Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain (GB PPP)

Bixlozone (F9600)

Volume 1

Great Britain

July 2022

Version History

When	What
July 2022	Initial DAR
September 2022	Updated post July 2022 Expert Committee on Pesticides (ECP) meeting Independent Scientific Advice (ISA)

Table of contents

Level 1

BIXLOZONE

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, pesticidal active substance F9600 (ISO provisionally approved name: bixlozone) and its formulated product F9600-4 SC. This dossier was submitted by FMC Corporation as FMC Chemical sprl ("FMC") for the first approval of this substance in Great Britain (GB) under Regulation No 1107 with the evaluation performed by the Chemicals Regulation Division of the Health and Safety Executive. FMC also have an ongoing application for the approval of bixlozone as a new active substance in the EU, with the evaluation being performed by the Netherlands as Rapporteur Member State (RMS).

Bixlozone (F9600) is a new active substance in the context of Regulation 1107/2009 and belongs to the isoxazolidinone family of herbicides. It is intended for pre- and early post emergence application to a number of crops, including cereals, oilseed rape and maize, for control of a range of broadleaf and grass weeds.

Data have been generated on the active substance and representative formulation pursuant to the requirements laid out in the Annexes to Regulations 283/2013 and 284/2013, and in accordance with the test guidelines defined under the associated communications (2013/C 95/01 and 2013/C 95/02 respectively).

FMC state that the application is made for approval of this new active substance in accordance with Article 4 and Article 5 of Regulation 1107/2009, and the submitted dossier is considered to demonstrate compliance with all of the relevant criteria set out therein.

FMC Corporation (as FMC Chemical sprl) are the sole applicant in support of the active substance and are the sole owner of the supporting data package.

Currently, bixlozone does not have an entry under Annex VI of Regulation (EC) No 1272/2008. However, classification and labelling is currently under evaluation and a mandatory classification and labelling report is being prepared under GB CLP by HSE, with HSE acting as the Agency. Therefore, this section will be completed at a later stage following the aligned evaluation process and when the report is complete.

1.1.2. Regulatory history for use in Plant Protection Products

Not relevant for the purpose of this submission as bixlozone is a new active substance and products containing it have not previously been authorised in Great Britain.

1.1.3. Evaluations carried out under other regulatory contexts

Bixlozone is a new, herbicidal active substance developed by the applicant (FMC). FMC provided a dossier in support of their application for the first approval of this pesticide in Great Britain in accordance with Regulation No. 1107. No registrations or authorisations of bixlozone-containing plant protection products currently exist in GB or EU Member States, however, there is an authorisation for a product in Australia.

There is an ongoing application for the approval of bixlozone as a new active substance in the EU, with the evaluation being performed by the Netherlands as Rapporteur Member State (RMS). The applicant has not provided details of any other evaluations by non-EU countries or international organisations, nor of any information exchange within the OECD. Furthermore, no other relevant EU-evaluations of the active substance have been carried out under other EU-legislation.

1.2. APPLICANT INFORMATION

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

Name:	FMC International Switzerland Sàrl
Address:	Chemin de Blandonnet 8 1214 Vernier Switzerland

1.2.2. Producer or producers of the active substance

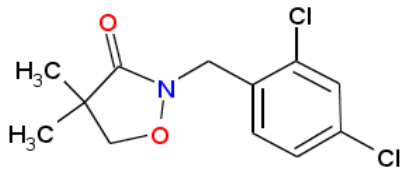
Name:	FMC Corporation
Address:	[redacted]
Location of plant:	Confidential information. Data provided separately (Document J)

1.2.3. Information relating to the collective provision of dossiers

The dossier is submitted for the purpose of approval of bixlozone (F9600), as a new active substance, with FMC as the sole applicant. Therefore, a joint dossier is not relevant for this active substance.

1.3. IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1. Common name proposed or ISO-accepted and synonyms	Bixlozone (Provisionally approved)
1.3.2. Chemical name (IUPAC and CA nomenclature)	
IUPAC	2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one
CA	2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-3-isoxazolidinone
1.3.3. Producer's development code number	F9600; [redacted]-57049
1.3.4. CAS, EEC and CIPAC numbers	
CAS	81777-95-9
EEC	Not assigned
CIPAC	Not assigned

1.3.5. Molecular and structural formula, molecular mass	
Molecular formula	$C_{12}H_{13}Cl_2NO_2$
Structural formula	
Molecular mass	274.14 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 DAR.
1.3.7. Specification of purity of the active substance in g/kg	Minimum 960 g/kg
1.3.8. Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1. *Additives*	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
1.3.8.2. *Significant impurities*	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
1.3.8.3. *Relevant impurities*	2,4-dichlorobenzyl alcohol: Maximum 1.5 g/kg
1.3.9. Analytical profile of batches	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.

1.4. INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1. Applicant	FMC International Switzerland Sàrl Chemin de Blandonnet 8 1214 Vernier Switzerland
1.4.2. Producer of the plant protection product	[redacted]
1.4.3. Trade name or proposed trade name and producer's development code number of the plant protection product	F9600-4 SC
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**	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
1.4.4.2. **Information on the active substances**	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
1.4.4.3. **Information on safeners, synergists and co-formulants**	Confidential information. Please refer to the Volume 4 (Confidential Information) section of the DAR.
1.4.5. Type and code of the plant protection product	Suspension Concentrate [Code: SC]
1.4.6. Function	Herbicide
1.4.7. Field of use envisaged	For the treatment of grasses and broad leaved weeds in winter wheat, winter barley, winter oilseed rape and maize.
1.4.8. Effects on harmful organisms	Bixlozone is a broadcast soil applied residual herbicide. After being absorbed by the roots and shoots, it is translocated upwards in water through the xylem tissue and then diffuses within the plant. It acts as a carotenoid biosynthesis inhibitor causing bleaching of weeds. Once in contact with light, the emerging seedlings of susceptible weed species express bleaching symptoms and die. Bixlozone does not appear to demonstrate downward systemic action or upward translocation from leaf to leaf. This may account for the inability to control larger weeds post-emergence, as well as explaining the appearance of chlorotic symptoms on contacted foliage with minimal or no effect on subsequent new growth.

1.5. DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1. Details of representative uses

1.5.1.1 Initial intended uses in Great Britain

Crop and/ or situation (a)	Region	Product code	F G or 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 as (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	kg as/hL min max	water L/ha min max	g as/ha min max		
Winter wheat Winter barley	**GB**	F9600-4SC	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 00-09	1	-	150-400	200	-	
Winter wheat	**GB**	F9600-4SC	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 11-13	1	-	150-400	200	-	
Winter Oilseed rape	**GB**	F9600-4SC	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 00-09	1	-	150-400	200-300	-	
Maize	**GB**	F9600-4SC	F	Grasses and broad leaved weeds	SC	400 g/L	Broadcast soil application	BBCH 00-09	1		150-400	250-375	-	

Remarks

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse 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 No 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 plants - type of equipment used must be indicated
(i) g/kg or g/l
(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) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

1.5.1.2 Representative uses covered in the EU dossier

Use-No.	Member state(s)	Crop and/ or situation (crop destination /purpose of crop)	F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/synergist per ha
					Method / Kind	Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	Timing	L product /ha a) max. rate per appl. b) max. total rate per crop/season	g, kg a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	Central & Southern zone	Winter wheat (TRZAW)	F	Annual Grass (3ANGWT) and broadleaved (3ANDIT) weeds	Broadcast soil application	00-09 Autumn (September-December) 11-13 Autumn (September-December)	1	Pre-emergence of the crop Early post-emergence of the crop	0.49 L/ha	200	150 to 400 L/ha	Not relevant	Professional use
2	Central & Southern zone	Winter barley (HORVW)	F	Annual Grass (3ANGWT) and broadleaved (3ANDIT) weeds	Broadcast soil application	00-09 Autumn (September-December)	1	Pre-emergence of the crop	0.49 L/ha	200	150 to 400 L/ha	Not relevant	Professional use
3	Central & Southern zone	Winter oilseed rape (BRSNW)	F	Annual Grass (3ANGWT) and broadleaved (3ANDIT) weeds	Broadcast soil application	00-09 Autumn (August-October)	1	Pre-emergence of the crop	0.49-0.73 L/ha	200-300	150 to 400 L/ha	Not relevant	Professional use
4	Central & Southern zone	Maize (ZEAMX)	F	Annual Grass (3ANGWT) and broadleaved (3ANDIT) weeds	Broadcast soil application	00-09 Spring (March-June)	1	Pre-emergence of the crop	0.61-0.91 L/ha	250-375	150 to 400 L/ha	Not relevant	Professional use

* 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

(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

(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
(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

Method of application

Bixlozone is applied as a broadcast soil application with a water volume of 150 to 400 l/ha using a tractor mounted boom sprayer. See table 1.5.1. above for the doses applied to each crop.

Number and timing of applications and duration of protection

Bixlozone is applied post-sowing pre-emergence in winter wheat, winter barley, winter oilseed rape and maize. Additionally, in winter wheat only, it may be applied early post-emergence (BBCH 11-13). Only one application of bixlozone can be made per crop. Bixlozone controls susceptible weeds during the early development period of these crops.

Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops

The sensitivity results from a seedling emergence glasshouse study discussed in Volume 3CP B3, combined with the high persistence of bixlozone in soil, indicate a high risk of phytotoxicity in various succeedings crops following an application of bixlozone at its proposed GAP. It is likely that many crops will not be acceptable as rotational or replacement crops and deep cultivation and/or long waiting periods may be necessary for some crops. A full risk assessment on succeeding crops, including field trials, will be conducted at the product authorisation stage.

Proposed instructions for use

Bixlozone is proposed for use in agriculture as a broadcast soil residual herbicide applied pre-emergence in winter wheat, winter barley, winter oilseed rape and maize, and also early post-emergence in winter wheat. See table 1.5.1. above for further details. Specific instructions for use, including the product label, will be considered in full at the product authorisation stage.

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

MRLs have been proposed based on GB uses (Table 1.5.1) of wheat, barley, maize and oilseed rape, and the subsequent possible residues in rotational crops and honey - see Volume 1, Section 2.7.10.

1.5.4. Overview on authorisations in EU Member States

Whilst bixlozone is not yet approved in the EU, an application is currently undergoing consideration for the approval of bixlozone as a new active substance (NAS) within the EU (the Netherlands are RMS). Therefore, there are currently no authorisations for the use of plant protection products containing bixlozone within EU Member States. The representative uses being considered in the EU bixlozone application are detailed under Volume 1, Section 1.5., Table 1.5.1.2 above.

Level 2

Bixlozone

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

2.1. IDENTITY

Acceptable data have been submitted to support the manufacturing sites of bixlozone and the proposed specification based on pilot scale manufacturing is considered supported by the available data. The following impurity identified in technical bixlozone is considered to be of toxicological or ecotoxicological relevance:

(2,4-dichlorophenyl)methanol (CAS 1777-82-8; 2,4-dichlorobenzyl alcohol): Maximum 1.5 g/kg.

Following scale-up from pilot plant to full scale manufacture data to confirm the commercial scale technical specification must be submitted. In addition, the toxicological significance of any changes in the impurity profile must be addressed.

2.2. PHYSICAL AND CHEMICAL PROPERTIES

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

Bixlozone (pure) is a white, crystalline solid with a melting range of 81.5-83.5°C. It has a vapour pressure of 1.1×10^{-3} Pa at 20°C and a moderate water solubility of 42.0 mg/L. It is readily soluble in organic solvents and has a Log P_{ow} of 3.3, indicating there is a possibility for bioaccumulation; it is not considered surface active. Bixlozone has a self-ignition temperature of 382°C and does not possess explosive or oxidising properties. There are no implications for classification, transport or storage on the basis of the physico-chemical properties.

Data to address the UV/visible absorption spectra of the relevant impurity (2,4-dichlorophenyl)methanol (CAS 1777-82-8; 2,4-dichlorobenzyl alcohol) are required.

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

'F9600-4SC' is a suspension concentrate (SC) formulation consisting of a uniform, cream liquid. The formulation is not explosive, oxidising or flammable. A 1% w/v dilution of the formulation has a pH of 7.18. It has a flash point of 107°C, an auto-ignition temperature of 423°C. The formulation has no explosive, flammable or oxidising properties. The surface tension, persistent foam, suspensibility and spontaneity of dispersion all meet the acceptable criteria. F-9600-4SC has been demonstrated to be stable in studies at 54 °C for 2 weeks and 25°C for 24 months, with no significant loss of active substance content. **Data to address the content of the relevant impurity (2,4-dichlorophenyl)methanol in the product before and after storage are required.** The packaging of the product remained free from any corrosion or degradation for the duration of the stability studies. The formulation also demonstrates acceptable physical and chemical properties after low temperature storage, with no significant weight change and acceptable results for suspensibility and wet sieve test. The physical and chemical properties submitted fulfil the requirements of a suspension concentrate formulation type.

2.3. DATA ON APPLICATION AND EFFICACY

2.3.1. Summary of effectiveness

The applicant has provided sufficient data to establish the appropriateness of the GAP and the effectiveness of the proposed formulation. In line with the guidance document SANCO/10054/2013, there is no requirement at this stage to submit a biological assessment dossier (BAD) and individual trials reports, since a full efficacy data package will be evaluated at the product authorisation stage. Overall, the data provided are sufficient to confirm that bixlozone and the associated representative formulation are sufficiently effective, and the proposed GAP is realistic and fulfils the needs of a risk envelope.

2.3.2. Summary of information on the development of resistance

Bixlozone belongs to the isoxazolidinone chemical family and will be classified in HRAC group 13 (previously F4). According to the website 'International Survey of Herbicide Resistant Weeds' (www.weedscience.org), globally there are two weed species which have been reported as resistant to HRAC group 13. Resistance has been reported in *Lolium rigidum* in Australia in 1982 and in *Echinochloa crus-galli var. crus-galli* in the USA in 2008. To date, no resistance cases to HRAC group 13 herbicides have occurred in Europe.

Overall, the risk of resistance developing to bixlozone is considered to be low. However, a full resistance risk analysis and consideration of appropriate management strategies must be conducted at the product authorisation stage.

2.3.3. Summary of adverse effects on treated crops

The applicant has provided sufficient data to examine the effects of the active substance and representative formulation on the treated crops, when applied in accordance with the proposed GAP. Bixlozone commonly causes phytotoxicity symptoms in the treated crops, predominantly low levels of bleaching and chlorosis, but these symptoms are usually transient and rarely result in yield losses. These effects are acceptable for a broad-spectrum agricultural herbicide used for the control of annual dicotyledonous and monocotyledonous weed species.

Overall, the proposed GAP is realistic in terms of its crop safety in the proposed crops. A detailed evaluation of all potential adverse effects on the treated crops, including phytotoxicity, yield quantity and quality, effects on plant parts for propagation and transformation processes, must be conducted at the product authorisation stage.

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

The risk assessments in Volume 3 CP B3 of the DAR indicate a low risk to adjacent crops from both spray and vapour drift. However, there appears to be a high risk to certain succeeding crops due to the persistence of bixlozone in soil. There is also a risk to crops subsequently treated using the same equipment previously used to apply bixlozone, and a cleaning method is likely to be needed. A detailed evaluation of all potential undesirable or unintended side-effects, including the impact on succeeding crops, other plants such as adjacent crops, tank cleaning and beneficial and non-target organisms must be conducted at the product authorisation stage.

2.4. FURTHER INFORMATION

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

Acceptable information has been provided to address these methods and precautions (see Volumes 3 CA and CP, section B.4).

2.4.2. Summary of procedures for destruction or decontamination

Acceptable information has been provided to address these methods and precautions (see Volumes 3 CA and CP, section B.4).

2.4.3. Summary of emergency measures in case of an accident

Acceptable information has been provided to address these methods and precautions (see Volumes 3 CA and CP, section B.4).

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 in the plant protection product. **A method for the determination of the relevant impurity (2,4-dichlorophenyl)methanol in the plant protection product is required.**

Acceptable methods have been submitted for the determination of bixlozone and selected metabolites in various studies used in support of the environmental fate, toxicology, ecotoxicology and physical chemical properties areas of the risk assessment.

For residues in plants, the proposed reside definition for risk assessment includes the compounds bixlozone and 2,4-dichlorobenzoic acid. The majority of studies submitted in support of residues studies used the methods of analysis CAM-0180/001 and CAM-0180/002 that is also proposed as the method for post authorisation control. It is considered fully validated for the determination of residues of bixlozone, and 2,4-dichlorobenzoic acid in wheat straw, oil seed rape, potato and grape. Additionally, it is validated in radish root, radish and lettuce leaves, wheat grain and wheat straw and hay in support of rotational crop studies.

The extraction efficiency of CAM-0180 was determined in wheat straw using samples with incurred radioactive residues from the plant metabolism studies. Levels of 2,4-dichlorobenzoic acid and 5'-0H-bixlozone were comparable to the levels found in the metabolism study when measured using radio HPLC techniques but were significantly lower when measured using LC-MS/MS. Bixlozone and 2,2-dimethyl-3 hydroxy propionic acid were not detected in the sample of wheat straw from the metabolism studies so no conclusions can be drawn on the extraction efficiency of the methods for these compounds.

2.5.2. Methods for post control and monitoring purposes

Methods have been submitted for the determination of bixlozone and selected metabolites in various matrices for use in post-approval monitoring and control. These methods are considered acceptable with the following exceptions:

For the determination of residues in plant and plant products methods are not fully validated for crops in the high water, high protein and high starch (dry) crop groups.

The following data are required:

Independent laboratory validation data for the method for the monitoring of residues in plants for the high water and high starch (dry) crop groups

Validation and ILV of the method for the monitoring of residues in plants for the high protein crop group

A summary of the available methods is given below.

Table 2.5.2.1 Summary of the available methods of analysis for monitoring residues of bixlozone

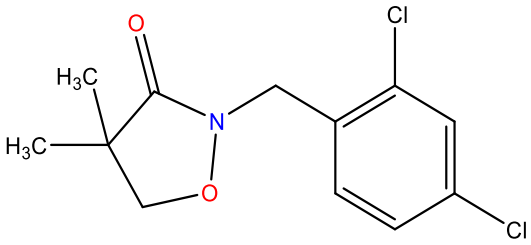
Matrix/Crop group	Analytes(s)	Method	LOQ	ILV?	Fully validated
Plants: High acid High oil High starch No group (cereal straw)	Bixlozone 2,4-dichlorobenzoic acid 5-hydroxy-bixlozone 2,2-dimethyl-3-hydroxy propionic acid 5'-hydroxy-bixlozone	CAM-0180 LC-MS/MS	0.01 mg/kg (0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid in high oil crops)	Yes	Yes. The proposed residue definition for monitoring is: Bixlozone
Plants: High water High starch (dry)	Bixlozone 2,4-dichlorobenzoic acid 5-hydroxy-bixlozone 2,2-dimethyl-3-hydroxy propionic acid 5'-hydroxy-bixlozone	CAM-0180 LC-MS/MS	0.01 mg/kg (0.05 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid in high oil crops)	No	**No.** The proposed residue definition for monitoring is: Bixlozone No ILV data for crops in the high water or high starch (dry) crop groups
Plants: High protein	-	-	-	-	**No.** No validation data in support of high protein crop group

Matrix/Crop group	Analytes(s)	Method	LOQ	ILV?	Fully validated
Products of animal origin: Egg Fat Kidney Liver Milk Meat (bovine)	Bixlozone 5-OH-bixlozone Bixlozone-3-OH propenamide Bixlozone-dimethyl malonamide	QuEChERS LC-MS/MS	0.01 mg/kg	Yes	Yes. The proposed residue definition for monitoring is: Bixlozone
Soil (Loam, clay)	Bixlozone 2,4-dichlorobenzoic acid Bixlozone-3-OH propenamide	CAM-0151 LC-MS/MS	0.005 mg/kg	Yes	Yes. The proposed residue definition for monitoring is: Bixlozone LOQ < end point for most sensitive soil organism (NOECcorr 11.25 mg a.s./kg dw soil; *Folsomia candida* derived from study conducted with F9600-4 SC)
Surface water Drinking water	Bixlozone 2,4-dichlorobenzoic acid Bixlozone-3-OH propenamide 4-carboxy-bixlozone Bixlozone-dimethyl malonamide	LC-MS/MS	0.1 µg/L	Yes	Yes. The proposed residue definition for monitoring is: Bixlozone LOQ < most sensitive effect concentration (5.1 µg/L) For groundwater the proposed residue definition is: 2,4-dichlorobenzoic acid LOQ < most sensitive effect concentration (1 mg/L)
Air	Bixlozone	LC-MS/MS	0.36 µg/m^3	n/a	Yes. The proposed residue definition for monitoring is: bixlozone LOQ < "c" (60 µg/m^3 based on $AOEL_{systemic}$ of 0.2 mg/kg bw)
Whole blood Urine	Bixlozone	LC-MS/MS	0.05 mg/L	n/a	Yes. The proposed residue definition for monitoring is: 5-keto-hydrate-bixlozone
Urine	5-keto-hydrate-bixlozone	LC-MS/MS	0.01 mg/kg	n/a	
Body tissues	Bixlozone 5-OH-bixlozone Bixlozone-3-OH propenamide Bixlozone-dimethyl malonamide	QuEChERS LC-MS/MS	0.01 mg/kg	Yes	Yes. The proposed residue definition for monitoring is: 5-keto-hydrate-bixlozone
Body tissues	5-keto-hydrate-bixlozone	LC-MS/MS	0.01 mg/kg	Yes	

2.6. EFFECTS ON HUMAN AND ANIMAL HEALTH

Bixlozone (2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one, also known as F9600, CAS 81777-95-9) is a new herbicidal active substance, developed by FMC Corporation. It is intended for pre- and early-post emergence application to a number of crops, including cereals, oilseed rape and maize, for control of a range of broadleaf and grass weeds.

The structure of bixlozone is presented below:



Bixlozone belongs to the isoxazolidinone chemical family and is a broadcast soil applied residual herbicide. Its mode of action is to inhibit the biosynthesis of carotenoids. After being absorbed by the roots and shoots, it is translocated upwards in water through the xylem tissue and then diffuses within the plant. Deprived of protective carotenoids, chlorophyll as well as other components of the photosynthetic apparatus becomes susceptible to photo-oxidation. Once in contact with light, these components are photodegraded and the emerging seedlings of express bleaching symptoms and die.

The representative product for bixlozone is F9600-4 SC which contains 400 g a.s./l of the active substance bixlozone. It acts as a carotenoid biosynthesis inhibitor causing bleaching of weeds. It is intended to be used as a selective herbicide for the control of annual monocotyledonous and dicotyledonous weed species in agricultural crops. The product will be applied after sowing but pre-emergence to winter wheat, winter barley, winter oilseed rape and maize or early post-emergence to winter wheat. Bixlozone does not appear to demonstrate downward systemic action or upward translocation from leaf to leaf. This may account for the inability to control larger weeds post-emergence, as well as explaining the appearance of chlorotic symptoms on contacted foliage with minimal or no effect on subsequent new growth.

This document uses the term 'bixlozone' when referring to the active substance. However, the development code F9600 has been used by the applicant within the individual study reports. The batches of bixlozone 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 regulatory purposes (see document CA B5 and the individual studies within this B6 document for further details).

The classification of bixlozone for Human Health effects has been addressed in an aligned MCL (Mandatory Classification and Labelling) dossier produced by HSE.

The data requirements of Regulation (EC) 1107/2009 and Regulation (EU) 283/2013 have been met and HSE concludes that there are no data gaps.

2.6.1. Summary of absorption, distribution and excretion in mammals

The absorption, distribution, metabolism and excretion of bixlozone in mammals have been extensively investigated in Sprague-Dawley (SD) rats following a single oral low dose (5 mg/kg bw), a single oral high dose (500 mg/kg bw and 1000 mg/kg bw), multiple oral low doses (5 mg/kg bw, 14 days) and a single low IV dose (3 mg/kg bw) of [^{14}C-Phenyl]-bixlozone. Moreover, a mass balance and excretion study was conducted with [^{14}C-Carbonyl]-bixlozone at a single low dose (5 mg/kg bw). In addition to the *in vivo* studies, two *in vitro* metabolism studies of bixlozone using cryopreserved hepatocytes of rats, mouse, dog and human were performed. Lastly, additional limited toxicokinetic data from repeated dose and carcinogenicity studies conducted in rats, mice and dogs are available. The table below presents an overview of all the available studies.

Table 2.6.1.1 Summary of the ADME studies of bixlozone

Method, Species, test substance, ***acceptability***	**Doses**	**Main findings**
Pharmacokinetics and metabolism (pilot study) **Rat** (Crl :CD9(SD), males & females, 3/sex/group) Not to GLP Not to OECD Guideline Deviations: N/A F9600 technical, batch G3773-17 Purity: 99.25 % Report no. FMC-R2838 [redacted] **(2014)** ***Supplementary only***	25 mg/kg bw Single oral (gavage)	One male was excluded from data analysis due to blockage of the catheter. Males (n=2): $T_{1/2}$ 3.11 h, T_{max} 0.5 h, C_{max} 169 ng/mL, AUC_{inf} 590 h.ng/mL Females (n=3): $T_{1/2}$ 1.94 h, T_{max} 0.67 h, C_{max} 315 ng/mL, $AUCi_{nf}$ 982 h.ng/mL Systemic exposure : 1.7 -1.9-fold greater for females compared to males. No F9600 detected in urine and rat faeces Extensive metabolism to various isoxazolidinone ring-opened/modified analogues in both urine and faeces.
Metabolism (pilot study) **Rat** (Crl :CD9(SD) 2 males Not to GLP OECD 417 (2010) Deviations: N/A **[^{14}C-phenyl]-F9600**, batch CFQ42017; purity 99.6 %; specific activity 56 mCi/mmol (202.9 µCi/mg) F9600 technical, batchPL14-0163; purity 99.8 % Study no. FMC-R3694 [redacted] **(2017a)** ***Supplementary only***	1000 mg/kg bw Single oral (gavage)	95 % of the dose was excreted in urine and faeces within 5 days with 72 % in urine and 24 % in faeces. Excretion through expired air was negligible. Bixlozone was extensively metabolised. Oxidation and ring-opening, followed by conjugation constituted the major metabolic reactions observed.

Method, Species, test substance, ***acceptability***	**Doses**	**Main findings**
Toxicokinetics **Rat** (Crl :CD9(SD) 2 males GLP OECD 417 (2010) Deviations: None of significance **[^{14}C-Phenyl]-F9600**, Batch CFQ42017; purity 99.6 %; specific activity 56 mCi/mmol (202.9 µCi/mg) F9600 technical, PL14-0163; purity 99.8 % Study no. FMC-P3773 [redacted] **(2016)** ***Acceptable***	Single oral low dose (5 mg/kg bw) Single oral high dose (1000 mg/kg bw) Multiple oral dose (5 mg/kg bw/day; 14 days) Intravenous bolus dose (IV; 3 mg/kg bw)	<u>Single oral low dose (5 mg/kg bw):</u> C_{max} of F9600 in plasma: 174 and 293 ng/mL at (Tmax) 0.25 h in male and female rats, respectively (M / F) $T_{1/2}$:1.4 h and 1.7 h in M / F $AUC_{0\text{-}inf}$: 145 and 221 ng x h/mL in M /F Bioavailability: 70 % and 86 % (total radioactivity) & 11 % and 18 % (F9600 in plasma) for M /F <u>Single oral high dose (1000 mg/kg bw):</u> C_{max} of F9600 in plasma: 9565 and 15060 ng/mL at (Tmax) 3.5 h in M / F $T_{1/2}$: 11 h and 14 h in M / F $AUC_{0\text{-}inf}$: $10.5x10^5$ and $35.9x10^5$ ng h/mL in M /F Bioavailability: 58 % and 60 % (total radioactivity) & 39 % and 100 % (F9600 in plasma) for M /F <u>Multiple oral dose (5 mg/kg bw/day; 14 days)</u> C_{max} of F9600 in plasma: 71 and 166 ng/mL in M / F at (Tmax) 0.0 h and 0.25 in M / F $T_{1/2}$: 11 h and 14 h in M / F $AUC_{0\text{-}inf}$: 65 and 162 ng h/mL in M /F Bioavailability: 58 % and 79 % (total radioactivity) & 5 % and 13 % (F9600 in plasma) for M /F No indications of accumulation of total radioactivity or F9600. <u>Intravenous bolus dose (IV; 3 mg/kg bw):</u> C_{max} of F9600 in plasma: 1317 and 1195 ng/mL at (Tmax) 0.08 h in M / F $T_{1/2}$: 2.0 h and 2.7 in M / F $AUC_{0\text{-}inf}$: 801 and 761 ng.h/mL in M /F Bioavailability: 58 % and 60 % (total radioactivity) & 39 % and 100 % (F9600 in plasma) for M /F <u>All groups</u>: Extensive metabolism of F9600 and limited partitioning of F9600 and its metabolites into red blood cells. Less than proportional increase in exposure with dose increase from 5 to 1000 mg/kg bw indicates non-linear kinetics in rats.
Tissue distribution **Rat** (Crl :CD9(SD), males & females, 4/sex/group) GLP OECD 417 (2010) Deviations: None **[^{14}C-Phenyl]-F9600** Batch 77874-3-19; purity 100 %; specific activity 56 mCi/mmol (202.9 µCi/mg)	Single oral low dose (5 mg/kg bw) Single oral high dose (500 mg/kg bw) Multiple oral dose (5 mg/kg bw/day; 14 days)	At T_{max} (0.5 h for single and repeated low dose group and 4 h for the single high dose group), highest tissue levels in gastrointestinal (GI) tract (~58 % of AD), carcass (up to 24 % of AD), liver (~5 % of AD) and blood (~1% of AD). No indication of preferential partition into whole blood cells. No indication of selective accumulation of bixlozone or its related metabolites in any of the tissues upon high dose administration compared to low dose. No indication of accumulation of bixlozone or its related metabolites after multiple dosing compared to single dosing. No clear differences in the distribution of bixlozone or its related metabolites between males and females.

Method, Species, test substance, ***acceptability***	**Doses**	**Main findings**
F9600 technical, PL14-0163; purity 99 % Study no. FMC-P4973 [redacted] **(2017b)** ***Acceptable***		
Radioactivity concentration in plasma and bone marrow Rat (Crl :CD9(SD) 4 males GLP OECD 417 (2010) Deviations: None **[^{14}C-Phenyl]-F9600** Batch CFQ43224; purity 99.8 %; specific activity 63 mCi/mmol (228.3 µCi/mg) Study no. FMC-P7354 [redacted] **(2017f)** ***Acceptable***	Single oral low dose 500 mg/kg bw	At T_{max} (4 h), total radioactivity concentration was 153.20 µg Eq/g ± 32.46 in plasma and 49.73 µg Eq/g ± 11.94 in bone marrow. The mean bone marrow to plasma ratio was 0.33. Results provide evidence for systemic exposure, in particular exposure of rat bone marrow, at doses used in the *in vivo* rat bone marrow micronucleus assay (Section B.6.4.2).
Excretion and Metabolism Rat (Crl :CD9(SD) 4 animals/sex/group GLP OECD 417 (2010) Deviations: None of significance **[^{14}C-Phenyl]-F9600**, batch 77874-3-19; purity 100 %; specific activity 56 mCi/mmol (202.9 µCi/mg) F9600 technical, batch PL14-0163; purity 99.8 % Study no. FMC-P3887 [redacted] **(2018b)**	Single oral low dose (5 mg/kg bw) Single oral high dose (1000 mg/kg bw for M, 500 mg/kg bw for F) Multiple oral dose (5 mg/kg bw/day; 14 days)	<u>Excretion</u> M: > 90 % of the AD recovered in 7 days Major route of excretion: urine (62 – 74 % of the AD) Faeces: 21 – 34 % of the AD F: > 92 % of the AD recovered in 7 days Major route of excretion: urine (79 – 88 % of the AD) Faeces: 10 – 13 % of the AD Excretion through expired air was negligible in all dose groups in both sexes. AD recovery in tissues and carcass was minimal (day 7). Estimated oral absorption (sum of radioactivity in urine and tissues at the low dose of 5 mg/kg bw excluding faeces): **65 %** in M and 88 % in F <u>Metabolism</u> F9600 was extensively metabolised; unchanged F9600 detected at levels < 1 % of the AD in male rat urine after high dose administration only (1000 mg/kg bw). No major sex differences observed in metabolite profiles. Major metabolites identified (> 10 % of the AD in both sexes in urine after single dose at 5 mg/kg bw): 2,4-dichlorohippuric acid (12 % in M; 14.5 % in F) and 5-keto-hydrate-bixlozone (18 % in M, 24 % in F).

Method, Species, test substance, ***acceptability***	**Doses**	**Main findings**
Acceptable		Proposed main metabolic pathway in rats: hydroxylation leading to the formation 5-OH-F9600 and its derivatives. Other routes of metabolism included a combination of oxidation, decarboxylation and deamination followed by conjugation of oxidative derivatives.
Excretion and Metabolism (pilot study) Rat (Crl :CD9(SD) 2 animals/sex/group Not to GLP OECD 417 (2010) Deviations: N/A **[^{14}C-Carbonyl]-F9600**, batch CFQ42018; purity 99.9 %; specific activity 59 mCi/mmol (213.8 µCi/mg) F9600 technical, batch G3773:62 (PL14-0163); purity 99.5 % Study no. FMC-R3449 [redacted] **(2018a)** ***Supplementary only***	Single oral low dose (5 mg/kg bw)	<u>Excretion</u> M: 91 % of the AD recovered in 7 days. Major route of excretion: urine (67 % of the AD) Faeces: 22 % of the AD F: > 94 % of the AD recovered in 7 days Major route of excretion: urine (74 % of the AD) Faeces: 17 % of the AD Excretion was rapid. Excretion through expired air was low for both sexes. AD recovery in tissues and carcass was minimal (day 7). <u>Metabolism</u> F9600 was extensively metabolised; unchanged F9600 was not detected in urine. No major sex differences observed in metabolite profiles. Major metabolites identified (> 10 % of the AD in both sexes in urine): carbamic acid (16 % in M; 22 % in F) and 5-keto-hydrate bixlozone (26 % in M, 23 % in F).
Excretion and Metabolism Rat (Crl :CD9(SD) 4 animals/sex/group GLP OECD 417 (2010) Deviations: None of significance **[^{14}C-Carbonyl]-F9600**, batch CFQ42476; purity 99.9 %; specific activity 59 mCi/mmol (213.8 µCi/mg)	Single oral low dose (5 mg/kg bw)	<u>Excretion</u> M: > 98 % of the AD recovered in 7 days. Major route of excretion: urine (62 % of the AD) Faeces: 34 % of the AD F: > 93 % of the AD recovered in 7 days Major route of excretion: urine (76 % of the AD) Faeces: 16 % of the AD Excretion was rapid for both sexes (> 88 % of the AD recovered after 48 h). Excretion through expired air was low for both sexes. AD recovery in tissues and carcass was minimal (day 7). <u>Metabolism</u> F9600 was extensively metabolised; unchanged F9600 was not detected in urine. No major sex differences observed in metabolite profiles. Major metabolites identified (> 10 % of the AD in both sexes in urine): carbamic acid (10 % in M; 18 % in F) and 5-keto-hydrate bixlozone (17 % in M, 23 % in F).

