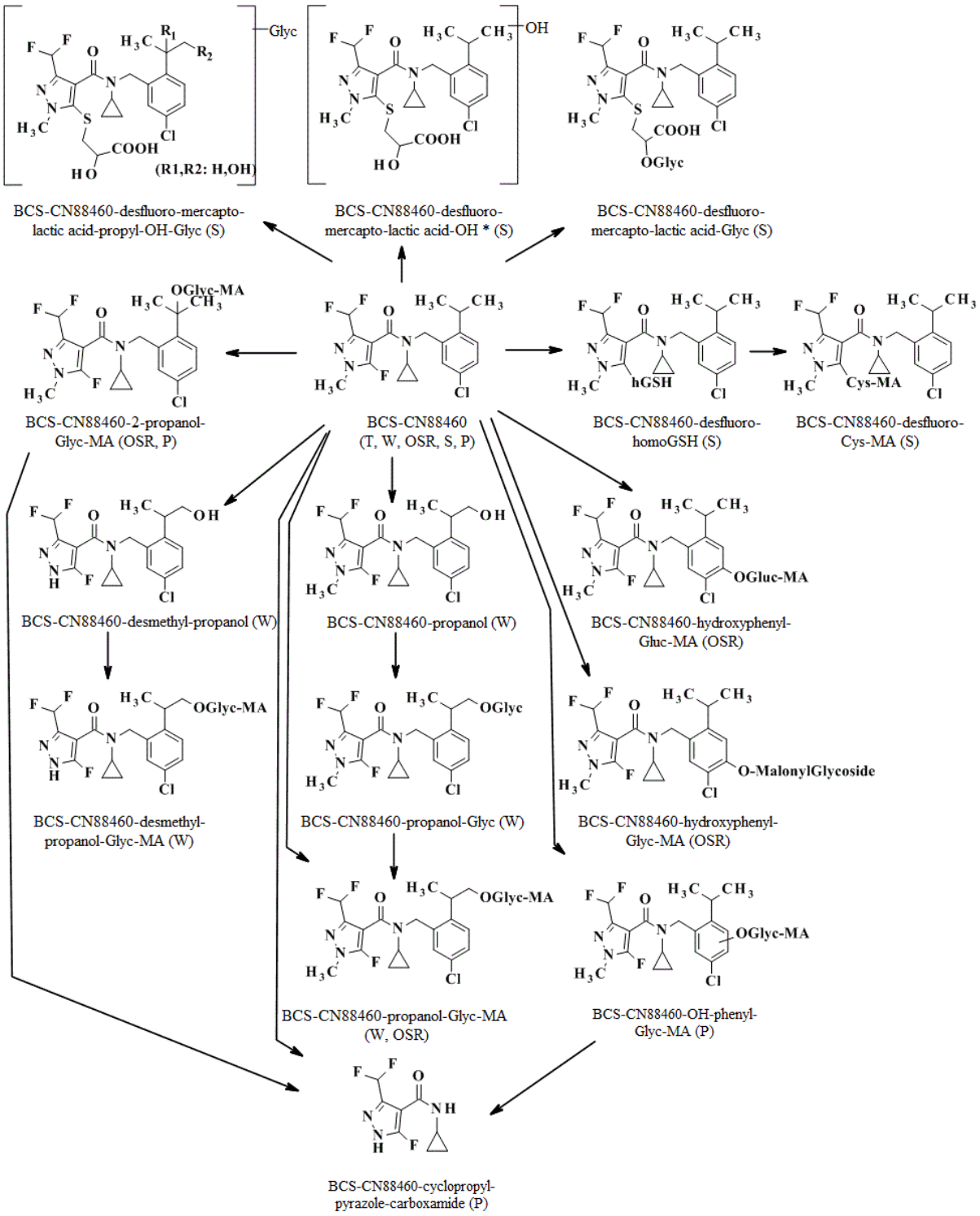
The proposed metabolic pathway for isoflucypram in primary crops (tomato, T; wheat, W; oilseed rape, OSR; soybean, S and potato, P) is presented in Figure 2.7.2-1, below.

The primary crops represent four different crop categories (fruit, cereals, oilseeds and root crops) covering foliar application and seed/tuber treatment. Except for soy and potato (seed treatment), the general metabolic steps in the primary crop metabolism studies were very similar. The divergent metabolism observed for soy is deemed specific to soybean as this was not observed in rape - which pertains to the same metabolism crop group as soybean – and for rape and wheat after foliar application common metabolic reactions were observed. Besides the common metabolic routes observed after foliar treatment (hydroxylation at the benzyl moiety followed by hexose and malonic acid conjugation), metabolism after seed/tuber treatment in potato further showed cleavage of isoflucypram followed by demethylation. This general metabolic route was also observed in confined rotational crops (CRCs) following soil application. In general, occurrence of pyrazole label specific metabolites in crops following soil or seed/tuber treatment with isoflucypram likely originates from uptake of label specific metabolites from the soil.

Therefore it is concluded that the nature of residues in primary crops after foliar application and seed/tuber treatment of **isoflucypram** is sufficiently understood and that no further studies are needed.

Based on the results of the metabolism studies in primary crops, only parent compound was measured in the residue trials.

Figure 2.7.2-1 : Proposed metabolic pathway of isoflucypram in primary crops (tomato, T; wheat, W; oilseed rape, OSR; soybean, S and potato, P)



Livestock

The metabolism of **isoflucypram** in livestock animals has been studied in **laying hens and lactating goats**. The TRR-values and even transfer factors for eggs and edible tissues in laying hens after dosing with [phenyl-UL-^{14}C]**isoflucypram** or [pyrazole-4-^{14}C]**isoflucypram** were very low with respect to the dose level and the dosing period of 14 days. This indicates that test compound related radioactivity does not accumulate during the time of feeding. The evaluations of the TRR-values should however

consider the fact that an exaggerated dose level of up to 18 mg a.s./kg feed/day was administered. Furthermore, the fact that the entire radioactivity was detected in the excreta and the relatively high TRR in kidney and liver at sacrifice approx. 6 hours after the last administration revealed that the test compound related residues are further metabolised and finally eliminated from the hen's bodies. In fat **isoflucypram** represented the major residue with an amount of up to 0.010 mg/kg. In eggs and leg muscle **isoflucypram** was detected in low amounts, only. While in thorax muscle and liver, unchanged **isoflucypram** was not found. **Isoflucypram** was metabolised most extensively in liver. The main metabolic reactions represented demethylation, hydroxylation followed by oxidation to carboxylic acid. Further metabolic reactions included conjugation with glucuronic acid.

The TRR-values and the transfer factors in milk, organs and tissues in lactating goats after dosing with [phenyl-UL-^{14}C]**isoflucypram** or [pyrazole-4-^{14}C]**isoflucypram** were very low compared to the dose level of up to 45 mg a.s./kg feed/day and a dosing period of five days. The highest TRR-value was detected for liver and was caused by the short time period of six hours between last dosing and sacrifice. It indicates the significance of this organ for metabolism. The elimination of radioactivity was mainly faecal and less than 10% of the dose was eliminated via urine, which was reflected by the low TRR-value for kidney. This excretion behaviour was similar to the findings in the ADME studies with rats. The TRR-values in the respective evening and morning milk samples showed a diurnal pattern as they declined slightly prior to the delivery of the next dose for most days. A continuous increase was observed before a residue plateau-level was reached at day three after the first administration. **Isoflucypram** was the main residue in fat. **Isoflucypram** was intensively metabolised in liver and kidney, where unchanged parent compound was only present in minor amounts. In milk and muscle unchanged parent compound was detected in low amounts but represented a major residue for both matrices. The main metabolism involves demethylation, hydroxylation followed by oxidation to carboxylic acid, and conjugation with glucuronic acid.

The metabolism in hens (poultry) and goat (ruminant) is similar with some minor varieties and includes:

1) hydroxylation of **isoflucypram** to isoflucypram-propanol (**M01**), isoflucypram-2-propanol (**M02**) or isoflucypram-1,2-propandiol (**M03**)
2) further oxidation to isoflucypram-carboxylic acid (**M12**) or to a lactic acid group
3) conjugation of isoflucypram-propanol (**M01**), isoflucypram-2-propanol (**M02**) or isoflucypram-1,2-propandiol (**M03**) with glucuronic acid or sulfate
4) demethylation of the pyrazole moiety and conjugation with glucuronic acid after demethylation
5) cleavage of the phenyl moiety in combination with cleavage of the cyclopropyl ring
6) dehydration after hydroxylation of the propyl group followed by conjugation with glucuronic acid

Based on the results of the metabolism studies in livestock, several compounds were proposed as residue definition for data collection and measured in the feeding studies.

Isoflucypram parent compound and its metabolites BCS-CN88460-propanol (**M01**), BCS-CN88460-2-propanol (**M02**), BCS-CN88460-desmethyl-propanol (**M06**), BCS-CN88460-desmethyl-carboxylic acid (**M11**) and BCS-CN88460-carboxylic acid (**M12**) were individually determined in milk, eggs and all tissues

Additionally, the sum of BCS-CN88460-2-propanol (**M02**) and its conjugate BCS-CN88460-2-propanol-GlucA (**M20**), and the sum of BCS-CN88460-propanol (**M01**) and its conjugate BCS-CN88460-propanol-GlucA (**M19**, isomer 1 and isomer 2) were determined in cow liver and kidney.

Moreover, the sum of BCS-CN88460-desmethyl-propanol (**M06**) and its conjugate BCS-CN88460-desmethyl-propanol-N-GlucA (**M37**) was determined in hen liver.

The proposed metabolic pathway for **isoflucypram** in poultry (H, laying hens) and ruminants (G, lactating goats) is shown in Figure 2.7.2-2, below:

The metabolic pathway of **isoflucypram** in laying hen, lactating goat and rat is evaluated to be similar since, generally, the same metabolic steps are involved and the same metabolites are found.
As the exact chemical structures of various metabolites could not be clarified in rats, some of the identified metabolites in livestock animals were not exactly observed in rats. Isoflucypram-2-propanol-GlucA (**M20**) is found in amount of 13% of the TRR in the liver of goats, but was not exactly detected in rat. Isoflucypram-propenol-GlucA (**M25**) is found in minor amounts in kidney and urine in the goats, but was not detected at all in rats. Isoflucypram-hydroxyphenyl (**M04**) was only detected in urine of goats, but not detected in rats, whereas other metabolites with the hydroxylated position 4 of the phenyl ring were detected in the rat.

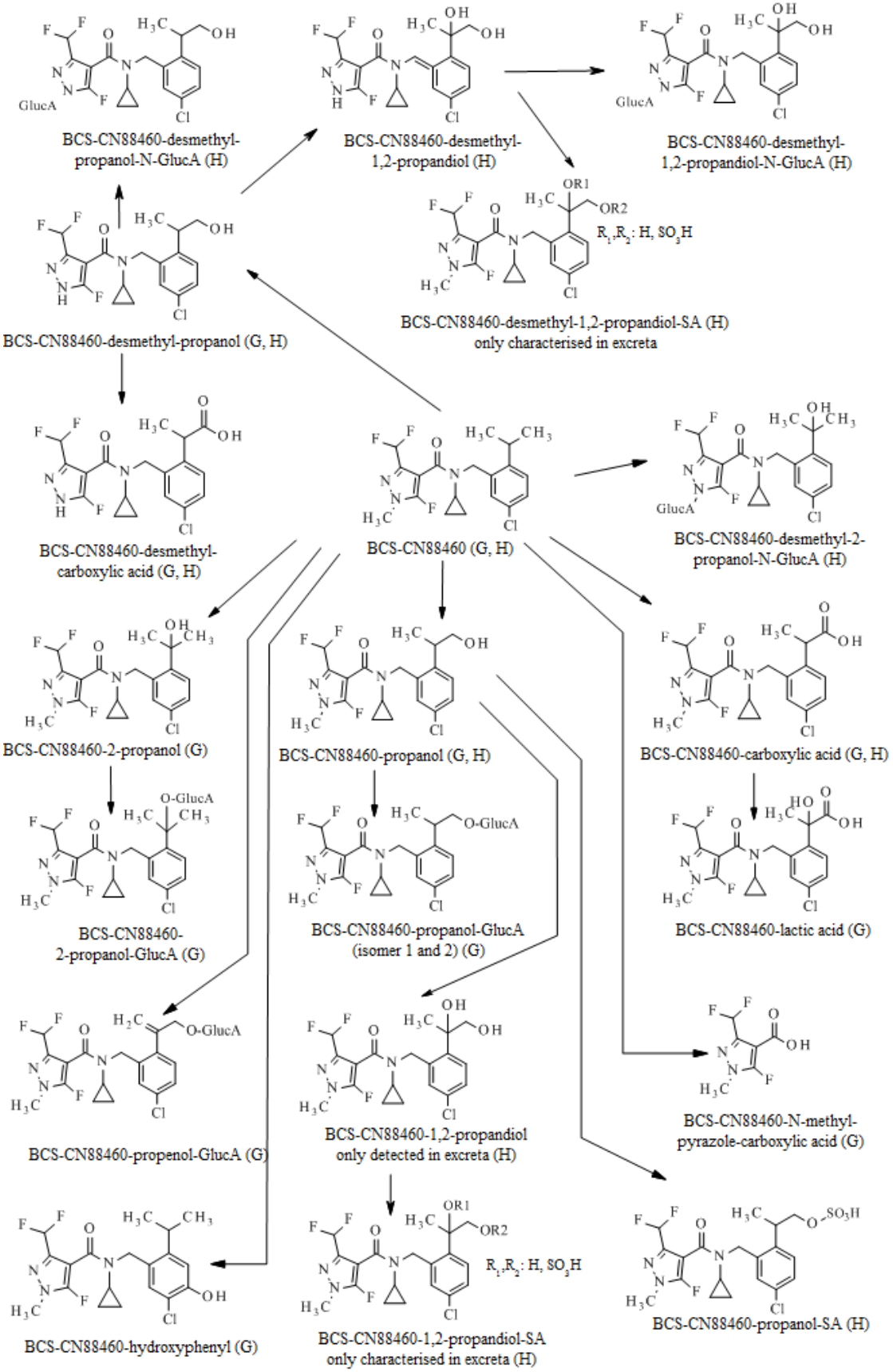
N-glucuronic acid conjugates and conjugates with SA-group were only detected in hens. Conjugates with SA-group were only present in minor amounts, and mainly in excreta.

The general metabolic reactions for conjugation with glucuronic acid and sulphuric acid were also observed in the metabolism study with rats and could be verified by enzymatic cleavage of the conjugates to their specific aglycons. Hydroxylation and demethylation leading to the specific aglycons were observed in the metabolic pathway of the rat.

All other metabolites including metabolites detected in fish were also detected in the rat.

For fish, the RMS notes that a metabolism study, dosing with pyrazole-labelled isoflucypram is available. o fish metabolism study dosing with phenyl-labelled isoflucypram has been provided. The details of the available study are provided in the DAR section B7 (AS); however, since, at the time of writing, there is no EU agreed guidance on conducting fish metabolism studies, it is not possible to evaluate it.

Figure 2.7.2-2 : Proposed metabolic pathway of isoflucypram in livestock (hens, H; goats, G)



2.7.3. Definition of the residue

The residue definitions for risk assessment and monitoring in plant and animal commodities are proposed as follows:

Plant commodities (including rotational crops and processed commodities)

RD-Mo: Isoflucypram

RD-RA (cereals and rotational crops): **Provisionally**: Isoflucypram

Please note that the RMS (UK) is aware of on-going studies on wheat and barley in which positive residues of M01 + conjugates and M06 + conjugates have been found ≥0.01 mg/kg in some matrices (barley grain, barley straw, wheat straw). At the time of writing (December 2018), this data is not available for evaluation and so, at the present time, it is not possible to take account of this new information in the DAR. On the basis of the above, once the on-going studies have been finalized, the RMS (UK) considers it likely that the residue definition for Risk Assessment in cereals may well need to be updated to: Sum of isoflucypram and its metabolites M01 and M06 and their conjugates, expressed as isoflucypram.

Animal commodities

The residue definition in primary crops is not yet finalised and so it is not possible to finalise the dietary burden estimate for livestock. However, based on the indicative dietary burden calculation and the available feeding studies, it would appear as though positive residues are not expected for the parent compound, or significant metabolites, in animal matrices at the 1N level. Hence, the proposal for the residue definitions for monitoring and risk assessment to be parent isoflucypram only.

RD-Mo: (Provisionally) Isoflucypram

RD-RA: (Provisionally) Isoflucypram

2.7.4. Summary of residue trials in plants and identification of critical GAP

The critical GAP for **isoflucypram** is defined as follows:

Description	F/G	No. of applications	Growth stage at application (BBCH Code)	Application rate per treatment (g a.s./ha) *	Water volume (L/ha)	Interval (days)	PHI (days)
Barley, oat	F**	1	30-61	75	100-400	--	n.a.
Wheat, Durum wheat, rye, spelt, triticale	F**	1	30-69	75	100-400	--	n.a.

F = field; G = greenhouse
* agricultural use based on the formulation Isoflucypram EC 50
** uses in both the Northern and Southern residue regions (EU-N and EU-S)
n.a.: not applicable; the timing is defined by the growth stage at application.

In order to support the EU "representative use" of **isoflucypram**, sets of GLP trials were conducted on barley and wheat in Northern and Southern European fields in 2015 and 2016.

Barley

A total of 25 independent supervised field trials on barley were conducted in Europe, 13 in Northern and 12 in Southern Europe. **Isoflucypram** was applied once at the latest intended growth stage of BBCH 61. Three EC formulations containing **isoflucypram** were tested at similar application rates (EC 050 or EC150 at 75 g **isoflucypram**/ha or EC250 at 62.5 g **isoflucypram**/ha). Residues of **isoflucypram** were determined.

Using statistical tests (Kruskal-Wallis H-Test and Mann-Whitney U-test, α = 0.05) the residue data obtained with the three formulations were not found significantly different. All the residue data were thus combined. A summary of the residue data is shown in the table below.

Crop	NEU/ SEU	Trial results relevant to the critical representative GAP (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded MRL; OECD (mg/kg)
Barley Grain	NEU	10 × <0.01; 0.013; 0.020; 0.041	<0.01	0.041	0.05
	SEU	9 × <0.01; 0.022; 0.027; 0.037	<0.01	0.037	0.05
	NEU + SEU	19 x <0.01; 0.013; 0.020; 0.022; 0.027; 0.037; 0.041	**<0.01**	0.041	**0.05**
Barley Straw	NEU	0.049; 0.11; 0.13; 0.16; 0.20; 0.24; 0.32; 0.40; 0.44; 0.51; 0.94; 0.96; 1.2	0.32	1.2	N/A
	SEU	0.021; 0.13; 0.16; 0.18; 0.24; 0.29; 0.29; 0.31; 0.85; 0.96; 1.0; 3.1	0.29	3.1	N/A
	NEU + SEU	0.021; 0.049; 0.11; 2 x 0.13;2 x 0.16; 0.18; 0.20; 2 x 0.24; 2 x 0.29; 0.31; 0.32; 0.40; 0.44; 0.51; 0.85; 0.94; 2 x 0.96; 1.0; 1.2; 3.1	**0.29**	**3.1**	N/A

Wheat

A total of 24 independent supervised field trials on wheat were conducted in Europe, 12 in Northern Europe and 12 in Southern Europe. **Isoflucypram** was applied once at the latest intended growth stage of BBCH 69. Three EC formulations containing **isoflucypram** were tested at similar application rates (EC 050 or EC150 at 75 g **isoflucypram**/ha or EC250 at 62.5 g **isoflucypram**/ha). Residues of **isoflucypram** were determined.

Using statistical tests (Kruskal-Wallis H-Test and Mann-Whitney U-test, α = 0.05) the residue data obtained with the three formulations were not found significantly different. All the residue data were thus combined. A summary of the residue data is shown in the table below.

Crop	NEU /SEU	Trial results relevant to the critical representative GAP (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded MRL; OECD (mg/kg)
Wheat Grain	NEU	12 × <0.01	<0.01	<0.01	0.05
	SEU	11 × <0.01; 0.042	<0.01	0.042	0.05
	NEU + SEU	23 x <0.01; 0.042	**<0.01**	0.042	**0.05**
Wheat Straw	NEU	0.054; 0.071; 0.12; 0.19; 0.38; 0.40; 0.82; 0.94; 1.5; 1.7; 3.3; 3.6	0.61	3.6	N/A
	SEU	0.22; 0.33; 0.41; 0.87; 1.3; 1.4; 1.6; 1.8; 1.9; 1.9; 2.3; 2.4	1.5	2.4	N/A
	NEU + SEU	0.054; 0.071; 0.12; 0.19; 0.22; 0.33; 0.38; 0.40; 0.41; 0.82; 0.87; 0.94; 1.3; 1.4; 1.5; 1.6; 1.7; 1.8; 1.9; 1.9; 2.3; 2.4; 3.3; 3.6	**1.12**	**3.6**	N/A

2.7.5. Summary of feeding studies in poultry, ruminants, pigs and fish

Isoflucypram (BCS-CN88460) is sought for use on cereals - parts of this crop might be fed to livestock (grain, straw and by-products of grain). The calculated maximum dietary burdens for different groups of livestock exceed the trigger value of 0.004 mg/kg bw/day for cattle (0.041 mg/kg bw/day), sheep (0.089 mg/kg bw/day) and poultry (0.029 mg/kg bw/day). Dairy cows and laying hens feeding studies were conducted.

Poultry

A feeding study was conducted with **isoflucypram** (BCS-CN88460) on poultry in order to elucidate the levels of relevant residues in poultry tissues and in eggs. **Isoflucypram** was administered orally (via capsule) to laying hens for 28 consecutive days at average dose rates of 0.03 mg/kg bw/day (1X EU dose), 0.12 mg/kg bw/day (4X) and 0.48 mg/kg bw/day (16X). Feed consumption, body weights, and egg production were not adversely affected by compound administration.

Prior to sacrifice, residues in eggs were measured at various intervals. After the final dose, the animals were sacrificed and the key edible tissues were analysed for the free residues of **isoflucypram** and its metabolites BCS-DC20298 (**M02**), BCS-CY26497 (**M12**), BCS-CY24813 (**M01**), BCS-DC22055 (**M06**) and BCS-CX99799 (**M11**) in all matrices. In addition, the sum of BCS-DC22055 (**M06**) and its conjugate **M37** was determined in liver.

Overall, free residues of **isoflucypram** parent, BCS-DC20298 (**M02**) and BCS-DC22055 (**M06**) were below the LOQ in eggs and tissues for all doses. Residues of BCS-CY26497 (**M12**), BCS-CY24813 (**M01**) and BCS-CX99799 (**M11**), as well as the sum of BCS-DC22055 (**M06**) and its conjugate **M37**, increase linearly with the dose level of **isoflucypram**. In the eggs, residues above the LOQ of 0.01 mg/kg were only found for BCS-CY24813 (**M01**) in the samples from the highest dose group of 16X (maximum 0.02 mg/kg). The plateau concentration in eggs was reached after approximately 9-11 days. After a depuration phase of 4, 7 and 14 days, all measured residues of **isoflucypram** and its metabolites had declined to below the LOQ of 0.01 mg/kg in eggs and tissues.

Ruminant

A feeding study was conducted with **isoflucypram** (BCS-CN88460) on cows in order to elucidate the levels of relevant residues in cow tissues and in milk. **Isoflucypram** was administered orally (via capsule) to cows for 28 consecutive days at average dose rates of 0.05 mg/kg bw/day test item for the dose group 1X, 0.15 mg/kg bw/day for the dose group 3X, 0.5 mg/kg bw/day for the dose group 10X and 1.5 mg/kg bw/day for the dose groups 30X and 30XE. Feed consumption, body weights, and milk production were not adversely affected by compound administration.

Prior to sacrifice, residues in milk were measured at various intervals. After the final dose, the animals were sacrificed and the key edible tissues were analysed for the free residues of **isoflucypram** and its metabolites BCS-DC20298 (**M02**), BCS-CY26497 (**M12**), BCS-CY24813 (**M01**), BCS-DC22055 (**M06**) and BCS-CX99799 (**M11**) in all matrices. In addition the sum of BCS-DC20298 (**M02**) and its conjugate **M20** and the sum of BCS-CY24813 (**M02**) and its conjugate **M19** were determined in liver and kidney.

Overall, residues of **isoflucypram** above the LOQ were found only in milk, fat, kidney and liver. Residues of BCS-CY26497 (**M12**) and BCS-CX99799 (**M11**) were found only in kidney and liver and free and conjugated residues of BCS-DC20298 (**M02**) and BCS-CY24813 (**M01**) were found in liver and kidney. The free residues of **isoflucypram**, BCS-CY26497 (**M12**) and BCS-CX99799 (**M11**) as well as free and conjugated residues of BCS-DC20298 (**M02**) and BCS-CY24813 (**M01**) in fat, liver and kidney were found to increase linearly with the dose level of **isoflucypram**.

Residues of **isoflucypram** were found in milk samples only in the 30X and 30XE groups up to 0.013 mg/kg, in cream samples up to 0.15 mg/kg in the 30X-group. The plateau concentration in milk was reached after approximately 9 days.

After a depuration phase of 4 days, the measured residues of **isoflucypram** had declined to below the LOQ of 0.005 mg/kg in milk and 0.01 mg/kg in tissues except for **isoflucypram** in fat (0.014 mg/kg). After a depuration phase of 4 and 14 days, all measured residues were found to be below their respective LOQ in all samples.

Fish

No residue study in fish was conducted. Currently, no test method or Guidance Document is available for conducting such study. In these cases, waiving of this particular data requirement is considered acceptable according to Regulations (EU) No 283/2013 and No 284/2013" (SANCO/10181/2013 rev. 3 of 12 December 2014).

2.7.6. Summary of effects of processing

Nature of the residues after processing

Isoflucypram was stable under all tested processing conditions. Based on these results, only the level of parent compound was measured in processing studies.

According to the data requirements of the Regulation (EC) No 1107/2009, studies investigating the magnitude of residues in processed commodities of cereals are not required. Nevertheless studies were conducted for barley and wheat.

Barley

Two residue trials were conducted in Northern and Southern Europe in 2015. Barley was treated at a growth stage of BBCH 61 (in one trial at BBCH 53) with an exaggerated dose rate (5X; 375 g **isoflucypram**/ha) to attempt to generate a commodity with quantifiable residues. All applications were at the required rates.

Barley grain was processed in order to obtain beer and pearl barley. The samples (RAC and processed fractions) were analysed for the residues of **isoflucypram** parent compound. The results of the study clearly indicate that residues of **isoflucypram** are diluted by the brewing processing.

When barley grain are processed into pearl barley, residues of **isoflucypram** remain to a large extent in pearl barley rub-off and can be removed from barley grain by hulling, resulting in lower residues in the end product, pearl barley.

Wheat

Two residue trials were conducted in Canada. Wheat was treated once at a growth stage of BBCH 69 with an exaggerated dose rate (5X; 375-382 g **isoflucypram**/ha) to attempt to generate a commodity with quantifiable residues.

Wheat grain was processed in order to obtain aspirated grain fraction, middlings, germ, white flour, shorts, bran, white bread, whole meal flour, whole meal bread, gluten, starch, fresh pasta, cooked fresh pasta, cooking water, dried pasta, dried and cooked pasta.
The samples (RAC and processed fractions) were analysed for the residues of **isoflucypram** parent compound.

The results of the study indicate that residues of **isoflucypram** are concentrated in the aspirated grain fraction, and in a lesser extent in bran and germ. Similar residue levels as in wheat grain are observed in shorts, whole meal flour and gluten, whereas a dilution of residues is observed in middlings, starch, white flour and the subsequent production of pasta (fresh, dried, fresh and cooked, and dried and cooked), as well as in bread (prepared from white and whole meal flours).

2.7.7. Summary of residues in rotational crops

Based on the Fate and Behaviour assessment, the following applies to isoflucypram:

Worst case field dissipation DT90 values: isoflucypram= 3090 days, M12 = 2370 days

For isoflucypram, the maximum PEC soil accumulation value is 0.0616 mg/kg, with a plateau concentration of 0.0416 mg/kg reached after 21 years.

For the M12 metabolite, the max PEC soil accumulation is: 0.0069 mg/kg, with a plateau of 0.00486 mg/kg reached after 20 years.

For isoflucypram, the maximum PEC soil value of 0.0616 mg/kg is equivalent to an application rate of:

10000 m^2 (1 ha) at a 0.05 m depth of soil = 500 m^3 soil; asuming a soil density of 1500 kg/m^3 (1.5 g/cm^3), this is 750,000 kg soil. Hence, a concentration of 0.0616 mg/kg is equivalent to 0.0616 x 750,000 = 46200 mg/ha, or 46.2 g/ha.

Metabolism in rotational crops

In the two confined rotational crop studies applied with an application rate of 198 - 202 g/ha of pyrazole and phenyl labelled **isoflucypram**, the residues of **isoflucypram** in rotational crops planted at all intervals were less than 0.08 mg/kg except in wheat hay and wheat straw of the pyrazole labelled confined rotational crop study where the content of **isoflucypram** was 0.114-0.220 mg/kg and 0.131-0.340 mg/kg, respectively. The TRRs in the different raw agricultural commodities (RACs) were generally low, increased slightly from the 1st to the 2nd rotation and stayed stable or declined to lower values in the 3rd rotation. The TRRs in the RACs of the phenyl labelled study were lower as the TRRs found in the study with the pyrazole label.

In the confined rotational crop study with **isoflucypram** labelled in the pyrazole moiety, unchanged parent compound was only detected in wheat forage, Swiss chard and turnip leaves with amounts of equal or less than 7.0% (0.003 mg/kg) of the TRR. Up to thirteen pyrazole derivative metabolites were identified. As the TRR values of the confined study was generally low, none of the identified metabolites accounted for more than 0.022 mg/kg and none of the unknown compounds accounted for more than 0.021 mg/kg.

The main metabolic reactions were the cleavage of the parent compound to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (**M49**, named as BCS-CR60082) and following conjugation of BCS-CR60082 (**M49**) with alanine (with or without defluorination) or the hydroxylation and defluorination of BCS-CR60082 (**M49**) followed by conjugation with cysteine or glutathione. Other metabolic reactions were demethylation, hydroxylation, deamination or defluorination of BCS-CR60082 (**M49**), followed by conjugation with glucose, lactic acid, acetic acid, cysteine or glutathione. The glutathione group was afterwards degraded to mercapto alcohol with an additional conjugation with malonic acid.

In the confined rotational crop study with **isoflucypram** labelled in the phenyl moiety, unchanged parent compound was only detected in wheat forage, wheat hay and Swiss chard with amounts of equal or less than 17.0% (0.004 mg/kg) of the TRR. Due to very low TRR values in the confined study, no further metabolites were identified in the conventional extracts as none of the unknown compounds was larger than 0.009 mg/kg. No label specific metabolites were detected in the CRC study with the phenyl label.

Isoflucypram labelled in the pyrazole moiety lead to higher residues than **isoflucypram** labelled in the phenyl moiety. This indicates that cleavage of the molecule more likely happens in soil, followed by uptake and subsequent conjugation in the plant.

The following metabolic reactions were observed:

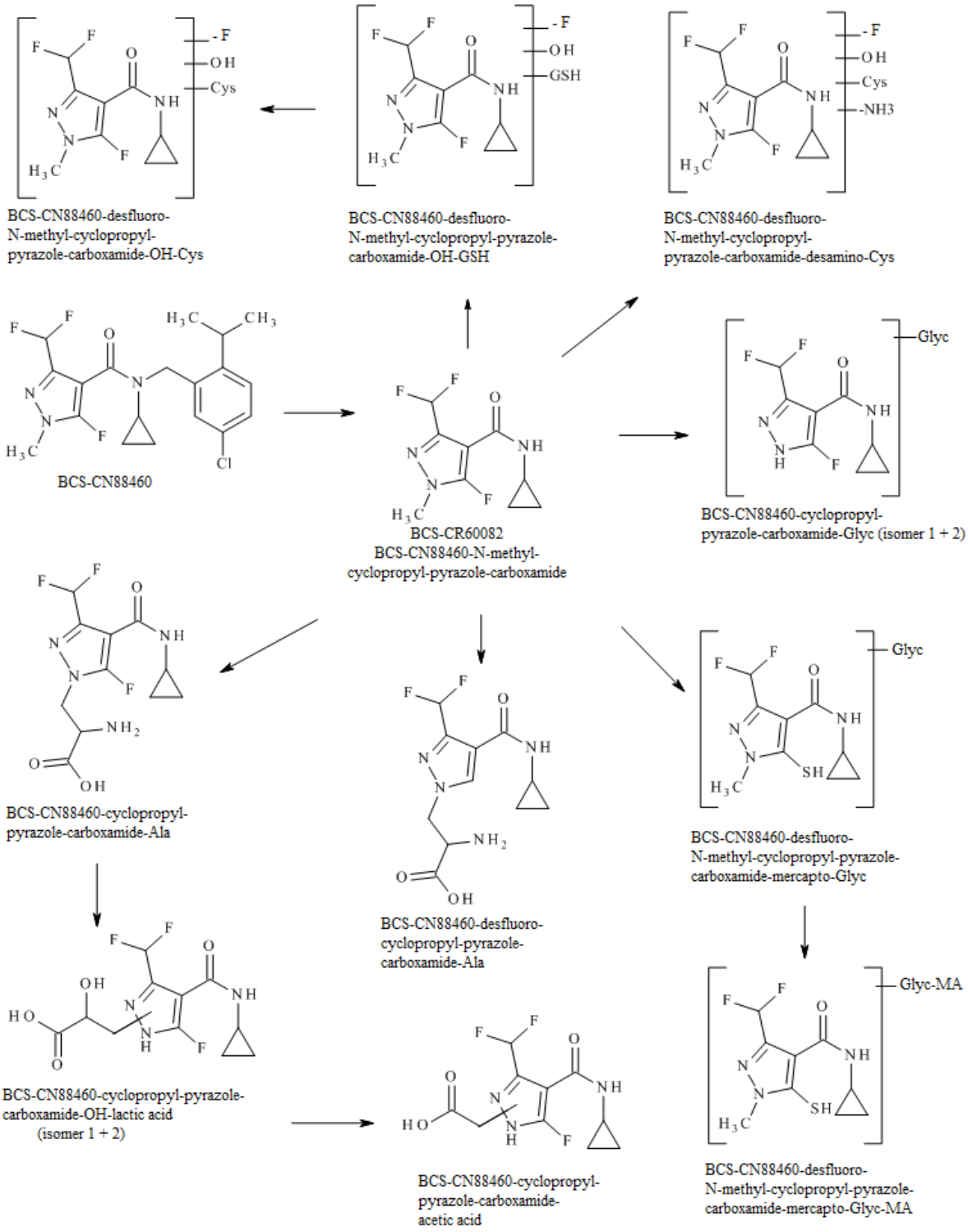
- cleavage of the parent compound leading to BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (**M49**, BCS-CR60082)
- conjugation of BCS-CR60082 (**M49**) with alanine, lactic acid or acetic acid with or without defluorination of the pyrazole ring
- demethylation of BCS-CR60082 (**M49**) followed by conjugation with glucose
- hydroxylation, deamination and defluorination of BCS-CR60082 (**M49**) followed by conjugation with cysteine or glutathione

- defluorination of BCS-CR60082 (**M49**) followed by conjugation with glucose and glutathione and degradation of the glutathione group to mercapto alcohol, additional conjugation with malonic acid

Based on these results, parent compound and its metabolite BCS-CN88460-N-methyl-cyclopropyl-pyrazole-carboxamide (BCS-CR60082; **M49**) were measured in the field rotational crop studies. Based on the identified metabolites, the metabolism of **isoflucypram** in confined rotational crops is adequately understood.

The proposed metabolic pathway for **isoflucypram** in rotational crops is presented in Figure 2.7.7-1, below.

Figure 2.7.7-1 : Proposed metabolic pathway of isoflucypram in confined rotational crops



Magnitude of residues in rotational crops

In order to support the use of **isoflucypram** in the EU for non-perennial crops, four multi-plant-back multi-crop rotational crop trials were conducted in Europe (2 each in the northern and southern residue regions) in 2015-2016. **Isoflucypram** (BCS-CN88460) was applied once as an EC 50 formulation to bare soil at an application rate of 3.6 L/ha (corresponding to 180 g active substance/ha). Crops representing 3 different botanical groups (roots, leafy vegetables, small grain cereals) were planted on

the plots at 3 intervals thereafter. All applications were at the required rates, and all trials were conducted according to GLP.

The residues of **isoflucypram** and BCS-CR60082 (**M49**) in the rotational crops were always found <LOQ with one exception.

In the trial 15-2501-01 (Germany), residues of **isoflucypram** reached levels of 0.057 to 0.075 mg/kg only in carrot leaves (2nd rotation, plot T-2A). These results – confirmed with re-analyses- were illogical because no residues were found in carrot roots from the same plot and no residues were found in the 1st rotation for carrot.

Five days before the first harvest of carrot leafs in plot T-2A, the adjoining plot dedicated to barley (T-1C) was sprayed with **Isoflucypram EC 50**. There was no buffer zone between the two plots, the carrot plot was not protected during the spray of the plot T-1C, and there was a very light wind in the direction of the carrot plot. This explanation supports the fact that the residue levels of **isoflucypram** found in carrot leaves in the plot T-2A are the result of a spray drift. These residue levels should not be considered because they are not attributable to residues arising from soil treatment.

Based on all the other results, it is concluded, that after an application of Isoflucypram EC 050 on bare soil at a rate of 180 g a.s./ha, the residues of **isoflucypram** and BCS-CR60082 (**M49**) are expected to be <0.01 mg/kg in barley, lettuce and carrot or turnip grown as rotational crops.

The dose rate of 180 g a.s./ha adequately covers the plateau concentration of **isoflucypram** in soil overtime. It is concluded that plant-back restrictions and MRLs proposals above 0.01 mg/kg based on residues in rotational crops are not necessary.

2.7.8. Summary of other studies

N/A

2.7.9. Estimation of the potential and actual exposure through diet and other sources

In order to evaluate the potential chronic and acute exposures to **isoflucypram** (BCS-CN88460) residues through the diet, calculations were conducted using the EFSA PRIMo model (revision 3), an ADI of 0.04 mg/kg bw/day and an ARfD of 0.7 mg/kg bw/day.

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI

TMDI calculations were conducted using the proposed MRLs for cereals and for animal commodities – see Table 2.7.9-1, below. The highest TMDI was estimated at ca. 2% of ADI for DK child.

The TMDI calculations are below the ADI. Therefore, no chronic health effects are expected for EU consumers as a result of the proposed representative uses of **isoflucypram** on cereals.

IEDI

Since the TMDI calculations demonstrate a large margin of safety the IEDI was not calculated.

IESTI

The IESTI/NESTI calculations were conducted using the proposed MRLs for cereals and for animal commodities – see Table 2.7.9-2, below. This is a very conservative approach for cereals since usually median residues can be applied for such blended commodities. The highest IESTI was estimated at ca. 0.1% ARfD for wheat consumed by children.

No exceedance of the ARfD was identified. Therefore, no acute health effects are expected for EU consumers as a result of the proposed representative uses of **isoflucypram** on cereals.