Method, Species, test substance, ***acceptability***	**Doses**	**Main findings**
F9600 technical, batch PL G3773-17; purity 99.5 % Study no. FMC-P4547 [redacted] **(2017c)** ***Acceptable***		Proposed main metabolic pathway in rats: the dimethylisoxazolidin-3-one ring moiety of bixlozone was the most susceptible site of metabolism in rats, with the phenyl ring remaining relatively well conserved. Combination of various metabolic reactions (oxidation, ring-scission, decarboxylation) lead to metabolites including oxidative ring-opened analogues and ring-cleaved analogues. The phase I metabolites, produced by various metabolic pathways, are subsequently conjugated as glucuronides in the urine.
Mass balance – bile cannulated rats Rat (Crl :CD9(SD) 5 males Not to GLP – OECD 417 (2010) Deviations: None of significance **[^{14}C-Phenyl]-F9600**, batch 77874-3-19; purity 100 %; specific activity 56 mCi/mmol (202.9 µCi/mg) F9600 technical, batch PL G3773-17; purity 99.5 % Study no. FMC-P5709 [redacted] **(2017d)** ***Not relied upon** since as IV administration was used instead of oral administration, extrapolation of the biliary excretion data to the oral route does not seem appropriate as kinetics (and in particular biliary excretion) following IV administration are likely to be different from those following oral administration.*	IV low dose (3 mg/kg bw)	Excretion M: > 90 % of the AD recovered within one day. Major route of excretion: urine (52 % of the AD) Faeces: 40 % of the AD Excretion was rapid for both sexes (> 88 % of the AD recovered after 48 h). Excretion through expired air was low for both sexes. AD recovery in tissues and carcass was minimal (day 7). Metabolism F9600 was extensively metabolised; unchanged F9600 was not detected in urine. Around 1 % of the AD was excreted through faeces, therefore gastric secretion was not significant. Predominant metabolite in bile: 5-OH-bixlozone-glucuronide (42 %) Predominant metabolite in urine: 5-OH-bixlozone-glucuronide (20 %) Proposed main metabolic pathway in rats: combination of oxidation (hydroxylation), ring-opening, and glucuronidation of oxidative products. No metabolite unique to the method employed in this study has been identified.

Method, Species, test substance, ***acceptability***	**Doses**	**Main findings**
In vitro **comparative interspecies metabolism (first study)** Mouse, rat, dog and human hepatocytes (males, females) Not to GLP however the scientific validity of such a qualitative study design is not compromised therefore this study is acceptable for regulatory purposes. **[^{14}C-Phenyl]-F9600**, batch 77874-3-19; purity 100 %; specific activity 56 mCi/mmol (202.9 µCi/mg) **[^{14}C-Carbonyl]-F9600**, Batch CFQ42476; purity 99.9 %; specific activity 59 mCi/mmol (213.8 µCi/mg) F9600 technical, batch PL G3773-17; purity 99.5 % Study no. FMC-R4547 [redacted] **(2017e)** ***Acceptable***	20 µM	[^{14}C]-bixlozone (phenyl and carbonyl) virtually completely metabolised after incubation for 4 hours in rat and dog hepatocytes. Metabolisation about 56-69 % in mouse hepatocytes and 62-86 % in human hepatocytes. Common metabolic reactions in all species: oxidation (hydroxylation) and conjugation (glucuronidation); the metabolic pathways drawn from the metabolism of bixlozone in hepatocytes are similar to those identified in rats after oral administration of [^{14}C]-bixlozone. No unique or label-specific metabolite was identified in human hepatocytes however a disproportionate production of 4-OH-Me-bixlozone was observed in human hepatocytes compared to the other species, especially the rat and dog where it was not detected. In the mouse, levels 5-8-times lower were measured. No significant sex differences or label specific metabolites were observed in human samples.
In vitro **comparative interspecies metabolism (second study)** Mixed-sex mouse, rat, dog and human hepatocytes GLP **[^{14}C-Phenyl]-F9600,** batch CFQ43508; purity 99.3 %; specific activity 61 mCi/mmol **[^{14}C-Carbonyl]-F9600, b**atch CFQ43509; purity 99.6 %; specific activity 56 mCi/mmol **F9600 technical,** batch PL14-0163; purity 99.8 %. Study no. FMC-53482	20 µM	[^{14}C]-bixlozone (phenyl and carbonyl) virtually completely metabolised after incubation for 4 hours in dog hepatocytes. Metabolisation about 72-87 % of the AR in the rat, 86-92 % in mouse hepatocytes and 49-51 % in human hepatocytes. Common metabolic reactions in all species: oxidation (hydroxylation) and conjugation (glucuronidation); the metabolic pathways drawn from the metabolism of bixlozone in hepatocytes are similar to those identified in rats after oral administration of [^{14}C]-bixlozone. No unique or label-specific metabolite was identified in human hepatocytes however a disproportionate production of 4-OH-Me-bixlozone was observed in human hepatocytes compared to the other species, especially the rat.

Method, Species, test substance, *acceptability*	Doses	Main findings
[redacted] (2020) *Acceptable*		

Absorption

The extent of absorption of total radioactivity in rats administered [^{14}C-U-Phenyl]-bixlozone or [^{14}C-Carbonyl]-bixlozone orally, measured in terms of percent excretion in urine of rats, was relatively high at ≈ **60-70** % of the administered dose (AD) in males and ≈ 80-90 % AD in females, with no significant differences in excretion pattern observed following low (single or repeated dosing) and high oral administration ([redacted] (2016) & [redacted] (2018b)). Comparing the oral and IV AUCs obtained from the pharmacokinetics study ([redacted] (2016)), it was shown that the bioavailability of total radioactivity was higher in females (86 %) compared to males (**70 %**) following low oral dosing. A possible saturation of absorption was observed following high dose administration with lower bioavailability values retrieved at 1000 mg/kg bw in males and females (58 % and 60 % respectively) and a less than proportional internal exposure to dose in plasma and blood in both sexes, suggesting non-linear kinetics in the rat ([redacted] (2017b)). No accumulation was observed in plasma following repeated dosing in both sexes.

Oral absorption was rapid following single or repeated oral low dose (5 mg/kg bw) with C_{max} reached in less than an hour post-dosing for both sexes. The rate of oral absorption was slower following oral high dose (1000 mg/kg bw), with C_{max} reached between 15-24 hours post-dosing ([redacted] (2016)).

A bile-cannulation study is available ([redacted] (2017d)), but this has been conducted following IV dosing. As the kinetics (and in particular the biliary excretion) of bixlozone following IV administration are likely to be different from those following oral administration, HSE is of the view that the biliary and urinary excretion profiles determined from this study cannot be directly extrapolated to the oral route.

Overall, therefore, taking into account the pharmacokinetics study where an oral bioavailability value of 70% was estimated comparing the IV and oral AUCs, **HSE proposes an oral absorption value and an oral systemic availability value of 70 %**. Although there no data available to determine the absorption of bixlozone and/or its metabolites across the respiratory tract, a default inhalation absorption value of 100 % is proposed based on the extensive oral absorption observed in the rats. The dermal absorption potential of bixlozone from its representative product is addressed in the CP-B6 document.

Distribution

Available toxicokinetics data showed that the plasma $AUC_{0\text{-}inf}$ levels of unchanged bixlozone in the IV dose group were only 2-3 % of the total radioactivity, indicating extensive metabolism of bixlozone ([redacted] (2016)). Limited information on plasma concentrations of bixlozone from repeated-dose and long-term toxicodynamic studies conducted in rats, mice and dogs confirmed there were low levels of unchanged bixlozone in blood and plasma following repeated exposure (Section B.6.3 & B.6.5).

Following oral administration bixlozone and its metabolites were widely distributed in all rat tissues by the T_{max}, and the distribution was similar between sexes ([redacted] (2017b)). Among all tissues analysed, the GI tract accounted for about 60 % of the adminisered dose, followed by the carcass (up to 24 %), the liver (~5 %) and blood (~1 %). There was no indication of accumulation of radioactivity following repeated dosing.

Metabolism

In vivo, bixlozone was extensively metabolised in rats following single, high or multiple oral gavage doses, resulting in rapid and extensive excretion via urinary, bile and faecal routes; low levels, if any, of unchanged bixlozone were noted in the urine and faeces from all dose groups ([redacted] (2018b) & [redacted] (2017c)).

The major metabolites identified in urine in both sexes were carbamic acid (identified using [^{14}C-Carbonyl]-bixlozone), 2,4-dichlorohippuric acid (identified using [^{14}C-Phenyl]-bixlozone) and 5-keto-hydrate-bixlozone (glucuronide) (identified using [^{14}C-Phenyl]-bixlozone or [^{14}C-Carbonyl]-bixlozone). Minor metabolites identified in urine were dihydroxy-isobutyramide-bixlozone, bixlozone-dehydro-malonamide, bixlozone-dimethyl-malonamide,

4-hydroxy-methyl-bixlozone, 4-carboxy-methyl- bixlozone, 5-keto bixlozone, bixlozone-cysteine derivative and 5-hydroxy-bixlozone (glucuronide).

Based on the metabolites identified in urine and faeces, it is proposed that the dimethyl-isoxazolidin-3-one moiety of bixlozone is the most susceptible site for metabolism in rats. A combination of reactions including oxidation, reduction, decarboxylation, ring opening/cleavage, and deamination lead to extensive metabolism of bixlozone and the formation of a variety of metabolites. Several of the metabolites are subjected to conjugation with glucuronic acid for subsequent excretion in urine. The metabolites found in faeces were primarily unconjugated and could have been derived from hepatic and/or intestinal metabolism of bixlozone.

In a non-GLP comparative *in* vitro metabolism study using rat, human, mouse and dog cryopreserved hepatocytes, bixlozone was virtually completely metabolised after incubation for 4 hours in rat and dog hepatocytes, whilst the extent of bixlozone conversion to metabolites was about 56-69 % of the applied radioactivity (AR) in mouse hepatocytes and 62-86 % of the AR in human hepatocytes ([redacted] (2017e)). No unique or label-specific metabolite was identified in human hepatocytes; however, a disproportionate production of 4-OH-Me-bixlozone was observed in human hepatocytes compared to the mouse (5-8-times higher), with none detected in the rat and dog. No significant sex differences or label specific metabolites were observed in human samples.

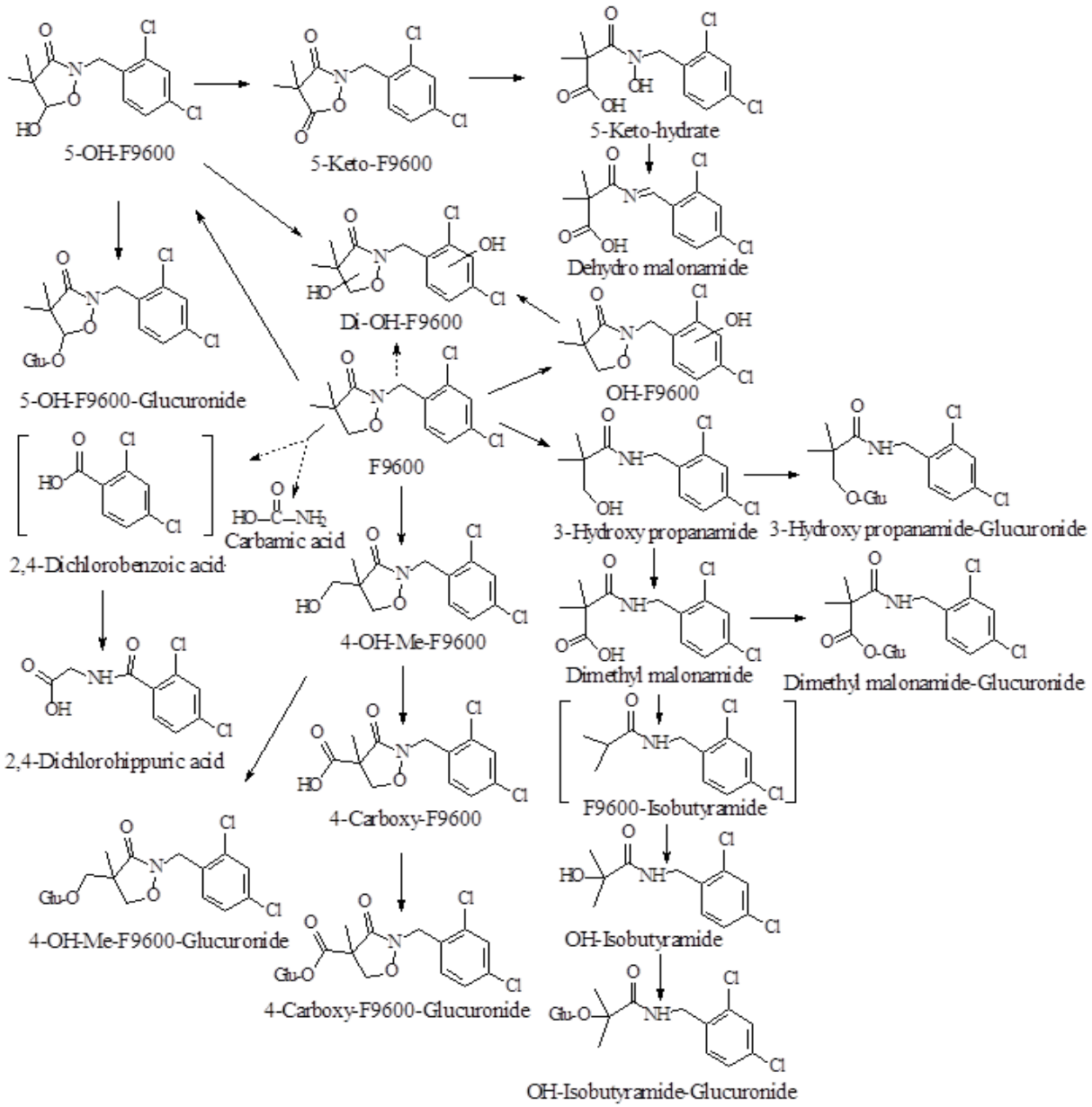
To address the reliability and toxicological significance of this disproportionate production of 4-OH-Me-bixlozone, the applicant submitted a second study (GLP compliant) and used hepatocytes from the same species selected for the first study; however mixed-sex hepatocytes were used instead of separated male and female hepatocytes ([redacted] (2020)). The findings from this study were broadly similar to the previous study and confirmed the disproportionate production of 4-OH-Me-bixlozone in human hepatocytes. Thus, the applicant provided HSE further information to evaluate the toxicological relevance of this finding. *In silico* genotoxicity comparative analysis, structural similarity analysis and a comparison of the physical-chemical properties of 4-OH-Me-bixlozone with those of the parent compound indicate the metabolite has a comparable toxicity profile to that of bixlozone. *In vivo* rat studies also showed the metabolite is rapidly eliminated in urine through oxidation to 4-COOH-Me-bixlozone and glucuronidation, suggesting that the metabolite is most likely less toxic than the parent substance. Therefore, the disproportionate production of this metabolite in human hepatocytes compared to the rat, the primary test species, is unlikely to lead to additional toxic effects beyond those already identified in the tested species as its toxicity profile is comparable to (and possibly less toxic than) that of the parent substance which has been fully tested in model experimental animals.

Lastly, due to the finding of the residues 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in plants, and the way that these metabolites feature in the livestock metabolism studies (hen and goat; Section B.7.2), it was important to establish whether both residues had been detected in the rat metabolism studies.

In the rat metabolism studies ((Sections B.6.1.1.5 & B.6.1.1.6)), more than 40 different metabolites were observed in urine samples, with fewer metabolites retrieved in faeces samples following single or multiple oral doses ([redacted] (2018b)). Several metabolites were detected in minor to trace amounts (< 2 % of the AD) and few unknown metabolites (RP2, RP5, RP10, and RP28) present at levels ≤ 3 % of the AD were observed in the radio-chromatograms of urine samples; the structures of these metabolites could not be identified by the LC/MS method used in the study. Thus, there were some metabolites at low levels that were not identified, however they did not actively seek M118/1 and M132/1. When comparing the metabolic pathways identified in the rat (figure B 6.1.4.1) with those identified in the goat (figure 2.7.2.5 Section 2.7.2) and hen (figure 2.7.2.6 Section B.2.7.2), it appears that the goat and hen metabolism profiles are subsets of what is occurring in the rat. No unique metabolite paths have been identified in the goat or the hen compared to the rat. Therefore the **livestock (goat, poultry) and rat metabolism pathways are considered qualitatively similar**. Hence, it is possible that either residues M118/1 and M132/1 were present in the rat samples but were not identified, or that qualitative differences in metabolite profiles between the three species considered (goat, poultry, rat) are in play to explain the interspecies variation highlighted. Thus, although they were not identified in the rat it is likely that both metabolites could be formed in the rat.

The proposed metabolic pathways of bixlozone in rats are presented in the figure below:

Figure 2.6.1.1: Proposed metabolic pathways of bixlozone in the rat



Elimination

Excretion after a low oral dose was rapid with 83-97 % of the administered dose (AD) being excreted within 48 hours via the urine and faeces, with a higher elimination rate in females ([redacted] (2018b)). Although the initial rate of excretion was slightly slower in rats that received the high oral dose (69-72 % AD within 48 hours), the excretion pattern was similar between the low and high dose groups. No significant label specific differences in excretion patterns were evident. In non-bile cannulated rats, urinary excretion was relatively high (64-88 % of the AD with the phenyl label and 62-76 % of the AD with the carbonyl label), with faecal elimination accounting for 11-27 % and 16-34 % of the AD for the phenyl and carbonyl label respectively. Elimination in expired air was very low with both labels. Biliary excretion was determined in bile cannulated rats following IV administration; however, HSE is of the view that the calculated value cannot be directly extrapolated to the oral route.

Proposed residue definition for monitoring purposes in body fluids and tissues

The applicant proposed to include the metabolite 5-hydroxy-bixlozone as the only marker for monitoring purposes in body fluids and tissues on the basis that it is mostly detected as a conjugate (glucuronide) form in rats, although a portion of unconjugated metabolite may circulate as well.

The proposal was considered further by HSE. Regarding the detection of metabolite 5-hydroxy-bixlozone in rats administered bixlozone, the available ADME data showed that this metabolite was mainly found in faeces samples with very low levels in urine samples; therefore systemic exposure is unlikely to be significant. This metabolite was also mainly present in urine in its conjugated form. In addition, the *in vitro* comparative metabolism studies showed that 5-hydroxy-bixlozone (unconjugated form) is not detected in human hepatocytes (males & females) whilst levels above 10 % of the applied radioactivity were reported in rat hepatocytes in both sexes. Thus, the available data suggest that in vivo human urine / blood samples may not contain 5-hydroxy-bixlozone.

Therefore, HSE is of the opinion that 5-hydroxy-bixlozone is not a suitable marker for monitoring purposes according to the data requirements of Regulation (EU) 283/2013. In addition, the inclusion of glucuronide and sulfate conjugates in the residue definition would hinder the monitoring process because of the need for methods requiring conjugate hydrolysis. Furthermore, it is understood that an analytical method is only validated for this metabolite for animal tissues but not for body fluids; therefore the recommendations of SANCO/825/00 rev. 8.1 (16/11/2010) are not fulfilled.

HSE propose as an alternative, to include the metabolite 5-keto-hydrate-bixlozone in the residue definition, based on the fact that 5-keto-hydrate-bixlozone, a downstream metabolite of 5-hydroxy-bixlozone, is a major metabolite consistently found at high levels in its unconjugated form in rat urine samples in both sexes. Moreover, it is also consistently found in abundance in all male and female species in vitro including in human hepatocytes samples and is not observed in the *in vivo* and *in vitro* samples in its conjugated form. The applicant agreed with the HSE proposal.

Therefore, the metabolite 5-keto-hydrate-bixlozone is considered to be a relevant analyte identified in the toxicological database and is suitable as a typical marker to be included in the residue definition for the monitoring of body fluids and tissues.

A validated analytical method for analysis of bixlozone (parent) and the marker metabolite 5-keto-hydrate-bixlozone in body fluids (plasma and urine) and tissues (liver) is available. Therefore, the data requirements of Regulation (EU) 283/2013 have been met.

2.6.2. Summary of acute toxicity

The acute toxicity of bixlozone was investigated *in vivo* via the oral, dermal and inhalation routes. The skin irritating potential of bixlozone was investigated in the *in vitro* skin irritation test (SIT) using the Epiderm™ skin model (OECD Guideline 439) and in the *in vivo* study in rabbits (OECD Guideline 404). Two studies were conducted to investigate the eye irritating potential of bixlozone: the *in vitro* Epiocular™ eye irritation test (OECD Guideline 492), aiming to identify test items not classified for eye irritation / damage, and the *in vivo* study in rabbits. The skin sensitisation potential of bixlozone was evaluated in the LLNA up to the maximum attainable concentration of 25 % w/w. Bixlozone showed no significant absorption of electromagnetic radiation above 290 nm and the ultraviolet/visible molar extinction/absorption coefficient of the substance was less than 10 L × mol $^{-1}$ × cm $^{-1}$; thus no *in vitro* phototoxicity testing is required.

All of the studies mentioned above were conducted according to standard OECD Test Guidelines and were GLP compliant. Bixlozone was shown to be of low acute toxicity via all the routes tested and thus no classification according to Regulation GB/NI Nº 1272/2008 is required for these endpoints. It was also demonstrated that bixlozone was not a skin or eye irritant and not a skin sensitiser according to CLP Criteria. A phototoxicity test is not required.

The table below provides an overview of the available acute toxicity studies.

Table 2.6.2.1: Summary of bixlozone acute toxicity data, with classification according to Regulation GB/NI No 1272/2008

Study and acceptability	Result	Reference	Classification according to Reg. GB/NI No 1272/2008
Acute oral, rat ***Acceptable***	Oral LD_{50} > 2000 mg/kg bw	[redacted] (2014a)	No classification
Acute dermal, rat ***Acceptable***	Dermal LD_{50} > 2000 mg/kg bw	[redacted] (2014b)	No classification
Acute inhalation, rat ***Acceptable***	Inhalation 4hr LC_{50} LC_{50} > 2.11 mg/L (maximum attainable concentration)	[redacted] (2014c)	No classification
Skin irritation, *in vitro* (Epiderm™ skin model) ***Acceptable***	Not a skin irritant	Costin, GE. (2018)	No classification
Skin irritation, rabbit ***Unnecessary and not relied upon***	Slightly irritating; cleared within 72 hrs; insufficient for classification	[redacted] (2014d)	No classification
Eye irritation *in vitro* (Epiocular™ eye model) ***Supplementary. Potential false positive with a requirement for further testing'***	Eye irritant	Wilt, N. (2018)	Not applicable
Eye irritation, rabbit ***Acceptable***	Minimal eye irritation observed, cleared within 24 hrs	[redacted] (2014e)	No classification
Skin sensitization, mice (LLNA) ***Acceptable***	Negative up to 25% w/w (maximum attainable concentration)	[redacted] (2014f)	No classification
Phototoxicity test ***Not required***	Not applicable	Not applicable	Not applicable

2.6.3. Summary of short-term toxicity

The short-term oral toxicity of bixlozone has been extensively investigated in GLP and OECD guideline compliant repeated-dose toxicity studies in rats, mice and dogs following 28- and 90-days' dietary exposure; a 12-month oral (capsule) study conducted in dogs is also available. This study, which is no longer required in Reg 283/2013, is considered acceptable and is relied upon as it has been used by HSE for a WoE assessment of the short-term toxicity of bixlozone. Moreover, 7-day palatability studies have been conducted in rats, mice and dogs; these studies are not GLP or OECD compliant, however they are reported in this Section as supplementary information. Considering other routes of exposure, a 21-day dermal study in rats is available for bixlozone. Further information on the short-term oral toxicity of bixlozone can also be extracted from the 2-generation reproductive toxicity study (see Section B.6.6.) and from the long-term toxicity studies (see Section B.6.5) conducted in rats and mice and have been taken into consideration in this summary.

The liver has been identified as a clear target organ in all species investigated: there were increases in relative and absolute liver weights accompanied in some instances with minimal to moderate hepatocellular hypertrophy. The toxicological significance of the effects on the liver has been assessed by HSE using a weight-of-evidence approach (WoE), with a clear distinction being made between effects that are clearly adverse and those which are potentially adaptive. This assessment has been carried out in line with the Technical Agreements for Biocides (TAB) entry,

agreed at the Biocide Working Group-IV-2018 meeting (WGIV2018_TOX_6-2); this paper describes a WoE approach for the evaluation of liver effects in repeated-dose toxicity studies based on several international reviews on liver effects (JMPR 2006 and 2015). 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 alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin or cholesterol). In line with the TAB entry, relative liver weight increases up to 15 %, that are not accompanied by other signs of liver dysfunction, have been considered by HSE to be an adaptive rather than an adverse response in the evaluation of the liver effects of bixlozone (see table below).

By means of these criteria it can be seen that the effects exerted upon the liver by bixlozone become adverse in the rat at 150 mg/kg bw/day (females, 90-days' exposure), at 583 mg/kg bw/day in the mouse (females, 90-days' exposure) and at 100 mg/kg bw/day in the dog (females, 90-days' exposure).

It would appear that the rat and the dog are more sensitive than the mouse to the liver effects of bixlozone, and that the female is the most sensitive sex across all species. These findings are generally concordant with the toxicokinetic evaluations which showed that systemic exposure was higher in female rats compared to male rats (the top dose was indeed set lower for females in the 90-day rat study) but was greater in male mice compared to female mice (please refer to Section B.6.1.1.3**Error! Reference source not found.** for more details). Furthermore, the effect on liver weights and histopathological incidence and severity did not appear to increase to any great extent with the duration of treatment in any species, which is supported by toxicokinetics evidence indicating that bixlozone and its metabolites did not accumulate in plasma or tissues following 14 days repeated dosing (Section B.6.1.4**Error! Reference source not found.** Summary of ADME studies).

Table 2.6.3.1: Summary of the liver effects of bixlozone observed after dietary repeated exposure in the rat, mouse and dog

Species	Sex	Duration of exposure	Dose at which effects become adverse (mg/kg bw/day)	Increase in relative weight at this dose (%)	Hepatocellular hypertrophy	Other adverse histopathological or biochemical findings
Rat	Male	28 days	182	15.5	3/5	None
Rat	Female	28 days	193	17	4/5	None
Rat	Male	90 days	505	37	10/10	↑ cholesterol, protein and calcium
Rat*	**Female**	**90 days**	**150**	**17**	**1/10**	**↑ cholesterol, protein and calcium**
Rat	Male F_0	2-generations (reproductive)	140	19	None	None
Rat	Female F_0	2-generations (reproductive)	187	21	18/25	None
Rat	Male F_1	2-generations (reproductive)	140	14	None	None
Rat	Female F_1	2-generations (reproductive)	187	21	20/25	None
Mouse	*Male*	*28 days*	*> 985*	*13*	*4/5*	*None*
Mouse	Female	28 days	984	21.5	2/5	None
Mouse	Male	90 days	930	23	10/10	None
Mouse*	**Female**	**90 days**	**583**	**17.5**	**3/9**	**None**
Dog	Male	90 days	750	20	2/4	None
Dog*	**Female**	**90 days**	**100**	**22**	**None**	**None**
Dog	Male	12 months	> 500	10	None	None
Dog	Female	12 months	> 500	10	None	None

* Lowest dose identified in the species for adverse liver effects

The kidney was also identified as a clear target organ in rats and dogs (but not in mice); increased kidney weights were observed in rats and dogs, with the rat being the more sensitive species and the male the more sensitive sex.

Rat

In the rat, the main target organs of toxicity identified were the liver and kidney. Additional effects were seen in the thyroid, prostate and uterus.

Adverse effects on the liver

Adverse increased liver weights (> 15 % compared to controls, with or without hepatocellular hypertrophy) were seen from 182 / 193 mg/kg bw/day (males / females) in the 28-day study ([redacted] (2015a)) and from 150 mg/kg bw/day (females) in the 90-day study ([redacted] (2016a)). In addition, similar liver effects were seen from ≈ 180 / 220 mg/kg bw/d (mean dose males / females) in the 2-generation study ([redacted] (2016c)) and at the top dose of 217 / 176 mg/kg bw/day (males / females) in the 2-year carcinogenicity study ([redacted], 2017) These liver effects were associated with alterations of some clinical-chemistry parameters indicative of liver toxicity (e.g. increased cholesterol, blood urea nitrogen (BUN), triglycerides) from 379 mg/kg bw/day (females) in the 28-day study, from 150 mg/kg bw/day (females) in the 90-day study and at the top-dose of 167 mg/kg bw/day in females in the 2-year carcinogenicity study.

Adverse effects on the kidney

Regarding adverse effects on the kidney, there were no histopathological or biochemical signs of adversity related to the kidney; however increased weights (absolute & relative to body weights > 10 % compared to control groups) indicative of an adverse effect were noted after 90 days' exposure from 121 mg/kg bw/day in males and 351 mg/kg bw/day in females; it was also noted that the relative weights remained high following 28 days of recovery. The effects on kidney weights after 28-days exposure were less pronounced. Treatment-related and adverse changes in kidney weights relative to body weight were also noted in the 2-generation reproductive toxicity study at the top dose in the F_0 generation (141 / 261 mg/kg bw males / females) and in the F_1 generation (140 / 187 mg/kg bw males / females). In contrast to these short-term studies, there were no adverse effects noted for the kidneys (including kidney

weights) in the 2-year carcinogenicity study at weeks 52 and 104 in both sexes up to the top-dose of 217 / 167 mg/kg bw/day (males / females). Overall, there were consistent adverse effects on kidney weights in both sexes in the rat short-term studies.

Other findings

Mild follicular cell hypertrophy of the thyroid was observed at the top dose of 505 / 351 mg/kg bw/day (males / females) in the 90-day study without associated changes in thyroid weights; no such finding was seen following a recovery period of 28 days. However there were no clear thyroid weight or histopathology changes noted in the 28-day study or the 2-year year carcinogenicity study. Females showed a slight increase in the incidence of follicular cell adenoma (benign tumours) in the thyroid gland at the top-dose of 167 mg/kg bw/day however these tumour findings were regarded as chance findings unrelated to treatment (B.6.5.1**Error! Reference source not found.**). Overall there were no clear adverse effects on the thyroid in the rat following repeated administration of bixlozone.

In addition, there was increased prostate inflammation at the top dose of 140 mg/kg bw/d in the rat 2-generation study. The toxicological significance of this finding on reproductive organs is discussed further in the summary of the reproductive toxicity section.

Furthermore, systemic toxicity characterised by decrease in body weight and/or body weight gain were observed in females from 193 mg/kg bw/day in the 28-day study (and at 740 mg/kg bw/day for males), at the top-dose of 351 / 505 mg/kg bw/day (males / females) in the 90-day study, at the top dose of 167 / 217 mg/kg bw/day in the 2-year carcinogenicity study and at the top dose in the F_0 generation (141 / 261 mg/kg bw males / females) and in the F_1 generation (140 / 187 mg/kg bw males / females) in the 2-generation reproductive toxicity study.

Female rats were more sensitive than males; this is consistent with the indication that females are more highly exposed to bixlozone than males since parallel toxicokinetics investigations showed higher concentrations of bixlozone in females' blood compared to males.

Mouse

In the mouse, the main target organ of toxicity was the liver. There were no adverse effects noted on the thyroid. Additional effects on kidney, epididymes and stomach were noted following chronic exposure.

Adverse effects on the liver

Increased liver weights with associated histopathology (enlarged individual hepatocytes with expanded eosinophilic cytoplasm) were seen at the top-dose of 984 mg/kg bw/day (females only) in the 28-day study ([redacted] [redacted] (2015b)), 930 / 583 mg/kg bw/day (males / females) in the 90-day study ([redacted](2016b)), and 647 / 834 mg/kg bw/day (males / females) in the 18-month carcinogenicity study ([redacted] (2017)). These effects were only associated with alterations of some clinical-chemistry parameters indicative of liver toxicity (e.g. increased ALT) at the top dose of 985 mg/kg bw/day (males) in the 28-day study. The adverse effects on the liver seen in the mouse occur at higher dose levels than the adverse effects observed in the rat.

Other findings

On chronic exposure, decreased sperm in the epididymes and chronic inflammation of the glandular stomach were seen in males from the mid dose of 126 mg/kg bw/day (1000 ppm), with kidney pelvis dilation noted in males at the top dose of 647 mg/kg bw/day (5000 ppm). Despite the uncertainties in these findings (sex-specificity, low biological plausibility), no robust argumentations (including appropriate historical control data (HCD)) have been provided by the applicant to discount their toxicological significance. The relevance of the reduced epididymal sperm counts observed in the 18-month chronic study is discussed further in the summary of the reproductive toxicity section.

In addition to the toxic effects seen in the liver and these other organs, decreases in body weight and/or body weight gain were observed in females only at the top-dose of 1384 mg/kg bw/day in the 28-day study and in the carcinogenicity study at the top dose of 834 mg/kg bw/day.

Dog

In the dog, the main target organ of toxicity identified was the liver. Additional effects were seen in the prostate and WBC.