Table 2.7.9-1: PRIMo 3 chronic risk assessment for isoflucypram

European Food Safety Authority

EFSA PRIMo revision 3.0; 2017/12/11

Isoflucypram

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	**0.04**	ARfD (mg/kg bw):	**0.7**
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment

Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Exposure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	2%	DK child	0.63	0.7%	Rye	0.6%	Wheat	0.2%	Milk: Cattle		2%
	1%	NL toddler	0.57	0.7%	Milk: Cattle	0.5%	Wheat	0.1%	Rye		1%
	1%	FR child 3 15 yr	0.40	0.6%	Wheat	0.3%	Milk: Cattle	0.0%	Bovine: Muscle/meat		1%
	1.0%	GEMS/Food G06	0.39	0.9%	Wheat	0.0%	Milk: Cattle	0.0%	Poultry: Muscle/meat		1.0%
	1.0%	DE child	0.39	0.5%	Wheat	0.2%	Milk: Cattle	0.1%	Rye		1.0%
	0.9%	NL child	0.37	0.5%	Wheat	0.3%	Milk: Cattle	0.0%	Swine: Muscle/meat		0.9%
	0.9%	UK infant	0.37	0.5%	Milk: Cattle	0.3%	Wheat	0.0%	Eggs: Chicken		0.9%
	0.9%	GEMS/Food G15	0.35	0.6%	Wheat	0.1%	Barley	0.1%	Milk: Cattle		0.9%
	0.9%	GEMS/Food G08	0.35	0.5%	Wheat	0.1%	Barley	0.1%	Rye		0.9%
	0.9%	FR toddler 2 3 yr	0.34	0.4%	Wheat	0.4%	Milk: Cattle	0.0%	Bovine: Muscle/meat		0.9%
	0.9%	RO general	0.34	0.6%	Wheat	0.1%	Milk: Cattle	0.0%	Swine: Muscle/meat		0.9%
	0.8%	ES child	0.34	0.6%	Wheat	0.2%	Milk: Cattle	0.0%	Bovine: Muscle/meat		0.8%
	0.8%	IT toddler	0.33	0.8%	Wheat	0.0%	Barley	0.0%	Oat		0.8%
	0.8%	UK toddler	0.33	0.5%	Wheat	0.3%	Milk: Cattle	0.0%	Bovine: Muscle/meat		0.8%
	0.8%	GEMS/Food G07	0.32	0.5%	Wheat	0.1%	Milk: Cattle	0.1%	Barley		0.8%
	0.7%	GEMS/Food G10	0.30	0.5%	Wheat	0.1%	Barley	0.1%	Milk: Cattle		0.7%
	0.7%	GEMS/Food G11	0.29	0.5%	Wheat	0.1%	Milk: Cattle	0.1%	Barley		0.7%
	0.7%	SE general	0.29	0.4%	Wheat	0.2%	Milk: Cattle	0.1%	Bovine: Muscle/meat		0.7%
	0.6%	DE general	0.24	0.2%	Wheat	0.2%	Milk: Cattle	0.1%	Rye		0.6%
	0.6%	DE women 14-50 yr	0.23	0.3%	Wheat	0.2%	Milk: Cattle	0.1%	Rye		0.6%
	0.5%	IT adult	0.21	0.5%	Wheat	0.0%	Barley	0.0%	Oat		0.5%
	0.5%	PT general	0.21	0.5%	Wheat	0.0%	Rye	0.0%	Barley		0.5%
	0.5%	ES adult	0.20	0.3%	Wheat	0.1%	Milk: Cattle	0.1%	Barley		0.5%
	0.5%	NL general	0.18	0.2%	Wheat	0.1%	Milk: Cattle	0.0%	Barley		0.5%
	0.4%	IE adult	0.18	0.3%	Wheat	0.1%	Milk: Cattle	0.0%	Oat		0.4%
	0.4%	FR adult	0.16	0.3%	Wheat	0.1%	Milk: Cattle	0.0%	Swine: Muscle/meat		0.4%
	0.4%	LT adult	0.15	0.1%	Rye	0.1%	Wheat	0.0%	Milk: Cattle		0.4%
	0.3%	FR infant	0.14	0.2%	Milk: Cattle	0.1%	Wheat	0.0%	Swine: Muscle/meat		0.3%
	0.3%	DK adult	0.13	0.1%	Wheat	0.1%	Milk: Cattle	0.1%	Rye		0.3%
	0.3%	UK vegetarian	0.13	0.3%	Wheat	0.0%	Milk: Cattle	0.0%	Eggs: Chicken		0.3%
	0.3%	FI 3 yr	0.12	0.1%	Wheat	0.1%	Rye	0.1%	Oat		0.3%
	0.3%	UK adult	0.12	0.2%	Wheat	0.0%	Milk: Cattle	0.0%	Bovine: Muscle/meat		0.3%
	0.2%	FI 6 yr	0.10	0.1%	Wheat	0.1%	Rye	0.0%	Oat		0.2%
	0.2%	IE child	0.08	0.1%	Wheat	0.0%	Milk: Cattle	0.0%	Swine: Muscle/meat		0.2%
	0.1%	FI adult	0.06	0.1%	Rye	0.0%	Wheat	0.0%	Oat		0.1%
		Column7			Grapefruits		Grapefruits		Grapefruits		

Conclusion:

The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.

The long-term intake of residues of Isoflucypram is unlikely to present a public health concern.

Table 2.7.9-2: PRIMo 3 acute risk assessment for isoflucypram

Acute risk assessment /children	Acute risk assessment / adults / general population	Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults	Hide IESTI new calculations	Show IESTI new calculations

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

IESTI new calculations:
The calculation is performed with the MRL and the peeling/processing factor (PF), taking into account the residue in the edible portion and/or the conversion factor for the residue definition (CF). For case 2a, 2b and 3 calculations a variability factor of 3 is used. Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.
Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.

Show results for all crops

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.1%	Wheat	0.05 / 0.05	0.72
0.09%	Milk: Cattle	0.01 / 0.01	0.62
0.05%	Rye	0.05 / 0.05	0.32
0.04%	Barley	0.05 / 0.05	0.28
0.02%	Poultry: Muscle/meat	0.01 / 0.01	0.17
0.02%	Eggs: Chicken	0.01 / 0.01	0.12
0.02%	Swine: Muscle/meat	0.01 / 0.01	0.12
0.02%	Milk: Goat	0.01 / 0.01	0.12
0.01%	Bovine: Liver	0.01 / 0.01	0.08
0.01%	Bovine: Edible offals	0.01 / 0.01	0.07
0.01%	Bovine: Muscle/meat	0.01 / 0.01	0.07
0.01%	Other farmed animals:	0.01 / 0.01	0.07
0.01%	Equine: Muscle/meat	0.01 / 0.01	0.06
0.01%	Oat	0.05 / 0.05	0.06
0.01%	Sheep: Muscle/meat	0.01 / 0.01	0.04

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.06%	Wheat	0.05 / 0.05	0.42
0.03%	Rye	0.05 / 0.05	0.24
0.03%	Barley	0.05 / 0.05	0.24
0.03%	Milk: Cattle	0.01 / 0.01	0.19
0.02%	Poultry: Muscle	0.01 / 0.01	0.12
0.01%	Milk: Goat	0.01 / 0.01	0.09
0.01%	Poultry: Edible offals (other	0.01 / 0.01	0.08
0.01%	Milk: Sheep	0.01 / 0.01	0.08
0.01%	Bovine: Muscle	0.01 / 0.01	0.06
0.01%	Other farmed animals:	0.01 / 0.01	0.06
0.01%	Equine: Muscle/meat	0.01 / 0.01	0.05
0.01%	Poultry: Liver	0.01 / 0.01	0.05
0.01%	Swine: Muscle/meat	0.01 / 0.01	0.05
0.01%	Eggs: Chicken	0.01 / 0.01	0.04
0.01%	Sheep: Muscle/meat	0.01 / 0.01	0.04

IESTI new
Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.1%	Wheat	0.05 / 0.05	0.72

Total number of commodities found exceeding the ARfD/ADI in children and adult diets (IESTI new calculation)

IESTI new
Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.06%	Wheat	0.05 / 0.05	0.42
0.03%	Rye	0.05 / 0.05	0.24
0.03%	Barley	0.05 / 0.05	0.24
0.03%	Milk: Cattle	0.01 / 0.01	0.19
0.02%	Poultry: Muscle	0.01 / 0.01	0.12
0.01%	Milk: Goat	0.01 / 0.01	0.09
0.01%	Poultry: Edible offals (other than	0.01 / 0.01	0.08
0.01%	Milk: Sheep	0.01 / 0.01	0.08
0.01%	Bovine: Muscle	0.01 / 0.01	0.06
0.01%	Other farmed animals:	0.01 / 0.01	0.06
0.01%	Equine: Muscle/meat	0.01 / 0.01	0.05
0.01%	Poultry: Liver	0.01 / 0.01	0.05
0.01%	Swine: Muscle/meat	0.01 / 0.01	0.05
0.01%	Eggs: Chicken	0.01 / 0.01	0.04
0.01%	Sheep: Muscle/meat	0.01 / 0.01	0.04

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.1%	Wheat / milling (flour)	0.05 / 0.05	0.60
0.0%	Wheat / milling (wholemea	0.05 / 0.05	0.28
0.0%	Rye / boiled	0.05 / 0.05	0.18
0.0%	Oat / boiled	0.05 / 0.05	0.18
0.0%	Barley / cooked	0.05 / 0.05	0.18
0.0%	Rye / milling (wholemeal)-l	0.05 / 0.05	0.18
0.0%	Oat / milling (flakes)	0.05 / 0.05	0.15
0.0%	Barley / milling (flour)	0.05 / 0.05	0.09

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0.1%	Barley / beer	0.05 / 0.01	0.36
0.0%	Wheat / bread/pizza	0.05 / 0.05	0.22
0.0%	Wheat / pasta	0.05 / 0.05	0.19
0.0%	Wheat / bread	0.05 / 0.05	0.08

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity. A short term intake of residues of Isoflucypram is unlil
For processed commodities, no exceedance of the ARfD/ADI was identified.

2.7.10. Proposed MRLs and compliance with existing MRLs

Plant matrices

The proposed residue definition for setting MRL-values in plants in the EU is **isoflucypram** parent only.

MRLs are calculated and proposed based on the available residue data in support of the representative uses of **isoflucypram** in cereals. Calculations of MRLs were carried out using the OECD MRL calculator.

Barley (extrapolated to oat)

An MRL of 0.05 mg **isoflucypram**/kg is proposed for barley grain, based on all EU residue data.

An extrapolation of this MRL of 0.05 mg/kg to oat grain is also permitted by the Guidance Document SANCO 7525/VI/95 rev. 10.3.

Wheat (extrapolated to rye, spelt and triticale)

An MRL of 0.05 mg **isoflucypram**/kg is proposed for wheat grain, based on all EU residue data.

An extrapolation of this MRL of 0.05 mg/kg to rye grain, triticale grain and spelt grain is also permitted by the Guidance Document SANCO 7525/VI/95 rev. 10.3.

Other crops

Residues of **isoflucypram** parent compound in rotational crops are expected to be <0.01 mg/kg. Thus, for all other crops, an MRL of 0.01 mg/kg (at the LOQ level of the enforcement method) is proposed.

Animal matrices

The proposed residue definition for setting MRL-values in animal commodities in the EU is **isoflucypram** parent only.

Based on the dairy cow feeding study, at the feeding dose of 0.15 mg/kg bw/day, residues of **isoflucypram** parent compound were <0.005 mg/kg (LOQ) in milk and <0.01 mg/kg in muscle, liver, kidney, perirenal fat and subcutaneous fat. The highest residue level of parent was 0.01 mg/kg in mesenteric fat (in one cow out of three) at the feeding dose of 0.15 mg/kg bw/day. Considering the maximum European anticipated dietary burdens of 0.089 mg/kg bw/day for sheep (lamb) and 0.041 mg/kg bw/day for cattle, it is concluded that residues of parent compound are expected to be <LOQ in milk and ruminant tissues.

Based on the laying hen feeding study, at the feeding dose of 0.03 mg/kg bw/day, residues of **isoflucypram** parent compound were found <0.01 mg/kg (LOQ) in eggs and poultry tissues. Considering the maximum European anticipated dietary burden of 0.029 mg/kg bw/day for laying poultry, it is concluded that residues of parent compound are expected to be <LOQ in eggs and poultry tissues.

Therefore the European MRLs in animal commodities resulting from exposure of livestock to feed crops treated with **isoflucypram** are proposed to be at the limit of quantification of the enforcement method as shown in the following table.

Commodities	**Code No.**	**MRL proposal**
Tissues from swine, bovine, sheep, goat, equine, poultry and other farmed terrestrial animals	1010000	0.01*
Milk	1020000	0.005*

Birds' eggs	1030000	0.01*

* at the limit of quantification of the enforcement method

2.7.11. Proposed import tolerances and compliance with existing import tolerances

N/A

2.8. FATE AND BEHAVIOUR IN THE ENVIRONMENT

Isoflucypram is a novel broad spectrum fungicide of the chemical class of N-cyclopropyl-N-benzyl-pyrazole-carboxamides with application to cereal crops (wheat, triticale, rye, barley and oats) evaluated as part of this submission. Isoflucypram is an SDH inhibitor fungicide the application scope of isoflucypram-containing products on cereals with only one foliar spray at a maximum of 75 g a.s./ha at BBCH 39-69.

Throughout the development of isoflucypram the following synonyms may have been used and also referred to in individual study reports: Bayer Code: BCS-CN88460, BCS-CN88460-a.s., ´460 and the Bayer-internal short Code: ISY. All chemical substances described by either of these codes refer to the same chemical name and structural formula. For the evaluation the Bayer common name Isoflucypram and code BCS-CN88460 are used. For the metabolites codes M12 are used for BCS-CN88460-carboxylic acid, M10 for BCS-CN88460-lactic acid and M11 *for* BCS-CN88460-desmethyl-carboxylic acid are used unless both name and code are presented.

The studies concerning the fate and behaviour of isoflucypram in the environment were conducted using two different radiolabel positions, [chlorophenyl-UL-^{14}C] and [pyrazole-4-^{14}C], as well as unlabelled isoflucypram. These radiolabel positions are sufficient to define the route of degradation of isoflucypram. Proposed pathways of degradation are given in figure B.2.8-1 for soil and figure B.2.8-2 for aerobic water.

Figure B.2.8-1: Proposed degradation pathway of isoflucypram in soil

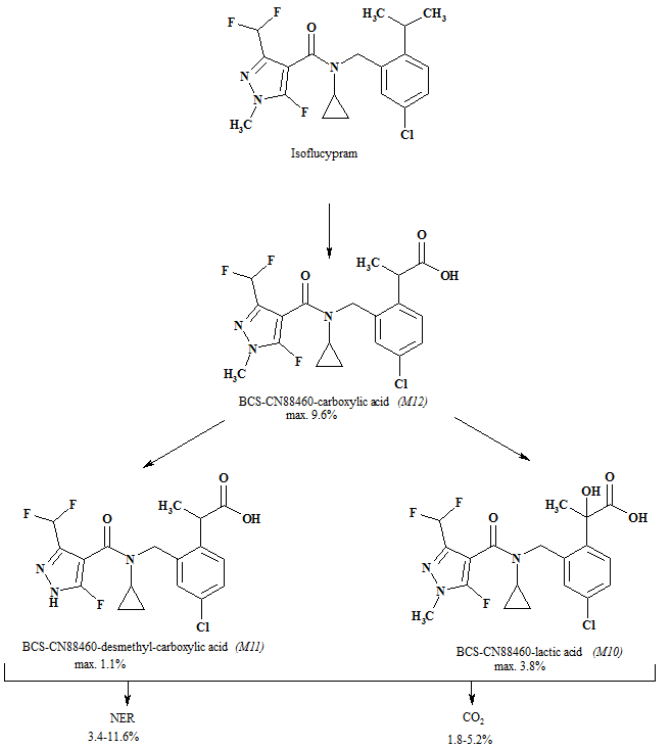
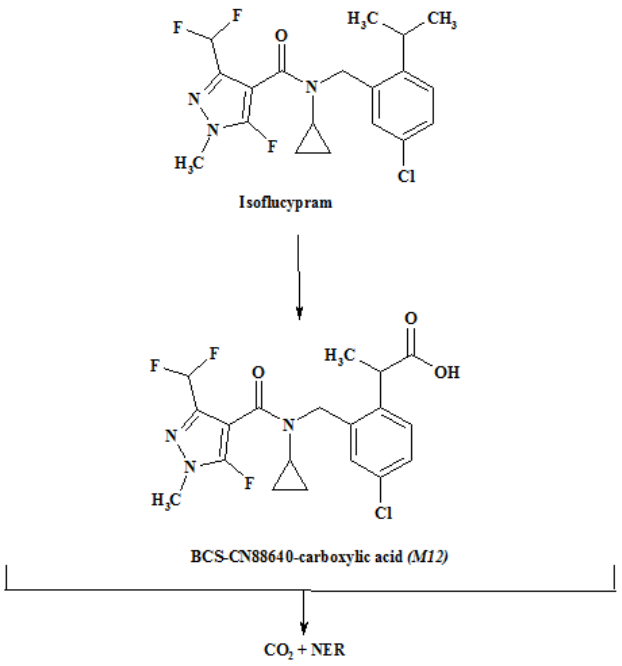


Figure B.2.8-2: Proposed degradation pathway of isoflucypram in water/sediment systems.



2.8.1. Summary of fate and behaviour in soil

Degradation in soil of isoflucypram

In soil metabolism studies under aerobic conditions, the metabolites were formed possibly via carboxylation of isoflucypram to result in M12 as major metabolite, hydroxylation of M12 to result in M10 and demethylation of M12 to result in M11 (see Figure B.2.8-1). No cleavage of the molecule was observed.
An addition aerobic soil metabolism study with the phenyl-label was also performed using one soil. In this study M12 was also found as major metabolite. In this study no split of the molecule could be observed.

From the studies on the route of degradation in soil it can be concluded that isoflucypram was slowly degraded in soil under aerobic conditions to the final degradation product carbon dioxide. Parallel to mineralisation, bound residues were formed. A total of three metabolites were identified in the soil extracts along with the parent compound and carbon dioxide. Two of the metabolites (M10 (BCS-CN88460-lactic acid) and M11 (BCS-CN88460-desmethyl-carboxylic acid)) were found only in amounts < 5% of the applied radioactivity (AR). The highest concentrations were found for the major metabolite M12, with a maximum of 9.6% AR (123 DAT). Carbon dioxide was formed at a maximum of 5.2% (125 DAT). Under anaerobic conditions no degradation products > 5% were found. Maximum non extracted residues values of 11.6% AR were seen at 104 DAT. Photodegradation does not play a role in the overall fate of isoflucypram.

A Normalised field geomean DT50 of 324.3 days has been calculated by the RMS using the K2 (slow phase) DT_{50} values for isoflucypram following EFSA (2014) guidance for use in PEC sw and PECgw calculations. For PECsoil and persistence calculations a worst case non-normalised field DT_{50} of 177 days has been calculated.

A combined laboratory and field geomean DT_{50} of 105.5 days has been calculated by the RMS for M12 with an arithmetic mean formation fraction of 0.192. A summary of maximum occurrences of the major metabolite M12, CO_2 and non-extractable residues in soil is given in Table B.2.8.1-1.

Table B.2.8.1-1: Summary of maximum occurrences of the major metabolite M12, carbon dioxide and non-extractable residues in soil (in percent of applied radioactivity)

Compound	Soil metabolism, aerobic [%]	Field	Soil metabolism, anaerobic [%]	Soil photolysis [%]
M12	9.6	4.3	-	-
Carbon dioxide	5.2	-	0.2	0.2
Non-extractable residues	11.6	-	4.2	1.2

The degradation of isoflucypram and its major metabolites under aerobic conditions has been addressed in both laboratory and field conditions. Non-normalised and normalised assessments were performed on all studies, three laboratory and 1 field dissipation. A summary of the results is presented in table B.2.8.1-2.

Table B.2.8.1-2: Summary of DT50 values of isoflucypram and the major metabolite M12 .

Compound	Soil metabolism, aerobic non normalised worst case field [%]	Soil metabolism, aerobic normalised, field Geomean [%]	Soil photolysis [%]	Soil metabolism anaerobic	Formation fraction
Isoflucypram	177 (DFOP)*	324.3 ^	1000	1000	-
M12	714 (DFOP-SFO)	105.5~	-	-	0.192

*K1=0.078 (88.9 days DT_{50}), K2=0.0007 (1020 DT_{50}) g =0.610.
^ field values only following EFSA (2014 guidance)
~ Combined laboratory and field data

Adsorption and desorption of isoflucypram and its metabolite M12

The soil adsorption properties of isoflucypram have been assessed. Five soils in total were considered to be acceptable by the RMS. A summary table of the values accepted by the RMS is included below see table B.2.8.1-3. Koc alues ranged from 1151.2 mL/g to 1569.1 mL/g with a geomean value of 1346.6 ml/g. 1/n values ranged from 0.869 to 0.9985 with an arethmetric mean value of 0.907.

The adsorption properties of metabolite M-12 have been assessed in 9 soils and were considered to be acceptable by the RMS. A summary of the values determined are listed in table B.8.2.8.1-4. Koc values ranged from 28.1 mL/g to 289.8 mL/g with a geomean 75.35 mL/g. . 1/n values ranged from 0.891 to 0.960 with an arethmetric mean of 0.922. Some pH dependence of the metabolite M12 was noted and this is explored further below

pH dependence of M12

The adsorption of M12 to soil was proposed by the applicant to be pH dependent following an S shaped curve which is typical for many ionic substances with a single ionisable functional group (Figure B.8.1.2.2.5- 1). A sigmoid correlation analysis of pH and K_{foc} values predicted with the German spreadsheet 'Input Decision Tool version 3.3' resulted in a K_{foc} for M12 of 37.7 mL/g considering an "apparent" pKa of 5.6 in soil. Please note that the "apparent" pKa in soil needs to be estimated as the pKa in soil is increased compared to the pKa determined in water as sorption processes are reducing the water available substance amount. UK RMS has validated this result using the same model and reaches the same conclusions, noting the lack of values for soil pH values above 8. Whilst the German tool is not agreed for use in EU-level approval assessments, it was considered to give useful assessment of the adsorption data. However, parameters calculated by the tool have not been proposed for use in risk assessment. pH in Cacl2 is converted into pH in H_2O by the model.

The geometric mean K_{foc} value for soils at pH 7.5 is 37.1 mL/g, with an arithmetic mean Freundlich exponent of 0.9424 (Table B.2.8.1- 6). The geometric mean K_{foc} value for weak acidic soils with a pH of about 5.4 is 153.2 mL/g, with an arithmetic mean Freundlich exponent of 0.9169 (Table B.2.8.1- 5).

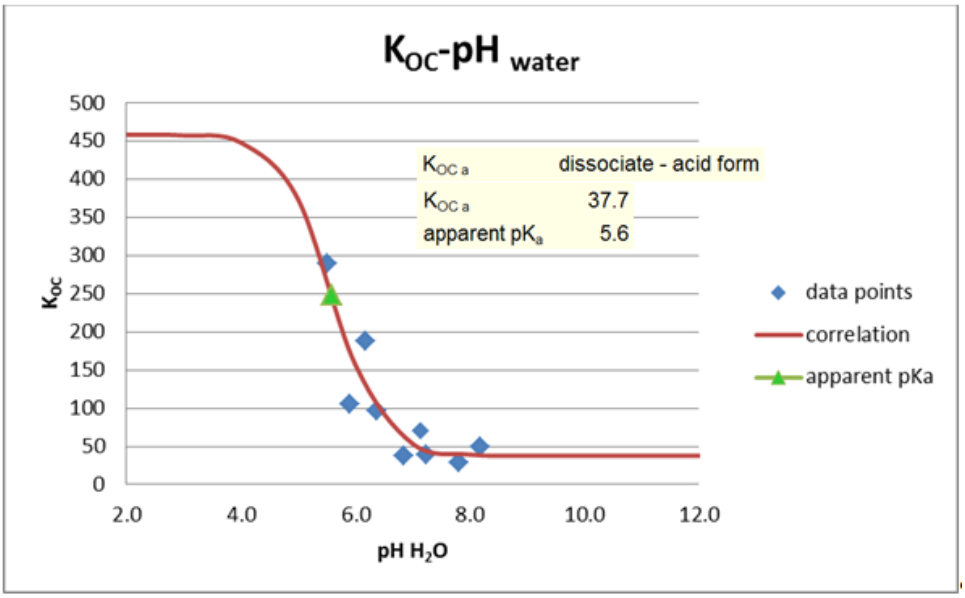
EFSA (2013) and FOCUS groundwater (2014) recommend that Tier 1 leaching simulations for consideration of EU approval should select adsorption values, chosen to represent a realistic worst case considering the pH of the soils in the EU that are used for the production of the pertinent crop, which in this case is cereals. For a compound with a single ionisable functional group that follows a typical S shaped relationship for adsorption with pH, such as a weak acid, two contrasting pH values for which realistic best case and realistic worst case adsorption estimates may be selected to consider the impact of variable soil pH values relevant for the crop growing situation (*e.g.* pH of 7.5 and pH of 5.4 as relevant range for cereal growing conditions; with an optimum pH of about 6.5 for cereals).

Thus, the adsorption of M12 can be described

- by the geometric mean K_{foc} value of 37.1 mL/g and the arithmetic mean Freundlich exponent 1/n of 0.9424 to represent a realistic worst case (neutral soil conditions around pH 7.5);
- by the geometric mean K_{foc} value of 153.2 mL/g and the arithmetic mean Freundlich exponent 1/n of 0.9169 to represent a realistic best case (weak acidic soil conditions around pH 5.4).

For the leaching assessment and for the $PEC_{sw/sed}$ assessment, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.

Figure B.2.8.1- 1: K_{oc}-pH_{water} correlation for M12 evaluated with Input Decision Tool version 3.3



For the leaching calculations, the adsorption of isoflucypram was described by the geometric mean K_{foc} value of 1346.6 mL/g (K_{fom} = 781.1 mL/g) and the arithmetic mean Freundlich exponent 1/n of 0.907. For the environmental exposure assessment of M12, only the realistic worst case (K_{foc} = 37.1 mL/g, 1/n = 0.9424) was taken into account.

No lysimeter or column leaching studies were submitted by the applicant as the OECD 106 sorption studies have addressed the data requirement.

The transpiration stream concentration factor of isoflucypram and the metabolite M12 has been assessed by a calculation method and in two laboratory assessments. In the desk study the log Pow was used in the Briggs equation (Briggs et al (1982)) to calculate the TSCF value of 0.10 for isoflucypram. The log Pow for M12 was determined to be pH dependant, therefore the default value of 0 was selected. In line with FOCUS (2014) guidance an experimentally determined PUF/ TSCF value was attempted. No E.U. guidance was available a the time of study conduct and evaluation and the studies were assessed on merit. UK RMS notes some potential deficiencies with the studies and proposes that neither study is acceptable for regulatory purposes. However the RMS notes that the experimentally derived TSCF value for isoflucypram of 0.14 and the value determined using the Briggs equation of 0.1 were similar. For Predicted Environmental Concentration calculations a TSCF value of 0.1 is agreed by the RMS for isoflucypram and 0 for the metabolite M12.

Summary tables

Table B.2.8.1-3: Summary of the adsorption values for isoflucypram.

Soil	**$K_{f(ads)}$ [mL/g]**	**1/n**	**$K_{oc(ads)}$ [mL/g]**	**r^2**
Laacher Hof AXXa	29.184	0.8904	1389.7	0.9963
Hoefchen am Hohenseh 4a	29.812	0.8788	1569.1	0.9979
Hanscheider Hof	32.430	0.8972	1410.0	0.9976
Dollendorf II	58.711	0.8690	1151.2	0.9976
Sanger	11.257	0.9985	1250.8	0.9891
Arith. mean	**32.28**	**0.907**	**1354.2**	**0.996**
Geo. mean			**1346.6**	

Table B.2.8.1-4: Summary of the adsorption values for M-12, all results without pH dependence considered.

Soil	**pH (Cacl2)**	**$K_{f(ads)}$ [mL/g]**	**1/n**	**$K_{oc(ads)}$ [mL/g]**	**r^2**
Wurmwiese	5.3	2.0	0.9297	105.8	0.9983
Hoefchen am Hohenseh 4a	6.3	0.8	0.8952	37.9	0.9978
Dollendorf II	7.3	1.3	0.9243	28.1	0.9988
Guadelupe	6.7	0.3	0.9311	38.4	0.9973
Springfield	6.6	1.2	0.9185	70.7	0.9983
END	4.9	2.724	0.9497	289.8	0.9989
MMN	7.7	1.178	0.9604	49.1	0.9992
SCA	5.6	0.544	0.8966	187.5	0.9997
SKS	5.8	1.727	0.8914	95.9	0.9995
Arith. mean		**1.31**	**0.922**	**100.36**	**0.999**
Geo. mean				**75.35***	

* Potential pH dependence is show

Table B.2.8.1-5: Soil adsorption/desorption for M12 at weak acidic soil conditions (around pH 5.4)

Soil name	Soil type	OC [%]	pH ($CaCl_2$)	K_f [mL/g]	K_{foc} [mL/g]	1/n
Wurmwiese, GER	sandy loam	1.9	5.3	2.0094	105.8	0.9297
Northwood, North Dakota, USA	loamy sand	0.94	4.9	2.724	289.8	0.9497
Sanger, California, USA	sandy loam	0.29	5.6	0.544	187.5	0.8966
Stilwell, Kansas, USA	silty clay loam	1.80	5.8	1.727	95.9	0.8914
Geometric mean (n=4)					**153.2**	
Arithmetic mean (n=4)						**0.9169**
pH-dependency					**yes – pH 5.4**	

Table B.2.8.1-6: Soil adsorption/desorption for M12 at neutral soil conditions (around pH 7.5)

Soil name	Soil type	OC [%]	pH ($CaCl_2$)	K_f [mL/g]	K_{foc} [mL/g]	1/n
Dollendorf II, GER	loam	4.5	7.3	1.2635	28.1	0.9243
Morris, Minnesota, USA	clay loam	2.40	7.7	1.178	49.1	0.9604
Geometric mean (n=2)					**37.1**	
Arithmetic mean (n=2)						**0.9424**
pH-dependency					**yes – pH 7.5**	

2.8.2. Summary of fate and behaviour in water and sediment

Isoflucypram is hydrolytically stable in sterile aqueous buffer solutions at three pH values (pH 4, 7 and 9) in the laboratory in the dark. No degradation products of isoflucypram were observed.

Hydrolytic degradation is unlikely to contribute to the degradation of isoflucypram under typical conditions of the environment.

Photodegradation is unlikely to contribute to the degradation of isoflucypram under typical light conditions of the environment. 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.

In surface water under aerobic conditions, isoflucypram does not degrade. Isoflucypram dissipated rapidly from the water in water/sediment systems under aerobic conditions. One degradation product of isoflucypram was identified: M12 with a maximum occurrence in the total system of 6.6% AR (water layer 5.4%; sediment 1.3%, respectively) at the end of the study (100 days). Formation of carbon dioxide accounted to ≤ 0.3% AR in both water/sediment systems. Non-extractable residues accounted for a maximum of 6.4% AR in both water/sediment systems. The proposed metabolic pathway of isoflucypram in the aerobic water/sediment systems is shown in Figure B.8.-2.

The dissipation rates in water have been assessed as 12.5 days and 26.9 days (DT_{50} geomean) of 18.33 days, dissipation rates are included for Member States wishing to rely on those values. The DT_{50} for the whole system degradation (SFO) of 354 days was determined.

Table B.2.8.2- 1: Degradation and dissipation in water / sediment systems: <u>trigger</u> endpoints of isoflucypram, Level P-I

Water / sediment system	Whole system			Water			Sediment		
	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation
Anglersee	222	736	SFO	2.79	74.2	FOMC recalc.	222	736	SFO
Wiehltalsperre	681	>1000	SFO	2.06	39.6	FOMC recalc.	n.r.	n.r.	-
Geometric mean	**388**			**2.40**			**222**		

Table B.8.2.8.2- 2: Degradation and dissipation in water / sediment systems: <u>modelling</u> endpoints of isoflucypram, Level P-I

Water / sediment system	Whole system			Water			Sediment		
	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation
Anglersee	222	736	SFO	1000	1000	Default.	222	736	SFO
Wiehltalsperre	681	> 1000	SFO	1000	1000	Default	681	> 1000	SFO
Geometric mean	**388**			**1000**			**388**		

Table B.8.2.8.2- 3: Degradation and dissipation in water / sediment systems: <u>modelling</u> endpoints and trigger of M12, Level P-I

Water / sediment system	Whole system			Water			Sediment		
	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation	DT_{50} [days]	DT_{90} [days]	Method of calculation
Anglersee*	1000	1000	default	1000	1000	Default.	1000	1000	SFO
Wiehltalsperre	1000	1000	default	1000	1000	Default	1000	1000	SFO
Geometric mean	1000			**1000**			1000		

* Formation fraction of 0.240 was calculated.

2.8.3. Summary of fate and behaviour in air

Based on the overall hydroxyl radical reaction rate constant in combination with the "long term" concentration of these radicals in the atmosphere (*i.e.*24 h day, 0.5 x 10^6 OH radicals/cm^3, 12 h day, 1.5 x 10^6 OH radicals/cm^3) the half-life ($t_{1/2}$) of isoflucypram in air is derived to:

- Half-life ($t_{1/2}$) = 0.344 days (24 h day)
- Half-life ($t_{1/2}$) = 0.229 days (12 h day)

That estimate should be regarded as worst-case assumption as the approach does not consider the contribution of any other reactive species to the overall atmospheric degradation of isoflucypram in air.

2.8.4. Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Not proposed, to be decided at the conclusion of the E.U. peer review procedure.

2.8.5. Definition of the residues in the environment requiring further assessment

Compartment	Residue definition for risk assessment
Soil	Isoflucypram and BCS-CN88460-carboxylic acid *(M12) (M10)*?*
Groundwater	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)* ***(M10)*?***
Surface water	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Sediment	Isoflucypram and BCS-CN88460-carboxylic acid *(M12)*
Air	Isoflucypram

* less than 5% AR but increasing very slightly at the last timepoint.

2.8.6. Summary of exposure calculations and product assessment

PECsoil, cereals, 1 application of 75g/ha

For the calculation of PECsoil biphasic endpoints were agreed in the CA document of this assessment. PECsoil modelling was conducted for the worst case GAP (early applications to both winter and spring sown cereals). The model ESCAPE v2 was used by the UK RMS to calculated PECsoil values as kinetic fits were biphasic. The fit endpoint values from the worst case DT50 in soil were used to calculate the maximum PEC in soil for a single year and the fit endpoint values from the soil with the worst case DT90 were used to calculate the worst case soil accumulation as a conservative assessment. Results for both Isoflucypram and its metabolite M12 are presented below in tables 2.8.6-2 and 2.8.6-4. . All assessments were made assuming a 5 cm incorporation depth for annual applications; this is to reflect an assessment of potential accumulation in soil under minimum/shallow tillage practice.

Table 2.8.6-1: Worst case GAP assessed for PECsoil.

Individual Crop	FOCUS crop used for Interception	Application				Amount reaching the soil per application [g a.s./ha]	Application Depth (cm)	Soil density kg/L
		Rate per Season [g a.s./ha]	Interval [days]	Plant Interception [%]	BBCH Stage			
Cereals	Cereals	1 × 75	-	80	30 - 69	1 × 15	5*	1.5

Table 2.8.6-2: PECsoil values isoflucypram, early applications to cereals.

Time (Days)	PECactual (mg/kg)	PECtwa (mg/kg)
PECmax	0.0200	-
1	0.0199	0.0200
2	0.0199	0.0199
4	0.0198	0.0199
7	0.0196	0.0198

14	0.0193	0.0197
21	0.0190	0.0195
28	0.0186	0.0193
42	0.0180	0.0190
50	0.0177	0.0188
100	0.0158	0.0177
Accumulation *	0.0616 after 21 years, steady state 0.0416	-

* Calculation performed with the worst case DT90 value parameters.

Table 2.8.6-3 : PECSsoil values M12, early applications to cereals.

Time (Days)	PECactual (mg/kg)	PECtwa (mg/kg)
PECmax	<0.0001	-
1	<0.0001	<0.0001
2	<0.0001	<0.0001
4	<0.0001	<0.0001
7	<0.0001	<0.0001
14	<0.0001	<0.0001
21	<0.0001	<0.0001
28	<0.0001	<0.0001
42	<0.0001	<0.0001
50	<0.0001	<0.0001
100	<0.0001	<0.0001
Accumulation *	0.0001 after 14 years, steady state <0.001	-

* Calculation performed with the worst case DT90 value parameters.

PECgroundwater, winter and spring cereals, 1 application of 75 g/ha either early or late season.

PECgw simulations were performed to address the GAP being winter or spring sown cereals for either early (BBCH 30-39) or late (BBCH40-69) applications. Crop interception was manually calculated using the agreed crop interception values from the EFSA DEGT50 guidance (2014). Details of the GAP assessed are presented in table 2.8.6-4.

Table 2.8.6-4

Individual Crop	**FOCUS crop used for Interception**	**Application**				**Amount reaching the soil per application [g a.s./ha]**	**Application Depth (cm)**
		Rate per Season [g a.s./ha]	**Interval [days]**	**Plant Interception [%]**	**BBCH Stage**		
Cereals, early	Cereals	1 × 75	-	80	30 - 39	1 × 15	0
Cereals, late	Cereals	1 × 75	-	90	40 - 69	1 × 7.5	0

The E.U. agreed models PEARL, PELMO and MACRO were used for the calculation of PECgw, the RMS took a 2 tier approach. At the first tier, two separate simulations were performed for each timing option (early cereals or late cereals), one using the fast phase of DFOP as the degradation rate for the parent and one using the slow phase of DFOP for the parent. Both simulations used a TSCF value of 0 for the parent. At the second tier, the TSCF value for the parent was changed to 0.1 following a calculation of TSCF using the Briggs equation. To assess the viability of this method as a refinement, an initial calculation was performed on the worst case results from the tier 1 assessment. PEC results are presented in the CP document in table B.8.3.1-9, but when compared to the results from table B.8.3.1-2 in the CP document, the addition of the PUF refinement for parent does not significantly refine

the metabolite PECgw values. Further simulations were therefore not performed. Metabolite M12 was noted to be pH dependant for sorption only, worst case Koc and 1/n values were proposed by the applicant to address all soils. UK RMS agreed to this approach in order to derive a conservative assessment for the concentration of M12 in groundwater.

It is noted by UKRMS that metabolite M10 does not formally trigger groundwater assessment according to E.U. guidance on assessment of metabolites in groundwater. However it is noted that EFSA have stated that identified metabolites increasing at the end of a soil route of degradation study but at less than 5% A.R. must be addressed for potential relevance if they were predicted to occur in shallow groundwater at greater than 0.1µg/L. With a maximum formation of 3.9% of A.R. M10 was increasing at the termination of the study and may need further consideration as noted above. No endpoints are available to perform groundwater exposure modelling and UKRMS suggests this be discussed at E.U. peer review as to whether a request for further information to the applicant, such that a groundwater assessment can be performed, is justified.

PECgroundwater assessments using either DFOP slow phase or fast phase DT50 for the active substance demonstrate that all FOCUS scenarios predict 80th percentile annual average concentrations <0.001 µg/l at all scenarios for the active substance at the first tier. Metabolite M12 was predicted to have concentrations >0.1 µg/l at a majority of simulated scenarios. A full implementation of DFOP kinetics for isoflucypram would not change the regulatory outcome for metabolite M12 as it would not have changed the number of scenarios where M12 would be predicted to occur at <0.1 µg/l. A higher tier assessment using the calculated TSCF value for the parent was attempted. This did not reduce the PEC values of M12 significantly compared to tier 1, therefore the first tier assessment is relied upon. At the first tier some scenarios predicted concentrations of M12 <0.1 µg/l, and these are detailed in table B..2.8.6-5.

Table 2.8.6-5: Summary table of FOCUSgw assessment.

Crop	timing	Scenarios passing	Maximum concentration Isoflucypram µg/L	Maximum concentration M12 µg/L passing	Maximum concentration M12 µg/L
Winter cereals	Early	Sevilla	<0.001	0.037	0.392
	Late	Porto, Sevilla	<0.001	0.093	0.189
Spring cereals	Early	-	<0.001	-	0.462
	Late	Porto	<0.001	0.090	0.232

PECsurface water, winter and spring cereals, 1 application of 75 g/ha either early or late season.

UK RMS remodelled the PECsw and PECsed assessment for the applicant's proposed application and scenarios as detailed in table 2.8.6-6. Steps 1-4 were required for isoflucypram and steps 1-3 were required for metabolite M12.