Adverse effects on the liver

Regarding adverse effects seen in the liver, increased absolute and relative liver weights to body weight with associated hepatocellular hypertrophy was observed in both sexes from 370 / 309 mg/kg bw/day (males / females) in the 28-day (oral, dietary) range-finding study ([redacted](2016b)). In the following 90-day study ([redacted](2017)), the method of oral administration was changed from dietary to capsule owing to palatability issues noted in the 7-day ([redacted]., (2015c)) and 28-day studies; in this study increased absolute and relative liver weights to body weight

were seen from 100 mg/kg bw/day in females and at the top-dose of 750 mg/kg bw/day in males, accompanied with minimal hepatocellular hypertrophy in males only. However, no liver-related adverse effects were noted in the 12-month (oral, capsule) study up to the top dose of 500 mg/kg bw/day. Overall the dog appears to be relatively less sensitive to the toxic effect of bixlozone on the liver compared to the rat.

Other findings

Thyroid weight was increased at the top dose of 750 mg/kg bw/day in females and from 300 mg/kg bw/day in males in the 90-day study, but no associated histopathology was seen. The thyroid was not affected in the 28-day study up to the top dose of approx. 1340/1080 mg/kg bw/day (M/F) or in the 1-year study up to 500 mg/kg bw/day. It is most likely these changes in thyroid weight are a spurious finding.

Changes in kidney weights were seen from 38 mg/kg bw/day in males in the dietary 28-day study. However, these changes were not reproduced after 90 days (up to 750 mg/kg bw/day) or 1 year treatment (up to 500 mg/kg bw/day) using capsules. It is possible that the kidney weight changes seen in the 28-day study were the consequence of the method of administration (dietary vs capsules) and associated severe toxicity due to palatability problems rather than the test substance itself. In addition, in the absence of any associated histopathology or changes in clinical-chemistry and urinalysis parameters indicative of kidney toxicity, these kidney weight changes are regarded as spurious findings.

Haematological changes (such as white blood cell (WBC), prothrombin time (PT), large unstained cell (LUC) and lymphocytes absolute) were also observed in females at the top-dose of 750 mg/kg bw/day in the 90-day study and in males at 500 mg/kg bw/day in the 12-month study.

Reductions in prostate weight with associated immaturity were seen in the 90-day study from 300 mg/kg bw/day, but not up to 500 mg/kg bw/day in the 1-year study. On this basis, these prostate findings are considered to be of minimal toxicological significance. The prostate findings in the dog are discussed further in the summary of the reproductive toxicity section.

In addition to toxic effects noted in the organs above, body weight and body weights gain were severely affected in dogs after 28 days' dietary exposure due to palatability issue with the test substance. Hence the mode of administration of bixlozone for the 90-day and 12-month studies was changed from dietary to capsule; following this change there was no effects seen in body weight or the body weight gain at any dose tested for both sexes.

A table summarising the main adverse effects observed in the repeated-dose toxicity studies of bixlozone is presented below:

Table 2.6.3.2: Summary of repeated-dose toxicity of bixlozone

Method, Species, test substance ***Acceptability***	**Doses**	**NOAEL**	**Main adverse effects**
Dietary 7-day **Rat** (Crl :CD9(SD), males & females, 5/sex/group) Not to GLP Not to OECD Guideline Deviations: None F9600 technical, batch PL13-0385 Purity: 99.2% ([redacted], (2015d) ***Supplementary***	0, 4000, 7000 and 12000 ppm Equivalent to : Males: 0, 441, 698 and 1067 mg/kg bw/day Females: 0, 434, 763 and 1250 mg/kg bw/day	No robust NOAEL can be derived from this non-GLP, non-OECD compliant study. *< 4000 ppm (441 / 434 mg/kg bw/day males / females)* Based on relative liver weight increases > 15 % in both sexes observed in both sexes at 4000 ppm	There were no reported deaths. **12000 ppm** ↓ body weight, body weight gain and/or food consumption (both sexes) ↑ relative liver weight > 15 % in both sexes **7000 ppm** ↓ body weight, body weight gain and/or food consumption (M) ↑ relative liver weight > 15 % in both sexes **4000 ppm** ↑ relative liver weight > 15 % in both sexes

Method, Species, test substance *Acceptability*	**Doses**	**NOAEL**	**Main adverse effects**
Dietary 7-day **Mouse** (Crl:CD-1(ICR)), males & females, 5/sex/group) Not to GLP Not to OECD Guideline Deviations: None F9600 technical, batch PL13-0385 Purity: 99.2% ([redacted]., (2015c) ***Supplementary***	0, 2000, 4000 and 6000 ppm Equivalent to : Males: 0, 404, 960 and 1348 mg/kg bw/day Females: 0, 476, 886 and 1460 mg/kg bw/day	No robust NOAEL can be derived from this non-GLP, non-OECD compliant study. *< 2000 ppm (404 / 476 mg/kg bw/day males / females)* Based on relative liver weight increases > 15 % in both sexes and reduced body weight / gain observed in both sexes	There were no reported deaths or clinical signs of toxicity. **6000 ppm** ↓ body weight gain (F): 42 % ↓ body weight (M): 11 % ↑ relative liver weight > 15 % in both sexes **4000 ppm** ↑ relative liver weight > 15% in both sexes **2000 ppm** ↑ relative liver weight > 15% in both sexes
Dietary 7-day **Dog** (Beagle), males & females, 2/sex/group) Not to GLP Not to OECD Guideline Deviations: None F9600 technical, batch PL13-0385 Purity: 99.2% ([redacted], (2015e) ***Supplementary***	0, 2500, 5000, 10000 and 30000 ppm Equivalent to : Males: 0, 67, 185, 292 and 818 mg/kg bw/day Females: 0, 79, 187, 244 and 716 mg/kg bw/day	No robust NOAEL can be derived from this non-GLP, non-OECD compliant study. *10000 ppm (292 / 244 mg/kg bw/day males / females)*	**30000 ppm** ↓ body weight & food consumption (first 3 days). **2500, 5000, 10000 ppm** No adverse effects observed.
Oral (capsule) 7-day **Dog** (Beagle), males & females, 2/sex/group) Not to GLP Not to OECD Guideline Deviations: None F9600 technical, batch PL14-0049 Purity: 96.0% ([redacted], (2016b) ***Supplementary***	0, 150, 350 and 550 mg/kg bw/day	*> 550 (males / females)* No robust NOAEL can be derived from this non-GLP, non-OECD compliant study.	No treatment-related findings were observed during the study period up to the highest dose tested.

Method, Species, test substance ***Acceptability***	**Doses**	**NOAEL**	**Main adverse effects**
28 day, dietary **Rat,** Crl :CD9(SD), males & females, 5/sex/toxicology group (Inc. control), 9/sex/toxicokinetic group (3/sex/control group) GLP OECD 407 (2008) Deviations : None F9600 technical, batch PL13-0385 Purity: 99.2% [redacted] (2015a) ***Acceptable***	0, 750, 2500, 5000, and 10000 ppm (for toxicology and toxicokinetic groups) Equivalent to : Males (M): 0, 57, 182, 359 and 740 mg/kg bw/d Females (F): 0, 61, 193, 379 & 733 mg/kg bw/d	750 ppm (equivalent to 57 mg/kg bw/d males & 61 mg/kg bw/d females) Based on relative liver weight increases > 15% in both sexes, adverse effect on body weight, body weight gain and food consumption in females at the LOAEL of 2500 ppm (The applicant proposed a NOAEL of 5000ppm)	There were no deaths or clinical signs of toxicity **10000 ppm (740 / 733 mg/kg bw/day M / F)** ↓ body weight (F): 18 %** ↓ body weight gain: 59 %** (F) & 14 % (M) ↓ food consumption (F): 41 %** (days 0-7), 17 %** (days 7-14) and 22 %** (days 14-27) ↓ food consumption (M): 20 % (days 0-7)** *Organ weights* ↑ absolute liver weights: 32 %** (F) & 56 %** (M) ↑ relative liver weights: 61 %** (F) & 65.5 %** (M) ↑ relative kidney weights: 14** % (F & M) *Histopathology - liver* Hepatocellular hypertrophy: 5/5 mild (F) & 4/5 mild + 1/5 moderate (M) *Clinical chemistry* ↑ total protein (9 % F &13 %** M), ↑ albumin (11 % M**), ↑ globulin (12 % M** & 15 %* F),↑ cholesterol (79 %** M & 91 %** F), ↑ BUN (45.5 %** F), ↑ triglyceride (86 %* F) **5000 ppm (359 / 379 mg/kg bw/day M / F)** ↓ food consumption (F): 23.5 %** (days 0-7) & 17 %* (days 7-21) ↓ food consumption (M): 16 %* (days 0-7) *Organ weights* ↑ absolute liver weight: 19 %* (F) ↑ relative liver weight: 29 %** (F) & 23 %** (M) *Histopathology - liver* Hepatocellular hypertrophy: 1/5 minimal & 4/5 mild (F); 3/5 minimal & 2/5 mild (M) *Clinical chemistry* ↑ cholesterol (43 %* F) **2500 ppm (182 / 193 mg/kg bw/day M / F)** ↓ food consumption in females: 12 %** (days 0-7) & 11 %* (days 7-14) *Organ weights* ↑ relative liver weight: 17 %** (F), 15.5 %** (M) *Histopathology - liver* Hepatocellular hypertrophy: 4/5 minimal (F) & 3/5 minimal (M) **750 ppm (57 / 61 mg/kg bw/day M / F)** No treatment-related findings.

Method, Species, test substance ***Acceptability***	**Doses**	**NOAEL**	**Main adverse effects**
28 day, dietary **Mouse,** Crl:CD-1, males & females, 5/sex/group GLP OECD 407 (2008) Deviations: none F9600 Technical, batch PL13-0385 Purity: 99.2% [redacted] (2015b) ***Acceptable***	0, 1000, 2000, 4000, and 5000 ppm Equivalent to: Males: 0, 187, 381, 788 & 985 mg/kg bw/day Females: 0, 289, 554, 984 & 1384 mg/kg bw/day	2000 ppm (554 mg/kg bw/day females) Based on relative liver weight increases > 15 % and hepatocellular hypertrophy from 4000 ppm in females (18.3 % absolute and 21.5% relative) (The applicant proposed a NOAEL of 5000ppm based on absence of adverse findings)	There were no treatment-related deaths. **5000 ppm (985 / 1384 mg/kg bw/day M / F)** ↓ body weight gain: 19 % (F) *Organ weights* ↑ absolute liver weight: 15 % (F) & 14 % (M) ↑ relative liver weight: 24 %** (F) & 13 %* (M) *Histopathology - liver* Hepatocellular hypertrophy: 3/5 F (2 minimal, 1 mild) & 4/5 M (2 minimal, 2 mild) *Clinical chemistry* ↑ ALT: 137 %* (M) **4000 ppm (788 / 984 mg/kg bw/day M / F)** *Organ weights* ↑ absolute liver weight: 18 %* (F) ↑ relative liver weight: 21.5 %** (F) *Histopathology - liver* Hepatocellular hypertrophy: 2/5 F (minimal) & 1/5 M (minimal) **2000 ppm (381 / 554 mg/kg bw/day M / F) & 1000 ppm (187 / 289 mg/kg bw/day M / F)** No treatment-related findings.
28 day, dietary **Dog**, Beagle, males & females, 2/sex/group Bixlozone technical, batch PL14-0049 Purity: 96% Vehicle: acetone GLP Dose-range finding study (loosely follows OECD 409) [redacted] (2016b) ***Supplementary***	0, 1000, 3000, 10000 & 30000 ppm Equivalent to control, 1000, 3000, and 10000 ppm groups: Males: 0, 38, 134 & 370 mg/kg bw/d Females: 0, 39, 108 & 309 mg/kg bw/d (test substance intake for 30000 ppm males and females could not be accurately calculated due to food supplementation)	No robust NOAEL and LOAEL could be set from this study as severe toxicity was seen as consequence of method of administration and palatability issues	There were no treatment related deaths No statistical analysis was performed **30000 ppm (≈ 1015 / 1110 mg/kg bw/day M / F)** Clinical signs: thin body condition (1 M), ↓ defecation (2 M) ↓ body weight: 17 % (M) and 9 % (F) ↓ body-weight gain: 116 % (M) and 90 % (F) ↓ food consumption led to food supplementation (M & F) *Organ weights* ↑ relative liver weight: 80 % (F) and 53 % (M) ↑ absolute liver weight: 30 % (M) and 63.5 % (F) ↑ relative kidney: 41 % (M) and 40 % (F) ↑ absolute kidney weight: 20 % (M) and 28 % (F) *Histopathology - liver* Hepatocellular hypertrophy in 2 / 2 M (1 minimal & 1 mild) Hepatocellular hypertrophy in 2/ 2 F (mild) **10000 ppm (370 / 309 mg/kg bw/day M / F)** ↓ body-weight gain: 17 % (M) and 54 % (F) ↓ food consumption in M & F *Organ weights* ↑ Relative liver weight: 28.5 % (F) and 20 % (M)

Method, Species, test substance *Acceptability*	**Doses**	**NOAEL**	**Main adverse effects**
			↑ absolute liver weight: 19 % (M) and 21 % (F) ↑ kidney weight in M: 22 % absolute and 23 % relative *Histopathology - liver* Hepatocellular hypertrophy in 2 / 2 M (minimal) Hepatocellular hypertrophy in 2 / 2 F (minimal) **3000 ppm (134 / 108 mg/kg bw/day M / F)** ↓ body weight gain: 45.5 % (F) *Organ weights* ↑ relative liver weight: 14 % (F) **1000 ppm (38 / 39 mg/kg bw/day M / F)** No treatment-related findings.
90-day, dietary (Includes neurotoxicity and recovery phase) **Rat,** Crl :CD9(SD), males & females, 21/sex/group or 16/sex group (including neurotoxicity phase) F9600 technical, batch PL14-0049 Purity: 96% Vehicle: acetone GLP OECD 408 (1998) & OECD 424 (1997) Deviations: None [redacted] (2016a) ***Acceptable***	0, 500, 2000, and 8000 ppm (males) Equivalent to: 0, 29, 121 & 505 mg/kg bw/day 0, 500, 2000, and 5000 ppm (females) Equivalent to: 0, 37, 150 & 351 mg/kg bw/day 90-days continuous dosing Recovery period: 28-days (5/sex group)	NOAEL 500 ppm (29/37 mg/kg bw/day in M/F) Based on liver weight increases in females (16 % absolute & 17 % relative) and increased kidney weights in males (15 % absolute &14.5 % relative) at 2000 ppm (Applicant also proposed a NOAEL of 2000 ppm)	One male (5000 ppm) was found dead on day 87 (undetermined cause); there were no clinical signs of toxicity at any dose. **8000 / 5000 ppm M/F (505 / 351 mg/kg bw/day M / F)** 1 death (M) ↓ body weight: 9 %* (M) & 9.5 %** (F) ↓ body weight gain: 18 %** (M) & 23 %** (F) food efficiency in M: - 14 %** (main group) & + 22 % (recovery group) food efficiency in F: - 11 %* (main group) & + 55 % (recovery group) *Organ weights* ↑ liver weights in M: 21.5 %** (absolute) & 37 %** (relative) ↑ liver weights in F: 22.5 %** (absolute) & 34 %** (relative) ↑ kidney weights in F: 17 %** (relative) *Histopathology - liver* Hepatocellular hypertrophy: 10/10 M (1 minimal, 6 mild, 3 moderate) and in 10/10 F (7 mild, 3 moderate) Macrovascular vacuolation 5/10 M (4 minimal, 1 moderate) *Histopathology - thyroid* Follicular cell hypertrophy (mild): 3/10 M & 5/10 F *Clinical chemistry* ↑ Cholesterol 40.5 %** (F) & 77 %** (M) ↑ globulin +11 %* and calcium +4.5 %* (F) **28-day recovery group (8000 / 5000 ppm)** ↑ food consumption 11 %** (M) & 4.5 % (F) ↑ relative liver weight 10 % (M) ↑ relative kidney weight 22 %** (M) Mild macro vascular vacuolation in liver 1/5 (M) ↑ cholesterol 31 %** (F)

Method, Species, test substance *Acceptability*	**Doses**	**NOAEL**	**Main adverse effects**
			2000 ppm (121 / 150 mg/kg bw/day M / F) *Organ weights* ↑ liver weights in females: 16 %* absolute & 17 %** relative ↑ kidney weights in males: 15 %* (absolute) & 14.5 %** (relative) *Histopathology - liver* Hepatocellular hypertrophy 1/10 F (mild) *Clinical chemistry* ↑ cholesterol +44 %**, globulin +11 % and calcium +4.5 %* (F) **500 ppm (29 / 37 mg/kg bw/day M / F)** No treatment-related findings.
90 day, dietary **Mouse**, Crl:CD1(ICR), males & females, 10/sex/ toxicology group, 12/sex/toxicokinetic group F9600 technical, batch PL14-0049 Purity: 96% Vehicle: acetone GLP OECD 408 (1998) Deviations: none [redacted] (2016b) ***Acceptable***	0, 1000, 2250, and 5000 ppm Equivalent to: Males: 0, 180, 414 & 930 mg/kg bw/day Females: 0, 257, 583 & 1185 mg/kg bw/day	1000 ppm (257 mg/kg bw/day) Based on relative liver weight increases in females at 2250 ppm (17.5 %) (The applicant proposed a NOAEL of 5000 ppm)	There were no test-substance related deaths or clinical signs of toxicity **5000 ppm (930 / 1185 mg/kg bw/day M/F)** ↑ relative liver weights: 23 % (M) & 21 % (F) ↑ absolute liver weights: 23 % (M) & 20 % (F) Hepatocellular hypertrophy in 10/10 M (1 minimal, 9 mild) Hepatocellular hypertrophy in 3/9 F (1 minimal, 2 mild) **2250 ppm (414 / 583 mg/kg bw/day M/F)** ↑ relative liver weights: 17.5 % (F) ↑ absolute liver weights: 13 % (F) Hepatocellular hypertrophy in 4/10 M (3 minimal, 1 mild) Hepatocellular hypertrophy in 3/9 F (minimal) **1000 ppm (180 / 257 mg/kg bw/day M/F)** No adverse effects observed **Neurotoxicity (90 days)** A higher incidence of alert females was noted at week 12 from the mid-dose of 2000 ppm (150 mg/kg bw/day), but, in isolation, this finding is not considered to represent a specific neurotoxic response. Please refer to Section 2.6.7 for more details.
90 day, capsule **Dogs**, Beagle, males & females, 4/sex/group F9600 technical, batch PL14-0049 Purity: 96 % Vehicle: none GLP OECD 409 (1998)	0, 30, 100, 300, and 750 mg/kg/day	30 mg/kg bw/day for females Based on liver weight increases in females at 100 mg/kg bw/day (27 % absolute & 22 % relative)	There were no treatment-related deaths or clinical signs of toxicity; there was no effect on body weight or food consumption at any dose levels. **750 mg/kg bw/day** *Organ weights* ↑ absolute liver weights: 54 %** (F) & 21 % (M) ↑ relative liver weights: 46 %** (F) & 20 %** (M) ↑ relative thyroid weight: 54 % (F) & 21 % (M) ↓ prostate weight: absolute 41 % and relative 43 % and associated immaturity

Method, Species, test substance ***Acceptability***	**Doses**	**NOAEL**	**Main adverse effects**
[redacted] (2016c) ***Acceptable***		(The applicant proposed a NOAEL of 750 mg/kg bw/day)	*Histopathology - liver* Hepatocellular hypertrophy in 2/4 males (minimal) *Clinical chemistry* ↑ WBC (37 %* wk. 6), ↑ lymphocytes (43 %* wk. 6 & 39 %* wk. 12/13), ↑ LUC (+150 % wk 6) in F **300mg/kg bw/day** *Organ weights* ↑ relative liver weight: 21.5 %** (F) ↓ abs (19%) and rel (26.5%) prostate weight and associated immaturity **100 mg/kg bw/day** *Organ weights* ↑ liver weights in F (27 %* absolute, 22 %** relative) **30 mg/kg bw/day** No treatment-related findings.
12 months, capsule **Dogs,** Beagle, males & females, 4/sex/group F9600 technical, batch PL14-0049 Purity: 96% Vehicle: none GLP OECD 409 (1998) Deviations: none [redacted] (2017) ***Acceptable***	0, 20, 100, and 500 mg/kg/day	100 mg/kg bw/day for females Based on haematological changes in males at 500 mg/kg bw/day (WBC, PT and lymphocytes absolute) (The applicant proposed a NOAEL of 500 mg/kg bw/day)	There were no treatment-related deaths or clinical signs of toxicity; there was no effect on body weight or food consumption at any dose levels. **500 mg/kg bw/day** ↑ WBC (+35 % week 26*; +27 % week 52) ↑ monocyte absolute (+55% week 26*;+15 % week 52) ↑ lymphocytes absolute (+34 % week 26; +55 % week 52**) ↑ PT (+8 % week 26*; +13 % week 52**) in males **100 & 20 mg/kg bw/day** No adverse effects observed.
21 day, dermal **Rat**, Crl :CD9(SD), males & females, 10/sex/group GLP OECD 410 (1981) Deviations : None F9600 technical, batch PL14-0049 Purity: 99.2% [redacted] (2016) ***Acceptable***	0, 100, 300, and 1000 mg/kg bw/day	> 1000 mg/kg bw/day based on no adverse effects observed at the highest dose tested (The applicant proposed a NOAEL of 1000 mg/kg bw/day)	There were no deaths or clinical signs of toxicity **1000, 300 and 100 mg/kg bw/day** No adverse effects observed.

Consideration of the classification of bixlozone for STOT-RE and setting of the overall/most sensitive NOAEL for short-term toxicity

The table below presents all the relevant NOAEL and LOAEL values identified in the available short-term studies. The overall / most relevant **NOAEL for short-term toxicity** is proposed to be set from the 90-day oral (dietary) repeated-dose toxicity conducted in the rat at **29 and 37 mg/kg bw/day** in males and females respectively (with a respective LOAEL of 121 / 150 mg/kg bw/day in males / females based on treatment-related and adverse increase in liver weights accompanied by increased cholesterol, protein and calcium and 1/10 hepatocellular hypertrophy in females and kidney weights in males). This NOAEL is consistent with the NOAEL of **30 mg/kg bw/day** from the 90-day dog study.

When compared with the classification criteria for STOT-RE, the liver and kidney were clear target organs at doses above the cut-off values for classification into category 2 for the oral route of exposure in the rat ($10 < dose \leq 100$ mg/kg bw/day). Therefore, HSE concludes that bixlozone should not be classified for STOT-RE 2 according to Regulation (EC) N°1272/2008 (see MCL report for further details).

Overall, the repeated-dose toxicity of bixlozone has been adequately investigated in studies in rats, mice and dogs; the critical target of organ of toxicity was identified as the liver followed by the kidney, and adverse effects observed in these organs could be relevant to humans. Classification for repeated-dose toxicity according to Regulation (EC) N°1272/2008 is not warranted (for more details please see aligned MCL Report).

Table 2.6.3.3. Summary of NOAEL values for the short-term toxicity of bixlozone

Study, guideline, reference, acceptability	**Species, doses tested**	**NOAEL mg/kg bw/d**	**LOAEL mg/kg bw/d**	***Adverse* effects at LOAEL**
Dietary 28-day GLP OECD 407 (2008) Deviations: None ([redacted] (2015a)) ***Acceptable***	**Rat** (Crl :CD9(SD), males & females) F9600 technical; Batch PL13-0385 Purity 99.2% 0, 750, 2500, 5000, and 10000 ppm Equivalent to : Males: 0, 57, 182, 359 and 740 mg/kg bw/day Females: 0, 61, 193, 379 & 733 mg/kg bw/day	57 (males) 61 (females) (750 ppm) (The applicant proposed a NOAEL of 740 / 733 for males / females)	182 (males) 193 (females) (2500 ppm)	Adverse ↓ on body weight, body weight gain and food consumption in females *Organ weights* ↑ relative liver weight: 17 %** (F), 15.5 %** (M) *Histopathology - liver* Hepatocellular hypertrophy: 4/5 minimal (F) & 3/5 minimal (M)
Dietary 28-day GLP OECD 407 (2008) Deviations: none ([redacted](2015b)) ***Acceptable***	**Mouse** (Crl:CD-1, males & females) F9600 technical; Batch PL13-0385 Purity 99.2% 0, 1000, 2000, 4000, and 5000 ppm Equivalent to: Males: 0, 187, 381, 788 & 985 mg/kg bw/day	381 (males) 554 (females) (2000 ppm) (The applicant proposed a NOAEL of 985 / 1384 mg/kg bw/day for males / females)	788 (males) 984 (females) (4000 ppm)	*Organ weights* ↑ absolute liver weight: 18 %* (F) ↑ relative liver weight: 21.5 %** (F) *Histopathology - liver* Hepatocellular hypertrophy: 2/5 F (minimal) & 1/5 M (minimal)

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	*Adverse* effects at LOAEL
	Females: 0, 289, 554, 984 & 1384 mg/kg bw/day			
Dietary 28-day Range-finding study GLP OECD 409 (1998) Deviations: Due to palatability issue at 30000 ppm animals were fed with food supplementation thus the mean achieved bixlozone consumption could not be calculated accurately ([redacted] (2016b)) ***Supplementary***	**Dog** (Beagle) F9600 Technical; batch PL14-0049 Purity 96 % 0, 1000, 3000, 10000, and 30000 ppm Equivalent to control, 1000, 3000, and 10000 ppm groups: Males: 0, 38, 134 & 370 mg/kg bw/day Females: 0, 39, 108 & 309 mg/kg bw/day (test substance intake for 30000 ppm males and females could not be accurately calculated due to food supplementation)	No robust NOAEL and LOAEL could be set from this study as severe toxicity was seen as consequence of method of administration and palatability issues	39 mg/kg bw/day (females) (1000 ppm)	↓ body weight gain: 45.5 % (females)
Dietary 90-day GLP OECD 408 (1998) & OECD 424 (1997) Deviations: None ([redacted] (2016a)) ***Acceptable***	**Rat** (Rat, Crl :CD9(SD), males & females) F9600 Technical; batch PL14-0049 Purity 96 % 0, 500, 2000, and 8000 ppm (males) Equivalent to: 0, 29, 121 & 505 mg/kg bw/day 0, 500, 2000, and 5000 ppm (females) Equivalent to: 0, 37, 150 & 351 mg/kg bw/day	**29** (males) **37** (females) (500 ppm) (The applicant proposed a NOAEL of 121 / 150 for males / females)	121 (males) 150 (females) (2000 ppm)	*Organ weights* ↑ liver weights in females: 16 %* absolute & 17 %** relative ↑ kidney weights in males: 15 %* (absolute) & 14.5 %** (relative) *Histopathology - liver* Hepatocellular hypertrophy 1/10 F (mild) *Clinical chemistry* ↑ cholesterol +44 %**, globulin +11 % and calcium +4.5 %* (F)
Dietary 90-day GLP OECD 408 (1998) Deviations: None ([redacted](2016a)) ***Acceptable***	**Mouse** (Crl:CD1(ICR), males & females) F9600 Technical; batch PL14-0049 Purity 96 % 0, 1000, 2250, and 5000 ppm	180 (males) 257 (females) (1000 ppm)	414 (males) 583 (females) (2250 ppm)	*Organ weights* ↑ absolute liver weights: 13 %* (F) ↑ relative liver weights: 17.5 %** (F) *Histopathology - liver* Hepatocellular hypertrophy in 4/10 M (3 minimal, 1 mild)

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	*Adverse* effects at LOAEL
	Equivalent to: Males: 0, 180, 414 &930 mg/kg bw/day Females: 0, 257, 583 & 1185 mg/kg bw/day	(The applicant proposed a NOAEL of 930 / 1185mg/kg bw/day for males / females)		Hepatocellular hypertrophy in 3/9 F (minimal)
90-day, capsule GLP OECD 409 (1998) Deviations: None ([redacted], 2015) ***Acceptable***	**Dog** (Beagle, males and females) F9600 technical; batch PL14-0049 Purity 96 % 0, 30, 100, 300, and 750 mg/kg/day	**30** (females) (The applicant proposed a NOAEL of 750 mg/kg bw/day)	100 (females)	*Organ weights* ↑ liver weights in females (27 %* absolute, 22 %** relative) There were no treatment-related deaths or clinical signs of toxicity; there was no effect on body weight or food consumption at any dose levels.
12-month, capsule GLP OECD 452 (1998) Deviations: None ([redacted] (2017)) ***Acceptable***	**Dog**, (Beagle, males & females), 4/sex/group F9600 technical, batch PL14-0049 Purity: 96% 0, 20, 100 and 500 mg/kg bw/day	100 (The applicant proposed a NOAEL of 500 mg/kg bw/day)	500	*Haematology* ↑ WBC (+35 % week 26*; +27 % week 52) ↑ monocyte absolute (+55% week 26*;+15 % week 52) ↑ lymphocytes absolute (+34 % week 26; +55 % week 52**) ↑ PT (+8 % week 26*; +13 % week 52**) in M
21-day, dermal GLP OECD 410 (1981) Deviations: None ([redacted] (2016)) ***Acceptable***	**Rat**, Crl :CD9(SD), males & females F9600 technical, batch PL14-0049 Purity: 99.2% 0, 100, 300 and 1000 mg/kg bw/day	> 1000 (The applicant proposed a NOAEL of 1000 mg/kg bw/day)	N/A	No adverse effects observed up to the highest dose tested

2.6.4. Summary of genotoxicity

The genotoxic potential of bixlozone was tested *in vitro* in a bacterial reverse mutation assay (Ames test), an *in vitro* chromosome aberration study using CHO cells, an *in vitro* mammalian cell gene mutation test (L5178Y/TK+/- Mouse Lymphoma Assay); and *in vivo* a rat micronucleus study was also conducted. The studies were all performed according to the relevant OECD TGs and were GLP compliant.

Bixlozone did not induce gene mutations in bacteria or mouse lymphoma cells *in vitro* but was clastogenic *in vitro* with metabolic activation (S9) at an exposure leading to significant cytotoxicity. However, when tested *in vivo* in a valid rat bone marrow micronucleus study up to the limit dose of 2000 mg/kg bw, the clastogenic activity seen *in vitro* was not evident *in vivo*. At this dose level, clinical signs of toxicity, reduction in body weight gain and toxicokinetic data all confirm exposure to the bone marrow occurred; these confirm optimal assay conditions were met. Nor was there any evidence of aneugenicity in the rat bone marrow micronucleus study.

Overall, it is concluded that bixlozone is not genotoxic *in vivo* and the data requirements of Regulation 283/2013 have been met. Therefore, classification of bixlozone for mutagenicity is not warranted (see also aligned MCL 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 bixlozone

Study ***Acceptability***	**Concentrations of Substance tested**	**Result**	**Reference**
In vitro assays			
Ames test OECD N° 471 (1997) GLP: yes ***Acceptable***	0, 5, 15, 50, 150, 500, 1500 and 5000 µg / plate ± S9	Negative	Bruce, S. (2018)
Chromosomal aberrations study in CHO cells OECD N° 473 (2016) GLP: yes **Acceptable**	0, 20, 40, 80, 100, 120, 140, 160, 180 µg/mL used for all three treatment conditions in the main experiment. Treatments: 4-h ± S9; 20-h - S9	Clastogenic following 4-h treatment with S9	Roy, S. (2018)
L5178Y/TK+/- Mouse Lymphoma cells mutagenicity study OECD N° 490 (2016) GLP: yes ***Acceptable***	0, 7.81, 15.6, 31.3, 62.5, 125, 175, 200 and 250 µg/mL 4-h treatment + S9 0, 15.6, 31.3, 62.5, 125, 150 and 200 µg/mL 4-h treatment - S9 0, 7.81, 15.6, 31.3, 62.5, 125, 175 and 200 µg/mL 24-h treatment – S9	Negative	Dutta, A. (2018)
***In vivo* assay**			
Rat micronucleus assay *in vivo* (oral gavage) OECD N° 474 (2016) GLP: yes ***Acceptable***	0, 500, 1000 and 2000 mg/kg bw/day Treatment on two consecutive days 24-h apart	Negative	[redacted] (2018)

2.6.5. Summary of long-term toxicity and carcinogenicity

The carcinogenicity potential of bixlozone administered orally was investigated in the rat and the mouse in two long-term OECD and GLP-compliant toxicity studies: a 2-year combined chronic toxicity/carcinogenicity was conducted in rats and an 18-month carcinogenicity in mice. Additional toxicokinetic measurements were also performed in parallel in both studies.

In the rat, there were no treatment-related tumours identified for both sexes up to the top dose tested in this study. Therefore, the NOAEL for carcinogenicity in the rat is set at the top dose of 5000 / 3000 ppm (167 / 217 mg/kg bw/day in males and females, respectively).

Systemic toxicity was observed at the top-dose in both sexes throughout the study (5000 ppm / 3000 ppm equating 217 / 167 mg/kg bw/day in males / females respectively); body weight and body weight gain were statistically significantly reduced compared to the control groups and, consistent with the findings of the short-term repeated-dose studies, the liver was identified as a target organ. Treatment-related increases in parameters indicative of adverse effects in the liver (serum cholesterol, albumin, calcium, total protein) were observed in the chronic toxicity top-dose females and correlated with the liver weight changes and associated hepatocellular hypertrophy findings observed in these animals. In males, treatment-related effects in the liver were observed from 1000 ppm (and considered adverse at 5000 ppm) and were characterised by liver weight changes at 5000 ppm and hepatocellular hypertrophy findings observed from 1000 ppm.

Overall, the NOAEL for systemic chronic toxicity in the rat is set at 1000 ppm (41 / 53 mg/kg bw/day in males / females respectively) with a LOAEL set at 5000/3000 ppm (217 / 167 mg/kg bw/day in males / females respectively)

based on adverse effects observed in the liver (serum chemistry changes, liver weight changes and histopathology findings), and effects on body weights in both sexes.

In the mouse, there were no neoplastic findings attributable to exposure to bixlozone up to the highest dose tested (5000 ppm).