Table 2.8.6-6: GAP modelled at steps 1 to 4 for the surface water and sediment assessment

Crop	BBCH stage	Rate [g a.s./ha]	Interval [days]	FOCUS crop (crop group)	Season	Crop cover
Cereals	30-69	1 × 75	-	Winter cereals	Spring (Mar. - May)	Average crop cover
		1 × 75		Winter cereals	Summer (Jun. – Sep.)	Full canopy
		1 × 75		Spring cereals	Spring (Mar. - May)	Average crop cover
		1 × 75		Spring cereals	Summer (Jun. – Sep.)	Full canopy

Full result tables are available in the CP document for steps 1 and 2, at step 3 2 sets of calculations were performed according to FOCUS Degradation Kinetics v 1.1, chapter 10 (on pages 193-194 and FOCUSsw generic guidance v 1.4, page 212 - 213 (particularly the footnote on p 213)), *at Step 3, substances with Koc values between 100 and 2000 ml/g should be modelled with the water/sediment whole system DT50 value in water and default value of 1000 days in sediment in one simulation and with the default value of 1000 days in water and the water sediment whole system DT50 in sediment in a separate simulation*. UK RMS performed a Step 3 assesment for the parent and metabolite according to this guidance, full results are available in the CP. A limited number of scenartio failed at step 3 for the active substance only, the maximum PECsw for M12 was 0.4823µg/L (D2 winter cereals early applications). The worst case result from this approach was taken to step 4 as a conservative assessment of the PECsw of isoflucypram.

Step 4 calculations were performed for the scenarios where the PEC at step 3 exceeds the proposed RAC of 0.948µg/L. Exceedance is noted in only the D1 scenario for early applications in winter and spring cereals and D1 and D2 for winter cereals. However entry is driven by drainage as the key route of entry. Current E.U. agreed models do not have any methods of mitigation to reduce entry via drainage. Step 4 calculations have been provided for a 20m spray drift buffer zone to demonstrate that any potential spray drift buffer will not provide sufficient mitigation. Calculations were performed for the worst case results predicted at step 3 using a DT50 in water of 388days and DT50 in sediment of 1000 days. Step 4 values are presented in table B.8.5.2-19.

The RAC is sediment was confirmed by eco-toxicology to be 10,000 µg/kg. Therefore assessment using the highest and lowest Koc for M12 was not considered to be necessary to assess the pH dependant nature. The use of the higher Koc value is unlikley to produce PECsed values that would fail the assessment (maxium PECsed 17.70 µg/kg). The use of the worst case value will be conservative with regards to the surface water assessment. Time weighted averages were not supportd by the applicant so were not listed by the RMS so as to present a more concise evaluation.

Table B.8.5.2-19: Step 4 values for all scenarios for a 20m spray drift buffer zone for isoflucypram.

Scenario **FOCUS**	**Application scenario**	**Waterbody**	**Max PEC_{sw} (µg/L)**	**Max PEC_{sed} (µg/kg)**
Step 3		Winter cereals early applications DT_{50} water 1000 days, DT_{50} sediment 388 days		
D1	Winter cereals early	Ditch	1.388	17.70
D2	Winter cereals early	Ditch	1.369	12.66
D1	Spring cereals early	Ditch	1.181	17.05

PEC AIR

The transport via air of isoflucypram was not studied since its vapour pressure is less than the FOCUS air trigger value for short-range transport exposure assessment of 10^{-5} Pa for substances applied to plants.

There are no other routes of exposure if the product is used according to good agricultural practice. Therefore no further estimations are considered necessary.

2.9. EFFECTS ON NON-TARGET SPECIES

2.9.1. Summary of effects on birds and other terrestrial vertebrates

Birds

- **Acute oral toxicity data –** Two studies were submitted to address this data point. While both studies were considered valid, the endpoint from the test on the species bobwhite quail was used for the risk assessment as this is the standard bird species for European registration. As such, the LD_{50} > 2000 mg a.s./kg bw. **As there were no mortalities at the limit dose of 2000 mg a.s./kg bw the endpoint has been extrapolated to 3776 mg a.s./kg bw.**
- **Short-term toxicity data –** Studies are available having been submitted. However, under Regulation (EC) 1107/2009 these data are not required and are not used in the relevant risk assessment. Study summaries provided by the applicant are available in Section B.9 (AS).

  Long-term toxicity – One mallard duck and two bobwhite quail long-term effects studies were performed. The bobwhite quail study ([redacted] 2017) was not considered reliable to derive an endpoint, therefore another bobwhite quail study was conducted [redacted], 2018). However, the endpoint from the mallard duck study was more critical, therefore this will be used to inform the long-term risk assessment for birds (**NOEL 60 mg a.s./kg bw/d**).

- Plant metabolites have been identified and a risk assessment was conducted considering the risk from the ecotoxicologically-relevant metabolites identified from the use of 'isoflucypram EC 50' (M21 and combined risk assessment from the active substance and M21 in conjunction). An assumption of 10 times parental toxicity has been assumed for the metabolite M21 in lieu of specific data.

Mammals

Toxicity data have been provided and considered within the human health assessment (see Section B.6 (CA) for details of the underlying studies). Endpoints for use in the mammalian risk assessment have been established for acute and long-term toxicity. The following endpoints have been used to perform the risk assessment:

- **Acute toxicity of the active substance –** The toxicity estimate used to address the toxicity of the active substance in the risk assessment is **LD_{50} > 2000 mg a.s./kg b.w.**

- **Long-term toxicity to the active substance –** The toxicity estimate used to address the toxicity of the active substance in the risk assessment is **NOAEL: 9.68 mg a.s./kg bw/day.** Discussion about how this endpoint was chosen is found in Section B.9.1.2. (PPP: 'isoflucypram EC 50')
- Note that due to evidence suggesting that metabolite M21 cleaves to M01 in the stomach of mammals and that M01 is covered by the risk from the parent isoflucypram (due to structural similarieties, see B.6_CA), the metabolite risk assessment has been removed from the mammal section of this assessment.

2.9.2. Summary of effects on aquatic organisms

Toxicity data to address the risk from isoflucypram, the represnettaive formulation and the relevant metabolites have been provided. The tier 1 and tier 2a toxicity data used in the risk assessments are summarised here in table B2.9.2-1. For full details of all the available toxicity data see the list of endpoints and Section B.9 (CA). Formulation toxicity data has also been submitted and evaluated in the relevant CP document.

Table B2.9.2-1: Tier 1 and tier 2a toxicity data relevant to the active substance isoflucypram, its metabolite and representative formulation isoflycypram EC 50

Test system/species	Test substance	Endpoint		Reference
Acute toxicity to fish				
Static, 96 hour *Pimephales promelas*	Isoflucypram	96 h LC_{50}	0.0861 mg a.s./L (nom)	[redacted] 2018; M-542897-02-1 KCA 8.2.1/01
Static, 96 hour *Oncorhynchus mykiss*	Isoflucypram	96 h LC_{50}	0.098 mg a.s./L (gmm)	[redacted] 2015; EBLNN024 KCA 8.2.1/02
Static, 96 hour *Cyprinodon variegatus*	Isoflucypram	96 h LC_{50}	0.544 mg a.s./L (mm)	[redacted] 2015; EBLNN023; KCA 8.2.1/03
Static, 96 hour *Oncorhynchus mykiss*	Isoflucypram EC50	96 h LC_{50}	1.29 mg/L (nom) (**~0.068 mg a.s./L**)[A]	[redacted] 2017; M-595274-01-1 KCP 10.2.1/01
Fish, acute *Oncorhynchus mykiss, Pimephales promelas, Cyprinodon variegatus*	Isoflucypram	96 h LC_{50}	**0.156 mg a.s./L**[B]	Geometric mean (EFSA Journal 2013;11(7):3290)
Static, 96 hour *Oncorhynchus mykiss*	BCS-CN88460-carboxylic acid (M12)	96 h LC_{50}	**> 33.5 mg p.m./L** (gmm)	[redacted] 2017; M-587655-01-1 KCA 8.2.1/04
Long-term toxicity to fish				
Flow-through, ELS *Pimephales promelas*	Isoflucypram	33 d NOEC (larval survival)	**0.01328 mg a.s./L** (mm)	[redacted] 2017; M-580247-01-1 KCA 8.2.2.1/01
Bioconcentration in fish				
BCF study flow through *Lepomis macrochirus*	Isoflucypram	BCF	**370** (kinetic BCF lipid normalized and growth corrected)	[redacted] 2017; M-610008-01-1 KCA 8.2.2.3/01
Acute toxicity to aquatic invertebrates				
Static, 48 hour *Daphnia magna*	Isoflucypram	48 h EC_{50}	0.201 mg a.s./L (gmm)	Kuhl, K.; 2016; M-574184-01-1 KCA 8.2.4.1/01
Static renewal, 96 hour *Americamysis bahia*	Isoflucypram	96 h EC_{50}	0.27 mg a.s./L (mm)	Brougher, D. S.; Siddiqui, A. I.; Gallagher, S. P.; 2016; 149A-257B; KCA 8.2.4.2/01;
Static, 48 hour *Daphnia magna*	Isoflucypram EC50	48 h EC_{50}	2.22 mg/L (nom) (**~0.117 mg a.s./L**)[A]	Kuhl, K.; 2017; M-607779-01-1 KCP 10.2.1/02
Invertebrate, acute *Daphnia magna Americamysis bahia*	Isoflucypram	EC_{50}	**0.203 mg a.s./L**[B]	Geometric mean (EFSA Journal 2013; 11 (7) 2013;11(7):3290)
Static, 48 hour *Daphnia magna*	BCS-CN88460-carboxylic acid (M12)	48 h EC_{50}	**> 24 mg p.m./L** (nom)	Riebschlaeger, T; 2016; M-573296-01-1 KCA 8.2.4.1/02
Long-term toxicity to invertebrates				

Test system/species	Test substance	Endpoint		Reference
Flow-through, 28 days *Americamysis bahia*	Isoflucypram	28 d NOEC (14 – 28 day adult mortality)	**0.020 mg a.s./L (mm)**	Milligan, A. L.; Siddiqui, A. I.; Gallagher, S. P.; Krueger, H. O.; 2016; 149A-256; KCA 8.2.5.2/01
Toxicity to sediment dwelling invertebrates				
Static renewal, 61 days *Chironomus dilutus*	Isoflucypram	61 d NOEC	**100 mg a.s./kg sediment (nom)**	Bradley, M. J.; 2017; M-596883-01-1 KCA 8.2.5.4/01
Toxicity to algae				
Static, 96 hour *Pseudokirchneriella subcapitata*	Isoflucypram EC50	72h E_rC_{50}	3.39 mg/L (nom) (**~0.179 mg a.s./L**)[A]	Kuhl, K.; 2017; M-600970-01-1 KCP 10.2.1/03
Static, 96 hour *Pseudokirchneriella subcapitata*	BCS-CN88460-carboxylic acid (M12)	72h-E_rC_{50}	> **35.1 mg p.m./L** (gmm)	Kuhl, K.; 2017; M-587659-01-1 KCA 8.2.6.1/02
Toxicity to aquatic macrophytes				
Semi-static, 7 days *Lemna gibba*	Isoflucypram	7d-E_rC_{50}	> **2.48 mg a.s./L** (gmm)	Kuhl, K.; 2017; M-593965-01-1 KCA 8.2.7/01

Bold: endpoints used in risk assessment

Nom = nominal concentrations, mm = mean measured concentration, gmm = geometric mean measured concentration

[A] Endpoints in the study report were reported based on the formulation only. For this table the endpoint is converted to mg a.s./L based on the reported content of isoflucypram of 5.28%.

[B] Endpoint based on geometric mean of the given relevant endpoints of acute studies with the active substance and the formulation. Detailed information given under‘ Selection of endpoints for Tier 2 risk assessments’ in section 9.4 of Volume 3 – B.9 (PPP).

Metabolite endpoints

The risk from the metabolite BCS-CN88460-carboxylic acid (M12) is considered below following the EFSA AGD (2013) stepwise approach:

- A complete acute experimental data set is available for the metabolite BCS-CN88460-carboxylic acid (M12); see table 2.9.2-1 above.
- Based on the acute data (> 10 times less toxic on a molar basis than the parent) it can be concluded that BCS-CN88460-carboxylic acid (M12) has lost its toxophore.
- Due to its limited formation in aquatic systems, no reliable degradation half-lives for BCS-CN88460-carboxylic acid can be derived. As a conservative approach it is assumed that the trigger for chronic risk assessment (DT_{90} > 1d) is met for BCS-CN88460-carboxylic acid (M12)

According to the AGD stepwise approach, the parent chronic endpoints can be used in the metabolite risk assessment as surrogate values for all Tier 1 taxonomic groups. Thus the chronic risk assessment for the metabolite BCS-CN88460-carboxylic acid (M12) is based on parent endpoints (see section 9.4 of Volume 3 – B.9 (PPP) for further details).

2.9.3. Summary of effects on arthropods

2.9.3.1. Bees

Tier 1 data

The following first tier studies for the active substance and the representative formulation were considered valid and acceptable for consideration in the risk assessment by the RMS:

Table B2.9.3-1: Toxicity of isoflucypram (technical and formulated products) to bees

Test substance	Test species/ study type	Endpoint		References
Isoflucypram tech.	Honeybee, 48 h	LD_{50} – oral LD_{50} – contact	> 106.3 µg a.s./bee > 100 µg a.s./bee	Schmitzer, S.; 2014; M-503824-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Bumble bee, 48 h	LD_{50} – oral	> 200.2 µg a.s./bumble bee	Taenzler, V.; 2015; M-542774-01-1 KCA 8.3.1.1.1/03
	Bumble bee, 48 h	LD_{50} – contact	> 100 µg a.s./bumble bee	Haupt, S.; 2015; M-509048-01-1 KCA 8.3.1.1.2/03
Isoflucypram EC 50	Honeybee, 72 h 96 h	LD_{50} – oral LD_{50} - contact	69.1 µg a.s./bee 14.1 µg a.s./bee	Ehmke, A.; 2016; M-571280-01-1 KCP 10.3.1.1.1/01
Isoflucypram SC 200	Honeybee, 10 day chronic adult feeding study	LDD_{50} NOEDD	> 89.7 µg a.s./bee/day 89.7 µg a.s./bee/day (equivalent to 3333 mg a.s./kg diet)	Gossmann, A.; 2015; M-540173-01-1 KCA 8.3.1.2/01

Higher tier data

Four semi-field tunnel tests using 'Isoflucypram EC50' were submitted for the registration of isoflucypram. The test item was sprayed onto flowering *Phacelia tanacetifolia* (BBCH 65) at a rate equivalent to the GAP (1 x 75 g a.s./ha). *Apis mellifera* were exposed for a total of 7 days and then monitored at a separate site for a minimum of 21 days.

All of the semi-field studies investigated the effects of the test item on honeybee mortality, foraging activity, colony condition, brood development and behaviour. Two of the studies also measured residue levels in pollen and nectar on the day of and day after application of the test item. Some transient effects on flight activity and behaviour were noted in two of the studies shortly after application of the test item. However no adverse effects on mortality, colony condition or brood development were noted.

2.9.3.2. Non-target arthropods other than bees

The following first tier studies were accepted by the RMS for the representative formulation 'isoflucypram EC 50':

Table B2.9.3.2-1:'isoflucypram': Ecotoxicological endpoints for arthropods other than bees: Tier 1 studies.

Test species, Reference	**Tested Formulation, study type, exposure**	**Ecotoxicological Endpoint**
Aphidius rhopalosiphi Waibel, J.; (2017)	Isoflucypram EC 50 Laboratory, glass plates 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75.0 g a.s./ha	LR_{50} 14.13 g a.s./ha Corr. Mortality [%] 10.2 32.2 100.0 100.0 100.0
Typhlodromus pyri Waibel, J.; (2017)	Isoflucypram EC 50 Laboratory, glass plates 7.5 g a.s./ha 13.3 g a.s./ha 23.7 g a.s./ha 42.2 g a.s./ha 75.0 g a.s./ha	LR_{50} 30.6 g a.s./ha Corr. Mortality [%] 2.3 -6.9[A] 27.6 80.5 96.6

The following Tier II studies were accepted by the RMS for the representative formulation 'isoflucypram EC 50':

Table B2.9.3.2-2: 'isoflucypram EC 50': Ecotoxicological endpoints for arthropods other than bees: Tier II studies

Test species, Reference	**Tested Formulation, study type, exposure**	**Ecotoxicological Endpoint**
Aphidius rhopalosiphi Waibel, J.; 2017	Isoflucypram EC 50 Extended Lab., exposure on barley seedlings	LR_{50} > 75 g a.s./ha; ER_{50} > 7.5 g a.s./ha Corr.Mortality [%] Effect on Reproduction [%] Wasps on plants [%]
	7.5 g a.s./ha	0 45.8 34.7
	13.3 g a.s./ha	0 53.8 21.5
	23.7 g a.s./ha	6.7 77.0 19.3
	42.2 g a.s./ha	6.7 62.8 25.2
	75 g a.s./ha	3.3 60.6 18.2
Typhlodromus pyri Waibel, J.; (2017);	Isoflucypram EC 50 Extended Lab., exposure on bean leaves	LR_{50} > 75 g a.s./ha; ER_{50} > 42.2 g a.s./ha Corr. Mortality [%] Effect on Reproduction [%]
	7.5 g a.s./ha	6.7 44.4
	13.3 g a.s./ha	9.0 31.3
	23.7 g a.s./ha	4.5 19.2
	42.2 g a.s./ha	19.9 21.2
	75 g a.s./ha	10.1 64.0
Chrysoperla carnea Waibel, J.; (2017)	Isoflucypram EC 50 Extended Lab., exposure on detached bean leaves	LR_{50} > 75 g a.s./ha; ER_{50} > 75.0 g a.s./ha Corr. Mortality [%] Effect on Reproduction [%]
	7.5 g a.s./ha	-5.3 6.2
	13.3 g a.s./ha	13.2 1.4
	23.7 g a.s./ha	0.0 -3.1
	42.2 g a.s./ha	13.2 1.2
	75 g a.s./ha	10.5 -2.5
Coccinella septempunctata Müller, R. U. (2017)	Isoflucypram EC 50 Extended Lab., exposure on detached bean leaves	LR_{50} > 75 g a.s./ha; ER_{50} > 75.0 g a.s./ha Corr. Mortality [%] Effect on Reproduction [%]
	7.5 g a.s./ha	-5.3 19.0
	13.3 g a.s./ha	-2.2 12.0
	23.7 g a.s./ha	-11..8 30.0
	42.2 g a.s./ha	2.9 31.0
	75 g a.s./ha	-2.9 24.0

Higher tier data

The following aged residue studies were submitted that the RMS considered acceptable for deriving an endpoint:

Table B2.9.3.2-3:'isoflucypram EC 50': Ecotoxicological endpoints for arthropods other than bees: Aged residue studies.

Aged residue studies		
Test species, Reference	**Tested Formulation, study type, exposure**	**Study details Ecotoxicological Endpoint**

Aphidius rhopalosiphi Jans (2017)	Isoflucypram EC 50 aged residues spray deposits on maize plants, 1 appl. of 75 g a.s./ha, residues aged for 0 d: residues aged for 14 d:	LR_{50} > 75 g a.s./ha; ER_{50} > 75.0 g a.s./ha Corr. Mortality [%] / Effect on Reproduction [%] / Wasps on plants [%] 3.3 / 44.7 / 32.8 sign. 0.0 / 13.8 / 46.7 n. sign.

2.9.4. Summary of effects on non-target soil meso- and macrofauna

The potential effects on non-target soil meso- and macrofauna were tested for the active substance, representative formulation and soil-relevant metabolites.