Overall, the NOAEL for carcinogenicity in the mouse is set at the highest dose tested of 5000 ppm equating to 647 and 834 mg/kg bw/day for males and females respectively, based on absence of carcinogenicity findings. Some systemic toxicity was observed in both sexes at 5000 ppm (the highest dose tested); the liver was identified as a target organ in both sexes, with the relative liver weight increased by > 15 % compared to controls; however, a clear association with histopathology was only found in males. Consistent with the findings from previous repeated-dose toxicity studies conducted in the mouse, the body weight gains and food consumption were not affected by treatment with bixlozone up to the top dose. Higher incidences of reduced epididymal sperm and inflammation of the glandular stomach were observed in males from 1000 ppm (126 mg/kg bw/day), with pelvis dilation of the kidney occurring in males at the top dose of 5000 ppm.

In conclusion, the LOAEL for systemic toxicity is set at the mid dose of 1000 ppm (126 mg/kg bw/day in males) based on adverse effects on sperm and stomach in males. The NOAEL for systemic chronic toxicity in the mouse is thus set at 250 ppm (32 mg/kg bw/day in males). The systemic toxicity NOAEL proposed by the applicant is 1000 ppm.

The overall/most sensitive **NOAEL for carcinogenicity is set at 217 / 167 mg/kg bw/day (5000 / 3000 ppm in M/F)** with a LOAEL of > **217 / 167 mg/kg bw/day** based on absence of neoplastic findings in the rat 2-year combined chronic toxicity / carcinogenicity study up to the highest dose tested.

The applicant proposed an overall NOEL for carcinogenicity at 5000 ppm for males and females, the highest dose level evaluated in the rat bioassay, corresponding to actual consumed dose levels of 217 and 167 mg/kg bw/day for males and females, respectively.

The overall/most sensitive **NOAEL for chronic systemic toxicity is 32 mg/kg bw/day** (250 ppm) identified for effects on epididymal sperm and stomach inflammation in males in the mouse 18-month carcinogenicity study at the LOAEL of 1000 ppm (126 mg/kg bw/day in males).

The applicant proposed an overall NOEL for chronic systemic toxicity at 53 mg/kg bw/day (1000 ppm) based on reduced body weight gain in females noted at 167 mg/kg bw/day (3000 ppm) in the rat 2-year combined chronic toxicity / carcinogenicity study.

Overall, long term oral administration of bixlozone was not carcinogenic in the rat or mouse. Therefore, classification of bixlozone for carcinogenicity is not required (see aligned MCL report).

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

Table 2.6.5.1: Summary of NOAELs from carcinogenicity studies with bixlozone

Study, guideline, reference	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
Dietary 24-month GLP OECD 453 (2009) Deviations: none ([redacted] (2017)) ***Acceptable***	**Rat** (Crl:CD (SD) rats, males and females) 0, 250, 1000, 5000/3000 ppm Equivalent to: Males: 0, 10, 41, 217 mg/kg bw/day Females: 0, 13, 53, 167 mg/kg bw/day	*Carcinogenicity* 217 / 167 (5000 / 3000 ppm in M/F)	*Carcinogenicity* > 217 / 167 (5000 / 3000 ppm in M/F)	*Carcinogenicity* No biologically relevant neoplastic findings
		Systemic chronic toxicity 41 (males) 53 (females) (1000 ppm)	*Systemic chronic toxicity* 217 (males) 167 (females) (5000/3000 ppm)	*Systemic chronic toxicity* Dermal atonia and thin body condition in F ↓ body weight gain for both sexes (9-14%** M and 14-24.5%** F) *Organ weights* ↑ relative liver weight > 15 %** (both sexes) ↓ cholesterol**, albumin*, calcium**, total protein* (F) *Histopathology findings - liver* Hepatocellular hypertrophy: 7/10 at 52 weeks & 79 % incidence at 104 weeks (M) 10/10 at 52 weeks & 74 % incidence at 104 weeks (F) Hepatocellular vacuolation: 7/10 at 52 weeks & 32 % incidence at 104 weeks (M) 10/10 at 52 weeks & 74 % incidence at 104 weeks (F)
Dietary 18-month GLP OECD 451 (2009) Deviations: none ([redacted] (2017)) ***Acceptable***	**Mouse** (Crl:CD1(ICR mice, males and females) 0, 250, 1000, 5000 ppm Equivalent to: Males: 0, 32, 126, 647 mg/kg bw/day Females: 0, 43, 164, 834 mg/kg bw/day	*Carcinogenicity* 647 (M) 834 (F) (5000 ppm)	*Carcinogenicity* > 647 (M) > 834 (F) (> 5000 ppm)	*Carcinogenicity* None attributable to exposure to bixlozone up to the highest dose tested (5000 ppm).
		Systemic chronic toxicity 32 (males) (250 ppm)	*Systemic chronic toxicity* 126 (males) (1000 ppm)	*Systemic chronic toxicity* ↑ incidence of reduced sperm in epididymes (M) ↑ incidence of inflammation of glandular stomach (M)

2.6.6. Summary of reproductive toxicity

A full assessment of the reproductive toxicity of bixlozone has been carried out with GLP and OECD guideline compliant studies: a 2-generation reproductive toxicity study in rats is available and developmental toxicity studies have been conducted in both rats and rabbits. Range-findings reproductive / developmental studies were also

conducted in the rat and rabbit and have been considered as supplementary information. Additional findings on reproductive organs from the short-term and long-term repeated dose toxicity studies are also discussed here.

Effects on Sexual Function and Fertility

The potential of bixlozone to adversely affect sexual function and fertility has been well investigated in a modern 2-generation dietary study, conducted in the rat ([redacted] (2016c)).

Bixlozone had no effect on male or female fertility or reproductive performance; gestation duration and spermatogenic endpoints were also unaffected by treatment up to the top-dose of 140 / 187 mg/kg bw/day (males / females) at which general systemic toxicity occurred. In addition, examination of the reproductive organs did not reveal any treatment-related changes except for mononuclear cell infiltration (chronic inflammation) in the prostate which was evident in the top-dose males of both generations. In the absence of a functional effect on fertility or reproductive performance, these findings on the prostate are considered of minimal toxicological significance but are accounted for by the parental NOAEL. There was also no effect on litter size, sex ratio or pup survival up to the highest dose tested in the study.

A delay in vaginal opening was seen in F_1 pups at 3000 ppm (33.6 days compared with 31.7 days in controls) whilst mean body weights of these female pups at the age of attainment were unaffected by treatment with bixlozone. Therefore, it can be concluded that the delay in vaginal opening was the secondary consequence of reduced post-weaning pup female body weight development and not a specific reproductive effect of bixlozone.

Therefore, a **NOAEL for reproductive toxicity of > 3000 ppm (140 / 187 mg/kg bw/day in males / females)** can be identified from this study, based on no adverse effect on reproduction up to the highest dose tested.

In relation to general toxicity in parental animals, adequate toxicity was achieved and in line with the findings of the repeated-dose toxicity studies, this was characterised by reductions in food consumption, body weight and body weight gain and increases in relative liver weights > 15 % compared to controls accompanied by histopathological findings (hepatocellular hypertrophy) at the top dose of 140 / 187 mg/kg bw/day (lowest dose males / females) in both sexes and both generations. Adverse effects on kidneys (increase in relative kidney weights in both sexes) were also noted at the top dose in both generations. Therefore, the top dose of ≈ 140 / 187 mg/kg bw/day constitutes the LOAEL for parental toxicity in this study. No adverse effects were observed at the lower dose of 34 / 49 mg/kg bw/day.

Consistent with the toxicity observed in the parental generations, body weights and body-weight gain of pups in the F_2 generation (but not in the F_1 generation) were affected by treatment with bixlozone at the top dose of 140 mg/kg bw/day, whilst liver weights were found to be adversely increased in male pups of the F_1 generation. Therefore, the top dose of 140 mg/kg bw/day constitutes the LOAEL for offspring toxicity in this study. No adverse effects were observed at the lower dose of 34 /49 mg/kg bw/day in both generations.

Therefore, for general **parental and offspring toxicity** a **NOAEL** of **750 ppm** (**34 / 49 mg/kg bw/d)** can be identified from this study.

This is consistent with the NOAELs that were proposed by the applicant.

Additional findings on reproductive organs from repeat dose toxicity studies

A slightly higher incidence of reduced epididymal sperm was seen in males from 126 mg/kg bw/day at terminal sacrifice in the 18-month mouse carcinogenicity study. No other reproductive organs were affected. No such findings were seen in the 90-day mouse study up to the top dose of 930 mg/kg bw/day. It is most likely that these mild and isolated changes occurring during the reproductive senescence of the male mouse are of minimal toxicological significance and of no relevance to the reproductive performance of the mouse.

In addition, reductions in prostate weight with associated immaturity were seen in the dog in the 90-day study from 300 mg/kg bw/day, but not up to 500 mg/kg bw/day in the 1-year study. On this basis, these prostate findings are considered to be of minimal toxicological significance and of no relevance to the reproductive performance of the dog.

Developmental toxicity

The developmental toxicity of bixlozone has been investigated in GLP and OECD guideline compliant gavage pre-natal developmental toxicity studies, conducted in the rat and rabbit. Additional information on the developmental toxicity potential of bixlozone can be extracted from the rat 2-generation study and has been taken into consideration in this summary.

In the rat developmental toxicity study ([redacted] (2016e)), maternal toxicity was noted from 225 mg/kg bw/day and was characterised by a higher incidence of clinical findings (red, yellow and/or clear material on various body

surfaces), reduced food consumption and a corresponding reduction in body weight and body weight gain. Reductions in body weight gain were most marked during the first 3 days of dosing. In addition, an adverse increase in liver weight with histopathological correlate was noted at the top dose. Thus, the dose of 225 mg/kg bw/day constitutes the LOAEL for maternal toxicity. A NOAEL of 75 mg/kg bw/day for maternal toxicity is thus proposed by HSE.

No evidence of developmental toxicity was observed in the rat at any dose tested and up to doses causing clear maternal toxicity. Therefore, a NOAEL for developmental toxicity of > 550 mg/kg bw/day is proposed. The proposed NOAELs are consistent with the NOAELs that were proposed by the applicant.

In the rabbit developmental toxicity study ([redacted] (2015)), the signs of maternal toxicity noted were a reduction in food consumption during the second week of dosing (GD 13-20) with a corresponding reduction in body weight gain and decrease in defecation at the highest dose tested of 400 mg/kg bw/day. The top-dose of 400 mg/kg bw/day thus constitutes the LOAEL for maternal toxicity in this study. No adverse maternal effects were noted at lower doses. Regarding developmental findings there was no developmental toxicity noted in the rabbit up to the highest dose tested.

In conclusion HSE proposes for the rabbit study a NOAEL of 200 mg/kg bw/day for maternal toxicity and a NOAEL of 400 mg/kg bw/d for developmental toxicity. The applicant proposed a NOAEL of 400 mg/kg bw/day for both maternal toxicity and developmental toxicity.

In addition, in the rat 2-generation study, there were no specific effects of treatment on pup survival, sex ratio, developmental landmarks or preputial separation up to the top dose of 140 mg/kg bw/day at which parental and offspring toxicity occurred.

Overall conclusions

The overall NOAELs for reproductive toxicity are set as follows:

A **NOAEL for reproductive toxicity of 3000 ppm (140 / 187 mg/kg bw/day in males / females)** can be identified from the 2-generation reproductive toxicity study in the rat, based on no specific adverse effect on reproduction up to the highest dose tested. **A NOAEL for parental toxicity and offspring toxicity of 750 ppm (34 / 49 mg/kg bw/day in males / females)** has also been identified.

The overall NOAELs for developmental toxicity are set as follows:

No evidence of developmental toxicity was observed in the rat and rabbit up the highest doses tested at which maternal toxicity occurred. HSE proposes to set the overall **NOAEL for developmental toxicity at 400 mg/kg bw/day** based on no adverse effects observed up to the highest dose tested in the rabbit developmental study.

The overall **NOAEL for maternal toxicity is 75 mg/kg bw/day** identified from the rat developmental toxicity study.

Overall, and in accordance with Regulation GB/NI Nº 1272/2008, classification of bixlozone for reproductive and developmental toxicity is not warranted (see also aligned MCL report).

The table below provides an overview of the NOAELs set from the reproductive toxicity studies.

Table 2.6.6.1: Summary of NOAELs from reproductive toxicity studies with bixlozone

Study, guideline, reference *Acceptability*	Species, doses tested	NOAEL	LOAEL	Adverse effects at LOAEL
2-generation dietary study GLP OECD Guideline 416 (2001) Deviations: None ([redacted] (2016c)) ***Acceptable***	**Rats** (Crl:CD(SD), males & females) 25/sex/group F9600 technical batch PL14-0049; purity 96 % 0, 150, 750, 3000 ppm Equivalent to doses expressed as mg/kg bw/day as in Table 6.6.1.2.1	**Parental:** **750 ppm** Equivalent to: 34/49 mg/kg bw/d in M/F **Offspring:** **750 ppm** Equivalent to 34/49 mg/kg bw/day in M/F **Reproductive:** **3000 ppm** Equivalent to 140/187 mg/kg bw/day in M/F	**Parental:** **3000 ppm** Equivalent to: 140/187 mg/kg bw/d in M/F **Offspring:** **3000 ppm** Equivalent to 140/187 mg/kg bw/day in M/F **Reproductive:** **> 3000 ppm** Equivalent to > 140/187 mg/kg bw/day in M/F	**Parental toxicity** ***F0 generation*** There were no treatment related deaths or clinical signs of toxicity. No treatment-related findings. ***F1 generation*** ↓ body-weight gains in M** & F ↓ body weights in M** & F* *Organ weights* ↑ absolute liver weights in F (+13 %**) ↑ relative liver weights: +14 %** (M) & +21 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %** (F) *Histopathology* Hepatocellular hypertrophy in F ↑ mononuclear cell infiltration (chronic inflammation) in the prostate* **Offspring toxicity** *F1 pups* ↑ relative liver weights: +18 % (M)* Delay in vaginal opening (33.6 days vs 31.7 days in controls) *F2 pups* ↓ mean body weights PND 14 (-8 %) No treatment-related findings. **Reproductive toxicity** No specific adverse effects up to top dose
Developmental gavage study GLP OECD Guideline 414 (2001) Deviations: None	**Rats** (Crl:CD(SD), females) 25/group F9600 Technical Batch PL14-0049	**Maternal:** 75 mg/kg bw/day	**Maternal:** 225 mg/kg bw/day	**Maternal toxicity:** Clinical signs: red, yellow and/or clear material on various body surfaces Early ↓ body-weight gain: -40 % (GD 6-9)*

Study, guideline, reference *Acceptability*	Species, doses tested	NOAEL	LOAEL	Adverse effects at LOAEL
([redacted] (2016e)) ***Acceptable***	Purity 96.0 % 0, 75, 225 & 550 mg/kg bw/day	**Developmental:** 550 mg/kg bw/day	**Developmental:** >550mg/kg bw/day	↓ food consumption: -8 % (GD 6-20)* **Developmental toxicity** No treatment-related findings.
Developmental gavage study GLP OECD Guideline 414 (2001) Deviations: None ([redacted] (2015)) ***Acceptable***	**Rabbits** (New Zealand White, females) 25/group F9600 Technical Batch PL14-0049 Purity 96.0 % 0, 25, 75, 200 & 400 mg/kg bw/day	**Maternal:** 200 mg/kg bw/day **Developmental:** 400 mg/kg bw/day	**Maternal:** 400 mg/kg bw/day **Developmental:** >400 mg/kg bw/day	**Maternal toxicity** ↓ defecation ↓ body-weight gain -32 % (GD 13-20) ↓ food consumption -18 % (GD 13-20) **Developmental toxicity** No adverse effects up to top dose

2.6.7. Summary of neurotoxicity

The neurotoxic potential of bixlozone has been investigated in Sprague Dawley rats in a guideline oral (gavage) acute neurotoxicity study (preceded by a range-finding study) as well as in a standard 90-day toxicity study which included a dedicated neurotoxicity phase; assessment of neurobehavioral parameters and histopathological examinations of central and peripheral nervous tissue were conducted following both acute or repeated administration of bixlozone.

In the acute neurotoxicity study, single oral (gavage) administration of bixlozone up to 2000 mg/kg bw resulted in no treatment-related changes in motor activity, functional observational battery (FOB) or neuropathology parameters. Therefore, HSE proposes a NOAEL for acute neurotoxicity of 2000 mg/kg bw. In addition, no clinical signs of toxicity and effects on body weights were seen up to the top dose. Therefore a NOAEL of 2000 mg/kg bw is also proposed for acute generalised toxicity.

Repeated neurotoxicity was investigated as part of a 90-day repeated-dose toxicity study in the rat. The FOB assessment did not show any relevant neurotoxic effect on home cage, handling, sensory or neuromuscular parameters. A higher incidence of alert females was noted at week 12 from the mid-dose of 2000 ppm (150 mg/kg bw/day), but, in isolation, this finding is not considered to represent a specific neurotoxic response. Motor activity patterns (mean ambulatory and total mean motor activity) were unaffected by treatment. There were no alterations to brain weight or length; however, a statistically significant higher group mean brain width in females at the top-dose of 5000 ppm (351 mg/kg bw/day) was noted; the finding is considered to be related to generalised toxicity rather than the expression of a specific neurotoxic effect. No test-substance related microscopic lesions or other unusual findings were noted in the central and peripheral nervous tissues.

Overall, HSE proposes a NOAEL for repeated neurotoxicity greater than the highest dietary dose tested of 8000 / 5000 ppm (equating to 505 mg/kg bw/day in males and 351 mg/kg bw/day in females respectively).

Relevant clinical findings potentially relating to neurotoxicity have also been searched in the other toxicological studies available for bixlozone, such as in acute, repeated-dose, long-term or reproductive toxicity studies conducted via the oral route in rodents (rats and mice).

Regarding the rat, the acute oral (gavage) toxicity study conducted by [redacted] (2014a) showed hypoactivity, reduced respiration (3-5 hours post administration) and decreased defecation in 2 out of 3 animals on the first day of treatment at 2000 mg/kg bw; these signs had fully reversed by day 2. These findings are considered to represent generalised toxicity at a very high dose rather than a specific neurotoxic response. No clinical signs indicative of neurotoxicity were found in the acute dermal toxicity study conducted in rats ([redacted] (2014b)). In the acute inhalation toxicity study ([redacted] (2014c), study no. 37947), irregular respiration following exposure was observed which had fully recovered by day 3; this finding is considered to be more specifically related to the route of exposure rather than the expression of a specific neurotoxic effect. Overall, there was no clear evidence of neurotoxicity in the acute toxicity studies; however, it should be noted that no specific neurobehavioural or neuropathology investigations are generally performed in these studies. No other relevant clinical findings potentially relating to neurotoxicity were noted in the

long-term or reproductive toxicity studies conducted via the oral route in the rat. In mice, no clinical findings potentially related to neurotoxicity were found in any of the studies conducted with this species.

Overall, it can be concluded that bixlozone is not neurotoxic after single or repeated administration.

Table 2.6.7.1: Summary of neurotoxicity studies in rodents

Study and Guideline *Acceptability*	**Species/ Strain/ Groups Doses**	**NOAEL (mg/kg bw) [ppm]**	**LOAEL (mg/kg bw) [ppm]**	**Effects at the LOAEL**
Dose-range finding acute neurotoxicity study, gavage Study no. [redacted]-105113 F9600 technical, batch PL13-0385 Purity: 99.2 % Vehicle: 0.5 % (w/v) carboxymethylcellulose in 5% Tween® 80 [redacted] 2014(b) GLP: no ***Supplementary***	Rats, Crl:CD(SD), males & females, 3/sex/group 0, 500, 1000, 1500 & 2000 mg/kg bw	A NOAEL was not set from this dose-range finding study	A LOAEL was not set from this dose-range finding study	N/A
Acute neurotoxicity study, gavage F9600 technical, batch PL14-0049 Purity: 96 % Vehicle: 0.5 % (w/v) carboxymethylcellulose in 5 % Tween® 80 Guideline: OECD 424 Deviations: none GLP [redacted] (2014c) ***Acceptable***	Rats, Crl:CD(SD), males & females, 10/sex/group 0, 500, 1000 & 2000 mg/kg bw	**Neurotoxicity**: 2000 mg/kg bw **Generalised toxicity:** 2000 mg/kg bw	**Neurotoxicity**: > 2000 mg/kg bw **Generalised toxicity:** >2000 mg/kg bw	**Neurotoxicity**: No specific findings **Generalised toxicity:** No adverse findings
Repeated-dose combined toxicity and neurotoxicity study, F9600 technical, batch PL14-0049 Purity: 96 % Vehicle: 0.5 % (w/v) carboxymethylcellulose in 5 % Tween® 80 Guideline: OECD 424 Deviations: none GLP [redacted] (2016a) ***Acceptable***	Rats, Crl:CD(SD), males & females, 10/sex/group Dietary doses: 0, 500, 2000 and 8000 ppm in males (0, 29, 121 and 505 mg/kg bw/day) 0, 500, 2000 and 5000 ppm in females (0, 37, 150 and 351 mg/kg bw/day).	**Neurotoxicity**: 8000/5000 ppm (505/351 mg/kg bw/day in M/F) **Generalised toxicity:** 500 ppm (29/37 mg/kg bw/day in M/F)	**Neurotoxicity**: >8000/5000 ppm (>505/351 mg/kg bw/day in M/F) **Generalised toxicity:** 2000 ppm (121/150 mg/kg bw/day in M/F)	**Neurotoxicity**: No specific effects **Generalised toxicity:** Kidney and liver effects

2.6.8. ummary of further toxicological studies on the active substance

Supplementary studies on the active substance

Palatability studies in mice, rats and dogs over 7-day (diet) repeated administration have been conducted and they are summarised in Section 2.6.3 for repeated-dose toxicity. These studies concluded that there were transient palatability-related issues seen in the dog, but not in the rat and mouse.

No other supplementary studies on the active substance have been submitted.

The review of the published literature for bixlozone and its metabolites did **not** reveal any studies considered to significantly affect the regulatory toxicological assessment of human health.

Endocrine disruption (ED)

An assessment for potential endocrine disrupting properties of bixlozone has been provided by the applicant. This assessment was conducted 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).

Estrogen, Androgen and Steroidogenic (EAS) modalities

Parameters relevant to assessing the endocrine disrupting potential of bixlozone for the EAS modalities include developmental effects, and effects on sexual/reproductive organs and performance in both Level 4 and Level 5 studies.

In males, weight changes and / or histopathology findings were observed in the prostate in the rat at the top dose (2-generation study) and dog from 300 mg/kg bw/day (90-day study). However the findings did not indicate a specific adverse effect on the prostate as they were inconsistent across studies; also they occurred concurrently to systemic toxicity and were not accompanied with any functional impairment of spermatogenesis or reproduction in the 2-generation reproduction toxicity (Level 5) study. In addition a slightly higher incidence of reduced epididymal sperm was seen in the mouse (from 126 mg/kg bw/day) in the 18-month carcinogenicity study; however this was an isolated occurrence with no other reproductive organs affected and no such findings were seen in the 90-day mouse study. Overall, there was no clear pattern of adversity for male EAS parameters and the reported changes on prostate in the rat and dog and on epididymis in the mouse are unlikely to be related to an endocrine mechanism.

There were no treatment-related effects on female EAS-mediated parameters. Bixlozone had no effect on male or female fertility or reproductive performance; gestation duration, oestrus cycle and spermatogenic endpoints were also unaffected by treatment. There was also no effect on litter size, sex ratio, pup survival and developmental landmarks.

The age of attainment of vaginal opening of F1 pups was statistically significantly greater at the top dose (187 mg/kg bw/day) compared to the corresponding controls (33.6 days compared with 31.7 days). The mean body weights of the female pups at the age of attainment were unaffected by treatment with bixlozone, which indicates that the delay in vaginal patency was the consequence of reduced pup body weight development, because, once the pup body weight was similar to that of the controls, vaginal opening was attained. Moreover the values seen at the top-dose were well within the laboratory HCD provided, although these cover a period of 10 years. In addition, there were no notable effects on other developmental landmarks and these females went on to mate successfully and produce the F2 generation. Overall, HSE considers this finding the secondary consequence of reduced post-weaning female pup body weight development and not a specific endocrine effect of bixlozone.

An isolated occurrence of higher relative adrenal weights (without histologic correlates) described in the dog 28-day range-finding oral (diet) toxicity study at the top dose in both sexes (1015 / 1110 mg/kg bw/day) was not reproduced in the capsules studies, indicating it was most likely the secondary consequence of general toxicity caused by the unpalatability of the diet. There were no treatment-related changes in adrenal weights and/or histopathology reported in any of the other species studied. Overall repeated exposure to bixlozone in the rat, mouse, dog and rabbit was not associated with any clear treatment-related effects on the adrenal gland.

Overall, there was no clear pattern of adversity for the EAS modalities identified on male and female reproductive organs and other endocrine organs related to EAS modalities (e.g. adrenal, pituitary, mammary) following repeated exposure to bixlozone in all species investigated (rat, mouse, dog). In addition, there were no specific adverse effects on reproduction in the rat and on development in the rat and the rabbit.

EAS-mediated adversity has been sufficiently investigated, based on a modern 2-generation reproduction toxicity study conducted in the rat; the study was fully compliant with the OECD Guideline No. 416 (2001) and followed GLP

standards. No EAS activity studies are available for bixlozone and none are necessary; it is in addition noted that bixlozone was predicted not to bind to estrogen receptors (ER) using the OECD Toolbox.

Thyroid (T) modalities

A dose-related and adverse increase in the thyroid/parathyroid weights was reported in the 90-day dog oral (capsule) study across all dose-groups (from 30 mg/kg bw/day) in both sexes however the thyroid was not affected in any of the other dog studies and up to the highest doses tested; thus the finding was likely to be a spurious finding. In addition there were no biologically relevant thyroid weight changes noted in the rat and the mouse in any of the relevant studies investigating potential adverse effects on the thyroid.

An increased incidence of mild follicular cell hypertrophy was noted in both sexes at the top dose compared to controls in the rat 90-day repeated dose toxicity study; excessive systemic toxicity occurred at that dose. The hypertrophy was not observed at the end of the 28-day recovery period. There were no similar occurrences reported in any of the other species investigated including in studies where comparable/higher dose levels of bixlozone were tested. Thus the relationship of this isolated histopathology observation to a specific effect of bixlozone on the thyroid was considered to be unlikely.

In the 2-year carcinogenicity study in the rat there was a non-statistically significant but dose-related increase in the incidence of follicular cell adenomas and follicular cell carcinomas in the thyroid gland of females at the top-dose of 3000 ppm (167 mg/kg bw/day) in comparison to controls. Considering the sex specificity of the response, the low incidence of the tumours and the low biological plausibility of the finding, HSE concluded that the thyroid tumours observed in female rats at the top dose are chance findings unrelated to treatment.

Overall, it was shown that repeated exposure to bixlozone in Level 5 and Level 4 studies in rats, mice and dogs was not associated with any clear or specific effects on the thyroid gland, with only isolated incidences of thyroid weight changes reported in the 90-day dog study or histopathology described in the 90-day rat study. Therefore there was no evidence of a clear pattern of adversity for the T modality. In addition, there was no indication of adverse pre- and post-natal neurological development of the offspring in the available 2-generation reproduction toxicity (Level 5) study in the rat and the Level 4 developmental toxicity studies in the rat and the rabbit. Therefore a potential concern for neurodevelopment was considered unlikely for bixlozone.

Overall bixlozone did not present a clear pattern of adversity for the T modality in relation to effects on the thyroid gland and/or neurodevelopment effects.

T-mediated adversity (thyroid weight and histopathology) has been sufficiently investigated, based on the following studies in which thyroid effects were investigated:

- 28-day oral toxicity studies in the rat, mouse, dog (OECD TG No. 407)
- 90-day oral toxicity studies in the rat, mouse, dog (OECD TG No. 408)
- Chronic toxicity / carcinogenicity studies in the rat and mouse (OECD TG No. 453)
- 2-generation reproductive toxicity study in the rat (OECD TG No. 416).

All were modern OECD compliant studies, however they were completed before the requirement to investigate additional thyroid-related parameters was added to OECD Guideline 408 (2018) (28-day study) and 414 (2018) (pre-natal developmental study). Overall, the available data showed that bixlozone did not present a clear pattern of adversity for the T modality in relation to effects on the thyroid gland and/or neurodevelopment effects. No thyroid activity studies are available for bixlozone and none are considered necessary.

Overall conclusion on ED

Overall, bixlozone does not meet the ED criteria of Regulation (EC) No 2018/605 of 19 April 2018, amending Annex II to Regulation (EC) No 1107/2009. HSE concludes that for the EATS-modalities bixlozone is not an ED and its ED potential has been sufficiently investigated and that no further information is required.

Immunotoxicity

No specific immunotoxicity study conducted with bixlozone is available. Nevertheless, potential biomarkers of immunotoxicity have been measured in the existing toxicology studies including short-term, chronic, carcinogenic and reproductive toxicity studies conducted in multiple species (rat, dog and mouse). In all of the relevant toxicology studies conducted, immunotoxicology parameters including haematology (blood neutrophil, mononuclear cells, eosinophils, white blood cell (WBC) counts), clinical chemistry (albumin and globin ratio), organ weights (spleen, thymus), gross and histopathological examination of immunological organs (thymus, spleen, bone marrow and lymph nodes) have been included. There was no indication that bixlozone had effects on the immune system in experimental animals from any of the studies conducted.

Overall, HSE concludes that bixlozone does not affect the immune system, and a specific *in vivo* immunotoxicity study is not required.

2.6.9. Summary of toxicological data on impurities and metabolites

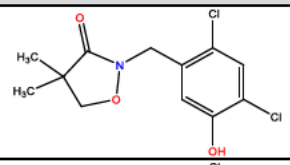
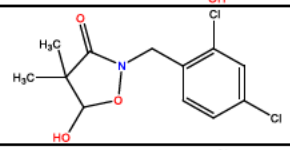
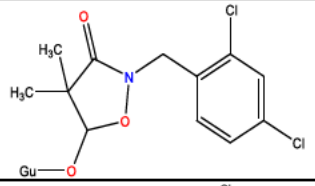
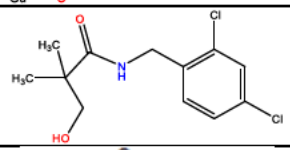
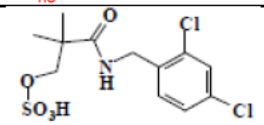
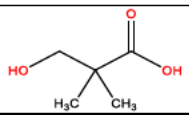
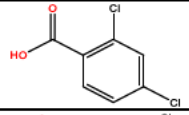
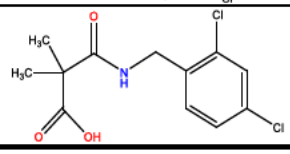
Relevant impurities

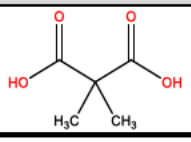
Following the toxicological assessment of the relevance of impurities present in bixlozone technical, including theoretical impurities, it was concluded that the proposed reference specification contains only one toxicologically relevant impurity:

- (2,4-dichlorophenyl)methanol (CAS 1777-82-8) specified at 0.15 % w/w (15 g/kg)

Metabolites

The following metabolites of bixlozone were selected for potential inclusion in the residue definitions based on their significant occurrence in the plant and livestock metabolism studies (Volume 3 Section B.7):

Compound name	Code (metabolism studies)	FMC Code[1]	Structure
5'-hydroxy-bixlozone	M289/3	FMC-077038	
5-hydroxy-bixlozone	M289/1	FMC-510226	
5-hydroxy-bixlozone-glucuronide	M465/1	N/A	
bixlozone-3-OH-propanamide	M275/1	FMC-510232	
bixlozone-3-OH-propanamide-sulfate	M355/1	N/A	
2,2-dimethyl-3-hydroxy propionic acid	M118/1	FMC-057089	
2,4-dichlorobenzoic acid [2]	M190/1	FMC-510224	
bixlozone-dimethyl-malonamide	M289/2	FMC-510233	

Compound name	Code (metabolism studies)	FMC Code[1]	Structure
Dimethyl-malonic acid	M132/1	FMC-043942	

[1] FMC Codes not generated for phase II metabolites

[2] Observed as the glycine conjugate: 2,4-dichlorohippuric acid

No specific toxicity studies are available on these metabolites, except for 2,4-dichlorobenzoic acid; in order to assess the toxicological properties of these metabolites all the available data (including data relating to bixlozone) were taken into account by HSE. These included:

- The presence of these metabolites in rat ADME and other relevant toxicity studies performed with bixlozone
- Structural similarity to bixlozone
- *In silico* genotoxicity assessment
- *In silico* assessment of general toxicity
- Publicly available data available on the metabolites

The metabolite 5'-hydroxy-bixlozone (M289/3) is not a major metabolite in rats. Although it is structurally very similar to bixlozone (it only differs from it by the presence of an additional hydroxy group on the phenyl ring) as confirmed by the comparative *in silico* analysis, it would be more prudent not to assume equivalence with the parent in relation to general toxicity as the reliability of QSAR predictions for complex endpoints is generally low. Having excluded genotoxicity by QSAR analysis, if a risk assessment were to be required for **5'-hydroxy-bixlozone, the Cramer class III TTC chronic value of 1.5 µg/kg bw/day and acute value of 5 µg/kg bw**[1] could be used in a conservative first-tier assessment.

The metabolite 5-hydroxy-bixlozone (M289/1) is a putative major rat metabolite considered to be covered via its downstream metabolite 5-keto-hydrate-bixlozone. On this basis, its toxicity profile could be considered 'covered' by the parent. It is structurally very similar to bixlozone since it only differs from it by the presence of an additional hydroxy group on the isoxazolidinone ring. No additional *in silico* alerts were flagged for this metabolite for genotoxicity or general toxicity hazards compared to bixlozone. In addition, the glucuronide conjugated form of 5-hydroxy-bixlozone (5-hydroxy-bixlozone-glucuronide) is expected to have a comparable or less severe toxicity profile than 5-hydroxy-bixlozone. Overall, the toxicological properties of **5-hydroxy-bixlozone** (M289/1) and **5-hydroxy-bixlozone-glucuronide (M465/1)**, as major rat metabolites, can be considered to have been intrinsically tested in the toxicological studies undertaken with bixlozone and thus these metabolites can be considered of **equivalent toxicity to the parent substance** and potential candidates for inclusion in the Residue Definition from a toxicological perspective. If a risk assessment were to be required for 5-hydroxy-bixlozone and 5-hydroxy-bixlozone-glucuronide, the **dietary reference values of bixlozone** could be used.