Earthworms

The endpoints used in tier 1 risk assessment for earthworms are presented below:

Table B2.9.4-1: Endpoints used in risk assessment for earthworms

Test item	**Test species, test design**	**Ecotoxicological endpoint**		**Reference**
Isoflucypram EC 50	*Eisenia fetida* reproduction 56 d, mixed	NOEC	560 mg formulation/ kg d.w.soil 29 mg a.s./ kg d.w.soil#	Frommholz, U.; 2016; M-574897-01-1 KCP 10.4.1.1/01
		$NOEC_{corr}$*	**280 mg formulation/ kg d.w.soil** **14.5 mg a.s./ kg d.w.soil#**	
Isoflucypram	*Eisenia fetida* reproduction 56 d, mixed	NOEC	56 mg a.s./ kg d.w.soil	Frommholz, U.; 2016; M-548749-01-1 KCA 8.4.1/01
		$NOEC_{corr}$*	**28 mg a.s./ kg d.w.soil**	
BCS-CN88460-carboxylic acid (M12)	*Eisenia fetida* reproduction 56 d, mixed	NOEC	100 mg p.m./kg dws	Frommholz, U.; 2017; M-579263-01-1 KCA 8.4.1/02
		$NOEC_{corr}$*	**50 mg p.m./kg dws**	

dws = dry weight soil; a.s. = active substance; p.m. = pure metabolite,
*Endpoint corrected due to lipophilic substance (log P_{OW} > 2)
Endpoint calculated on the basis of analysed isoflucypram content in the formulation (5.18% w/w; as given in study report)
Endpoints in **Bold** used in risk assessment

Other soil macro-organisms

The endpoints used in the tier 1 risk assessment for other soil-macro-organisms are presented below:

Table B2.9.4-2: Endpoints used in risk assessment

Test substance	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Isoflucypram EC 50	*Folsomia candida* reproduction 28 d, mixed	NOEC	100 mg prod./kg dws 5.18 mg a.s./kg dws#	Larnaudie Lopez, M. I.; 2017; M-591834-01-1 KCP 10.4.2.1/01
		$NOEC_{corr}$*	**50 mg prod./kg dws** **2.59 mg a.s./kg dws#**	
		EC_{10}	98 mg prod./kg dws 5.08 mg a.s./kg dws#	
		EC_{10corr}*	49 mg prod./kg dws 2.54 mg a.s./kg dws#	
		EC_{20}	127 mg prod./kg dws 6.58 mg a.s./kg dws#	
		EC_{20corr}*	63.5 mg prod./kg dws 3.29 mg a.s./kg dws#	
Isoflucypram	*Folsomia candida* reproduction 28 d, mixed	NOEC	99 mg a.s./kg dws	Frommholz, U.; 2015; M-522863-01-1 KCA 8.4.2.1/01
		$NOEC_{corr}$*	**49.5 mg a.s./kg dws***	
BCS-CN88460-carboxylic acid (M12)	*Folsomia candida* reproduction 28 d, mixed	NOEC	18 mg p.m./kg dws	Friedrich, S.; 2017; M-587760-01-1 KCA 8.4.2.1/02
		$NOEC_{corr}$*	**9 mg p.m./kg dws**	
		EC_{10}	13 mg p.m./kg dws	
		EC_{10corr}*	6.5 mg p.m./kg dws	
		EC_{20}	20 mg p.m./kg dws	
		EC_{20corr}*	10 mg p.m./kg dws	
Soil mites, reproduction				
Isoflucypram EC 50	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	316 mg prod./kg dws 16.37 mg a.s./kg dws#	Larnaudie Lopez, M. I.; 2017; M-592571-01-1 KCP 10.4.2.1/02

		**$NOEC_{corr}$* **	**158 mg prod./kg dws** **8.18 mg a.s./kg dws#**	
		EC_{10}	362 mg prod./kg dws 18.75 mg a.s./kg dws#	
		EC_{10corr}*	181 mg prod./kg dws 9.38 mg a.s./kg dws#	
		EC_{20}	422 mg prod./kg dws 21.86 mg a.s./kg dws#	
		EC_{20corr}*	211 mg prod./kg dws 10.93 mg a.s./kg dws#	
Isoflucypram	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC **$NOEC_{corr}$***	990 mg a.s./kg dws 495 mg a.s./kg dws	Larnaudie-Lopez, M.; 2015; M-528194-01-1 KCA 8.4.2.1/03
BCS-CN88460-carboxylic acid (M12)	*Hypoaspis aculeifer* reproduction 14-d, mixed	NOEC **$NOEC_{corr}$***	990 mg a.s./kg dws 495 mg a.s./kg dws	Larnaudie-Lopez, M. I.; 2015; M-524464-01-1 KCA 8.4.2.1/04

dws = dry weight soil, a.s. = active substance, p.m. = pure metabolite

* Endpoint corrected due to lipophilic substance (log P_{OW} > 2)

Endpoint calculated on the basis of analysed isoflucypram content in the formulation (5.18% w/w; as given in study report)

2.9.5. Summary of effects on soil nitrogen transformation

Effects on nitrogen transformation in soil were tested for the active substance, representative formulation and relevant metabolites. Endpoints for use in the risk assessment are summarised in Table 2.9.5-1.

Table B2.9.5-1: Endpoints used in risk assessment

Test substance	Test species, test design	Ecotoxicological endpoint	Reference
N-transformation			
Isoflucypram EC 50	Study duration, 28 days	no unacceptable effects at a rate of 9.74 mg prod./kg (7.5 L prod./ha) equivalent to 0.5 mg a.s./kg soil (375 g a.s./ha)	Schulz, L.; 2016; M-574633-02-1 KCP 10.5/01
Isoflucypram	Study duration, 28 days	no unacceptable effects at an application rate of 0.53 mg a.s./kg soil (375 g a.s./ha)	Schulz, L.; 2015; M-532055-01-1 KCA 8.5/01
BCS-CN88460-carboxylic acid (M12)	Study duration, 28 days	no unacceptable effects at an application rate of 0.54 mg p.m./kg soil (403 g p.m./ha)	Schulz, L.; 2015; M-538059-01-1 KCA 8.5/02

a.s. = active substance; p.m. = pure metabolite

2.9.6. Summary of effects on terrestrial non-target higher plants

The endpoints for vegetative vigour and seedling emergence studies following exposure to 'Isoflucypram EC 50' at the GAP rate of 75 g a.s./ha are summarised in B.2.9.6-1:

Table B.9.2.6-1: Effect values relevant for the risk assessment for non-target terrestrial plants for the product Isoflucypram EC 50

Test organism	Study type	Max. effects	Most sensitive species	References
Maximum application rate: 75 g a.s./ha (equivalent to 1.5 L product/ha)				
Terrestrial non-target plants; 10 species	Vegetative vigour; Tier 2 dose response 21 days	No effects ≥ 50 % at a rate of 75 g a.s./ha	Corn (*Zea mays*)	Koehler, P.; 2017; M-589028-01-1 KCP 10.6.2/01
Maximum application rate: 75 g a.s./ha (equivalent to 1.5 L product/ha)				
Terrestrial non-target plants; 10 species	Seedling emergence; Tier 1 single dose 21 days	No effects ≥ 50 % at a rate of 75 g a.s./ha	Onion (*Allium cepa*)	Koehler, P.; 2017; M-596298-01-1 KCP 10.6.2/02
Terrestrial non-target plants; 4 species	Seedling emergence; Tier 2 dose response 21 days	No effects ≥ 50 % at a rate of 75 g a.s./ha	Soy bean (*Glycine max*)	Köhler, P.; 2017; M-607264-01-1 KCP 10.6.2/03

2.9.7. Summary of effects on other terrestrial organisms (flora and fauna)

No data submitted.

2.9.8. Summary of effects on biological methods for sewage treatment

No studies were submitted with the formulation; only tests conducted with the active substance are considered necessary to indicate the potential risk to biological sewage treatment systems.

A study was submitted which measured the respiration rate of activated sludge exposed to Isoflucypram (active substance).

The study summary is in section B.9.8 of Volume 3 – B.9 (AS). The following table (Table B.2.9.8)-1 lists the respiration rate endpoint for activated sludge exposed to Isoflucypram (as a.s) for use in the risk assessment.

Table B.2.9.8-1 : Endpoint for activated sludge exposed to Isoflucypram (as a.s.)

Test item	Test system	Endpoint (µg a.s/L)		Reference
Isoflucypram	Activated sludge respiration rate	EC50 based on respiration rate	1 000 000	Neuhalm (2018)

2.9.9. Summary of product exposure and risk assessment

2.9.9.1. Risk assessment for birds

Acute risk assessment

The acute dietary screening risk assessment was conducted with the indicator species "small omnivorous bird" on cereal crops at a rate of 0.075 kg a.s./ha. The extrapolated acute endpoint of 3776 mg a.s./kg bw was compared to a daily dietary dose (DDD) of 11.91 mg a.s./kg bw/d to give a TER of 317 which is greater than the trigger value of 10. **Therefore, an acceptable acute risk to birds was demonstrated.**

Long-term/reproductive risk

The long-term dietary screening risk assessment was conducted with the indicator species "small omnivorous bird" on cereal crops at a rate of 0.075 kg a.s./ha. The long-term endpoint of 60 mg a.s./kg bw was compared to a daily dietary dose (DDD) of 2.58 mg a.s./kg bw/d to give a TER of 23.3 which is greater than the trigger value of 5. **Therefore, an acceptable long-term risk to birds was demonstrated**.

Risk assessment for birds drinking contaminated water

No calculations of exposure and TER are necessary for the risk assessment for birds drinking contaminated water, when the ratio of the application rate (in g/ha) to the relevant endpoint (in mg a.s./kg bw/day) does not exceed 3000 when the Koc of the test item is > 500 L/kg. The acute ratio is 0.02 and the long term ratio is 1.25 **therefore no further consideration is required** (the Koc of isoflucypram is 1346.6 L/kg).

Risk assessment for secondary poisoning

Isoflucypram, has a $logP_{OW}$ of 4, indicating that further consideration of the risk from secondary poisoning and biomagnification is required.

The risk assessment for earthworm-eating birds via secondary poisoning used peak $PEC_{soil\ accumulation}$ values to calculate a daily dietary dose. The TER was 208 (trigger value of 5) which **indicates an acceptable risk to earthworm-eating birds.**

The risk assessment for fish-eating birds via secondary poisoning used peak PEC_{sw} values to calculate a daily dietary dose. The TER was 105 (trigger value of 5) which **indicates an acceptable risk to fish-eating birds.**

Metabolites of isoflucypram

BCS-CN88460-propanol-Glyc-MA (M21) was identified as a potentially-relevant plant metabolite in Section B.7.2 as it occurred at >10% total radioactive residue (TRR). The risk from M21 is not considered to be covered by the active substance risk assessment because it was not identified in bird studies used in the active substance risk assessment. Therefore, the risk of M21 to birds was assessed.

Note that due to evidence suggesting that metabolite M21 cleaves to M01 in the stomach of mammals and that M01 is covered by the risk from the parent isoflucypram (due to structural similarieties, see B.6_CA), the metabolite risk assessment has been removed from the mammal section of this assessment. However, due to uncertainties about extrapolation of this theory to birds, the M21 risk assessment has been retained for birds, with the conservative assumption of 10x toxicity of the parent isoflucypram.

The application rate of the residue study conducted in hay is 2 x 65 g a.s./ha; whereas the application rate of the GAP for 'Isoflucypram' is 1 x 75 g a.s./ha. Therefore, the proposed GAP for the parent (0.075 kg a.s./ha) was multiplied by the maximum formation fraction of the metabolite (0.103) to calculate an effective applied rate of metabolite residue of 0.0077 kg/ha. This rate was used in a Tier 1 risk assessment to calculate a daily dietary dose (DDD), which is then used to calculate a TER. As there is no toxicity data on this metabolite, 10 x the toxicity of the parent active substance is assumed.

As the active substance was present at 50% at the same time as M21 in the hay residue study, a combined risk assessment is also provided. The rate of the active substance that was present in combination with the metabolite is calculated by multiplying the GAP rate in the test (0.075 kg a.s./ha) by the fraction that was present at the time of the peak metabolite formation (0.5) to calculate a residue of 0.0375 kg a.s./ha. This is then used to calculate the DDD for the active substance.

When the acute and long term risk assessment for M21 was conducted separately, the TER values exceeded the relevant triggers and acceptable risks were demonstrated. When the combined risk to both M21 and the active substance was assessed, the acute and long-term TER values exceeded the relevant triggers. Therefore, **the acute and long-term risk from M21 and isoflucypram separately and in combination is acceptable to birds.**

2.9.9.2. Risk assessment for mammals

Acute risk assessment

The acute dietary screening risk assessment was conducted with the indicator species "small herbivorous mammal" on cereal crops at a rate of 0.075 kg a.s./ha. The acute endpoint of >2000 mg a.s./kg bw was compared to a daily dietary dose (DDD) of 8.88 mg a.s./kg bw/d to give a TER of 225 which is greater than the trigger value of 10. **Therefore, an acceptable acute risk to mammals was demonstrated.**

Long-term/reproductive risk

The long-term dietary screening risk assessment was conducted with the indicator species "small herbivorous mammal" on cereal crops at a rate of 0.075 kg a.s./ha. The long-term endpoint of 9.68 mg a.s./kg bw was compared to a daily dietary dose (DDD) of 1.92 mg a.s./kg bw/d to give a TER of 5.0 which is greater than the trigger value of 5.

As the TER value calculated was above the relevant trigger value of 5, **the long-term/reproductive risk to mammals is resolved at the screening stage. Therefore, no further consideration of the risk is required.**

Risk assessment for birds drinking contaminated water

No calculations of exposure and TER are necessary for the risk assessment for mammals drinking contaminated water, when the ratio of the application rate (in g/ha) to the relevant endpoint (in mg a.s./kg bw/day) does not exceed 3000 when the Koc of the test item is > 500 L/kg. The acute ratio is 0.038 and the long term ratio is 7.7 **therefore no further consideration is required** (the Koc of isoflucypram is 1346.6 L/kg).

Risk assessment for secondary poisoning

Isoflucypram, has a $logP_{OW}$ of 4, indicating that further consideration of the risk from secondary poisoning and biomagnification is required.

The risk assessment for earthworm-eating mammals via secondary poisoning used peak $PEC_{soil\ accumulation}$ values to calculate a daily dietary dose. The TER was 27.5 (trigger value of 5) which **indicates an acceptable risk to earthworm-eating mammals.**

The risk assessment for fish-eating mammals via secondary poisoning used peak PEC_{sw} values to calculate a daily dietary dose. The TER was 19.1 (trigger value of 5) which **indicates an acceptable risk to fish-eating mammals.**

Metabolites of isoflucypram

Note that due to evidence suggesting that metabolite M21 cleaves to M01 in the stomach of mammals and that M01 is covered by the risk from the parent isoflucypram (due to structural similarieties, see B.6_CA), the metabolite risk assessment has been removed from the mammal section of this assessment.

2.9.9.3. Risk assessment for aquatic organisms

The results of the risk assessments for the representative formulation are summarised here. Risk assessments were conducted according to the guidance document EFSA (2013).

Tier 1 aquatic risk assessment for isoflucypram

Table B2.9.9.3-1 shows the FOCUS Step 3 assessment for surface water and sediment for the proposed uses of 'Isoflucypram EC50' on Spring and Winter cereals, considering both early (March – May) and late applications (June – September) at BBCH 30 – 69 at an application rate of 1 x 75 g a.s./ha.

Table B2.9.9.3-1 : Tier 1 aquatic risk assesment FOCUS Step 3 risk assessment for the proposed uses of 'Isoflucypram EC50'

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates-chronic	Algae	Higher plant	PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*		*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0		10000 µg a.s./kg sediment)
Winter cereals BBCH 30 – 69 – early application (March – May) at 1 x 75 g a.s./ha									
FOCUS Step 3		PEC/RAC ratio							PEC/RAC ratio
D1 ditch	1.387	**2.04**	**1.04**	**1.19**	0.69	-[a]	-[a]		-[a]
D1 stream	0.869	**1.28**	0.65	0.74	0.43	-[a]	-[a]		-[a]
D2 ditch	1.369	**2.01**	**1.03**	**1.17**	0.68	-[a]	-[a]		-[a]
D2 stream	0.8551	**1.26**	0.64	0.73	0.43	-[a]	-[a]		-[a]
D3 ditch	0.4745	0.70	0.36	0.41	0.24	-[a]	-[a]		-[a]
D4 pond	0.1005	0.15	0.08	0.09	0.05	-[a]	-[a]		-[a]
D4 stream	0.3652	0.54	0.28	0.31	0.18	-[a]	-[a]		-[a]
D5 pond	0.1182	0.17	0.09	0.10	0.06	-[a]	-[a]		-[a]
D5 stream	0.3798	0.56	0.29	0.32	0.19	-[a]	-[a]		-[a]
D6 ditch	0.7443	1.09	0.56	0.64	0.37	-[a]	-[a]		-[a]
R1 pond	0.0425	0.06	0.03	0.04	0.02	-[a]	-[a]		-[a]
R1 stream	0.3124	0.46	0.24	0.27	0.16	-[a]	-[a]		-[a]
R3 stream	0.4415	0.65	0.33	0.38	0.22	-[a]	-[a]		-[a]
R4 stream	0.4364	0.64	0.33	0.37	0.32	-[a]	-[a]		-[a]
Winter cereals BBCH 30 – 69 – late application (June - September) at 1 x 75 g a.s./ha									

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates-chronic	Algae	Higher plant	PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*		*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0		10000 µg a.s./kg sediment)
FOCUS Step		PEC/RAC ratio							
D1 ditch	0.6537	0.96	0.49	0.56	0.33	-[a]	-[a]		-[a]
D1 stream	0.4216	0.62	0.32	0.36	0.21	-[a]	-[a]		-[a]
D2 ditch	0.8686	**1.28**	0.65	0.74	0.43	-[a]	-[a]		-[a]
D2 stream	0.5859	0.86	0.44	0.50	0.29	-[a]	-[a]		-[a]
D3 ditch	0.4755	0.70	0.36	0.41	0.24	-[a]	-[a]		-[a]
D4 pond	0.0529	0.08	0.04	0.05	0.03	-[a]	-[a]		-[a]
D4 stream	0.4103	0.60	0.31	0.35	0.21	-[a]	-[a]		-[a]
D5 pond	0.0600	0.09	0.05	0.05	0.03	-[a]	-[a]		-[a]
D5 stream	0.4426	0.65	0.33	0.38	0.22	-[a]	-[a]		-[a]
D6 ditch	0.4768	0.70	0.36	0.41	0.24	-[a]	-[a]		-[a]
R1 pond	0.0531	0.08	0.04	0.05	0.03	-[a]	-[a]		-[a]
R1 stream	0.3133	0.46	0.24	0.27	0.16	-[a]	-[a]		-[a]
R3 stream	0.4415	0.65	0.33	0.38	0.22	-[a]	-[a]		-[a]
R4 stream	0.3921	0.58	0.30	0.34	0.29	-[a]	-[a]		-[a]
Spring cereals BBCH 30 – 69 – early application (March – May) at 1 x 75 g a.s./ha									

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates-chronic	Algae	Higher plant	PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*		*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		0.68	1.328	1.17	2.0	17.9	248.0		10000 µg a.s./kg sediment)
FOCUS Step 3									
D1 ditch	1.181	**1.74**	0.89	**1.01**	0.59	-[a]	-[a]		-[a]
D1 stream	0.7401	**1.09**	0.56	0.63	0.37	-[a]	-[a]		-[a]
D3 ditch	0.4746	0.70	0.36	0.41	0.24	-[a]	-[a]		-[a]
D4 pond	0.1224	0.18	0.09	0.10	0.06	-[a]	-[a]		-[a]
D4 stream	0.3880	0.57	0.29	0.33	0.19	-[a]	-[a]		-[a]
D5 pond	0.1113	0.16	0.08	0.10	0.06	-[a]	-[a]		-[a]
D5 stream	0.3992	0.59	0.30	0.34	0.20	-[a]	-[a]		-[a]
R4 stream	0.4179	0.61	0.31	0.36	0.21	-[a]	-[a]		-[a]
Spring cereals BBCH 30 – 69 – Late application (June – September) at 1 x 75 g a.s./ha									
FOCUS Step 3									
D1 ditch	0.6891	**1.01**	0.52	0.59	0.34	-[a]	-[a]		-[a]
D1 stream	0.4226	0.62	0.32	0.36	0.21	-[a]	-[a]		-[a]
D3 ditch	0.4750	0.70	0.36	0.41	0.24	-[a]	-[a]		-[a]
D4 pond	0.0699	0.10	0.05	0.06	0.03	-[a]	-[a]		-[a]
D4 stream	0.4090	0.60	0.31	0.35	0.20	-[a]	-[a]		-[a]
D5 pond	0.0635	0.09	0.05	0.05	0.03	-[a]	-[a]		-[a]
D5 stream	0.4142	0.61	0.31	0.35	0.21	-[a]	-[a]		-[a]
R4 stream	0.4361	0.64	0.33	0.37	0.22	-[a]	-[a]		-[a]

[a] Acceptable risk demonstrated at FOCUS Step 1/2
Values in **bold** are > 1 and therefore high risk

Conclusion at Tier 1: For Winter cereals (early application) there is an unresolved acute and chronic risk to fish and an unresolved acute risk to aquatic invertebrates at FOCUS Step 3; for late application in Winter cereals there is an unresolved acute risk to fish at FOCUS Step 3.