The metabolite bixlozone-3-OH-propanamide (M275/1) shares some structural similarity to bixlozone; however additional alcohol and carboxylic acid amide functional groups are formed when the isoxazolidinone ring of bixlozone is opened up. Although no additional *in silico* alerts were flagged for this metabolite for genotoxicity or general toxicity compared to bixlozone, the reliability of QSAR predictions for complex general toxicity endpoints is low. Its conjugate form, bixlozone-3-OH-propanamide-sulfate (M355/1) is expected to have a comparable or less severe toxicity. Its downstream metabolite bixlozone-dimethyl-malonamide (M289/2) is structurally close to bixlozone-3-OH-propanamide; both shared the same comparative *in silico* findings. None of these metabolites is a major rat metabolite. However, having excluded genotoxicity by QSAR analysis, if a risk assessment were to be required for **bixlozone-3-OH-propanamide (M275/1), bixlozone-3-OH-propanamide-sulfate (M355/1) and bixlozone-dimethyl-malonamide (M289/2), the Cramer class III TTC chronic value of 1.5 µg/kg bw/day and acute value of 5 µg/kg bw** could be used in a conservative first-tier assessment. Given their close structural similarity, a combined risk assessment of these three metabolites against the TTC values should be performed, if required.

The metabolite 2,4-dichlorobenzoic acid (M190/1) is a putative major rat metabolite considered to be covered via its downstream glycine conjugate 2,4-dichlorohippuric acid, the latter being recovered in rat urine at levels > 10 % of the

[1] EFSA (2012) Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07):2799

AD in both sexes following single low dose oral exposure. On this basis, its toxicity profile could be considered 'covered' by the parent. However, specific data (acute oral toxicity studies in rat and mouse and modern in vitro genotoxicity studies) are available on this metabolite. These data take precedence on the kinetic prediction and indicate that 2,4-dichlorobenzoic acid is not genotoxic in vitro in modern studies but may be approximately 2-fold more toxic than bixlozone. On this basis, it is concluded that **2,4-dichlorobenzoic acid (M190/1) is more toxic than the parent** and a likely candidate for inclusion in the Residue Definition from a toxicological perspective. If a risk assessment were to be required, **the dietary acute and chronic reference values of bixlozone should be used**, adjusting the residue estimate of 2,4-dichlorobenzoic acid for a relative potency factor of 2. In addition, a modifying factor of 1.435 should also be applied to account for the molecular weight conversion between the metabolite and the parent. This will allow to express 2,4-dichlorobenzoic acid into parent bixlozone equivalents.

The two metabolites 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl-malonic acid (M132/1) are not structurally similar to bixlozone but are closely related to each other. Both substances are not major rat metabolites. No additional *in silico* alerts were flagged for these metabolites for genotoxicity compared to bixlozone; they both have classification notifications indicating a more severe toxicity profile compared to bixlozone however these general toxicity hazards (local irritant effects on skin, eye and respiratory tract) are of no relevance to the dietary route of exposure. Having excluded genotoxicity by QSAR analysis, if a risk assessment were to be required for **2-dimethyl-3-hydroxy propionic acid and dimethyl-malonic acid, the Cramer class I TTC chronic value of 30 µg/kg bw/day could be used in a conservative first-tier assessment. This TTC value can also be used for the acute exposure assessment for these metabolites (when performing an initial 'screen' versus the TTC (CCI)).** Given their close structural similarity, a combined risk assessment of these two metabolites against the TTC value should be performed, if required.

2.6.10. Summary of medical data and information

Bixlozone is a new herbicidal active ingredient, which has not yet been sold commercially. It has been handled by only a limited number of employees or contract scientists involved in regulatory and field biological testing. Therefore, human data is still limited at this time. As of 11 January 2018, there were no reports of diseases or adverse health effects attributed to exposure associated with the handling, testing or manufacture of bixlozone and formulations containing bixlozone. At the time of submission of the bixlozone dossier, there were no reports of clinical cases and poisoning.

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

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

Table 2.6.11.1 : Summary of all studies relevant to setting of reference values for bixlozone

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
REPEATED-DOSE TOXICITY				
Dietary 28-day GLP OECD 407 (2008) Deviations: None ([redacted](2015a)) ***Acceptable***	**Rat** (Crl :CD9(SD), males & females) F9600 technical; Batch PL13-0385 Purity 99.2% 0, 750, 2500, 5000, and 10000 ppm Equivalent to: Males: 0, 57, 182, 359 and 740 mg/kg bw/day	57 (males) 61 (females) (750 ppm) (The applicant proposed a NOAEL of 740 / 733 for males / females)	182 (males) 193 (females) (2500 ppm)	↓ food consumption in females: 12 %** (days 0-7) & 11 %* (days 7-14) *Organ weights* ↑ relative liver weight: 17 %** (F), 15.5 %** (M) *Histopathology - liver* Hepatocellular hypertrophy: 4/5 minimal (F) & 3/5 minimal (M)

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
	Females: 0, 61, 193, 379 & 733 mg/kg bw/day			
Dietary 28-day GLP OECD 407 (2008) Deviations: none ([redacted] (2015b)) ***Acceptable***	**Mouse** (Crl:CD-1, males & females) F9600 technical; Batch PL13-0385 Purity 99.2% 0, 1000, 2000, 4000, and 5000 ppm Equivalent to: Males: 0, 187, 381, 788 & 985 mg/kg bw/day Females: 0, 289, 554, 984 & 1384 mg/kg bw/day	381 (males) 554 (females) (2000 ppm) (The applicant proposed a NOAEL of 985 / 1384 mg/kg bw/day for males / females)	788 (males) 984 (females) (4000 ppm)	*Organ weights* ↑ absolute liver weight: 18 %* (F) ↑ relative liver weight: 21.5 %** (F) *Histopathology - liver* Hepatocellular hypertrophy: 2/5 F (minimal) & 1/5 M (minimal)
Dietary 90-day GLP OECD 408 (1998) & OECD 424 (1997) Deviations: None ([redacted] (2016a)) ***Acceptable***	**Rat** (Rat, Crl :CD9(SD), males & females) F9600 Technical; batch PL14-0049 Purity 96 % 0, 500, 2000, and 8000 ppm (males) Equivalent to: 0, 29, 121 & 505 mg/kg bw/day 0, 500, 2000, and 5000 ppm (females) Equivalent to: 0, 37, 150 & 351 mg/kg bw/day	**29** (males) **37** (females) (500 ppm) (The applicant proposed a NOAEL of 121 / 150 for males / females)	121 (males) 150 (females) (2000 ppm)	*Organ weights* ↑ liver weights in females: 16 %* absolute & 17 %** relative ↑ kidney weights in males: 15 %* (absolute) & 14.5 %** (relative) *Histopathology - liver* Hepatocellular hypertrophy 1/10 F (mild) *Clinical chemistry* ↑ cholesterol +44 %**, globulin +11 % and calcium +4.5 %* (F)
Dietary 90-day GLP OECD 408 (1998) Deviations: None ([redacted] (2016a)) ***Acceptable***	**Mouse** (Crl:CD1(ICR), males & females) F9600 Technical; batch PL14-0049 Purity 96 % 0, 1000, 2250, and 5000 ppm Equivalent to: Males: 0, 180, 414 &930 mg/kg bw/day Females: 0, 257, 583 & 1185 mg/kg bw/day	180 (males) 257 (females) (1000 ppm) (The applicant proposed a NOAEL of 930 / 1185mg/kg bw/day for males / females)	414 (males) 583 (females) (2250 ppm)	↑ relative liver weights: 17.5 % (F) ↑ absolute liver weights: 13 % (F) Hepatocellular hypertrophy in 4/10 M (3 minimal, 1 mild) Hepatocellular hypertrophy in 3/9 F (minimal)

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
90-day, capsule GLP OECD 409 (1998) Deviations: None [redacted] (2016c) ***Acceptable***	**Dog** (Beagle, males and females) F9600 technical; batch PL14-0049 Purity 96 % 0, 30, 100, 300, and 750 mg/kg bw/day	**30** (females) (The applicant proposed a NOAEL of 750 mg/kg bw/day)	100 (females)	*Organ weights* ↑ liver weights in F (27 %* absolute, 22 %** relative)
12-month, capsule GLP OECD 452 (1998) Deviations: None ([redacted] (2017)) ***Acceptable***	**Dog,** (Beagle, males & females), 4/sex/group F9600 technical, batch PL14-0049 Purity: 96% 0, 20, 100 and 500 mg/kg bw/day	100 (The applicant proposed a NOAEL of 500 mg/kg bw/day)	500	↑ WBC (+35 % week 26*; +27 % week 52) ↑ monocyte absolute (+55% week 26*;+15 % week 52) ↑ lymphocytes absolute (+34 % week 26; +55 % week 52**) ↑ PT (+8 % week 26*; +13 % week 52**) in males
21-day, dermal GLP OECD 410 (1981) Deviations: None ([redacted] (2016)) ***Acceptable***	**Rat,** Crl :CD9(SD), males & females F9600 technical, batch PL14-0049 Purity: 99.2% 0, 100, 300 and 1000 mg/kg bw/day	> 1000 (The applicant proposed a NOAEL of 1000 mg/kg bw/day)	N/A	No adverse effects observed up to the highest dose tested
CARCINOGENICITY STUDIES				
Dietary 24-month GLP OECD 453 (2009) Deviations: none ([redacted] (2017)) ***Acceptable***	**Rat** (Crl:CD (SD) rats, males and females) 0, 250, 1000, 5000/3000 ppm Equivalent to: Males: 0, 10, 41, 217 mg/kg bw/day Females: 0, 13, 53, 167 mg/kg bw/day	*Carcinogenicity* 217 / 167 (5000 / 3000 ppm in M/F)	*Carcinogenicity* > 217 / 167 (5000 / 3000 ppm in M/F)	*Carcinogenicity* No biologically relevant neoplastic findings
		Systemic chronic toxicity 41 (males) 53 (females) (1000 ppm)	*Systemic chronic toxicity* 217 (males) 167 (females) (5000/3000 ppm)	*Systemic chronic toxicity* Dermal atonia and thin body condition in F ↓ body weight gain for both sexes (9-14%** M and 14-24.5%** F) *Organ weights* ↑ relative liver weight > 15 %** (both sexes) ↓ cholesterol**, albumin*, calcium**, total protein* (F) *Histopathology findings - liver* Hepatocellular hypertrophy: 7/10 at 52 weeks & 79 % incidence at 104 weeks (M)

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
				10/10 at 52 weeks & 74 % incidence at 104 weeks (F) Hepatocellular vacuolation: 7/10 at 52 weeks & 32 % incidence at 104 weeks (M) 10/10 at 52 weeks & 74 % incidence at 104 weeks (F)
Dietary 18-month GLP OECD 451 (2009) Deviations: none ([redacted] (2017)) ***Acceptable***	**Mouse** (Crl:CD1(ICR mice, males and females) 0, 250, 1000, 5000 ppm Equivalent to: Males: 0, 32, 126, 647 mg/kg bw/day Females: 0, 43, 164, 834 mg/kg bw/day	*Carcinogenicity* 647 (M) 834 (F) (5000 ppm)	*Carcinogenicity* > 647 (M) > 834 (F) (> 5000 ppm)	*Carcinogenicity* None attributable to exposure to bixlozone up to the highest dose tested (5000 ppm).
		Systemic chronic toxicity **32** (males) (250 ppm)	*Systemic chronic toxicity* 126 (males) (1000 ppm)	*Systemic chronic toxicity* ↑ incidence of reduced sperm in epididymes (M) ↑ incidence of inflammation of glandular stomach (M)
REPRODUCTIVE TOXICITY				
2-generation dietary study GLP OECD Guideline 416 (2001) Deviations: None ([redacted] (2016c)) ***Acceptable***	**Rats** (Crl:CD(SD), males & females) 25/sex/group F9600 technical batch PL14-0049; purity 96 % 0, 150, 750, 3000 ppm Equivalent to doses expressed as mg/kg bw/day as in Table 6.6.1.2.1	**Parental toxicity: 750 ppm** Equivalent to: 34/49 mg/kg bw/d in M/F **Offspring toxicity: 750 ppm**	**Parental toxicity: 3000 ppm** Equivalent to: 140/187 mg/kg bw/d in M/F **Offspring toxicity: 3000 ppm**	**Parental toxicity** ***F0 generation*** There were no treatment related deaths or clinical signs of toxicity. No treatment-related findings. ***F1 generation*** ↓ body-weight gains in M** & F ↓ body weights in M** & F* *Organ weights* ↑ absolute liver weights in F (+13 %**) ↑ relative liver weights: +14 %** (M) & +21 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %** (F) *Histopathology* Hepatocellular hypertrophy in F ↑ mononuclear cell infiltration (chronic inflammation) in the prostate* **Offspring toxicity** *F1 pups*

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
		Equivalent to 34/49 mg/kg bw/day in M/F **Reproductive: 3000 ppm** Equivalent to > 140/187 mg/kg bw/day in M/F	Equivalent to 140/187 mg/kg bw/day in M/F **Reproductive: > 3000 ppm**	↑ relative liver weights: +18 % (M)* Delay in vaginal opening (33.6 days vs 31.7 days in controls) *F2 pups* ↓ mean body weights PND 14 (-8 %) No treatment-related findings. **Reproductive toxicity** No specific adverse effects up to top dose
Developmental dietary study GLP OECD Guideline 414 (2001) Deviations: None ([redacted] (2016e)) ***Acceptable***	**Rats** (Crl:CD(SD), females) 25/group F9600 Technical Batch PL14-0049 Purity 96.0 % 0, 75, 225 & 550 mg/kg bw/day	**Maternal toxicity:** **75 mg/kg bw/day** **Developmental toxicity:** 550 mg/kg bw/day	**Maternal toxicity:** 225 mg/kg bw/day **Developmental toxicity:** >550 mg/kg bw/day	**Maternal toxicity:** Clinical signs: red, yellow and/or clear material on various body surfaces Early ↓ body-weight gain: -40 % (GD 6-9)* ↓ food consumption: -8 % (GD 6-20)* **Developmental toxicity:** No adverse effects up to top dose
Developmental dietary study GLP OECD Guideline 414 (2001) Deviations: None ([redacted] (2015)) ***Acceptable***	**Rabbits** (New Zealand White, females) 25/group F9600 Technical Batch PL14-0049 Purity 96.0 % 0, 25, 75, 200 & 400 mg/kg bw/day	**Maternal toxicity:** 200 mg/kg bw/day **Developmental toxicity:** 400 mg/kg bw/day	**Maternal toxicity:** 400 mg/kg bw/day **Developmental toxicity:** > 400 mg/kg bw/day	**Maternal toxicity:** ↓ defecation ↓ body-weight gain -32 % (GD 13-20) ↓ food consumption -18 % (GD 13-20) **Developmental toxicity:** No adverse effects up to top dose
NEUROTOXICITY				
Acute neurotoxicity study, gavage GLP Guideline: OECD 424 Deviations: none [redacted] (2014c) ***Acceptable***	**Rats,** Crl:CD(SD), males & females 10/sex/group F9600 technical, batch PL14-0049 Purity: 96 % 0, 500, 1000 & 2000 mg/kg bw	**Neurotoxicity:** 2000 mg/kg bw **Generalised toxicity:** 2000 mg/kg bw	**Neurotoxicity:** >2000 mg/kg bw **Generalised toxicity:** >2000 mg/kg bw	**Neurotoxicity:** No specific adverse findings up to the top dose **Generalised toxicity:** No specific adverse findings up to the top dose

Study, guideline, reference, acceptability	Species, doses tested	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL
Repeated-dose combined toxicity and neurotoxicity study, diet GLP Guideline: OECD 424 Deviations: none [redacted] (2016a) ***Acceptable***	**Rats**, Crl:CD(SD), males & females 10/sex/group F9600 technical, batch PL14-0049 Purity: 96 % 0, 500, 2000 and 8000 ppm in males (0, 29, 121 and 505 mg/kg bw/day) 0, 500, 2000 and 5000 ppm in females (0, 37, 150 and 351 mg/kg bw/day).	**Neurotoxicity** 8000 / 5000 ppm (505/531 mg/kg bw/day in M/F) **Generalised toxicity** 500 ppm (29/37 mg/kg bw/day in M/F)	**Neurotoxicity** >8000 / 5000 ppm (>505/531 mg/kg bw/day in M/F) **Generalised toxicity** 2000 ppm (121/150 mg/kg bw/day in M/F)	**Neurotoxicity** No specific adverse findings up to the top dose **Generalised toxicity** Kidney and liver effects

An acceptable daily intake (ADI) is usually established from long-term, repeated-dose and reproductive toxicity studies. The toxicological profile of bixlozone has been investigated in long-term toxicity / carcinogenicity studies in rats and mice, in repeated-dose toxicity studies in rats, mice and dogs, and in reproductive and developmental toxicity studies in rats and rabbits.

The critical NOAEL and LOAEL values extracted from these studies demonstrated that the liver was a clear target organ in all three species, with the rat and dog being the most sensitive species. Reduction in body weight / body weight gain, increased liver weight, clinical-chemistry changes and hepatocellular hypertrophy were the lead effects observed in the rat. The kidney is also a target organ in the rat.

The lowest NOAEL extracted from all the relevant studies is set at **29**/37 mg/kg bw/day (males/females) identified in the **rat 90-day dietary** oral repeated-dose toxicity study ([redacted] (2016a)). Adverse effects on the liver were observed at the LOAEL of 121/150 mg/kg bw/day (males/females) and were characterised by liver weight changes, mild histopathology findings and serum chemistry changes indicative of liver function (cholesterol, total protein, calcium); absolute and relative kidney weights were also affected at this dose.

This value is supported by the NOAEL of 34/49 mg/kg bw/day (males/females) for parental toxicity (effects on body weight and body-weight gain and an increase in liver and kidney weights which correlated with histopathology findings for the liver) identified in the dietary 2-generation reproductive toxicity study ([redacted] (2016c)), the NOAEL of 30 mg/kg bw/day for effects on the liver from the 90-day oral (capsule) study in dogs ([redacted] (2016c)) and the NOAEL of 32 mg/kg bw/day (males) for chronic systemic toxicity (effects on epididymal sperm and inflammation of stomach in males) in the 18-months study in mice ([redacted] (2017)).

Therefore, HSE considers that the value of 29 mg/kg bw/day from the rat dietary oral 90-day repeated-dose toxicity study, supported by the 90-day dog study (NOAEL of 30 mg/kg bw/day), is the most relevant to use to derive the ADI. The application of the standard factors of 10 for each of intra- and inter-species differences results in an **ADI of 0.3 mg/kg bw/day**. There is no evidence to suggest it is necessary to deviate from these standard assessment factors.

The applicant proposed an ADI of 0.34 mg/kg bw/day, based on their lowest NOAEL set at 34 mg/kg bw/day from the 2-generation reproductive toxicity study in rats.

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

An Acute Reference Dose (ARfD) is usually established from acute and short-term toxicity studies. Bixlozone is not acutely toxic by the oral route, is not a neurotoxicant and is not a developmental toxicant. In principle, the setting of an Acute Reference Dose (ARfD) is thus not necessary.

However, in the rat (oral gavage) developmental toxicity study ([redacted] (2016e)) there was an initial reduction in maternal body weight at the start of dosing (-40 % for GD 6-9, compared to control group, statistically significant) at 225 mg/kg bw/day. A NOAEL for maternal toxicity of **75 mg/kg bw/day** was proposed by HSE.

This NOAEL is an appropriate starting point for the derivation of the ARfD. By applying a standard assessment factor of 100, an **ARfD of 0.75 mg/kg bw** is derived.

The applicant did not propose an ARfD based on low acute toxicity, no adverse developmental effect in gavage studies and no findings in the acute neurotoxicity study.

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

The systemic acceptable operator exposure level (AOEL) is the maximum level of active substance to which spray operators, workers, bystanders of resident population can be exposed, without incurring any adverse health effects, and is usually derived from short-term toxicity studies in rats (including the two-generation study), mice and dogs. The lowest NOAEL from the short-term repeated-dose toxicity studies was set at **29**/37 mg/kg bw/day (males/females) from the 90-day rat study ([redacted] (2016a)); adverse effects on the liver were observed at the LOAEL of 121/150 mg/kg bw/day (males/females) and were characterised by liver weight changes, mild histopathology findings and serum chemistry changes indicative of liver function (cholesterol, total protein, calcium). Absolute and relative kidney weights were also affected at this dose.

This value is supported by the NOAEL of 34/49 mg/kg bw/day (males/females) identified in the dietary 2-generation reproductive toxicity study ([redacted](2016c)) for parental toxicity (effects on body weight, liver, kidney and prostate), the NOAEL of 30 mg/kg bw/day for effects on the liver from the 90-day oral (capsule) study in dogs ([redacted] (2016c)) and the NOAEL of 32 mg/kg bw/day (males) for chronic systemic toxicity (effects on epididymal sperm and inflammation of stomach in males) in the 18-months study in mice ([redacted](2017)).

HSE therefore considers it appropriate to use the NOAEL of 29 mg/kg bw/day from the 90-day rat study to derive the AOEL.

Since an AOEL is an internal (systemic) dose, it should be adjusted according to the extent of systemic bioavailability. A **systemic bioavailability value of 70 %** has been determined in the rat (Section B.6.1).

Overall, by applying the standard factors of 10 for each of intra- and inter-species differences and the bioavailability value of 70 %, the overall **systemic AOEL of 0.2 mg/kg bw/day (rounded value of (29 / 100) × 70 %)** is proposed.

2.6.14. Toxicological end point for assessment of the Acute Acceptable occupational, bystander and residents risks – AAOEL

HSE is of the view that an Acute Acceptable Operator Exposure Level (AAOEL) should not be derived from the ARfD as the effects driving its NOAEL (initial reduction in maternal body weight at the start of dosing at 225 mg/kg bw/day in the rat (oral gavage) developmental toxicity 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.

2.6.15. Summary of product exposure and risk assessment

Operator exposure

A non-dietary human exposure risk assessment for bixlozone has been conducted based on the representative product 'F9600-4SC' containing 400 g a.s./L. Exposure was estimated using the EFSA guidance (EFSA Journal 2014;12(10):3874) and the respective EFSA Calculator (EFSA Calculator version: 30 March 2015). Bixlozone does not have significant acute toxicity or the potential to exert toxic effects after a single exposure, therefore no acute risk assessment is required. 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. Thus, long-term exposure assessment also covers acute exposure assessment.

An acceptable long-term systemic operator exposure equal to 7.5 % of the AOEL of bixlozone is predicted for an operator that applies the product 'F9600-4SC' without using PPE.

The product 'F9600-4SC' is not classified for human health effects, therefore, no additional PPE is required.

Bystander and resident exposure

Exposure to bystanders and residents has been calculated using the EFSA Calculator. The exposure assessment for inhalation of vapour has been conducted using the EFSA Guidance default value for average concentration in air in the 24 hours after application of 1 µg/m³ for low volatile substances with a vapour pressure between $<5 \times 10^{-3}$ Pa. Bixlozone has a vapour pressure of 1.1×10^{-3} Pa at 20°C and 2.3×10^{-3} Pa at 25 °C according to DAR04 Volume 3(AS) Section B2. The vapour pressure of Bixlozone is therefore within the specified range for low volatile substances at both 20°C and 25°C.

The longer term exposure assessment to residents indicates that the proposed outdoor uses of 'F9600-4SC' will result in an acceptable risk of exposure to an unprotected adult and child. The longer term exposure to residents from the sum of all pathways is acceptable and estimated to be 12% and 5% of the AOEL for bixlozone for a child and adult resident respectively.

Bixlozone does not have significant acute toxicity or the potential to exert toxic effects after a single exposure, therefore no bystander risk assessment is required.

Worker exposure

For the proposed uses of the product 'F9600-4SC' an acceptable worker exposure equal to 6.3 % of the AOEL of bixlozone is predicted for a worker that performs crop inspection or irrigation activities wearing normal workwear (arms, legs and body covered).

Conclusion

The operator, bystander, resident and worker risk assessment demonstrates an acceptable risk of exposure to bixlozone under conditions of intended uses of the representative product 'F9600-4SC', thus it is concluded that all the proposed uses of the representative product are safe.

2.7. RESIDUE

2.7.1. Summary of storage stability of residues

Stability in a representative crop from four crop groups has been considered: high oil (oilseed rape seed), high acid (grape), high water (lettuce) and high starch (potato tuber, radish root, wheat grain), as well as wheat straw which falls into no specific grouping, for at least 24 months (18 months for 5-hydroxy-bixlozone). The analytes chosen to study reflects the analytes investigated in the primary crop trials (bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone, 2,2-dimethyl-3-hydroxy propionic acid and 5'-hydroxy-bixlozone) and rotational crop field trials (bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone). The metabolite codes for these are stated in Table 2.7.1.1.

Table 2.7.1.1 Stability of residues in samples stored at -18°C

Commodity	**Crop Group**	**Sufficient stability of residues observed (months)**							
		Bixlozone [F9600]	2,4-dichlorobenzoic acid [M190/1]	5-hydroxy-bixlozone [M289/1]	2,2-dimethyl-3-hydroxy propionic acid [M118/1]	5'-hydroxy-bixlozone [M289/3]	Bixlozone-dimethyl-malonamide [M289/2]	Bixlozone-OH-isobutyramide [M261/1]	4-hydroxymethyl-bixlozone [M289/4]
		Metabolites investigated in relation to residues in primary crops (Method CAM 0154)					*Metabolites investigated in relation to residues in rotational crops* (Method CAM 0180)		
Oilseed rape seed	High oil	24	24	18	24	24	-	-	-
Potato tuber	High starch	24	24	18	24	24	-	-	-
Grapes	High acid	24	24	18	24	24	-	-	-
Wheat straw	Not listed in OECD 506 but representative of dry commodities	24	24	18	24	24	24	24	24
Radish root	High starch	-	-	-	-	-	24	24	24
Leaf lettuce	High water	-	-	-	-	-	24	24	24
Wheat grain	High starch	-	-	-	-	-	24	24	24

There was no indication of instability in the matrices tested. All analytes were sufficiently stable for at least 24 months (at least 18 months for 5-hydroxy-bixlozone).

A mixed standard was used to fortify the test samples in the study determining stability of bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid and in the study determining stability of bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone. This approach is not recommended in accordance with OECD Guideline 506. However, as no clear instability or decline has been observed in this case, there are no concerns with the use of mixed standards in these studies.

In both the residues trials and the storage stability studies, the samples extracts were not analysed immediately. This is not ideal as Regulation (EU) 283/2013 states that: "*the stability of extracts shall be investigated if extracts are not analysed immediately*." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required.

Considering the proposed uses and the analytes expected in primary crops, all relevant matrices have been tested except cereal grain. This is not ideal; primary crop trials analysing for grain have stored samples for long periods (> one year and almost two years in a number of the primary crop trials). It is noted also that processing studies are available that consider the magnitude of residues over processing and these studies consider residues in processed grain fractions. In these processing studies, samples of the raw agricultural commodity (RAC) and processed fractions were stored for long periods of frozen storage (e.g. around 300 days in the wheat magnitude of residue studies). However, based on the low residues found in crops for the intended uses for the analytes that are proposed to be included in the residue definition for dietary risk assessment, RD-RA (bixlozone and 2,4-dichlorobenzoic acid, please see section 2.7.3) the studies on magnitude of the residues over processing do not need to be fully relied upon for the currently intended uses.

In terms of a comprehensive approach to cover all commodities, the data are limited. Considering the analytes relevant to primary crops, whilst no decline was observed, only one representative from the high oil, high starch and high acid groups, plus cereal straw, have been tested. A representative commodity from the high water and high protein groups have not been tested for the analytes relevant to primary crops. For future uses, it will be necessary to judge whether the currently available data suitably cover new trials data and future crop uses (also consideration of needed analytes to address dietary risk assessment and enforcement), or whether further stability data would be needed if field trial samples are stored long term.

To support cereal uses (and other crop uses), storage stability data on either cereal grain or representatives of the high water and high protein commodity groups (to cover all crops; data on representatives of all five commodity categories) should be generated in accordance with the OECD guideline 506 and using suitably validated analytical methods. These data should be provided to support a product authorisation for cereal crops. If stability in cereal grain (or a broad range of raw agricultural commodity types) is demonstrated, then the principle of extrapolation of these data to cereal processed fractions (or a range of broad commodity types) is considered reasonable.

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

Plant metabolism studies have been submitted on wheat, canola (oilseed rape), sugar beet and rice. Livestock metabolism studies have been submitted dosing with parent bixlozone in goats and hens. These studies are summarised in this section.

Comments relating to various metabolism studies - Sample storage periods

The main metabolism studies, on primary crops, rotational crops, and livestock (hen and goat), all involved undertaking the studies over a longer period than is desirable for the conduct of radiolabelled metabolism studies.

Samples were stored frozen for periods in the range of up to 1.5 years (livestock studies) and up to 3.4 years (for wheat metabolism studies). During the studies, samples, either as raw samples, or homogenised powders or extracts, were stored frozen during the course of the studies. OECD Guidelines 501, 502 and 503 indicate that metabolism studies

should be completed within an analysis period of six months, or otherwise be appropriately supported by storage stability investigations performed in the context of the metabolism studies.

The storage stability investigations performed within the scope of the metabolism studies (only in the following studies: wheat, sugar beet, and poultry) have been written up in each of the metabolism study evaluations (Vol 3, section B.7.2 and section B.7.6.1) but are considered limited. It is considered that the comparisons made (based on chromatographic comparison of extracts or samples stored and reanalysis after a period) do not support firm conclusions on the stability of residues over the course of these studies.

All the metabolism studies stated the dates until 'initial analyses' which were either within 6 months of the samples being taken or not very far away from a six-month timeframe. HSE sought to obtain more specific information from the applicant about timings of various aspects of the work in each metabolism study to determine whether the main pathways of metabolism had been elucidated in good time and to support the validity of the results of the studies. The response from the applicant lead to some further uncertainties in the understanding of the data.

The enquiry to the applicant also sought to find out whether there were further chromatographic data to inform the situation and that could help support storage stability comparisons. The applicant responded that the analytical contractor had supplied mostly detector raw data to the applicant, and it seemed that determining the extent of the work near to the 'initial HPLC analyses' versus later on in the study from chromatographic raw data was not possible. The attempts to provide support for stability from the existing data had proved challenging.

Further details on the time period aspects for each of the metabolism studies is presented in Vol 3 at the end of section B.7.2.1.

In order to further address this issue, the applicant provided some new GLP reports (2021 reports) providing retrospective stability analysis of incurred residues in the primary crop commodities of wheat, canola, sugar beet and rice, in the context of radiolabelled metabolism work. Such studies were not submitted for livestock or the rotational crops study. Based on the data submitted overall, the applicant is of the viewpoint that the residues metabolism package as a whole can be relied upon to understand the metabolic pathway of bixlozone and to demonstrate the lack of exposure clearly excluding consumer and livestock health risk.

HSE notes that the livestock metabolism studies do not need to be relied upon at this time, as the livestock dietary burden estimation (worst case) concludes that livestock intakes do not exceed the 'trigger' of 0.004 mg/kg bw/day.

These new (retrospective) storage stability reports (2021) are evaluated in Vol 3 at the end of section B.7.2.1. Further to the evaluation of these studies, HSE considers that there are certainly radioactive residues that remain after this long period, 4- 6 years, of frozen storage and there is also some evidence of marked loss of residues (e.g. wheat grain, where the chromatograms suggests [from the ratio of the main peak to the baseline variation] that there has been a considerable loss of amount of radioactive residue analysed). Quantitative analysis is not available, and to a varying extent there are some qualitative differences in metabolite peaks (chromatograms show patterns and are not labelled to identify specific peaks). Some chromatographic comparisons are better than others and support the view that only some of the metabolite residues are retained. As such HSE considers it is not possible to conclude that these data demonstrate good stability of residues in the radiolabelled metabolism context, when comparing these samples re-extracted and analysed much later on in time after frozen storage.

Therefore, the evaluation write up of each of the metabolism studies in Vol 3 elaborates on the timeframes for samples as well as reporting any stability investigations performed within the scope of the metabolism studies (wheat, sugar beet and hens), and in the additional retrospective additional chromatographic comparisons done on the retrospective analysis (2021 reports) for the samples from the primary crop metabolism (wheat, canola, sugar beet, and rice).

It is noted that **none** of the storage stability data available for metabolites investigated in the context of non-radiolabelled residues studied over freezer storage over a time-course, as written up in section B.7.1 (see also summary in Vol 1, section 2.7.1) suggests that there is a concern with any instability of residues. These studies in section B.7.1 and summarised in section 2.7.1 above are for a number of metabolites of differing structures relating to bixlozone. Some of these residues are primary crop residues (bixlozone, 2,4-dichlorobenzoic acid, 5-hydroxy-bixlozone, 2,2-dimethyl-3-hydroxy propionic acid and 5'-hydroxy-bixlozone) and some of these residues are potential rotational crop metabolites (bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone). These studies involved spiking of untreated samples prepared by homogenisation and with dry ice and storing in the freezer over a time course prior to analysis.

Extract stability was not studied in the context of non-radiolabelled stability studies evaluated in section B.7.1 or in the residue field trials. Extracts were stored (frozen) in residues studies (the non-radiolabelled stability studies and field primary and rotational crop residues trials between extraction and analysis). Sample extracts for the various metabolites were stored for up to 7 days (up to 5 for 5'-hydroxy-bixlozone and up to 2 days for bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone). As such, in both the residues trials (section B.7.3 and B.7.6.2) and the storage stability trials here, the samples extracts were not analysed immediately. This is not ideal as Regulation (EU) 283/2013 states that: "*the stability of extracts shall be investigated if extracts are not analysed immediately*." As the samples which were analysed for procedural recoveries were stored under the same conditions, for the same time period, gave acceptable results (although results were variable and not always close to 100% for every analyte and matrix combination) and there is no evidence of instability, no further consideration of the storage stability of these residues in extracts is required. As such there is no indication that there is any decline of residues in the first few days of extracts having been made, for the metabolites that have been studied in non-radiolabelled residues studies.

HSE would not wish to suggest that, without the evidence to support this, residues in radiolabelled studies might be more stable over frozen storage than non-radiolabelled residues (we do not know).

Considering the issue overall (of the metabolism studies having been performed, using some samples stored for a long time), HSE has presented all the data to describe the understanding of the metabolic profile. However, HSE considers that all the metabolism data have a higher degree of uncertainty associated with them, than would normally be the case for a metabolism data package if the samples had been all analysed close to a 6 month timeline, or if a longer storage time had been suitably supported by comparative chromatograms demonstrating the stability of residues in the samples. This greater uncertainty seems especially to be the case for the plant samples, the primary crop and rotational crop metabolism samples. The final analyses of the frozen livestock samples were all completed within 1.5/1.6 years after sampling (so shorter in time than for the plant samples- the range of final analyses for the frozen plant samples was 2.1 to 3.4 years after sampling). If the applicant wishes to conduct new livestock metabolism studies, then this should be discussed with HSE before commencement of studies.

The following aspects provide some reassurance when considering the additional uncertainties for the metabolism package:

- The non radiolabelled storage stability results showing no observed decline provide reassurance. No issues with instability were observed in these studies up to a period of two years for six of the metabolites that have been studied, and a period of up to 18 months for a further metabolite 5-hydroxy-bixlozone that was also studied.

- The lack of decline seen in extracts stored in non radiolabelled residues studies, stored frozen over a number of days to a week. Sometimes issues of instability can be evident in the early stages.

- Although the metabolism studies have examples of specimens stored for a time as raw samples, or processed powdered homogenates, most of the samples kept for the extended periods in metabolism studies will have been in the form of frozen extracts.

- The overall toxicity profile of bixlozone does not indicate that the residues are of particular concern (the currently estimated dietary intakes take up <1% of the ADI of 0.3 mg/kg bw/day and <1% of the ARfD of 0.75 mg/kg bw/day). The main metabolite found (in cereals) in residues trials, 2,2-dimethyl-3-hydroxy propionic acid (M118/1), is considered to be of low toxicological significance, as it has been considered (see section 2.6.9 and section B.6.8.1) that the residues can be assessed in terms of an exposure assessment in the TTC (Threshold of Toxicological Concern) scheme by the assignation to the Cramer Class I (presumption of low toxicological significance). This CCI assignation is also the case for dimethyl malonic acid (M132/1); the evaluation of the metabolism studies suggests the presence of this component in cereals in broadly equal amounts to 2,2-dimethyl-3-hydroxy propionic acid (M118/1).

- The main analytes found in plant metabolism studies, aside from dimethyl malonic acid (M132/1), were sought in the field trials and were included in the non radiolabelled freezer storage stability studies. The results of the field trials indicate the finding of the metabolite M118/1 (2,2-dimethyl-3-hydroxy propionic acid) as the main residue found in cereal grain and straw; this fits with a general understanding gained from the plant metabolism studies. The other main metabolites sought in trials (M190/1, 2,4-dichlorobenzoic acid

and M289/3, 5'-hydroxy-bixlozone) were found only in cereals as an infrequent low finding; these metabolites also are well supported by the non radiolabelled freezer storage stability studies.

Comments relating to various metabolism studies – General remarks

Some general evaluation remarks have been made at the start of Volume 3 section B.7.2 to introduce all the metabolism studies.

All metabolism studies involved use of bixlozone labelled in the phenyl and carbonyl ring, as two different treatments. The molecule was radiolabelled as follows: in the dichloro-phenyl ring ([phenyl-U-^{14}C]-label) or on the carbonyl carbon of the dimethyl-isoxazolidin-3-one ring ([carbonyl-C5-^{14}C]-label). The results for both labels were reported for all plant and livestock studies.

All mg/kg expression of residues in all the metabolism studies are as mg parent equivalents/kg (mg parent eq./kg).

See Volume 3 CA Section B.5.2.1, for an evaluation of the extraction efficiency of the residues. The efficiency of the extraction procedures for the major components of the residue in plants has been considered only for wheat straw (to compare extraction of the proposed enforcement method CAM-180) with the extractability observed in the metabolism study. The conclusions made in section B.5.2.1 are in regard of 2,4-dichlorobenzoic acid or 5'-hydroxy-bixlozone. Due to the lack of residues of bixlozone and 2-2-dimethyl-3-hydroxy propionic acid in the samples tested in the efficiency extraction work (section B.5.2.1), no conclusions are made in regard of these components. Extraction efficiency of analytical methods for residues of bixlozone in animal products has been considered, see section B.5.2.6.

Some errors and inconsistencies were identified within the initially submitted metabolism studies. After correspondence with the applicant, GLP study amendments for all of the metabolism studies (primary crops, rotational crops, and livestock metabolism studies) were submitted to HSE for evaluation to update and correct needed aspects. The data presented, and subsequent conclusions drawn, within this DAR consider the final GLP amendment versions of the study reports. Where it has been necessary to refer to correspondence between the applicant and HSE this has been stated in the evaluation.

Wheat

Metabolism of bixlozone in wheat was investigated after early post emergence application at around 1 × 300 g as/ha. Comparisons to the GAP 'N' rates for the achieved application rates were around 1.5N with regard to the wheat and barley GAP and around 0.8N with regard to the maize GAP. Wheat forage was sampled 28 days after application, hay sampled at 48 days after application and wheat grain and straw were sampled at harvest 60 days after application.

The overall residue levels (TRR) in the phenyl-labelled forage, hay, straw and grain were 0.97, 1.90, 1.40 and 0.14 mg/kg respectively. For carbonyl-labelled forage, hay, straw and grain the levels were 0.94, 1.01, 1.09 and 0.09 mg/kg.

Solvent extraction of the samples involved thrice extraction with acetonitrile/water (80:20) followed by thrice extraction with methanol: water (50:50) and extracts combined for assay. For both labels, solvent extractability) was high for forage, hay and straw (at least 85% TRR). Sequential enzyme hydrolysis of straw and hay released 7-12% of the TRR and then acid hydrolysis of straw and hay released 0.8-6% of the TRR. In forage sequential enzyme hydrolysis released ~2% TRR. For grain, solvent extraction retrieved 60% TRR and 55% TRR for the phenyl- and carbonyl-labels respectively. In the phenyl-label sequential enzyme hydrolysis steps released a further 17% TRR and then sequential acid and base hydrolysis released a further 11% TRR. In the carbonyl-label sequential enzyme hydrolysis released 29% TRR and then further sequential acid and base hydrolysis released a further 4% TRR. In grain the final unextracted residue was 6 to 8 %TRR (0.007 to 0.009 mg/kg).

A key aspect of the study was that the organic solvent extractable residues were also processed by acid hydrolysis and this led to the identification of a number of metabolites that had not been 'seen' in the organic solvent extracts. It was proposed that some deconjugation behaviour was observed, e.g., high amounts of metabolite 5'-hydroxy-bixlozone (M289/3) were found in the acid hydrolysed extract (not found in the organic solvent extract) whereas instead a proposed 5'-hydroxy-bixlozone (M289/3) conjugate was instead found in the organic extract. Mostly information on metabolites, is therefore taken from determination of residues in the acid hydrolysis extracts.

For both labels, unchanged parent bixlozone was not detected in any of the commodities. Metabolism of bixlozone includes primarily the hydroxylation at the 5'-position and oxidative ring opening. In forage, hay and straw the metabolites 5'-hydroxy-bixlozone (M289/3) and its conjugate accounted for the highest proportion of the radioactive residue (accounting for ~31-49% TRR). In contrast, the metabolite 5'-hydroxy-bixlozone (M289/3) is only detected at very low levels in grain (~0.5-2% TRR), and the major metabolites detected in grain were 2,4-dichlorobenzoic acid (M190/1) and 2,2-dimethyl 3 hydroxy propionic acid (M118/1) accounting for 25% and 44% TRR respectively after the acid hydrolysis of the organic solvent extract. Additional hydroxylations at the 4 and 5 positions of the 5-membered ring, were also observed, with these metabolites (4-hydroxymethyl-5'-hydroxyl-bixlozone (M305/1) and 5-hydroxy-5'-hydroxy-bixlozone (M305/2)) individually being found at a maximum level of up to 12% TRR in straw, hay and forage. The metabolites formed upon oxidative ring opening of the isoxazolidin-3-one ring (2,2-dimethyl-3-hydroxypropionic acid (M118/1) and 2,4-dichlorobenzoic acid (M190/1)) which had been identified in grain, were also found in forage, hay and straw, especially after acid hydrolysis of the organic extracts (up to 22%TRR).

A high portion of the TRR in grain was unidentified (<47% TRR). The majority of the unknown regions detected in the organic extracts were not detected after acid hydrolysis of the sample, perhaps indicating that these might be conjugates that were released via the acid treatment of the extract. The proportion remaining unidentified was far less in the acid hydrolysed extracts. The highest proportion of unidentified residues in the acid hydrolysed extracts was for wheat grain at 32.2%TRR, however this was a number of different unknown chromatographic regions (12).

The levels of identified metabolites, especially in the acid hydrolysed extracts, were higher in forage, hay and straw (representing 65% to 95% TRR identified in these commodities in the acid hydrolysed fractions). The difference in residues between the acid hydrolysed extracts and the organic solvent extracts, and the way that post extraction solids could be further worked on to then release further radioactivity (when treated sequentially with various enzymes then acid and base) are suggestive of conjugated residues being present, and possibly some natural incorporation of residues.

Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in wheat. Overall, this study has enabled metabolism in wheat to be reasonably well-elucidated (see the applicant's proposed metabolic pathway below). The summary of metabolism tables in section 'definition of residue' in section 2.7.3 provide a full overview of metabolites found and their levels in the metabolism studies, including the comparison of metabolites in the acid hydrolysed extract compared to the organic solvent extracts analysed.

Canola/(Oilseed rape)

The metabolism of bixlozone was investigated in canola by applying phenyl-labelled or carbonyl-labelled bixlozone as an early post emergence application at around 1 × 300 g as/ha. The actual application rates achieved were slightly low (compared to the target rate of 300 g as/ha), so the actual achieved application rate was 0.92 to 0.96 with respect to the oilseed rape GAP. Immature forage was sampled 36 days after application, oilseed rape seed and straw were sampled at harvest 70-71 days after application.

The TRR in the phenyl-labelled forage, straw and seeds were 0.017, 0.058 and 0.015 mg/kg respectively. For carbonyl-labelled forage, straw and seeds the levels were 0.026, 0.074 and 0.009 mg/kg. Hence, TRR for forage, straw and seeds were broadly similar across both labels.

Solvent extraction of the samples involved repeated extractions with acetonitrile/water (80:20, three times) then re-blending with a solution of acetonitrile/water (80:20) and extraction process repeated four more times, followed by repeated extractions with methanol: water (50:50, three times). For both labels, solvent extractability was high for forage and straw (at least 90% TRR) For seeds, solvent extraction retrieved 65% TRR and 42% TRR for the phenyl- and carbonyl-labels respectively. In seed the final unextracted residue was 35.1 %TRR (0.005 mg/kg) for the phenyl-label and 58.2% TRR (0.005 mg/kg) for the carbonyl-label. The actual concentrations of the post extraction solids (PES) were low and therefore no further extraction techniques were investigated. The organic solvent extracts were hydrolysed using a 1N HCL (reflux) treatment to investigate the nature of residues before and after such an acid hydrolysis step.

Due to the overall only low (seed and forage) to moderate (straw) levels of radioactivity as mg/kg amounts, and relatively large number of unknown fractions (albeit with individual components being present at low levels), the metabolic pathway is based on only a relatively small proportion of overall identified metabolites. Most information on the known metabolites comes from the elucidation of metabolites in the acid hydrolysed extracts of forage and

straw. It was suggested that some metabolites (in the organic solvent extract) prior to acid hydrolysis might be present as conjugates.

For both labels, unchanged parent bixlozone was not detected in any of the commodities.

In forage and straw, the metabolites bixlozone-hydroxy-isobutyramide (M261/1) and 2,2-dimethyl-3-hydroxypropionic acid (M118/1) accounted for the highest proportion of the radioactive residue in the phenyl-label and carbonyl-label respectively (2,2-dimethyl-3-hydroxypropionic acid (M118/1) up to 31%TRR and 0.023 mg/kg in straw and bixlozone-hydroxy-isobutyramide (M261/1) up to 15%TRR and 0.009 mg/kg in straw).

In contrast, these metabolites were not detected in seeds, and the major metabolite in seeds detected was 2,4-dichlorobenzoic acid (M190/1) (accounting for 34.7% TRR, 0.005 mg/kg). In seeds, a high portion of the TRR was unextracted (35%TRR) and 26% TRR was extracted but unidentified (with very low levels for any individual unidentified regions).

Metabolism of bixlozone in canola includes primarily the reduction and subsequent oxidation, decarboxylation and hydroxylation of the dimethyl-oxazolidone ring and oxidative ring opening. Overall, metabolism of bixlozone in canola, seems to have been adequately studied, however the degree of identification of residues is not as high in this study (due to low residue levels) compared to other crop metabolism studies (such as wheat metabolism). The elucidated metabolism for canola (oilseeds) is outlined in the applicant's metabolic pathway (see below).

Both the wheat metabolism study and the canola (oilseed rape) metabolism study contained a relatively large number of unknown metabolic fractions. It seems that the relatively low TRR in the canola (oilseed rape) metabolism study (up to 0.07 mg/kg in straw) constrained the extent to which the metabolic pathway in canola could be more fully elucidated. The differences in the proposed metabolic pathways therefore (between wheat and canola) might be reflective of the different degrees of identification rather than there being significant differences in the metabolic profile.

Sugar Beet

The metabolism of bixlozone was investigated in sugar beet by applying phenyl-labelled or carbonyl-labelled bixlozone as an early post emergence application at around 1 × 300 g as/ha. Immature sugar beet tops were sampled 28 days after application, and mature sugar beet roots and tops were sampled at harvest 173 days after application.

The overall residue levels (TRR) in the phenyl-labelled immature tops, mature tops and roots were 0.159, 0.007 and 0.031 mg/kg respectively. For carbonyl-labelled immature tops, mature tops and roots the levels were 0.176, 0.011 and 0.014 mg/kg respectively.

For both labels and all matrices, solvent extractability was high (at least 91% TRR). In all commodities the post extraction solids (PES) contained less than 9% TRR, furthermore the actual concentrations of the PES were low (up to 0.015 mg/kg) and therefore no further extraction techniques/investigation of the PES was taken forward.

For both labels, unchanged parent bixlozone was not detected in any of the commodities.

In immature sugar beet tops, the most significant metabolite after solvent extraction was 5-hydroxy-bixlozone (M289/1) conjugate for both labels, with 22.9% TRR (0.036 mg/kg) and 24.7% TRR (0.043 mg/kg) for the phenyl label and the carbonyl label respectively. Four other metabolites were identified in the organic solvent extract at a maximum level of 11.2% TRR (0.02 mg/kg) [dihydroxy-bixlozone conjugate, M467/1]. For the phenyl-label, five metabolites were observed following acid hydrolysis of the organic extracts. 2,4-dichlorobenzoic acid (M190/1) was found at 35.3% TRR (0.056 mg/kg), bixlozone-dimethyl-malonamide (M289/2) was found at 29.7% TRR (0.047 mg/kg) and 5-hydroxy-bixlozone (M289/1) was found at 16.4% TRR (0.026 mg/kg). For the carbonyl label, three metabolites were observed following acid hydrolysis of the organic extracts. 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was found at 64.7% TRR (0.114 mg/kg) after acid hydrolysis. This had been present at only 4.4% in the initial organic solvent extract. The metabolite bixlozone-dimethyl-malonamide (M289/2) was also found in the acid hydrolysate at 10.5% TRR (0.018 mg/kg) and 5-hydroxy-bixlozone (M289/1) was identified at 5.3% TRR (0.009 mg/kg).

In mature sugar beet tops (phenyl-label), the most significant metabolite was the 5-hydroxy-bixlozone (M289/1) conjugate at 30.3% TRR (0.002 mg/kg). Two other metabolites were tentatively characterised at >10% TRR: 4-

hydroxy-methyl-bixlozone (M289/4) (18.8% TRR, 0.001 mg/kg) and a hydroxy glucoside conjugate of bixlozone (M451/1) (17.5% TRR, 0.001 mg/kg). In the carbonyl-label, the most significant peak was tentatively characterised as a hydroxy glucoside conjugate of bixlozone (metabolite M451/1) which was detected at 23.5% TRR (0.003 mg/kg). 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was also detected at 15.8% TRR (0.002 mg/kg). Following acid hydrolysis, the profiles had changed. For the phenyl label (following acid hydrolysis), the most significant metabolite was bixlozone-dimethyl-malonamide (M289/2) which was detected at 34.0% TRR (0.002 mg/kg), followed by 2,4-dichlorobenzoic acid (M190/1) at 19.0% TRR (0.001 mg/kg), dihydroxy-bixlozone conjugate (M467/1) was tentatively characterised at 17.3% TRR (0.001 mg/kg) and the 5-hydroxy-bixlozone (M289/1) conjugate was postulated as a region of 11.1% TRR (0.001 mg/kg). 5-hydroxy-bixlozone (M289/1) was found at a maximum of 6.2% TRR (<0.001 mg/kg). For the carbonyl label, four metabolites were detected after acid hydrolysis. As seen in the phenyl label, the most significant metabolite identified was bixlozone-dimethyl-malonamide (M289/2), at 54.6% TRR (0.006 mg/kg). The 5-hydroxy-bixlozone (M289/1) conjugate was postulated at 13.2% TRR (0.001 mg/kg). Dihydroxy-bixlozone conjugate (M467/1) was tentatively characterised at 12.8% TRR (0.001 mg/kg) and 5-hydroxy-bixlozone (M289/1) was also identified at 11.4% TRR (0.001 mg/kg) respectively.

In mature sugar beet roots: In the phenyl-label, the most significant metabolite present after organic solvent extraction was bixlozone-dimethyl-malonamide (M289/2) which was detected at 41.1% TRR (0.013 mg/kg). Dihydroxy-bixlozone conjugate (M467/1) was tentatively characterised at 10.5% TRR (0.003 mg/kg). In the carbonyl-label, dimethyl malonic acid (M132/1) was the most significant metabolite present after organic solvent extraction at 40.8% TRR (0.006 mg/kg). Two other metabolites were found at a maximum level of 6.6% TRR (0.001 mg/kg) [2,2-dimethyl-3-hydroxypropionic acid, M118/1]. Acid hydrolysis of the organic extracts for the phenyl label revealed five identified metabolites in the acid hydrolysate. The most significant metabolite was 2,4-dichlorobenzoic acid (M190/1) which was detected at 34.8% TRR (0.011 mg/kg), followed by bixlozone-dimethyl-malonamide (M289/2) at 29.8% TRR (0.009 mg/kg). For the carbonyl label, three identified metabolites were found after acid hydrolysis of the organic extracts. The most significant metabolite identified was 2,2-dimethyl-3-hydroxypropionic acid (M118/1) at 43.4% TRR (0.006 mg/kg). Dimethyl malonic acid (M132/1) was also identified at 34.0% TRR (0.005 mg/kg).

Across all matrices, the metabolites 5-hydroxy bixlozone (M289/1), bixlozone-dimethyl-malonamide (M289/2), 2,4-dichlorobenzoic acid (M190/1), 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) accounted for the majority proportion of the radioactive residues, with glucoside conjugates of bixlozone (M451/1 and M467/1) and 4-hydroxy-methyl-bixlozone (M289/4) representing smaller amounts of radioactivity.

A high portion of the TRR before acid hydrolysis was unidentified (<46% TRR in immature tops and up to 57% TRR in roots). However, based on both low %TRR levels and the way in which the unknowns are representing a number of different regions, seem to indicate that the unknowns are mostly very low (<0.01 mg/kg or <0.005 mg/kg in many cases). An exception to this was in sugar beet tops (immature), where the highest concentration of an unknown residue fraction was 0.022 mg/kg (in the solvent organic fraction), however the majority of the unknown regions detected in the organic extracts were not detected after acid hydrolysis (and the highest level of any individual level of an unknown in the acid hydrolysed extract was 0.009 mg/kg) indicating that there is a potential for conjugation within the sugar beet.

Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism in foliar applied sugar beet. Metabolism of bixlozone in sugar beet includes primarily the hydroxylation of the 5 position in the dimethyl-oxazolidone ring and oxidative ring opening. Bixlozone is also metabolised in sugar beet via other hydroxylation reactions, reduction and oxidation (to form the main metabolite bixlozone-dimethyl-malonamide (M289/2)). This was present as in higher amounts in sugar beet tops after acid hydrolysis of the initial solvent extract, so it could be present as a conjugate.

Overall, metabolism of bixlozone in sugar beet is considered reasonably well-elucidated (see the applicants proposed pathway below).

Rice

Metabolism of bixlozone in rice was investigated in dry land and paddy rice after early post emergence application at around 1 × 350 g as/ha. Comparisons to the GAP 'N' rates for the achieved application rates were around 1.75N with regard to the wheat and barley GAP and around 0.93N with regard to the maize GAP. Rice grain and straw were sampled at harvest around 152 days after application.

The overall total radioactive residue levels (TRR) in dry land rice in the phenyl-labelled grain and straw were 0.112 and 0.908 mg/kg respectively and in the carbonyl-label grain and straw the levels were 0.078 and 0.525 mg/kg respectively. In paddy rice the overall residue levels (TRR) in the phenyl label were 0.077 and 0.712 mg/kg in grain and straw respectively and in the carbonyl label the levels were 0.038 and 0.243 mg/kg in grain and straw respectively.

Solvent extraction of the samples involved thrice extractions with acetonitrile/water (80:20) followed by thrice extractions with methanol: water (50:50). For dry land rice, in both labels, solvent extractability was high for straw (>90% TRR) and in grain the extractability was lower at *ca*. 73% TRR. In all commodities the non-extractible post extraction solids (PES) contained less than 10% TRR after sequential enzyme, acid and base hydrolysis steps, indicating efforts to release radioactivity. For paddy rice, in both labels, solvent extractability was high for straw (>88% TRR) and in grain the extractability was slightly lower at around 55 and 73% TRR for the phenyl and carbonyl labels respectively. In all commodities the non-extractible PES contained less than 16% TRR (0.006 mg/kg grain) and less than 0.035 mg/kg in straw after sequential enzyme, acid and base hydrolysis steps, indicating efforts to release radioactivity.

For both labels, unchanged parent bixlozone was not detected in any of the commodities (paddy and dry land rice).

Results were not vastly different between paddy rice and dry land rice; however, a summary for each sample matrix is given below for completeness.

Dryland rice – Grain The most significant metabolite found after acid hydrolysis of the organic extracts for the phenyl label was 2,4-dichlorobenzoic acid (M190/1) which was detected at 38.3% TRR (0.043 mg/kg). Bixlozone-dimethyl-malonamide (M289/2) was also detected at 7.9% TRR (0.009 mg/kg). For the carbonyl label, the most significant region found after acid hydrolysis of the organic extracts was 2,2-dimethyl-3-hydroxypropionic acid (M118/1). Two co-eluting peaks were postulated to be 2,2-dimethyl-3-hydroxypropionic acid (M118/1) – together these contained a total of 21.9% TRR (0.017 mg/kg). Free dimethyl malonic acid (M132/1) was found at 13.9% TRR (0.011 mg/kg) and the coeluting peak/region considered to be 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) conjugates were determined as 12.8% TRR (0.010 mg/kg) – total for both the conjugates.

Paddy rice- Grain The most significant metabolite found after acid hydrolysis of the organic extracts for the phenyl label was 2,4-dichlorobenzoic acid (M190/1) which was detected at 21.4% TRR (0.016 mg/kg), followed by bixlozone-3-hydroxy-propanamide (M275/1) at 13.5% TRR (0.010 mg/kg). Bixlozone-dimethyl-malonamide (M289/2) was also identified at a maximum level of 3.8% TRR (0.003 mg/kg). The most significant metabolite identified for the carbonyl label after acid hydrolysis of the organic extracts was dimethyl malonic acid (M132/1) at 19.6% TRR (0.008 mg/kg). A peak containing a total of 17.2% TRR (0.007 mg/kg) was postulated to be metabolite 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and bixlozone-3-hydroxy-propanamide (M275/1) was identified at 12.3% TRR (0.005 mg/kg). A further region (coeluting peaks) was considered to be a conjugate of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) at a total level of 8.0% TRR (0.003 mg/kg). Bixlozone-dimethyl-malonamide (M289/2) was identified at 2.0% TRR (0.001 mg/kg).

Dry land rice – Straw For the phenyl-label, two metabolites were identified in the acid hydrolysis fraction of the organic extracts: 2,4-dichlorobenzoic acid (M190/1) was identified at 67.5% TRR (0.613 mg/kg) and bixlozone-dimethyl-malonamide (M289/2) was identified at 12.7% TRR (0.115 mg/kg). After the acid hydrolysis of the organic extracts for the carbonyl-label, two co-eluting peaks were postulated to be 2,2-dimethyl-3-hydroxypropionic acid (M118/1) – together these contained a total of 27.4% TRR (0.144 mg/kg) and the conjugates of dimethyl malonic acid (M132/1) and 2,2-dimethyl-3-hydroxypropionic acid (M118/1) indicated to be overlapping regions containing 26.9% TRR (0.141 mg/kg) in total. A different peak (14.8% TRR (0.078 mg/kg)) was identified as dimethyl malonic acid (M132/1).

Paddy rice – Straw After acid hydrolysis of the organic extracts for the phenyl label, the most significant metabolite was 2,4-dichlorobenzoic acid (M190/1) which was detected at 24.8% TRR (0.177 mg/kg), followed by bixlozone-3-hydroxy-propanamide (M275/1) at 20.4% TRR (0.145 mg/kg) and bixlozone-dimethyl-malonamide (M289/2) at 11.0% TRR (0.078 mg/kg). For the carbonyl label, the most significant region detected after acid hydrolysis of the organic extracts was a co-eluting peak that was postulated to be conjugate of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and conjugate of dimethyl malonic acid (M132/1) at a total level of 31.1% TRR (0.076 mg/kg). 14.5% TRR (0.035 mg/kg) was also tentatively identified as 2,2-dimethyl-3-hydroxypropionic acid (M118/1).

Metabolism of bixlozone in rice includes primarily oxidative ring opening. In all commodities, the metabolites 2,4-dichlorobenzoic acid (M190/1), dimethyl malonic acid (M132/1) and 2,2-dimethyl-3-hydroxypropionic acid (M118/1) accounted for the majority proportion of the identified radioactive residues.

Data obtained with phenyl-label and carbonyl-label taken together show a similar picture of the metabolism in both paddy and dry land rice. Overall, metabolism of bixlozone in rice is considered well-elucidated (see the applicant's proposed metabolic pathway below). Some differences in metabolism in rice and wheat metabolism studies were observed; this might in part be reflective of the use of some different reference standards used in the rice versus wheat metabolism study (dimethyl malonic acid (M132/1) was not determined as a residue in the wheat metabolism study). It is proposed that the metabolic profile information for cereals should be taken from both the wheat and rice metabolism studies.

General concluding remarks on plant metabolism studies: HSE made some remarks at the end of each metabolism study, specific to each study. Please see section B.7.2.1 of Volume 3, just prior to the Figures on the applicant's proposed metabolic pathways (also at the end of the rotational crop metabolism study in section B.7.6.1).

The applicant has postulated that residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) might be present in the residues studies because it is 'natural' (this is discussed further in Vol 1 in section 2.7.4). It is noted that radiolabelled residues of this component were determined in all of the primary crop metabolism studies, so it seems that this metabolite does form as a direct result of the proposed pesticide treatment. Whilst 2,2-dimethyl-3-hydroxy propionic acid (M118/1) is reported as a residue in primary crop field trials samples, in both treated samples and untreated control samples (field trial plots), residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) were not reported in the untreated controls in any of the metabolism studies. Across the metabolism studies, there tended to be good separation between the treated and untreated (control) containers (across studies mostly at least 60m from the treated containers, and often plastic sheeting was used as a guard around treated plots at the time of the pesticide application).

Proposed metabolic pathways- plants

The applicant's proposed metabolic pathways for primary crops are presented below in Figures 2.7.2.1 (wheat), 2.7.2.2 (canola), 2.7.2.3 (sugar beet)) and 2.7.2.4 (rice) respectively.

Figure 2.7.2.1 Applicant's proposed metabolic pathway of bixlozone (F9600) in wheat

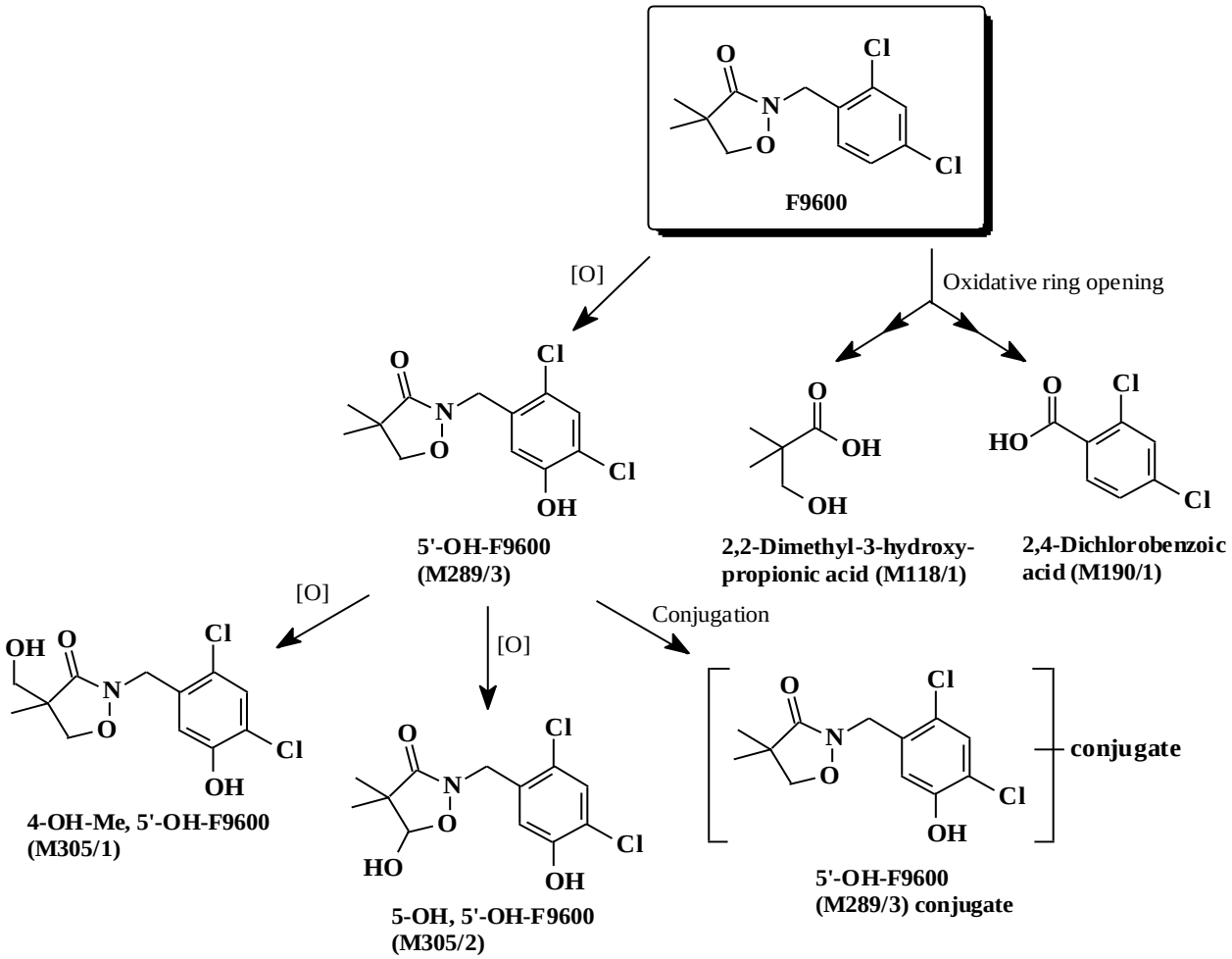


Figure 2.7.2.2 Applicant's proposed metabolic pathway for bixlozone (F9600) in canola

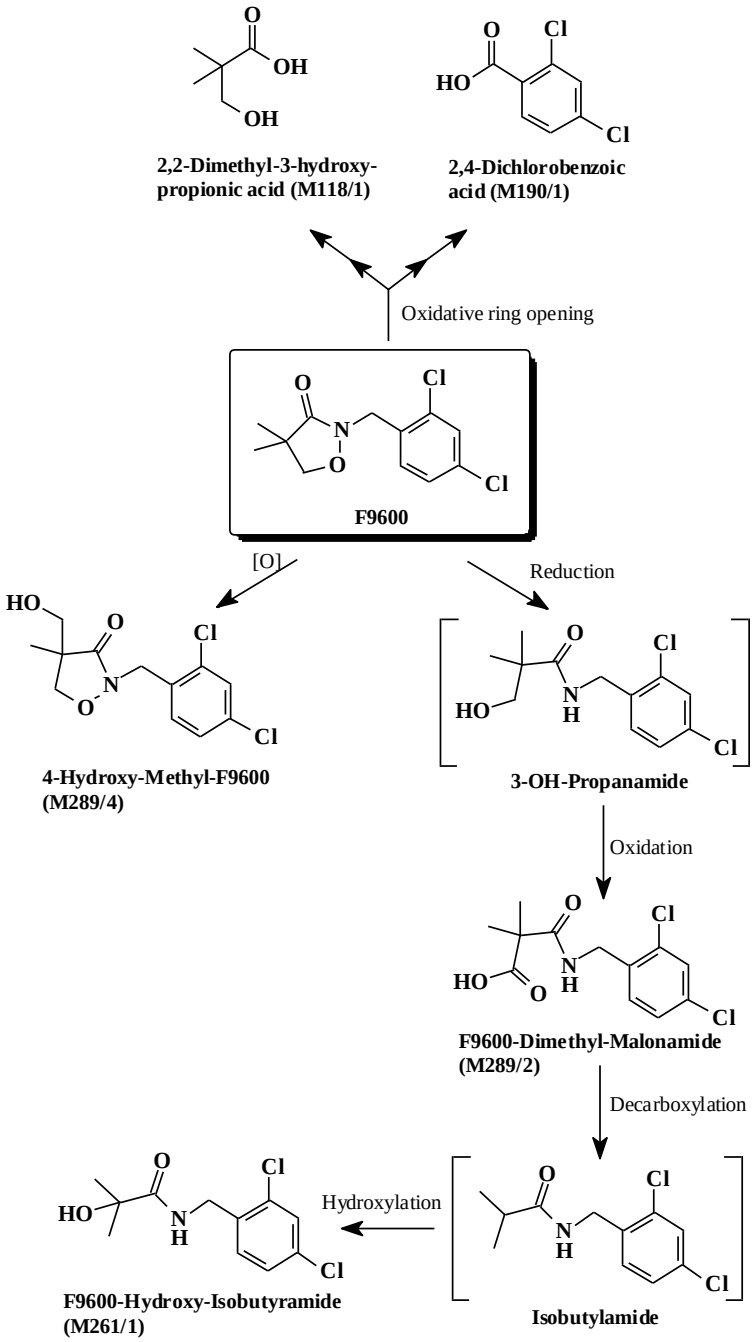


Figure 2.7.2.3 Applicant's proposed metabolic pathway for bixlozone (F9600) in sugar beet