For Spring cereals (early application) there is an unresolved acute risk to fish and an unresolved acute risk to aquatic invertebrates at FOCUS Step 3; for late application in Spring cereals there is an unresolved acute risk to fish at FOCUS Step 3.

Therefore refinement of the endpoints following a geometric mean approach has been considered below at tier 2.

Tier 2 aquatic risk assessment for isoflucypram

As the acute risk to aquatic invertebrates and the acute and chronic risk to fish was not resolved at FOCUS Step 3, refinement of the endpoints considering a tier 2a geomean approach has been conducted.

For the acute risk to fish and aquatic invertebrates it was possible to refine the tier 1 endpoints based on further valid studies submitted that exceeded the data requirements at tier 1. For the chronic risk to fish, although two fish early life stage studies were submitted, the studies were not considered comparable and as such it was not possible to refine the tier 1 chronic fish endpoint. See section 9.4 of the Volume 3 CP PPP dossier for further details.

The FOCUS step 3 assessment considering the tier 2 endpoints is presented below in table B2.9.9.3-2.

Table B2.9.9.3-2 : Tier 2 aquatic risk assesment FOCUS Step 3 risk assessment for the proposed uses of 'Isoflucypram EC50'

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})
		1.56	1.328	2.03
Winter cereals BBCH 30 – 69 – early application (March – May) at 1 x 75 g a.s./ha				
FOCUS Step 3		PEC/RAC ratio		
D1 ditch	1.387	0.89	**1.04**	0.68
D1 stream	0.869	0.56	0.65	0.43
D2 ditch	1.369	0.88	**1.03**	0.67
D2 stream	0.8551	0.55	0.64	0.42
D3 ditch	0.4745	0.30	0.36	0.23
D4 pond	0.1005	0.06	0.08	0.05
D4 stream	0.3652	0.23	0.28	0.18
D5 pond	0.1182	0.08	0.09	0.06
D5 stream	0.3798	0.24	0.29	0.19
D6 ditch	0.7443	0.48	0.56	0.37
R1 pond	0.0425	0.03	0.03	0.02
R1 stream	0.3124	0.20	0.24	0.15
R3 stream	0.4415	0.28	0.33	0.22
R4 stream	0.4364	0.28	0.33	0.21
Winter cereals BBCH 30 – 69 – late application (June - September) at 1 x 75 g a.s./ha				

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})
		1.56	1.328	2.03
FOCUS Step 3			PEC/RAC ratio	
D1 ditch	0.6537	0.42	-[a]	-[a]
D1 stream	0.4216	0.27	-[a]	-[a]
D2 ditch	0.8686	0.56	-[a]	-[a]
D2 stream	0.5859	0.38	-[a]	-[a]
D3 ditch	0.4755	0.30	-[a]	-[a]
D4 pond	0.0529	0.03	-[a]	-[a]
D4 stream	0.4103	0.26	-[a]	-[a]
D5 pond	0.06	0.04	-[a]	-[a]
D5 stream	0.4426	0.28	-[a]	-[a]
D6 ditch	0.4768	0.31	-[a]	-[a]
R1 pond	0.0531	0.03	-[a]	-[a]
R1 stream	0.3133	0.20	-[a]	-[a]
R3 stream	0.4415	0.28	-[a]	-[a]
R4 stream	0.3921	0.25	-[a]	-[a]
Spring cereals BBCH 30 – 69 – early application (March – May) at 1 x 75 g a.s./ha				
FOCUS Step 3			PEC/RAC ratio	
D1 ditch	1.181	0.76	[a]	0.49
D1 stream	0.7401	0.47	[a]	0.31
D3 ditch	0.4746	0.30	[a]	0.23
D4 pond	0.1224	0.08	[a]	0.04
D4 stream	0.388	0.25	[a]	0.20
D5 pond	0.1113	0.07	[a]	0.04
D5 stream	0.3992	0.26	[a]	0.20
R4 stream	0.4179	0.27	[a]	0.19
Spring cereals BBCH 30 – 69 – late application (June - September) at 1 x 75 g a.s./ha				
FOCUS Step 3			PEC/RAC ratio	
D1 ditch	0.6891	0.44	-[a]	-[a]
D1 stream	0.4226	0.27	-[a]	-[a]
D3 ditch	0.475	0.30	-[a]	-[a]
D4 pond	0.0699	0.04	-[a]	-[a]
D4 stream	0.409	0.26	-[a]	-[a]
D5 pond	0.0635	0.04	-[a]	-[a]
D5 stream	0.4142	0.27	-[a]	-[a]
R4 stream	0.4361	0.28	-[a]	-[a]

Values in **bold** are > 1 and therefore high risk

[a] Acceptable risk demonstrated at FOCUS Step 3

Overall conclusion for isoflucypram: For early application of isoflucypram in Winter cereals, there is an unresolved chronic risk to fish for the D1 and D2 ditch scenarios; for late application in Winter cereals the acute risk to fish is resolved at FOCUS Step 3 following consideration of a tier 2a geometric mean.

For early application of isoflucypram in Spring cereals, the acute risk to fish and aquatic invertebrates is resolved at FOCUS Step 3 following consideration of a tier 2a geometric mean. The acute risk to fish from late application of isoflucypram in Spring cereals is also resolved following consideration of a tier 2a geomean.

As the main entry route for the D1 and D2 scenarios is driven by drainage, it is not possible to mitigate the risk under current EU models. **As such, member states may wish to further consider the risk to these aquatic organism groups at national registration.**

Metabolites of isoflucypram

Tier 1 aquatic risk assessment for BCS-CN88460-carboxylic acid (M12)

Table B2.9.9.3-3 shows the FOCUS Step 3 assessment for surface water and sediment for the proposed uses of 'Isoflucypram EC50' on Spring and Winter cereals, considering the worst-case application (early application in March – May) at an application rate of 1 x 75 g a.s./ha.

Table B2.9.9.3-3 : Tier 1 aquatic risk assesment FOCUS Step 3 risk assessment for the proposed uses of 'Isoflucypram EC50'

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates-chronic	Algae	Higher plant	PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*		*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		335	1.328	240	2.0[1]	3510	248.0[1]		10000[1] µg a.s./kg sediment)
Winter cereals BBCH 30 – 69 – early application (March – May) at 1 x 75 g a.s./ha									
FOCUS Step 3		PEC/RAC ratio							PEC/RAC ratio
D1 ditch	0.4044	-	0.30	-[a]	-[b]	-[a]	-[a]		-[a]
D1 stream	0.2584	-	0.19	-[a]	-[b]	-[a]	-[a]		-[a]
D2 ditch	0.487	-	0.37	-[a]	-[b]	-[a]	-[a]		-[a]
D2 stream	0.3164	-	0.24	-[a]	-[b]	-[a]	-[a]		-[a]
D3 ditch	0.1650	-	0.12	-[a]	-[b]	-[a]	-[a]		-[a]
D4 pond	0.4367	-	0.33	-[a]	-[b]	-[a]	-[a]		-[a]
D4 stream	0.2194	-	0.17	-[a]	-[b]	-[a]	-[a]		-[a]
D5 pond	0.3007	-	0.23	-[a]	-[b]	-[a]	-[a]		-[a]
D5 stream	0.1334	-	0.10	-[a]	-[b]	-[a]	-[a]		-[a]
D6 ditch	0.1417	-	0.11	-[a]	-[b]	-[a]	-[a]		-[a]
R1 pond	0.0187	-	0.01	-[a]	-[b]	-[a]	-[a]		-[a]
R1 stream	0.0081	-	0.01	-[a]	-[b]	-[a]	-[a]		-[a]
R3 stream	0.03184	-	0.02	-[a]	-[b]	-[a]	-[a]		-[a]
R4 stream	0.0159	-	0.01	-[a]	-[b]	-[a]	-[a]		-[a]
Spring cereals BBCH 30 – 69 – early application (March – May) at 1 x 75 g a.s./ha									

Scenario	PECsw global max (µg L)	Fish acute	Fish chronic	Aquatic invertebrates	Aquatic invertebrates-chronic	Algae	Higher plant	PECsed global max (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Pimephales promelas*	*Daphnia magna*	*Americamysis bahia*	*Pseudokirchneriella subcapitata*	*Lemna gibba*		*Chironomus riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (EC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (ErC_{50})		RAC (NOEC)
		335	1.328	240	2.0[1]	3510	248.0[1]		10000[1] µg a.s./kg sediment)
FOCUS Step 3									
D1 ditch	0.3904	-	0.29	-[a]	-[b]	-[a]	-[a]		-[a]
D1 stream	0.2486	-	0.19	-[a]	-[b]	-[a]	-[a]		-[a]
D3 ditch	0.2270	-	0.17	-[a]	-[b]	-[a]	-[a]		-[a]
D4 pond	0.4145	-	0.31	-[a]	-[b]	-[a]	-[a]		-[a]
D4 stream	0.1973	-	0.15	-[a]	-[b]	-[a]	-[a]		-[a]
D5 pond	0.2859	-	0.22	-[a]	-[b]	-[a]	-[a]		-[a]
D5 stream	0.1329	-	0.10	-[a]	-[b]	-[a]	-[a]		-[a]
R4 stream	0.0072	-	0.01	-[a]	-[b]	-[a]	-[a]		-[a]

[1]Based on parental toxicity
[a] Acceptable risk demonstrated at FOCUS Step 1
[b] Acceptable risk demonstrated at FOCUS Step 2
Values in **bold** are > 1 and therefore high risk

Overall conclusion for BCS-CN88460-carboxylic acid (M12): An acceptable risk to all aquatic organisms from the metabolite BCS-CN88460-carboxylic acid (M12) was demonstrated at FOCUS Step 3 following application of Isoflucypram in Winter and Spring cereals.

2.9.9.4. Risk assessment for bees

Risk assessment for the proposed use of 'Thiacloprid OD 240'

First tier: Assessment of the acute risk to bees from the active substance (isoflucypram) and the representative formulation ('Isoflucypram EC50') was conducted in accordance with Regulation (EC) No. 1107/2009, and the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). The critical acute contact and oral LD_{50} values were compared with the maximum individual application rate for the representative use to derive a Hazard Quotient (HQ) for each exposure route (oral or contact exposure). HQ values of ≤ 50 indicate a low acute risk to honeybees. Table B2.9.9.4-1 shows the oral and contact acute HQs for the proposed use of 'Isoflucypram EC50'on cereals.

Table B2.9.9.4-1: Acute risk assessment for bees for the proposed use of 'Isoflucypram EC50'

Compound	**Exposure route**	**Oral LD_{50} [µg a.s./bee]**	**Max. application rate [g/ha]**	**Hazard quotient Q_{HO}**	**Trigger**
Isoflucypram tech.	Oral	> 106.3	75	< 0.71	50
	Contact	> 100.0		< 0.75	
Isoflucypram EC 50	Oral	69.1		1.09	
	Contact	14.1		5.32	

All of the hazard quotients in table B2.9.9.4-1 are below the trigger of 50; therefore an acceptable acute risk to adult bees from the active substance and representative formulation can be concluded.

The Applicant also submitted chronic and larval studies. These studies were evaluated; the chronic study was considered valid by the RMS; however the larval study did not meet the validity criteria, although it is noted that study indicated a potential low toxicity. Due to the lack of an EU-agreed risk assessment scheme, these data have not been used in a formal risk assessment. Despite this, there may be some indication that the chronic risk to bees is acceptable because the level of exposure to isoflucypram-contaminated pollen and nectar is shown to be much lower than the NOEC from the available studies (see B.9.6.1 in the CP dossier).

Higher tier: Several higher tier semi-field tunnel studies were submitted (see B.9.5.1 in the CP dossier) which have been evaluated by the RMS and considered valid. Noting that the acute risk assessment for 'Isoflucypram EC50' passes at first tier, these higher tier studies were considered further to establish if they support the result of this risk assessment (see section B.9.6.1 of the CP dossier). The studies have been considered in a weight of evidence approach to the chronic risk assessment for 'Isoflucypram EC50'.

The studies investigating the effects of 'Isoflucypram EC50'were conducted at application rates equivalent to the GAP (1 x 75 g a.s./ha). The available data indicate that exposure to 'Isoflucypram EC50' could result in behavioural effects such as reduced foraging shortly following application. However, this did not appear to have an adverse effect on the survival on the bees, the brood or colony strength, and therefore the behavioural effects are considered acceptable. As such, the risk to bees from 'Isoflucypram EC50' is considered acceptable.

2.9.9.5. Risk assessment for other non-target arthropods

Tier I assessment

The acute risk to non-target arthropods from 'isoflucypram EC 50' was assessed in accordance with ESCORT II (2000) guidance and is presented below for the in-field and off-field risk:

Table B.2.9.9.5-1:**:** Tier 1 in-field risk assessment for non-target arthropods

Crop	Species	In-field PER [g a.s./ha]	LR_{50} [g a.s./ha]	HQ	Trigger
Cereals	*A. rhopalosiphi*	75	14.13	**5.3**	2
	T. pyri		30.6	**2.5**	2

For the standard species, the in-field scenario is above the trigger of concern, therefore further refinements are required.

Table B.2.9.9.5-2: Tier 1 off-field risk assessment for non-target arthropods

Crop	Species	Off-field PER [g a.s./ha]	LR_{50} [g a.s./ha]	HQ	Trigger
Cereals	*A. rhopalosiphi*	2.08	14.13	0.15	2
	T. pyri		30.6	0.07	2

For the standard species, the off-field HQ values are below the trigger of concern, indicating an acceptable risk for non-target arthropods.

Tier II assessment

For the standard species, the in-field scenario is above the trigger of concern in the Tier 1 risk assessment Therefore, a Tier 2 risk assessment is presented with the two standard species and the two additional species *Chrysoperla carnea* and *Coccinella septempunctata.*

Table 10.3.2- 1: Tier 2 in-field risk assessment for non-target arthropods

Crop	Species	In-field PER [g a.s./ha]	ER_{50} [g a.s./ha]	Risk acceptable if:	Refined assessment required?
Cereals	*A. rhopalosiphi*	75	> 7.50	Effects are <50%	**Yes**
	T. pyri		> 42.2	Effects are <50%	**Yes**
	C. carnea		> 75	Effects are <50%	No
	C. septempunctata		> 75	Effects are <50%	No

For the standard species *A. rhopalosiphi* and *Typhlodromus pyri* at Tier 2, the in-field risk assessment reveals effects >50% at the in-field rate of 75 g a.s./ha. Therefore, further refinements are necessary.

Refined in-field risk assessment for *Aphidius rhopalosiphi*

An extended aged residue laboratory study was performed with the most sensitive species *Aphidius rhopalosiphi.* Isoflucypram EC 50 was applied to potted maize plants at a rate of 1.46 L product/ha. An application rate of 1.46 L product/ha is equivalent to 75 g a.s./ha under the conditions of the test.
The exposure of the test organisms to fresh residues (0DAT1) resulted in a mortality of 3.3%. No mortality occurred when test organisms were exposed to aged residues (14DAT1).
A statistically significant reduction in reproductive success relative to the control of 44.7% was found after exposure to fresh residues (0DAT1). Although these effects were <50%, they were coupled with a significant repellance effect. This creates uncertainty over the results being representative of true exposure to the test item.

After exposure to aged residues (14DAT1) a reduction in reproduction of 13.8% was observed which was not statistically significantly different to the control. This indicates that recolonization can begin to occur within 2 weeks. This is supported by a low off-field risk to non-target arthropods at tier 1, which indicates recolonization populations are likely to be available. Furthermore, at the 14DAT1 bioasssay, there was no significant repellance effect, therefore the reproduction results can be used with more confidence. Therefore, no unacceptable effects on non-target arthropods in the in-field area are expected from the intended use of isoflucypram EC 50.

2.9.9.6. Risk assessment for non-target soil meso- and macrofauna

The risk assessment for non-target soil meso- and macrofauna was conducted following the SANCO terrestrial guidance document (2002). The soil PEC values calculated in the fate volume 3 CA/CP B.8 dossiers were used in the assessment. The chronic TER values for the active substance, representative formulation and metabolite are presented below:

Earthworms

Table B2.9.9.6-1: TER calculations for earthworms

Compound	Species, study type	Endpoint [mg/kg]		$PEC_{soil\ accumulation}$ [mg/kg]	TER_{LT}	Trigger
Isoflucypram EC 50	Earthworm, reproduction	NOEC	14.5[1]*	0.0616[1]	235	5
Isoflucypram	Earthworm, reproduction	NOEC	28[1]	0.0616[1]	455	5
BCS-CN88460-carboxylic acid (M12)	Earthworm, reproduction	NOEC	50[2]	0.0001[2]	500000	5

[1]mg a.s./kg dws
[2]mg pure metabolite/kg dws
*Expressed as a.s. content of the formulation

Earthworm TER values exceed the trigger value of 5 for the active substance, representative formulation and metabolite (BCS-CN88460-carboxylic acid; M12). An acceptable risk can therefore be concluded.

Other soil macro-organisms

Table B2.9.9.6-2: TER calculations for other soil macro-organisms

Compound	**Species**	**Endpoint [mg/kg]**		**PEC_{soil} accumulation [mg/kg]**	**TER**	**Trigger**
Isoflucypram EC 50	*Folsomia candida*	NOEC	2.59[1]*	0.0616[1]	42	5
	Hypoaspis aculeifer	NOEC	8.18[1]*	0.0616[1]	133	5
Isoflucypram, a.s.	*Folsomia candida*	NOEC	49.5[1]	0.0616[1]	804	5
	Hypoaspis aculeifer	NOEC	495[1]	0.0616[1]	8036	5
BCS-CN88460-carboxylic acid (M12)	*Folsomia candida*	NOEC	9.0[2]	0.0001[2]	90000	5
	Hypoaspis aculeifer	NOEC	495[2]	0.0001[2]	4950000	5

[1]mg a.s./kg dws
[2]mg pure metabolite/kg dws
*Expressed as a.s. content of the formulation

All TER values exceed the trigger value of 5 for the active substance, representative formulation and metabolite (BCS-CN88460-carboxylic acid; M12). An acceptable risk can therefore be concluded.

2.9.9.7. Risk assessment for soil nitrogen transformation

The risk assessment for soil nitrogen transformation was conducted following the SANCO/10329 terrestrial guidance document (2002). Table 2.9.9.7-1 shows the results of the risk assessment for the active substance, representative formulation and metabolite.

Table B2.9.9.7-1 : Nitrogen transformation risk assessment

Compound	**Species**	**Endpoint (< 25 % effects after 28 days) [mg/kg]**	**$PEC_{soil,max}$ [mg/kg]**
Isoflucypram EC 50	Soil micro-organisms	0.50[1]*	0.0616[1]
Isoflucypram	Soil micro-organisms	0.53[1]	0.0616[1]
BCS-CN88460-carboxylic acid (M12)	Soil micro-organisms	0.54[2]	0.0001[2]

[1]mg a.s./kg dws
[2]mg pure metabolite/kg dws
*Expressed as a.s. content of the formulation

The available soil nitrogen transformation studies demonstrated less than 25% effects at rates higher than the maximum predicted PEC_{soil} values. A low risk to soil nitrogen transformation from the proposed uses can therefore be concluded.

2.9.9.8. Risk assessment for terrestrial non-target higher plants

Seedling emergence screening data (single dose of 75.0 g a.s./ha) on 10 species showed that there were no effects ≥ 50 % at a rate of 75 g a.s./ha, showing an acceptable risk. Despite this, the applicant provided a dose-response seedling emergence study on 4 species (TER conducted on this data below). The applicant did not provide reasoning for this further test but it is noted that the 4 species test were the only ones that showed a significant inhibition on either shoot dry weight or shoot length in the Tier 1 test.

There was no screening data for vegetative vigour. Instead, a dose-response study was conducted on 10 species and this is detailed in Table B.2.9.9.8-1, alongside a tier II deterministic risk assessment for both pre- and post-emergence exposure to non-target plants.