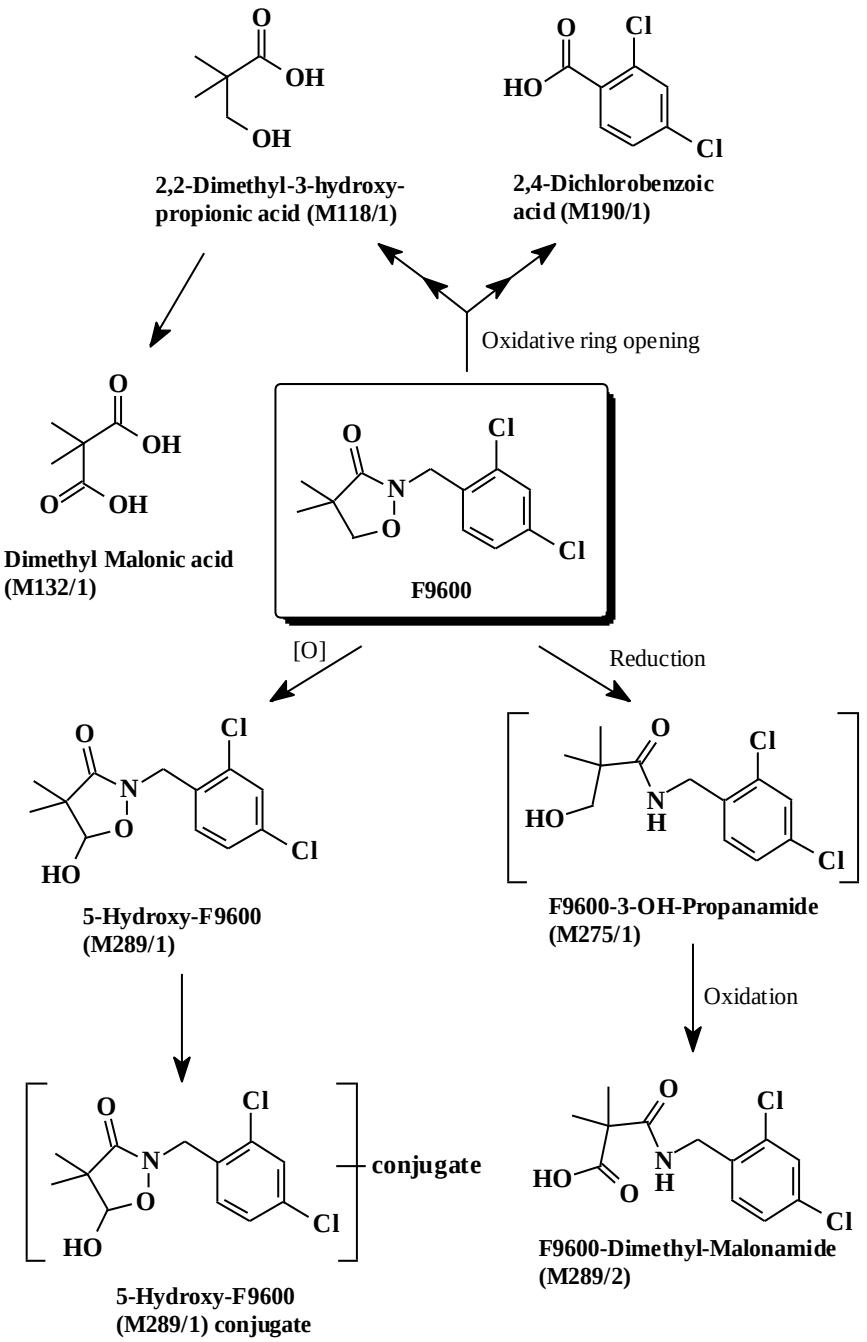
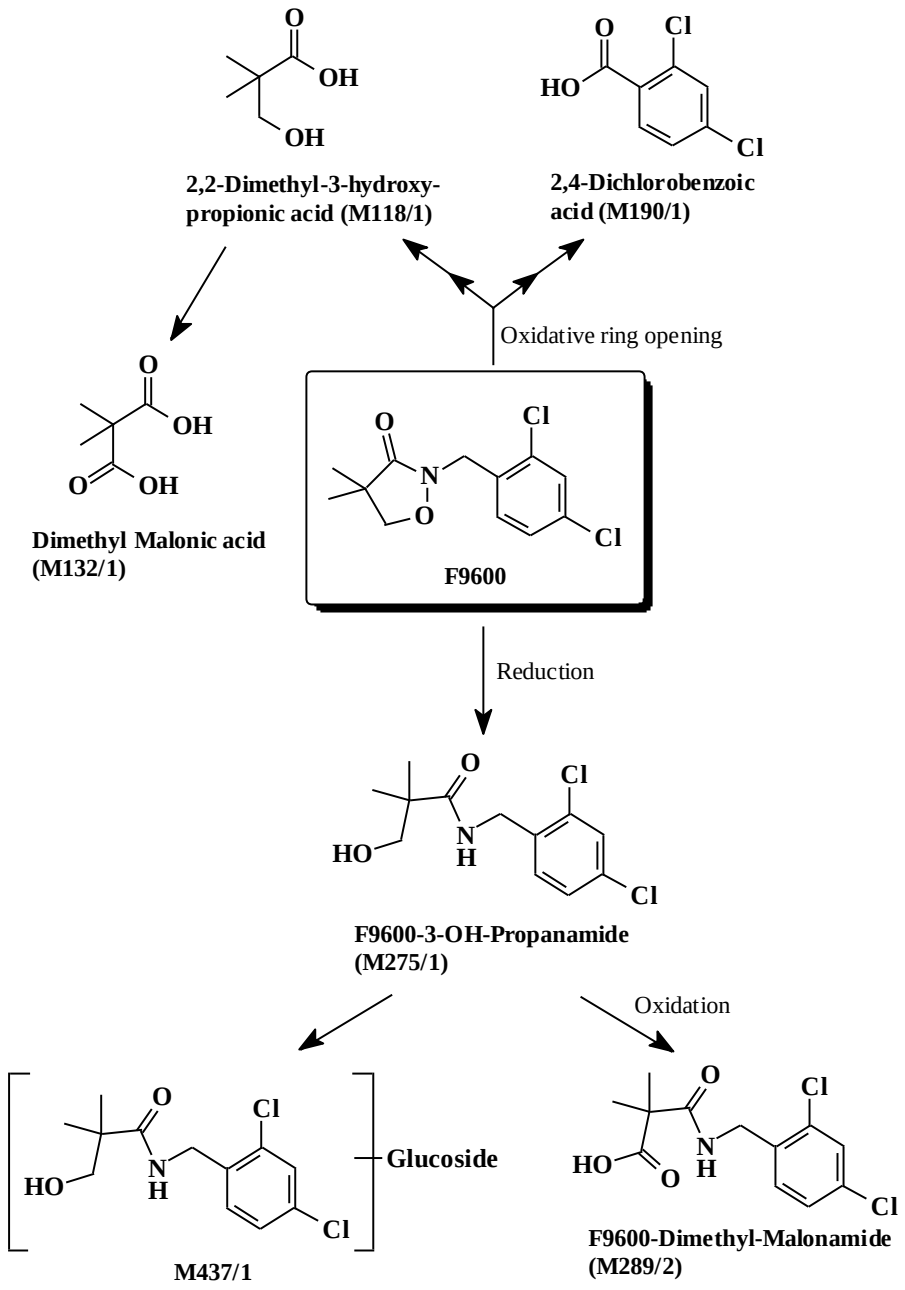


Figure 2.7.2.4 Applicant's proposed metabolic pathway for bixlozone (F9600) in rice



Goat (ruminant)

The metabolism of bixlozone was investigated in ruminants by dosing goats with phenyl-labelled or carbonyl-labelled bixlozone (one goat each label). Dosing was over 7 days at nominal doses of 15 mg/kg feed (administered in oral gelatine capsules once daily). The achieved daily dose administered was 0.41-0.49 mg/kg bw/d.

The overall residue levels (TRR) in the phenyl-labelled liver, kidney, muscle, fat and milk were 0.133, 0.343, 0.010, 0.022 and 0.072 mg/kg respectively. For carbonyl-labelled liver, kidney, muscle, fat and milk the levels were 0.120, 0.369, 0.011, 0.013 and 0.069 mg/kg. It was considered that a plateau in residue levels in milk might have been reached in around 2 days (data were variable so it was difficult to conclude to an exact day).

For both labels, solvent extractability (ERR) was high for liver, kidney, muscle, milk, skim milk and milk cream (at least 90% TRR). For fat, solvent extraction retrieved 67% TRR and 54% TRR for the phenyl- and carbonyl-labels respectively. As only <0.01 mg/kg of radioactivity remained in the post extraction solids (PES) in fat commodities, no additional characterisation was carried out.

For both labels, unchanged parent bixlozone was not detected in any of the commodities.

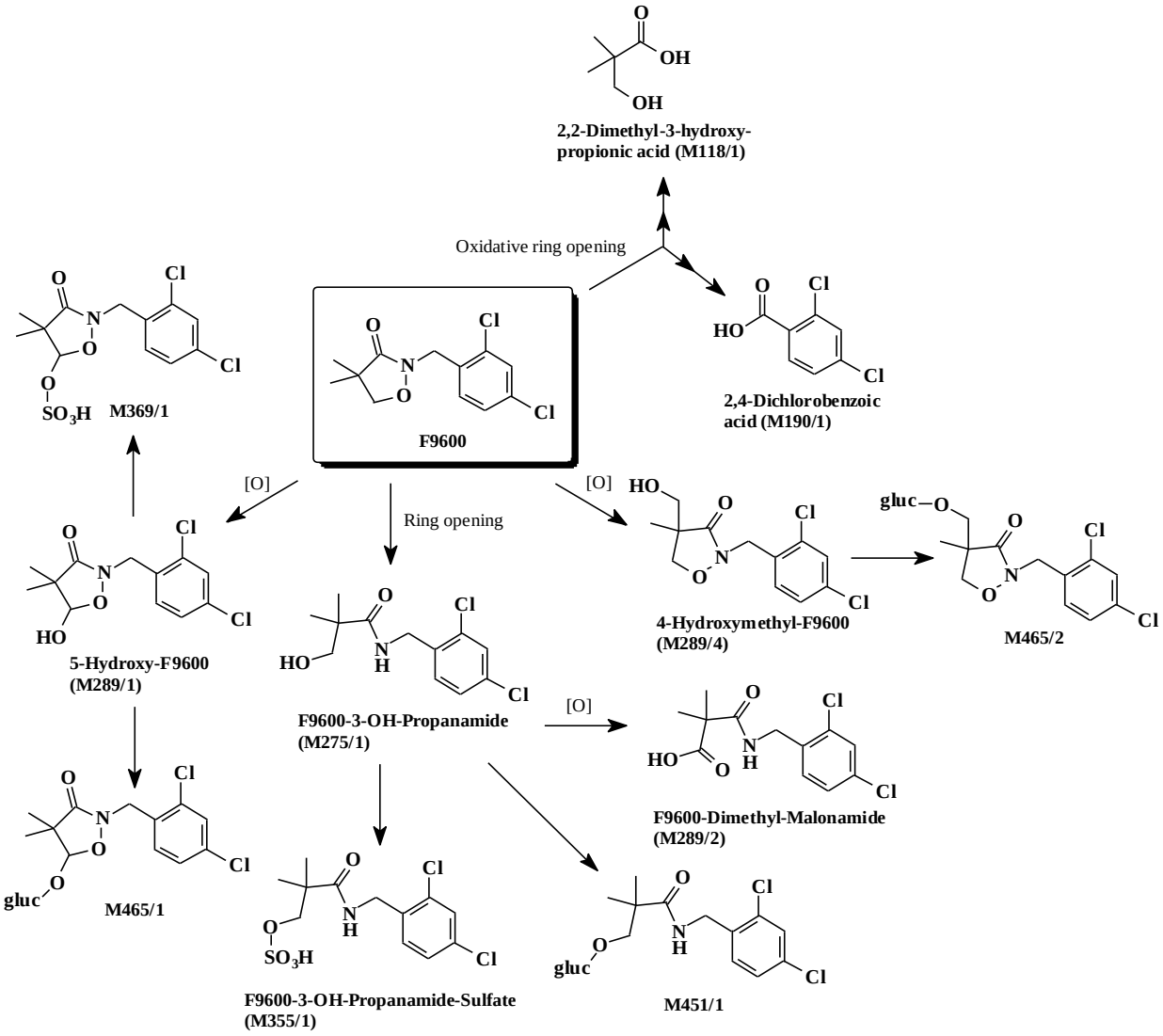
Metabolism of bixlozone in ruminant tissues includes primarily the hydroxylation at the 5-position (5-hydroxy-bixlozone, M289/1) and subsequent conjugation with glucuronide (5-hydroxyl-bixlozone-glucuronide, M465/1), as well as oxidative ring opening of the dimethyl-oxazolidone ring with subsequent oxidation (bixlozone-dimethyl-malonamide, M289/2). In muscle, liver and kidney, both 5-hydroxy-bixlozone (M289/1) and the glucuronide conjugate (M465/1) each accounted for >30%TRR [with 5-hydroxy-bixlozone (M289/1) featuring at this level in either the acid or enzyme hydrolysed extract, and with 5-hydroxyl-bixlozone-glucuronide (M465/1) featuring at this level in the initial (unhydrolyzed) organic extract]. In milk, the sulfate conjugate (M369/1) of 5-hydroxy-bixlozone (M289/1) accounted for the majority of the radioactivity (>73% TRR). Kidney and liver contained relatively large amounts of the metabolites bixlozone-3-hydroxy-propanamide (M275/1) (30% TRR in kidney and 16% TRR in liver) and bixlozone-dimethyl-malonamide (M289/2) (20% TRR in kidney and 21% TRR in liver); these metabolites were also found at >10% TRR in muscle. 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was also found in muscle (30% TRR), liver (17% TRR) and kidney (17% TRR) in samples from the carbonyl labelled study.

Therefore, additional metabolic pathways identified included hydroxylation and conjugation with glucuronide at the 4 position of the 5-membered ring, as well as oxidative ring opening with subsequent conjugation. Data obtained with phenyl-label and carbonyl-label taken together; show a consistent picture of the metabolism. Overall, metabolism of bixlozone in lactating ruminants is considered reasonably well-elucidated. The applicant's proposed metabolic pathway is presented in Figure 2.7.2.5.

Dimethyl malonic acid (M132/1) was not reported as a residue in the goat metabolism. However, it is not clear how thoroughly the metabolite dimethyl malonic acid (M132/1) had been sought in the goat metabolism study. It was included as a reference standard to initial HPLC scoping but no residues were reported in the study and its presence or absence was not explained or discussed in the goat metabolism study. Dimethyl malonic acid (M132/1) is a main metabolite in the poultry metabolism study, so it is considered that this should be clarified, if possible, ideally with supporting chromatograms, by the applicant if this study needs to be relied upon. It is noted in the current evaluation that dimethyl malonic acid (M132/1) has been assessed, in the human dietary intake assessment for the proposed uses (TTC exposure consideration), as not being of toxicological significance. However, this may require re-consideration for future uses of bixlozone in terms of human exposure and livestock dietary intakes.

The applicant's proposed metabolic pathways for goats are presented below in Figure 2.7.2.5 below.

Figure 2.7.2.5 Applicant's proposed metabolic pathway for bixlozone (F9600) in lactating goats



Hens (poultry)

The metabolism of bixlozone was investigated in poultry by dosing laying hens with phenyl-labelled or carbonyl-labelled bixlozone. Dosing was over 13 days at nominal doses of 15 mg/kg feed (administered in oral gelatine capsules once daily). The achieved daily dose administered was 1.15 mg/kg bw/d. The overall residue levels (TRR) in the phenyl-labelled pooled fat, thigh muscle, breast muscle, liver and eggs were 0.263, 0.027, 0.019, 0.608 and 0.089 mg/kg respectively. For carbonyl-labelled pooled fat, thigh muscle, breast muscle, liver and eggs the levels were 0.058, 0.058, 0.057, 0.491, 0.103 mg/kg. It was considered that a plateau in residue levels in eggs had been reached in 7-9 days.

For both labels, solvent extractability was high for pooled fat, thigh and breast muscles (at least 70% TRR). For liver, solvent extraction retrieved ca. 45% TRR and ca. 40% TRR for the phenyl- and carbonyl-labels respectively. In both labelled samples of liver multiple microwave extractions released a further 50-52% TRR in total.

Metabolism of bixlozone includes primarily the hydroxylation at the 5-position and oxidative ring opening. Unchanged parent bixlozone was only detected at fairly low levels in pooled fat and egg samples (<7.5% TRR).

Dimethyl malonic acid (M132/1) accounted for a high proportion of the TRR in all matrices (accounting for ~25-60% TRR). In eggs and fat, the metabolite 5-hydroxy-bixlozone (M289/1) was also detected at high levels of the radioactive residue (accounting for ~30% TRR). Fat samples also contained 12% TRR, 0.03 mg/kg of 2,4-dichlorobenzaloxime (M189/1). A significant unidentified region was detected in the carbonyl-labelled egg sample (36.5% TRR,

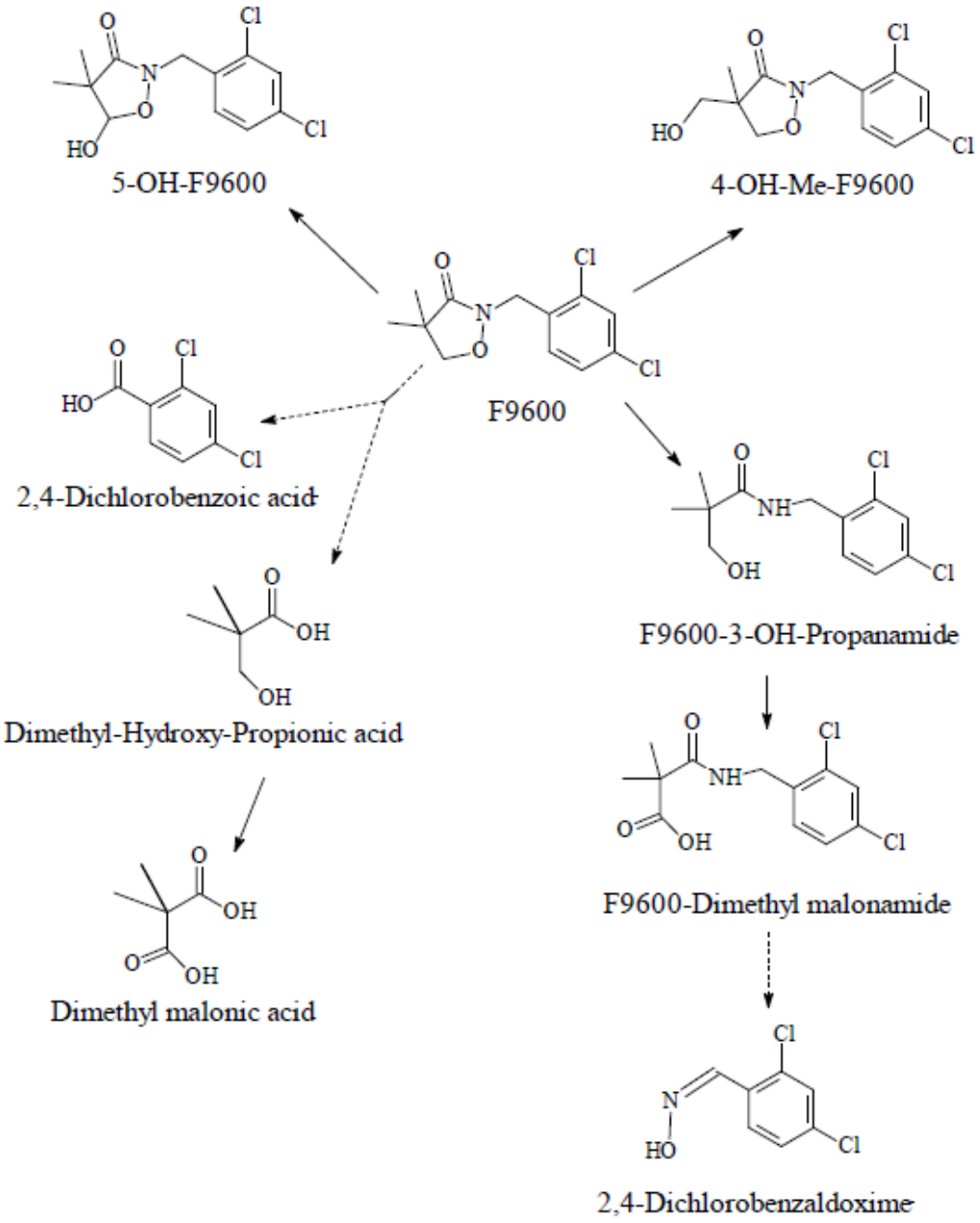
0.037 mg/kg). Identification of this region would have been beneficial. In eggs, other metabolites (>10%TRR) were 2,2-dimethyl-3-hydroxypropionic acid (M118/1) (10%TRR, 0.01 mg/kg), and 2,4-dichlorobenzamine (M175/1) (11%TRR, 0.01 mg/kg). Muscle contained bixlozone-dimethyl-malonamide (M289/2) at 26%TRR (0.007 mg/kg). The following residues >10%TRR were found in liver: dimethyl malonic acid (M132/1) (40%TRR and 0.2 mg/kg), 2,4-dichlorobenzamine (M175/1) (up to 14 %TRR) and 2,4-dichlorobenzaloxime (M189/1) (10%TRR).

Additional conjugates were also observed in all matrices, individually found at a maximum TRR of 14.4% in thigh muscle samples, and at a maximum residue of 0.074 mg/kg (12.2% TRR) in liver samples. Data obtained with phenyl-label and carbonyl-label taken together show a consistent picture of the metabolism in laying poultry. Overall, metabolism of bixlozone in poultry is considered reasonably well-elucidated.

The applicant's proposed metabolic pathways for hens are presented below in Figure 2.7.2.6 below.

Figure 2.7.2.6 Applicant's proposed metabolic pathway for bixlozone (F9600) in poultry

Names and metabolite codes to support the pathway diagram:
F9600 = bixlozone
5-OH-F9600 = 5-hydroxy bixlozone (M289/1)
4-OH-Me-F9600 = 4-hydroxy methyl bixlozone (M289/4)
2,4-Dichlorobenzoic acid (M190/1)
Bixlozone-3-OH-Propanamide (M275/1)
Dimethyl-hydroxy-propionic acid (M118/1)
Bixlozone-Dimethyl malonamide (M289/2)
Dimethyl malonic acid (M132/1)
2,4-Dichlorobenzaldoxime (M189/1)



Comparability of residues in the rat metabolism compared to the goat (ruminant) and hen (poultry) metabolism (and comment regarding the fat solubility of residues):

The livestock metabolism studies involved dosing with parent bixlozone (as per the rat metabolism studies). Overall, based on the identified metabolites and the applicant's proposed metabolic pathways for each species, it is generally concluded that the rat and livestock metabolism studies show comparable metabolism of parent bixlozone. The goat and poultry metabolism appear to be 'subsets' of metabolism in the rat, and no unique metabolite paths have been identified in the goat or poultry compared to the rat. It can be concluded that livestock and rat metabolism of parent bixlozone are similar. It is possible that there were low level residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) in the rat, which were not identified in the rat metabolism. They were not actively sought in the rat metabolism work (Section B.6.1.4), but there were some low level unidentified components. The current assessment proposes (see the TTC exposure assessment in section 2.7.3 on the human dietary exposure) that residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) do not need to be included in the residue definition for dietary risk assessment (consumers) and also that these residue components, for the current time, do not need to be included in the assessment of livestock dietary burden (as explained in section 2.7.3).

It is concluded that the residues found in the livestock studies (and the rat metabolism studies) can be regarded as mainly not fat soluble. Residues were not high in fat in the livestock metabolism studies.

It seems in livestock animals that bixlozone (parent) is metabolised (with only low amounts of parent found in poultry tissues and no detectable amounts of parent in the ruminant study) with 5-hydroxy-bixlozone (M289/1) (and conjugates) being 'major' in ruminants and poultry. Based on the log Pow (estimation) of 5-hydroxy-bixlozone (M289/1) [section B.2.7 logPow estimate of 1.97 for 5-hydroxy-bixlozone is provided] there is not a particular expectation that 5-hydroxy-bixlozone would be particularly fat-soluble. Residues were low in the ruminant fat and were therefore not as extensively worked on compared to other matrices (such as kidney and liver). Estimations for log Pow for other metabolites are also reported in section B.2.7 (on physical and chemical properties) indicating a tendency for metabolite to have lower log Pow values, than parent bixlozone itself.

In the rat, bixlozone is quickly and extensively metabolised following oral administration (low dose, high dose or repeated dose). In the tissue distribution and metabolism studies only low amounts of bixlozone were found in the rat. In addition, only low % of the administered dose was found in fat (less than 1 % of the dose) compared to the GI tract, liver and kidney. Hence what was observed in the livestock animal metabolism studies is similar to what was observed in the rat. Therefore, as the residue in livestock animals (like in rats) are not bixlozone itself, but metabolites with LogPow < 3, and considering that in the rat, levels of radioactivity were very low in fat, it is considered that residues in animals and in rats as per livestock, are not fat-soluble.

Fish

At present there is no agreed guidance on how to conduct fish metabolism studies to determine the residue definition for risk assessment and enforcement, and there are no agreed guidance documents on how then to conduct a fish feeding study. It is also the case that there is no agreed diet for farmed fish. Since no agreed guidance is available, and there is no agreed data on the diets of fish (to address fish dietary burden) at this time, it is considered that the residue requirements for fish do not need to be addressed in the current evaluation.

The applicant did not submit any more detailed position paper since they considered that residues in crops were insignificant, and the dietary intake for fish would be very low. Depending on the residues in crops, further information to address this data requirement (nature of residues in fish [metabolism]), and if needed, magnitude of the residues in fish [feeding studies]) will be required when guidance becomes available.

2.7.3. Definition of the residue

Plant

Primary crops

The available primary crop metabolism data are for wheat, canola (oilseed rape), sugar beet and rice.

The most relevant metabolism data for the intended uses are the studies on wheat and canola (oilseed rape). The way they were conducted is reflective of the GAP in terms of timings of application (and also broadly in terms of proposed application rates, the N rates). Rice metabolism data should also be considered. As rice is not grown in the UK, it does not seem as directly relevant to UK cereals as 'wheat', however in terms of metabolism both rice and wheat are used to inform on metabolism in cereals, and metabolism data on rice should also be considered as well as wheat. The information on sugar beet (see section 2.7.2 Vol 1 and below) is considered supporting information on metabolism for the current time. The current proposals for the residue definitions are dependent on the intended uses. Threshold of Toxicological Concern (TTC) calculations and animal dietary burden estimates are such that the assessment would need to be revisited in the case of future extended and wider crop uses. Currently a universal residue definition is therefore not proposed even though metabolism studies are available on three different crop groups (cereals, pulses/oilseeds and root and tuber vegetables).

Based on metabolism studies generally, the applicant selected the following residue analytes for study in all the GAP compliant field trials:

F9600 (bixlozone)
5'-hydroxy bixlozone (M289/3)
2,4-dichlorobenzoic acid (M190/1); and
2,2-dimethyl-3-hydroxy propionic acid (M118/1)

Therefore, there is quantitative information available on parent and these three metabolites in each of the crops for the representative uses. This information is valuable in considering the residue definition proposals.

Oilseed rape:

See the 'overview of metabolism' in canola (oilseed rape) (Table 2.7.3.1). All commodities are included to show the range of metabolites in the whole crop, including forage and straw, and to show the information that informs an understanding of overall metabolic profile across crops. As the intended use is for oilseed rape (not fodder use) the residues in the seed are the most important residues.

The overall identification of residues in canola/oilseed rape seed was limited (the low TRRs in seed, up to 0.015 mg/kg, will have been an influencing factor). The N rate was close to the GAP rate (0.92 or 0.96N).

No parent bixlozone was found in the canola (oilseed rape) metabolism study in any of the matrices.

The only metabolite in oilseed rape seed at >10%TRR was 2,4-dichlorobenzoic acid (M190/1) at 35%TRR (and 0.005 mg/kg). The only other metabolite reported in oilseed rape seed was at 4% TRR (0.001 mg/kg), bixlozone-dimethyl-malonamide (M289/2). Only residues in the phenyl labelled seeds were analysed based on low TRRs in the carbonyl labelled seeds.

Considering the other matrices in the canola/oilseed rape metabolism, the only finding of a metabolite both >10%TRR and >0.01 mg/kg was metabolite 2,2-dimethyl-3-hydroxypropionic acid (M118/1) in straw at 30.6%TRR and 0.023 mg/kg. There were a small number of metabolites which were found at >10% and <0.01 mg/kg (2,2-dimethyl-3-hydroxypropionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1), bixlozone-hydroxy-isobutyramide (M261/1), bixlozone-dimethyl-malonamide methyl ester (M303/1) and 4-hydroxy-methyl-bixlozone (M289/4)). Of these 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and 2,4-dichlorobenzoic acid (M190/1) were the most prevalent (they both featured above 30% TRR in at least one of the oilseed rape matrices).

Therefore, the field trials covering **at least** the metabolites 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and 2,4-dichlorobenzoic acid (M190/1) are considered an effective consideration of potential for any residues in oilseed rape.

Table 2.7.3.1 Summary table of the overview of metabolism of bixlozone in canola/oilseed rape

Crop	Canola/oilseed rape																	
Study reference	CA 6.2.1-02, Desai, M., 2019						CA 6.2.1-02, Desai, M., 2019				CA 6.2.1-02, Desai, M., 2019				CA 6.2.1-02, Desai, M., 2019			
Year	2014						2014				2014				2014			
Rate	1 x 300 g a.s./ha						1 x 300 g a.s./ha				1 x 300 g a.s./ha				1 x 300 g a.s./ha			
N rate	1N						1N				1N				1N			
Label	Phenyl (organic extracts)						Phenyl (acid hydrolysis)				Carbonyl (organic extracts)				Carbonyl (acid hydrolysis)			
BBCH at application	Pre-emergence						Pre-emergence				Pre-emergence				Pre-emergence			
PHI	36		70-71				36		70-71		36		70-71		36		70-71	
Crop part	Forage		Straw		Seeds		Forage		Straw		Forage		Straw		Forage		Straw	
%TRR / mg eq./kg	100	0.017	100	0.058	100	0.015	100	0.017	100	0.058	100	0.026	100	0.074	100	0.026	100	0.074
M118/1															14.3	0.004	30.6	0.023
M190/1					34.7	0.005	5.4	0.001	10.7	0.006								
M289/5																		
M261/1	7.4	0.001	6.8	0.004			14.8	0.002	15.0	0.009	5.4	0.001	5.0	0.004	6.8	0.002	6.8	0.005
M289/2			7.4	0.004	4.0	0.001	4.1	0.001	7.2	0.004			7.5	0.006	6.7	0.002	6.2	0.005
M303/1							**5.7**	**0.001**	**11.4**	**0.007**								
M289/4			**4.9**	**0.003**			**10.9**	**0.002**	**14.7**	**0.009**					**6.2**	**0.002**	**3.8**	**0.003**
Total identified	7.4	0.001	19.1	0.011	38.7	0.006	40.9	0.007	59.0	0.034	5.4	0.001	12.5	0.01	34.0	0.01	47.4	0.036
Unassigned	81.4	0.013	70.6	0.041	26.4	0.003	46.0	0.007	30.4	0.02	84.6	0.021	78.5	0.059	55.1	0.015	41.8	0.031
Total (sum of unknown and identified extracted residues)	88.8	0.014	89.7	0.052	65.1	0.009	86.9	0.014	89.4	0.054	90	0.022	91	0.069	89.1	0.025	89.2	0.067

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M190/1: 2,4-dichlorobenzoic acid; M289/5: 6'-hydroxy-bixlozone; M261/1: bixlozone-hydroxy-isobutyramide; M289/2: bixlozone-dimethyl-malonamide; M303/1: bixlozone-dimethyl-malonamide methyl ester; M289/4: 4-hydroxy-methyl-bixlozone

The GAP compliant trials in oilseed rape showed no residues above the LOQ for any of the analytes sought (see summary in section 2.7.4) in seed. Residues of 5'-hydroxy bixlozone (M289/3) and 2,4-dichlorobenzoic acid (M190/1) were not found in all other oilseed rape matrices in the trials (Vol 3, section B.7.3.2). In these trials and some matrices other than seed, some positive residues of bixlozone and 2,2-dimethyl-3-hydroxy propionic acid were observed in samples of whole plant (bixlozone up to around 0.024 mg/kg), pods (2,2-dimethyl-3-hydroxypropionic acid up to 2.2 mg/kg), plants without pods (2,2-dimethyl-3-hydroxypropionic acid up to 0.59 mg/kg) and flowers (bixlozone up to 0.012 mg/kg and 2,2-dimethyl-3-hydroxypropionic acid up to 0.06 mg/kg).