Table B.2.9.9.8-1:: Dose-response data and risk assessment for non-target terrestrial plants treated with 75.0 g a.s./ha 'Isoflucypram EC 50'

Species	Test substance	ER_{50} (g a.s./ha) vegetative vigour	ER_{50} (g a.s./ha) emergence	Exposure[1] (g a.s./ha)	TER	Trigger
Beta vulgaris	'Isoflucypram EC 50'	> 75.0	> 75.0	2.0775	36.1	5
Brassica napus						
Cucumis sativus[2]						
Glycine max						
Helianthus annuus[2]						
Solanum lycopersicum[2]						
Allium cepa						
Avena sativa[2]						
Lolium perenne[2]						
Zea mays[2]						
Extended laboratory studies : None submitted. Semi-field and field test: None submitted.						

[1] exposure has been estimated based on maximum application rate x drift factor of 0.0277 (based on Ganzelmeier *et* al, 1995 drift data)

[2] This species was not included in the seedling emergence dose-response study.

Conclusion

The maximum application rate from the GAP was tested on more than 6 species of terrestrial plants, encompassing both dicotyledonous and monocotyledonous species for both seedling emergence and vegetative vigour effects and no effects ≥ 50% were observed, meaning endpoints defined for tisk assessment were pre- and post-emergence ER_{50} > 75 g a.s./ha. These endpoints were considerd in a tier II deterministic risk assessment and resultant TER values were greater than 5, indicating acceptable risk to non-target terrestrial plants. No further refinements are necessary.

2.9.9.9. Risk assessment for biological methods for sewage treatment

The max PEC_{SW} at FOCUS step 1 (as confirmed in the dossier for Environmental Fate and Behaviour B8 for 'Isoflucypram EC 50') is 9.63 µg a.s/L. As the EC_{50} (1000000 µg a.s/L) is much greater than the PEC_{SW} value , no adverse effects are expected with regard to activated sewage sludge and the risk does not require further consideration.

2.10. CLASSIFICATION AND LABELLING

Proposed classification according to Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures

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	Not classified – conclusive but not sufficient for classification		Not available	
2.2.	Flammable gases	hazard class not applicable		Not available	
2.3.	Flammable aerosols	hazard class not applicable		Not available	
2.4.	Oxidising gases	hazard class not applicable		Not available	
2.5.	Gases under pressure	hazard class not applicable		Not available	
2.6.	Flammable liquids	hazard class not applicable		Not available	
2.7.	Flammable solids	Not classified – conclusive but not sufficient for classification		Not available	
2.8.	Self-reactive substances and mixtures	hazard class not assessed		Not available	
2.9.	Pyrophoric liquids	hazard class not applicable		Not available	
2.10.	Pyrophoric solids	Not classified – conclusive but not sufficient for classification		Not available	
2.11.	Self-heating substances and mixtures	Not classified – conclusive but not sufficient for classification		Not available	
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified – conclusive but not sufficient for classification		Not available	
2.13.	Oxidising liquids	hazard class not applicable		Not available	
2.14.	Oxidising solids	Not classified – conclusive but not sufficient for classification		Not available	
2.15.	Organic peroxides	hazard class not applicable		Not available	

2.16.	Substance and mixtures corrosive to metals	hazard class not assessed		Not available	
3.1.	Acute toxicity - oral	Not classified – conclusive but not sufficient for classification		Not available	
	Acute toxicity - dermal	Not classified – conclusive but not sufficient for classification		Not available	
	Acute toxicity - inhalation	**Acute Tox 4; H332**		Not available	
3.2.	Skin corrosion / irritation	Not classified – conclusive but not sufficient for classification		Not available	
3.3.	Serious eye damage / eye irritation	Not classified – conclusive but not sufficient for classification		Not available	
3.4.	Respiratory sensitisation	No data		Not available	
3.4.	Skin sensitisation	**Skin Sens 1B; H317**		Not available	
3.5.	Germ cell mutagenicity	Not classified – conclusive but not sufficient for classification		Not available	
3.6.	Carcinogenicity	Not classified – conclusive but not sufficient for classification		Not available	
3.7.	Reproductive toxicity	Not classified – conclusive but not sufficient for classification		Not available	
3.8.	Specific target organ toxicity –single exposure	Not classified – conclusive but not sufficient for classification		Not available	
3.9.	Specific target organ toxicity – repeated exposure	Not classified – conclusive but not sufficient for classification		Not available	

3.10.	Aspiration hazard	Not classified – conclusive but not sufficient for classification		Not available	
4.1.	Hazardous to the aquatic environment	Acute category 1; H400 Chronic category 1; H410	M-factor = 10 M-factor = 1	Not available	
5.1.	Hazardous to the ozone layer				

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

Labelling: Signal word:
Warning

Hazard statements:
H332 – Harmful if inhaled
H317 – May cause an allergic skin reaction
H400 - Very toxic to aquatic life
H410 – Very toxic to aquatic life with long-lasting effects

Precautionary statements:
P273 - Avoid release to the environment
P391 - Collect spillage
P501 - Dispose of contents/ container to ... (in accordance with local/ regional/ national/ international regulation - to be specified).

Proposed notes assigned to an entry:
Notes in accordance with CLP Regulation, Annex VI, Section 1.1.3

2.11. RELEVANCE OF METABOLITES IN GROUNDWATER

The parent substance, isoflucypram does not leach into groundwater at levels > 0.1 µg/L. With the exception of M12 (isoflucypram carboxylic acid), no other metabolites of isoflucypram have been identified in groundwater at levels > 0.1 µg/L from the application of the representative product according to the GAP. For M12, the trigger value is not breached in all scenarios. The highest concentration (0.462 µg/L) has been predicted for the eraly application on spring cereals. An evaluation of the relevance of metabolite M12 in groundwater is therefore required. No biological activity or toxicological data has been submitted by the applicant. A data gap is therefore identified.

2.11.1. STEP 1: Exclusion of degradation products of no concern

2.11.2. STEP 2: Quantification of potential groundwater contamination

2.11.3. STEP 3: Hazard assessment – identification of relevant metabolites

2.11.3.1 STEP 3, Stage 1: screening for biological activity

2.11.3.2 STEP 3, Stage 2: screening for genotoxicity

2.11.3.3 STEP 3, Stage 3: screening for toxicity

2.11.4. STEP 4: Exposure assessment – threshold of concern approach

2.11.5. STEP 5: Refined risk assessment

2.11.6. Overall conclusion

2.12. CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.12.1. Identity and physical chemical properties

Not relevant; isoflucypram does not show isomerism.

2.12.2. Methods of analysis

Not relevant; isoflucypram does not show isomerism.

2.12.3. Mammalian toxicity

Not relevant; isoflucypram does not show isomerism.

2.12.4. Operator, Worker, Bystander and Resident exposure

Not relevant

2.12.5. Residues and Consumer risk assessment

2.12.6. Environmental fate

Not relevant

2.12.7. Ecotoxicology

Not relevant

2.13. RESIDUE DEFINITIONS

2.13.1. Definition of residues for exposure/risk assessment

Food of plant origin: (Provisional) Cereals: Isoflucypram (pending additional field trial data on the level of metabolites in cereal commodities)

Food of animal origin: (Provisional): Isoflucypram

Soil: *Isoflucypram, M12*

Groundwater: *Isoflucypram, M12 and possibly M10 following discussion during the peer review.*

Surface water: *Isoflucypram, M12*

Sediment: *Isoflucypram, M12*

Air: *Isoflucypram*

2.13.2. Definition of residues for monitoring

Food of plant origin: Isoflucypram

Food of animal origin: (Provisional): Isoflucypram

Soil: *to be decided at E.U. peer review*

Groundwater: *to be decided at E.U. peer review*

Surface water: *to be decided at E.U. peer review*

Sediment: *to be decided at E.U. peer review*

Air: *to be decided at E.U. peer review*

Level 3

ISOFLUCYPRAM

3. PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1. BACKGROUND TO THE PROPOSED DECISION

3.1.1. Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1. Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		Fate and Ecotoxicology: Based on the ecotoxicological risk assessments conducted the representative use of isoflucypram is expected to be possible, noting that for early uses on winter cereals certain further consideration may be required by some member states with regards to aquatic risk.
3.1.1.2. Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted		X	*[If no go to ii immediately below]*
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.	X		Ecotoxicology: Due to the finalization of scientific criteria and EU-level guidance after the submission of the dossier to the RMS there was not sufficient data provided in order to reach a decision on the Endocrine Disrupting properties with regards to non-target organisms (see (a)). Toxicology: Biological activity and toxicological data are required for metabolite M12 (isoflucypram carboxylic acid) identified in groundwater in some scenarios at levels > 0.1 µg/L to exclude relevance. Physical and Chemical properties: Persistent foam data at the lowest in-use concentration of 0.375 %v/v and ambient storage stability data are required. Methods of Analysis: Method details and validation data are required to support the analysis of diet and plasma samples from the 2nd generation rat toxicity study (Renaut, R., 2018; M-612750-02-1). Methods of Analysis: Extaction efficiency data are required for the proposed post approval monitoring method for te determination of BCS-CN88460 in high acid crops.
3.1.1.3. Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.			*[If yes –clearly specify the nature of the proposed restriction(s) i.e.* *(a) the minimum degree of purity of the active substance;*

				(b) the nature and maximum content of certain impurities; *(c) restrictions arising from the evaluation of the information referred to in Article 8 of 1107/2009 taking account of the agricultural, plant health and environmental, including climatic, conditions in question;* *(d) type of preparation;* *(e) manner and conditions of application;* *(f) submission of further confirmatory information to Member States, the Commission and the European Food Safety Authority, (the Authority), where new requirements are established during the evaluation process or as a result of new scientific and technical knowledge;* *(g) designation of categories of users, such as professional and non-professional;* *(h) designation of areas where the use of plant protection products, including soil treatment products, containing the active substance may not be authorised or where the use may be authorised under specific conditions;* *(i) the need to impose risk mitigation measures and monitoring after use;* *(j) any other particular conditions that result from the evaluation of information made available in the context of Regulation 1107/2009.* *Explain if some of the information to be submitted relates only to specified products/uses/use scenarios]*
3.1.1.4. Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		Robust ADI, AOEL and ARfD have been established.
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops			*[Insert brief overall summary of consideration of residues & consumer assessment here]* *[Explain if this applies to all or some of the representative uses/use scenarios/products]*

	(c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		*[Explain if this applies to all or some of the representative uses/use scenarios/products]*
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		*Brief summary of efficacy Cross refer to level 2 as necessary* *[Explain if this applies to all or some of the representative uses use scenarios/products]*
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.		X	There is insufficient information for metabolite M12 to establish its non-relevance in groundwater.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		*[Insert brief overall summary on identify here. Cross refer to level 2 as necessary]*
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			No FAO specification
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			No FAO specification
Methods of analysis				
		Yes	No	

	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		*[Insert brief overall summary here. Cross refer to level 2 as necessary]*
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	Acceptable methods have been submitted for all matrices with the exception of plants (high acid crop group), for which extraction efficiency data is outstanding. It is expected that these data can be generated during Peer Review.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		An **ADI value of 0.04 mg/kg bw/d** has been derived from the NOAEL of 4.2 mg/kg bw/d for liver effects from the 12-month dog study. An **ARfD value of 0.7 mg/kg bw** has been derived from the NOAEL of 70 mg/kg bw/d for maternal toxicity (initial body weight effects) from the rabbit developmental toxicity study. An **AOEL value of 0.04 mg/kg bw/d** has been derived from the NOAEL of 4.2 mg/kg bw/d for liver effects from the 12-month dog study. An **AAOEL** has not been derived.
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Isoflucypram did not induce gene mutations in bacteria or mammalian cells *in vitro* but was clastogenic *in vitro* with and without metabolic activation. However, when tested *in vivo* in a valid mouse bone marrow micronucleus study up to the limit dose of 2000 mg/kg bw, at which clinical signs of toxicity occurred, the clastogenic activity seen *in vitro* was not expressed *in vivo.*

				Overall, it can be concluded that isoflucypram is not genotoxic *in vivo* and the data requirements of Regulation 283/2013 have been met. Classification of isoflucypram for mutagenicity is not warranted (see also aligned CLH report).
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	Isoflucypram in not carcinogenic in rats or mice. No classification for carcinogenicity is warranted (see aligned CLH report).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable.
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	Isoflucypram has no adverse effects on fertility, reproductive function or development. No classification for reproductive toxicity is required (see aligned CLH report).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food			Not applicable

	and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	Isoflucypram is not classified for carcinogenicity or reproductive toxicity. In addition, it is considered that isoflucypram is not an endocrine disruptor with regard to human health in line with the criteria in Reg 605/2018.
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	Isoflucypram is not classified for reproductive toxicity.
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable.
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.			Soil, water and Sediment: Meets criterion for persistence.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	P: met for soil, water and sediment B: Not met (BCF_{fish} <2000) T: Not met: lowest NOEC ≮ > 0.01 mg/L = 0.01328 mg a.s./L (long-term toxicity to fish)

Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	vP: met for water and sediment vB: Not met (BCF_{fish} <5000)
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		An acceptable ecotoxicological risk has been demonstrated for all relevant organism groups for the proposed representative use pattern of the active substance, noting that not all aquatic exposure scenarios were shown to fully result in a low risk under the assessments conducted.
	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.			Not applicable : On the basis of the information available at the time of submission it is not possible to conclude whether or not the substance has endocrine disrupting properties in the context of the applicable COMMISSION REGULATION (EU) 2018/605 of 19 April 2018, with regards to non-target organisms.
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.		X	
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		Sufficient information has been submitted to demonstrate an acceptably low risk to honeybees.

Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.			*[Insert brief overall summary of residue definition here. Cross refer to level 2]*
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		Isoflucypram does NOT exceed the groundwater protection value. For metabolite M12 exceedance of 0.1 µg/L trigger value is shown to occur in certain scenarios only.

3.1.2. Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	*[If yes identify the criteria considered met by the substance* *i.e.* *its ADI, ARfD or AOEL is significantly lower than those of the majority of the approved active substances within groups of substances/use categories, Not met* *— it meets two of the criteria to be considered as a PBT substance Not Met* *— there are reasons for concern linked to the nature of the critical effects (such as developmental neurotoxic or immunotoxic effects) which, in combination with the use/exposure patterns, amount to situations of use that could still cause concern, for example, high potential of risk to groundwater; even with very restrictive risk management measures (such as extensive personal protective equipment or very large buffer zones), Not met* *— it contains a significant proportion of non-active isomers,* Not met

				— it is or is to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B, if the substance has not been excluded in accordance with the criteria laid down in point 3.6.3, Not met *— it is or is to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 1A or 1B if the substance has not been excluded in accordance with the criteria laid down in point 3.6.4, Not met* *— if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, reviewed by the Authority, it is considered to have endocrine disrupting properties that may cause adverse effects in humans if the substance has not been excluded in accordance with the criteria laid down in point 3.6.5.] Not met*

3.1.3. Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.		X	In relation to human health, isoflucypram is sensitising. With regards to the environment the substance is considered to be: - Persistent (soil, surface water and sediment) - BCF_{fish} > 100

3.1.4. List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	**Relevance in relation to representative use(s)**	**Study status**		
		No confirmation that study available or on-going.	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.1. Identity of the active substance or formulation				
3.1.4.2. Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
Persistent foam data at the lowest in-use concentration (0.375 %v/v)		X		
Ambient storgage stability study				X
3.1.4.3. Data on uses and efficacy				
Biological activity data on groundwater metabolite M12	Relevant to certain scenarios	X		
3.1.4.4. Data on handling, storage, transport, packaging and labelling				
3.1.4.5. Methods of analysis				
Method of analysis and validation data (Envigo study numbers DNM0082 and DNM0085) for the				X

determination isoflucypram in the diet and plasma as reported in the rat 2-generation study (Envigo study number DNM0081; Renaut, R., 2018; M-612750-02-1).				
Extraction efficiency data for the post approval monitoring method proposed for high acid crops (Method 01520; Uceda, L.; 2017; M-588974-01-1).		X		
3.1.4.6. Toxicology and metabolism				
Toxicological data on groundwater metabolite M12	Relevant to certain scenarios	X		
3.1.4.7. Residue data				
3.1.4.8. Environmental fate and behaviour				
3.1.4.9. Ecotoxicology				
Sufficient data to address data requirement 8.2.3 of (EU) No. 283/2013 – endocrine disrupting properties in aquatic non-target organisms				

3.1.5. Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
The assessment for endocrine disruption for non-target organisms according to the scientific criteria of Commission regulation (EU) 2018/605	All representative uses
The assessment of non-relevance for groundwater metabolite M12	Relevant only to certain scenarios
The long-term risk to fish from the active substance (FOCUS scenarios D1 ditch, D2 ditch)	Early applications to winter cereals

3.1.6. Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**

None identified.	

3.1.7. Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Use Winter cereals 1 x 75 g a.s./ha (X^1)	Use Spring cereals 1 x 75 g a.s./ha (X^1)
Operator risk	Risk identified		
	Assessment not finalised		
Worker risk	Risk identified		
	Assessment not finalised		
Bystander risk	Risk identified		
	Assessment not finalised		
Consumer risk	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial vertebrates	Risk identified		
	Assessment not finalised		
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified		
	Assessment not finalised		
Risk to aquatic organisms	Risk identified	X – Scenarios D1, D2 ditch	
	Assessment not finalised		
Groundwater exposure active substance	Legal parametric value breached		
	Assessment not finalised		
Groundwater exposure metabolites	Legal parametric value breached	X	X
	Parametric value of 10µg/L[(a)] breached		
	Assessment not finalised	X	X
Comments/Remarks			

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8. Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
	[specify the reasons why expert consultation is considered necessary]

3.1.9. Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
Tox: NOAEL for maternal toxicity in the rabbit developmental toxicity study	The 12% reduction in overall body weight gain at the mid-dose of 70 mg/kg bw/d should be considered adverse and the NOAEL set at 10 mg/kg bw/d.	As the effect is not statistically significant; was seen in isolation; there is a large dose spacing; and given the magnitude of the change; the effect is not considered adverse and the NOAEL for maternal toxicity should be 70 mg/kg bw/d.
Tox: Endocrine disruption	There are adverse effects potentially related to EAS modalities and Level 2 studies are required. Effects on vaginal opening are considered treatment-related and the uterotrophic/pubertal assay only supplementary. In addition, further mechanistic data are required to exclude the human relevance of the thyroid effects.	There are no adverse effects potentially related to EAS modalities and Level 2 studies are not required. Effects on vaginal opening are considered un-related to treatment and the uterotrophic/pubertal assay acceptable as a mechanistic assay. In addition, no further mechanistic data are required to exclude the human relevance of the thyroid effects.

Tox: ARfD	An ARfD of 0.1 mg/kg bw is proposed.	An ARfD of 0.7 mg/kg bw is proposed.
Tox: AAOEL	An AAOEL of 0.1 mg/kg bw is proposed.	The data are not adequate to derive an AAOEL.
Tox: Tox batches	The tox batches are not considered representative of the proposed technical specification.	The tox batches are considered representative of the proposed technical specification.

3.2. PROPOSED DECISION

[redacted]

3.3. RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1. Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
	[redacted]

3.4. APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSMENT

Ecotoxicology:

- Guidance of EFSA : Risk Assessment for Birds and Mammals: EFSA Journal 2009; 7(12):1438
- Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters: EFSA Journal 2013;11(7):3290
- Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods ESCORT II (2000)
- Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC: SANCO/10329/2002
- Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009: EFSA Journal 2011;9(2):2092
- Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA/ECHA, 2018). EFSA Journal, Vol 16, Issue 6, June 2018, e05311 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311.

Toxicology

- Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009: EFSA Journal 2011;9(2):2092.

- Guidance on Dermal Absorption; EFSA Panel on Plant Protection Products and their Residues (PPR). EFSA Journal 2012; 10(4): 2665.

- Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC Sanco/221/2000 rev.10.

- Guidance on the application of the CLP criteria; guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures Version 4.0 June 2015.

- Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA/ECHA, 2018). EFSA Journal, Vol 16, Issue 6, June 2018, e05311 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311.

3.5. REFERENCE LIST

None cited.