Whilst residues were not found in seeds in the trials, based on the prevalence of residues in the metabolism studies (2,2-dimethyl-3-hydroxypropionic acid (M118/1) and 2,4-dichlorobenzoic acid (M190/1) seeming to be the most major) it is not surprising that residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) were found in some of the other (not seed) plant matrices, especially those harvested at earlier times than would be done in the case of the intended OSR GAP.

With reference to these main metabolites, the following conclusions are made on the toxicology (section 2.6.9 of Vol 1):

Metabolite	**Covered by parent**	**Tox. compared to parent**	**Ref. value**	**Tox. relevant**
2,4-dichlorobenzoic acid M190/1	'covered'- putative major rat metabolite	may be approximately 2-fold more toxic	Parent (but include a 2 x potency factor)	Y
2,2-dimethyl-3-hydroxypropionic acid M118/1	N	Not known	None (Cramer class I TTC value of 30 µg/kg bw/day can be used to assess the chronic and acute exposure assessments) A combined assessment with dimethyl malonic acid (M132/1) is needed (structural similarity)- £	N (following exposure assessment versus the TTC CCI for the intended uses, see the calculations later in this section).

£- Considering the potential presence of dimethyl malonic acid (M132/1) not reported as a residue in the canola metabolism study - In the canola metabolism, the highest number of reference standards was used for initial scoping e.g. where dimethyl malonic acid (M132/1), was included but it is not clear how comprehensively this reference standard was used to check against metabolite fractions, since this component (dimethyl malonic acid, M132/1) was not designated as an identified residue in canola, and this metabolite (and its identification) was not discussed in the canola metabolism study report. As explained in Vol 3 [canola (OSR)], the unknown radioactive regions in grain, were individually very small <0.001 mg/kg).

In view of no residues of any metabolites sought being found in seeds in the OSR trials (which seemed to cover a good selection of analytes to test for potential residues in oilseed rape) it is proposed that a more general consideration of residue definition for risk assessment (RD-RA) should be based on prevalence in cereals, where positive residues of some metabolites were determined in the GAP compliant trials.

In consideration of the requested use on oilseed rape, then based on the main metabolite in oilseed rape seed (metabolism study) being 2,4-dichlorobenzoic acid (M190/1), then it would be possible to consider a proposed residue definition for risk assessment that includes bixlozone and 2,4-dichlorobenzoic acid (M190/1). Whilst 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was not sought **in seed** in the metabolism study (as the carbonyl labelled TRRs in the seed- carbonyl were too low for further analysis, at <0.01 mg/kg), 2,2-dimethyl-3-hydroxypropionic acid (M118/1) was sought and not found in the seeds in the oilseed rape residues trials. See the exposure assessment for M118/1 versus the TTC, which is provided later in this section 2.7.3.

Cereals (consideration of wheat then rice):

Wheat:

See the 'overview of metabolism' in wheat (Table 2.7.3.2). All commodities are included to show the range of metabolites in the whole crop, including grain, forage, hay and straw, and to show the information that informs an understanding of overall metabolic profile across crops.

The overall degree of identification of residues in wheat was higher than in the canola (OSR) metabolism study due to generally a higher TRR level of residues found. The N rate of the wheat metabolism study was around 1.5 N (with reference to the wheat and barley GAP intended use) and around 0.8 N (with reference to the higher application rate maize GAP intended use).

Table 2.7.3.2 Summary table of the overview of metabolism of bixlozone in wheat

Crop	Wheat								Wheat							
Study reference	CA 6.2.1-01, Desai, M., 2019								CA 6.2.1-01, Desai, M., 2019							
Year	2014								2014							
Rate	1 x 300 g a.s./ha								1 x 300 g a.s./ha							
N rate	1.5N								1.5N							
Label	Phenyl (organic extracts)								Phenyl (acid hydrolysis)							
BBCH at application	Pre-emergence								Pre-emergence							
BBCH at harvest	39		73		89				39		73		89			
PHI	28		48		60				28		48		60			
Crop part	Forage		Hay		Straw		Grain		Forage		Hay		Straw		Grain	
%TRR / mg eq./kg	100	0.969	100	1.896	100	1.399	100	0.135	100	0.969	100	1.896	100	1.399	100	0.135
M190/1	10.768	0.104	7.87	0.149	7.776	0.109	8.621	0.012	20.709	0.201	18.42	0.349	14.541	0.203	25.503	0.034
M305/1									12.322	0.119	4.544	0.086				
M305/2									10.611	0.103	8.241	0.156	12.064	0.169		
M289/3									49.359	0.478	43.6	0.827	47.878	0.67	0.51	0.001
M289/3 Conjugate	**38.411**	**0.372**	**37.502**	**0.711**	**39.22**	**0.549**										
M289/5									2.328	0.023	4.97	0.094	4.572	0.064		
Total identified	49.179	0.447	45.17	0.856	46.996	0.658	8.621	0.012	95.33	0.924	79.78	1.51	79.05	1.106	26.013	0.035
Unassigned	46.151	0.447	40.34	0.764	38.69	0.541	47.016	0.065	0	0	5.73	0.109	6.636	0.093	32.236	0.044
Total (sum of unknown and identified extracted residues)	95.33	0.894	85.51	1.62	85.686	1.199	55.637	0.077	95.33	0.924	85.51	1.619	85.686	1.199	58.249	0.079

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M190/1: 2,4-dichlorobenzoic acid; M305/1: 4-hydroxymethyl-5'-hydroxyl-bixlozone; M305/2: 5-hydroxy-5'-hydroxy-bixlozone; M289/3: 5'-hydroxy-bixlozone; M289/5: 6'-hydroxy-bixlozone

Table 2.7.3.2 continued Summary table of the overview of metabolism of bixlozone in wheat

Crop	Wheat								Wheat							
Study reference	CA 6.2.1-01, Desai, M., 2019								CA 6.2.1-01, Desai, M., 2019							
Year	2014								2014							
Rate	1 x 300 g a.s./ha								1 x 300 g a.s./ha							
N rate	1.5N								1.5N							
Label	Carbonyl (organic extracts)								Carbonyl (acid hydrolysis)							
BBCH at application	Pre-emergence								Pre-emergence							
BBCH at harvest	39		73		89				39		73		89			
PHI	28		48		60				28		48		60			
Crop part	Forage		Hay		Straw		Grain		Forage		Hay		Straw		Grain	
%TRR / mg eq./kg	100	0.938	100	1.013	100	1.088	100	0.085	100	0.938	100	1.013	100	1.088	100	0.085
M118/1							6.902	0.006	18.578	0.174	17.174	0.174	22.408	0.244	44.438	0.038
M305/1									7.375	0.069	5.991	0.061				
M305/2											5.442	0.055	9.008	0.098		
M289/3							1.803	0.002	46.989	0.441	31.502	0.319	30.48	0.332		
M289/3 Conjugate	**40.511**	**0.38**	**33.99**	**0.344**	**36.467**	**0.397**					**2.996**	**0.03**	**3.003**	**0.033**		
M289/5							0.684	0.001	1.67	0.016	1.797	0.018	1.70	0.018		
Total identified	40.511	0.38	33.99	0.344	36.467	0.397	9.389	0.009	74.612	0.7	64.902	0.657	66.599	0.725	44.438	0.038
Unassigned	54.41	0.511	51.33	0.52	48.742	0.53	45.391	0.039	20.317	0.19	20.418	0.207	18.611	0.202	10.34	0.009
Total (sum of unknown and identified extracted residues)	94.921	0.891	85.32	0.864	85.209	0.927	54.78	0.048	94.929	0.89	85.32	0.864	85.21	0.927	54.778	0.047

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M305/1: 4-hydroxymethyl-5'-hydroxyl-bixlozone; M305/2: 5-hydroxy-5'-hydroxy-bixlozone; M289/3: 5'-hydroxy-bixlozone; M289/5: 6'-hydroxy-bixlozone

No parent bixlozone was found in the wheat metabolism study in any of the matrices.

In grain, the only metabolites >10%TRR were also found at >0.01 mg/kg. These were 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (at up to 44%TRR at 0.038 mg/kg) and 2,4-dichlorobenzoic acid (M190/1) (25.5%TRR and 0.034 mg/kg). The only other (low level %TRR) metabolites identified in grain were 5'-hydroxy-bixlozone (M289/3) (low level of up to 2%TRR and 0.002 mg/kg) and 6'-hydroxy-bixlozone (M289/5), <1%TRR).

In the GAP compliant wheat/barley trials and maize trials, the applicant sought 2,2-dimethyl-3-hydroxy propionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1) and 5'-hydroxy-bixlozone (M289/3) in grain and straw. Therefore, at least in regard of grain, this is considered an effective consideration of potential for any residues in wheat grain based on wheat metabolism data (however please see information below, with regard to rice in relation to finding of dimethyl malonic acid (M132/1)).

In the plant (wheat) metabolism these metabolites (those sought in the trials, 2,2-dimethyl-3-hydroxy propionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1) and 5'-hydroxy-bixlozone (M289/3)) had featured too as major residues in forage, hay and straw (2,2-dimethyl-3-hydroxy propionic acid (M118/1) [up to 22.4% TRR and 0.244 mg/kg], 2,4-dichlorobenzoic acid (M190/1) [up to 20%TRR and 0.035 mg/kg] and 5'-hydroxy-bixlozone (M289/3) [up to 49%TRR and 0.83 mg/kg).

The following two indented paragraphs serve to discuss the findings of these 'key' metabolites (M118/1- 2,2-dimethyl-3-hydroxy propionic acid, M190/1 - 2,4-dichlorobenzoic acid and M289/3 - 5'-hydroxy-bixlozone) in the trials for comparative purposes (comparing metabolism outcomes with field trial outcomes for these metabolites).

Grain: The GAP compliant trials for wheat and barley showed that some residues of 2,4-dichlorobenzoic acid (M190/1) (very infrequently found in grain only) and 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (commonly found) were found in wheat/barley grain. No residues of bixlozone or 5'-hydroxy-bixlozone (M289/3) were found in wheat/barley grain. The only residues found to be present in maize grain were 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (commonly found). The applicant has stated their views about the residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) potentially being present as 'natural'; this is discussed further in section 2.7.4. HSE considers that the residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) are likely arising due to the pesticide treatment.

Barley and wheat **grain**	Bixlozone	13 x <0.01
	5'-hydroxy-bixlozone	13 x <0.01
	2,4-dichlorobenzoic acid	12 x <0.01, 0.01
	2,2-dimethyl-3-hydroxy propionic acid	<0.05, 0.064, 0.072, 0.075, 0.076, 0.077, 0.09, 0.096, 5 x <0.2

Maize **grain**	Bixlozone	4 x <0.01
	5'-hydroxy-bixlozone	4 x <0.01
	2,4-dichlorobenzoic acid	4 x <0.01
	2,2-dimethyl-3-hydroxy propionic acid	<0.05, 0.11, 0.13, 0.17

Straw: The GAP compliant trials for maize showed that none of these residues were found above the LOQs in straw. Some positive residues were observed in wheat and barley straw from GAP compliant trials, mostly 2,2-dimethyl-3-hydroxy propionic acid (M118/1) which were frequently found in straw samples, with infrequent finding of 2,4-dichlorobenzoic acid (M1901/1) and 5'-hydroxy-bixlozone (M289/3) as follows:

Barley and wheat **straw**	Bixlozone	13 x <0.01
	5'-hydroxy-bixlozone	12 x <0.01, 0.015
	2,4-dichlorobenzoic acid	12 x <0.01, 0.028
	2,2-dimethyl-3-hydroxy propionic acid	4 x <0.05, 0.058, 0.064, 0.069, 0.072, 0.077, 0.10, 0.14, 0.198, 0.261

The trials show that the residues of 5'-hydroxy-bixlozone (M289/3) and 2,4-dichlorobenzoic acid (M190/1) were infrequent (and low) arising from GAP use, and the levels seen in the wheat metabolism in straw (2,4-dichlorobenzoic acid (M190/1), 0.1 or 0.2 mg/kg and 5'-hydroxy-bixlozone (M289/3), 0.2 or 0.7 mg/kg) were not such a feature in the GAP complaint trials. It is noted that the wheat metabolism studies are a limited number of plants and were container grown rather than field plots.

Conversely the levels of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) seen in the trials in straw (up to 0.261 mg/kg) are reflective of the levels seen in straw in the wheat metabolism study (there is only one sample result in the wheat metabolism study for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) the acid hydrolysis/carbonyl labelled fraction so there is only limited information): 0.244 mg/kg in straw (22%TRR). It is noted that the analytical method for the assessment of residues in the field trials also uses an acid step in the extraction of the residues.

It is considered reasonable to use the information from the GAP compliant trials for quantitative purposes, and in terms of these analytes sought in the trials (2,2-dimethyl-3-hydroxy propionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1) and 5'-hydroxy-bixlozone (M289/3)) the available wheat/barley and maize trials are considered an effective consideration of potential for these residues all cereal matrices based on wheat metabolism data (see rice below, in consideration of the potential for another metabolite dimethyl malonic acid (M132/1) to be found).

As higher residues are found in straw, compared to grain, the 'overview of metabolism' (Table 2.7.3.2) was considered in regard of any **other metabolites** (in addition to 2,2-dimethyl-3-hydroxy propionic acid (M118/1), 2,4-dichlorobenzoic acid (M190/1) and 5'-hydroxy-bixlozone (M289/3)) found in the wheat metabolism study straw at >10%TRR that were not sought in the field trials:

> 5-hydroxy-5'-hydroxy-bixlozone (M305/2) was found at 12.1%TRR and 0.17 mg/kg. This metabolite is present at a lower %TRR than 5'-hydroxy-bixlozone (M289/3) (which was sought in trials and not found in 12 out of 13 trials, and at 0.015 mg/kg when found). This metabolite is considered unlikely to contribute significantly to the overall dietary burden as it is found at not far above 10%TRR in straw and was not found in grain. It is not proposed to include it in the residue definition for risk assessment or in the livestock dietary assessment, and it is not of concern that this analyte was not sought after in the current cereal trials.
>
> 5'-hydroxy-bixlozone (M289/3) conjugate (unspecified conjugate form) was found in the wheat metabolism at 36 to 39% TRR in the initial organic extract. However it seems that following acid hydrolysis this was converted to the aglcone of 5'-hydroxy-bixlozone (M289/3) (a similar amount of the free counterpart was found in the acid hydrolysis extract compared to the amount of conjugate found in the initial solvent extract). The free aglycone was not present in the initial solvent extract (not yet subject to acid hydrolysis), so that it seems that de-conjugation (following acid hydrolysis) is the reason for the appearance of 5'-hydroxy-bixlozone (M289/3) in samples. It is noted that the analytical method for residues in the trials, does involve an acid step in the extraction of the residues. It is, therefore, proposed that the field trials analysing for 5'-hydroxy-bixlozone (M289/3) would have ideally picked up on any residues derived from the conjugate, and this is evidenced from the data on extraction efficiency for the acid reflux step for the analytical method CAM -180/002 (section B.5.2.1). In the trials 5'-hydroxy-bixlozone (M289/3) was only found at a low level in straw in one trial (wheat) out of 13 at 0.015 mg/kg (<0.01 mg/kg in straw the other 12 trials). Residues of 5'-hydroxy-bixlozone (M289/3) were not found (<0.01 mg/kg) in wheat grain, oilseed rape seed, and maize grain and maize straw. Whilst the toxicological relevance of 5'-hydroxy-bixlozone (M289/3) is not fully known (see the table below), based on its low prevalence in trials, this metabolite is considered unlikley to contribute significantly to the overall dietary burden and it is not proposed to include it in the residue definition for risk assessment or in the livestock dietary assessment.

With reference to the above discussed main metabolites, the following conclusions are made on the toxicology (section 2.6.9 of Vol 1):

Metabolite	Covered by parent	Tox. compared to parent	Ref. value	Tox. relevant
M190/1 (2,4-dichlorobenzoic acid)	'covered' putative major rat metabolite	may be approximately 2-fold more toxic	Parent (but include a 2 x potency factor)	Y
M118/1 (2,2-dimethyl-3-hydroxy propionic acid)	N	Not known	None (Cramer class I TTC value of 30 µg/kg bw/day can be used to assess the chronic and acute exposure assessments) A combined assessment with dimethyl malonic acid (M132/1) is needed (structural similarity)- £	N (following exposure assessment versus the TTC CCI for the intended uses, see the calculations later in this section).
M289/3 (5'-hydroxy-bixlozone)	Not a major metabolite in rats	Equivalence to parent not assumed	None (Cramer class III TTC) chronic value of 1.5 µg/kg bw/day and acute value of 5 µg/kg bw can be used for the exposure assessments	Presumed Yes (TTC exposure assessment has not been undertaken based on its low prevalence in field trials- low infrequent occurrence in straw only)

£- Considering the potential presence of dimethyl malonic acid (M132/1) not reported as a residue in the wheat metabolism study - In the wheat metabolism, the highest number of reference standards was used for initial scoping e.g. where dimethyl malonic acid, was included but it is not clear how comprehensively this reference standard was used to check against metabolite fractions, since this component (dimethyl malonic acid, M132/1) was not designated as an identified residue in canola, and this metabolite (and its identification) was not discussed in the wheat metabolism study report.
Dimethyl malonic acid (M132/1) was identified in the rice metabolism, see below section on rice

The residue definition proposal for cereals are further discussed after the consideration for rice below.

Rice:

As stated above, although the wheat metabolism is especially relevant for the requested cereal uses (considering the GAP and the application regime in the metabolism study), it is also necessary to consider the nature of residues observed in the available rice metabolism study, as both rice and wheat are representative of cereals.

See the 'overview of metabolism' in rice (Table 2.7.3.3**Error! Reference source not found.**). The study considered dry land rice and paddy rice, grain and straw in each, and distribution of metabolite residue results were only presented in the rice metabolism study for the residues in the extracts following acid hydrolysis.

Grain: The most prevalent residues in rice grain were 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (17%TRR or 22%TRR), 2,4-dichlorobenzoic acid (M190/1) (21%TRR or 38%TRR), and dimethyl malonic acid (M132/1) at 20%TRR or 14%TRR).

Table 2.7.3.3 Summary table of the overview of metabolism of bixlozone in rice

Crop	Paddy Rice				Paddy Rice				Dry land rice				Dry land rice			
Study reference	CA 6.2.1-02, Desai, M., 2019				CA 6.2.1-02, Desai, M., 2019				CA 6.2.1-02, Desai, M., 2019				CA 6.2.1-02, Desai, M., 2019			
Year	2014				2014				2014				2014			
Rate	1 x 375 g a.s./ha				1 x 375 g a.s./ha				1 x 375 g a.s./ha				1 x 375 g a.s./ha			
Label	Phenyl (acid hydrolysis)				Carbonyl (acid hydrolysis)				Phenyl (acid hydrolysis)				Carbonyl (acid hydrolysis)			
BBCH at application	Pre-emergence				Pre-emergence				Pre-emergence				Pre-emergence			
BBCH at harvest	39				39				39				39			
PHI	151-153				151-153				151-153				151-153			
Crop part	Straw		Grain		Straw		Grain		Straw		Grain		Straw		Grain	
%TRR / mg eq./kg	100	0.712	100	0.077	100	0.243	100	0.038	100	0.908	100	0.112	100	0.525	100	0.038
M118/1					*14.5*	*0.035*	*17.2*	*0.007*					*27.4*	*0.144*	*21.9*	*0.017*
M190/1	24.8	0.177	21.4	0.016					67.5	0.613	38.3	0.043				
M289/2	11.0	0.078	3.8	0.003	2.5	0.006	2.0	0.001	12.7	0.115	7.9	0.009			1.7	0.001
M132/1					*8.9*	*0.022*	*19.6*	*0.008*					*14.8*	*0.078*	*13.9*	*0.011*
M275/1	20.4	0.145	13.5	0.013			12.3	0.005							0.04	<0.001
M132/1 & M118/1 conjugate					*31.1*	*0.076*	*8.0*	*0.003*					*26.9*	*0.141*	*12.8*	*0.01*
Total identified	56.2	0.4	38.7	0.029	57.0	0.139	59.1	0.024	80.2	0.728	46.2	0.052	69.1	0.363	50.3	0.04
Unassigned	30.2	0.215	16.4	0.013	25.9	0.065	14	0.005	12.3	0.112	25.8	0.029	21.6	0.114	23.6	0.02
Total (sum of unknown and identified extracted residues)	86.4	0.615	55.1	0.042	82.9	0.204	73.1	0.029	92.5	0.84	72	0.081	90.7	0.477	73.9	0.06

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M132/1: dimethyl malonic acid ; M275/1: bixlozone-3-hydroxy-propanamide

A peak was reported in the study assigned to two conjugates: dimethyl malonic acid (M132/1) conjugate and 2,2-dimethyl-3-hydroxy propionic acid (M118/1) conjugate, and this was found at 8% TRR or 13% TRR in grain. It was a sole peak that the applicant considers corresponds to conjugates of dimethyl malonic acid (M132/1) and 2,2-dimethyl-3-hydroxy propionic acid (M118/1) together. The free aglocone of dimethyl malonic acid (M132/1) was reported as a separate component in grain at 20% TRR or 14% TRR. The chromatographic analysis of these components in rice was especially challenging (the assignation of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) was two co-eluting peaks that the applicant considers are both 2,2-dimethyl-3-hydroxy propionic acid (M118/1), see discussion further in Vol 3, section B.7.2.1.4 rice metabolism). These determinations are plausible suggestions (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in view of these metabolites found in other metabolism studies, such as sugar beet) but based on the chromatography are not considered to be fully robust. 2,2-dimethyl-3-hydroxy propionic acid (M118/1) was found in cereal field trials in grain (maize, wheat and barley), and dimethyl malonic acid (M132/1) was not a sought analyte in these field trials. It is therefore postulated that dimethyl malonic acid (M132/1) could have also been present in cereal grain samples in the field trials following the requested use rates. Dimethyl malonic acid (M132/1) was stated as a reference standard in the wheat metabolism study (initial scoping HPLC work), but the wheat metabolism report was not clear about whether this reference standard was used to check against metabolite fractions, since this component (dimethyl malonic acid, M132/1) was not designated as an identified residue, and this metabolite (and its identification) was not discussed in the wheat metabolism study report. Even if it could be definitively concluded that dimethyl malonic acid (M132/1) was not found after being sought in the wheat metabolism study, from the rice metabolism data there is a suggestion that dimethyl malonic acid (M132/1) is also a major metabolite in grain. Due to the above mentioned issues with the challenges in chromatography, it is difficult to assign comparative levels of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in rice grain. It can only be very generally concluded that dimethyl malonic acid (M132/1) could be found in almost equal proportions to metabolite 2,2-dimethyl-3-hydroxy propionic acid (M118/1). It is appreciated that this estimate is very uncertain. There is currently no toxicological data available for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1), and based on similarity in structure of these molecules, the toxicology evaluation has indicated that a first tier screening risk assessment could be performed using Cramer Class I (CCI, TTC classification), and that a combined risk assessment should be performed for dimethyl malonic acid (M132/1) and 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (section 2.6.9).

Metabolite	Covered by parent	Tox. compared to parent	Ref. value	Tox. relevant
M132/1 (dimethyl-malonic acid)	N	Not known	None (Cramer class I TTC value of 30 µg/kg bw/day can be used to assess the chronic and acute exposure assessments) A combined assessment with 2,2-dimethyl-3-hydroxy propionic acid (M118/1) is needed (structural similarity)	N (following exposure assessment versus the TTC CCI for the intended uses, see the calculations later in this section).

A further metabolite in rice grain was bixlozone-3-hydroxy-propanamide (M275/1) (found at >10% TRR and >0.01 mg/kg, at a maximum level in grain of 13.5% and 0.013 mg/kg (in the phenyl labelled paddy rice). This metabolite has not been sought in cereal trials. Since 2,4-dichlorobenzoic acid (M190/1) was present in the same paddy rice sample at a higher level of 21.4% TRR, and given the very low residues found in grain in the cereal residues trials for 2,4-dichlorobenzoic acid (M190/1), it is proposed that this metabolite (bixlozone-3-hydroxy-propanamide (M275/1)) will likely not be found at >LOQ levels in grain. In concluding this, it is noted that the rice metabolism study application rate (at 350 g a.s./ha) was 0.93N with regard to the intended maize GAP and 1.75N with regard to the intended wheat and barley GAP rate.

A lower level residue in grain (minor <10% and <0.01 mg/kg) was bixlozone-dimethyl-malonamide (M289/2), and again as per bixlozone-3-hydroxy-propanamide (M275/1) above, it is not expected that this component would be found in cereal grain arising from GAP intended uses in cereals.

Therefore, based on the metabolism in rice grain, the data are suggestive that the major metabolites 2,2-dimethyl-3-hydroxy propionic acid (M118/1), dimethyl malonic acid (M132/1) and 2,4-dichlorobenzoic acid (M190/1) should be addressed based on their prevalence. As no toxicological or residues field trials data are available for dimethyl malonic acid (M132/1) and the metabolism assignations are somewhat uncertain, then assumptions need to be made about dimethyl malonic acid (M132/1) in order to cover it in the assessment. HSE proposes, that in this evaluation, when exposure of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) is estimated (and compared to the threshold of toxicological concern (TTC)), dimethyl malonic acid (M132/1) should be included as a combined exposure assessment assuming equal proportions of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) are present in cereal grains).

Straw: As per grain, the most prevalent metabolites in rice straw seem to be 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (14.5%TRR or 27%TRR), 2,4-dichlorobenzoic acid (M190/1) (25%TRR or 67.5%TRR), and dimethyl malonic acid (M132/1) (15%TRR or 9%TRR). The above reported issues with chromatography also occurred for straw and the level of the conjugate peak reported for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) (a peak thought to be covering two conjugates – conjugate of 2,2-dimethyl-3-hydroxy propionic acid and dimethyl malonic acid) were 31%TRR or 27%TRR. These components taken together in straw samples cover 25%, 55%, 68 and 69% of the TRR (four different straw extracts). As per grain, the estimation of a level dimethyl malonic acid (M132/1) relative to 2,2-dimethyl-3-hydroxy propionic acid (M118/1) is very uncertain. Without considering the conjugate peak, the ratio of 'free' dimethyl malonic acid (M132/1) : 'free' 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in straw is around 0.6 : 1. As uncertain (and in view of the single peak for the conjugates of these two metabolites), it would not be unreasonable, in the absence of more specific data, to assume roughly equal presence of dimethyl malonic acid (M132/1) and 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in cereal straw samples, where dimethyl malonic acid (M132/1) has not been sought in primary crop cereal field trials (and where data on 2,2-dimethyl-3-hydroxy propionic acid (M118/1) are available).

Other metabolites found in rice straw were bixlozone-dimethyl-malonamide (M289/2) (2 to 13%TRR), and bixlozone-3-hydroxy-propanamide (M275/1) (only found in paddy rice straw in the phenyl labelled sample, so one out of the four straw sample extracts) at 20%TRR. 2,4-dichlorobenzoic acid (M190/1) was found in a sample of paddy rice (phenyl label) that contained the following three metabolites together in the following proportions 2,4-dichlorobenzoic acid (M190/1) 25%TRR: bixlozone-dimethyl-malonamide (M289/2) 11%TRR: bixlozone-3-hydroxy-propanamide (M275/1) 20%TRR. In the cereal GAP compliant field trials 2,4-dichlorobenzoic acid (M190/1) was infrequently found and at low levels in straw (<LOQ of 0.01 mg/kg in all maize trials, and 12 x <0.01 mg/kg, 0.028 mg/kg in the 13 wheat and barley trials). It is therefore presumed that the contribution of bixlozone-dimethyl-malonamide (M289/2) and bixlozone-3-hydroxy-propanamide (M275/1) to the overall dietary burden from consumption of cereal straw is likely to be very small.

Sugar beet:

The currently intended uses do not encompass sugar beet. As the proposal on the residue definition includes a TTC assessment (for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1)) considering the currently intended uses, it is not possible to currently propose a universal residue definition (across all crops).

The metabolism in sugar beet is not distinctly different to the metabolism observed in oilseed rape and wheat/rice. The metabolites >10%TRR and >0.01 mg/kg in the sugar beet metabolism study are 2,4-dichlorobenzoic acid (M190/1), 2,2-dimethyl-3-hydroxy propionic acid (M118/1), 5-hydroxy-bixlozone (M289/1) (and its conjugate), bixlozone-dimethyl-malonamide (M289/2), and dimethyl malonic acid (M132/1). Dimethyl malonic acid (M132/1) was found in sugar beet at up to 41%TRR (<0.01 mg/kg) in sugar beet roots. For a complete picture, see the 'overview of metabolism' in sugar beet (Table 2.7.3.4).

Table 2.7.3.4 Summary table of the overview of metabolism of bixlozone in sugar beet

Crop	Sugar beet						Sugar beet					
Study reference	CA 6.2.1-02, Desai, M., 2019						CA 6.2.1-02, Desai, M., 2019					
Year	2014						2014					
Rate	1 x 300 g a.s./ha						1 x 300 g a.s./ha					
N rate	No GAP on sugar beet or root crops						No GAP on sugar beet or root crops					
Label	Phenyl (organic extracts)						Phenyl (acid hydrolysis)					
BBCH at application	Pre-emergence						Pre-emergence					
BBCH at harvest	BBCH 39		BBCH 49				BBCH 39		BBCH 49			
PHI	28		173				28		173			
Crop part	Immature tops		Mature tops		Roots		Immature tops		Mature tops		Roots	
%TRR / mg eq./kg	100	0.159	100	0.007	100	0.031	100	0.159	100	0.007	100	0.031
M190/1	4.0	0.006					35.3	0.056	19.0	0.001	34.8	0.011
M289/2	5.9	0.009	4.5	<0.001	41.1	0.013	29.7	0.047	34.0	0.002	29.8	0.009
M303/1												
M467/1	**9.2**	**0.015**	**4.5**	**<0.001**	**10.5**	**0.003**	**3.3**	**0.005**	**17.3**	**0.001**		
M289/1 conjugate	**22.9**	**0.036**	**30.3**	**0.002**	**5.4**	**0.002**	**4.0**	**0.006**	**11.1**	**0.001**	**9.0**	**0.003**
M289/1							16.4	0.026	6.2	<0.001	2.8	0.001
M451/1			**17.5**	**0.001**	**3.2**	**0.001**						
M289/6												
M289/4			**18.8**	**0.001**							**2.7**	**0.001**
Total identified	42.0	0.066	75.6	0.005	60.2	0.019	88.7	0.14	87.6	0.005	79.1	0.025
Unassigned	47.1	0.075	21.5	0.002	32.3	0.01	3.1	0.005	9.5	0.001	17.7	0.006
Total (sum of unknown and identified extracted residues)	89.1	0.141	97.1	0.007	92.5	0.029	91.8	0.145	97.1	0.006	96.8	0.031

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M303/1: bixlozone-dimethyl-malonamide methyl ester; M467/1: Dihydroxy-bixlozone conjugate; M289/1: 5-hydroxy-bixlozone; M451/1: bixlozone hydroxy glucoside conjugate; M289/6: 3'-hydroxy-bixlozone; M289/4: 4-hydroxy-methyl-bixlozone

Table 2.7.3.4 continued Summary table of the overview of metabolism of bixlozone in sugar beet

Crop	Sugar beet						Sugar beet					
Study reference	CA 6.2.1-02, Desai, M., 2019						CA 6.2.1-02, Desai, M., 2019					
Year	2014						2014					
Rate	1 x 300 g a.s./ha						1 x 300 g a.s./ha					
N rate	No GAP on sugar beet or root crops						No GAP on sugar beet or root crops					
Label	Carbonyl (organic extracts)						Carbonyl (acid hydrolysis)					
BBCH at application	Pre-emergence						Pre-emergence					
BBCH at harvest	BBCH 39		BBCH 49				BBCH 39		BBCH 49			
PHI	28		173				28		173			
Crop part	Immature tops		Mature tops		Roots		Immature tops		Mature tops		Roots	
%TRR / mg eq./kg	100	0.176	100	0.011	100	0.014	100	0.176	100	0.011	100	0.014
M118/1	4.4	0.008	15.8	0.002	6.6	0.001	64.7	0.114			43.4	0.006
M305/1			2.5	<0.001								
M289/2	5.0	0.009	2.5	<0.001			10.5	0.018	54.6	0.006	3.5	<0.001
M467/1	**11.2**	**0.02**							**12.8**	**0.001**		
M289/1 conjugate	**24.7**	**0.043**	**4.7**	**0.001**					**13.2**	**0.001**		
M289/1	7.2	0.013			1.7	<0.001	5.3	0.009	11.4	0.001		
M451/1			**23.5**	**0.003**								
M289/6					**3.7**	**<0.001**						
M289/4			**9.1**	**0.001**								
M132/1			9.7	0.001	40.8	0.006					34.0	0.005
Total identified	52.5	0.084	67.8	0.008	51.1	0.007	80.5	0.141	92	0.009	80.9	0.011
Unassigned	39.4	0.068	26.8	0.003	56.7	0.008	11.1	0.02	0	0	14.3	0.002
Total (sum of unknown and identified extracted residues)	91.9	0.152	94.6	0.011	107.8	0.015	91.6	0.161	92	0.009	95.2	0.013

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

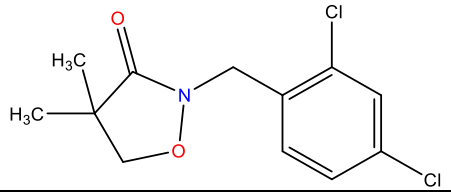
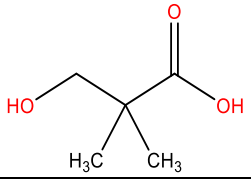
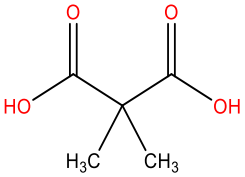
Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M305/1: 4-hydroxymethyl-5'-hydroxyl-bixlozone; M289/2: bixlozone-dimethyl-malonamide; M467/1: Dihydroxy-bixlozone conjugate; M289/1: 5-hydroxy-bixlozone; M451/1: bixlozone hydroxy glucoside conjugate; M289/6: 3'-hydroxy-bixlozone; M289/4: 4-hydroxy-methyl-bixlozone; M132/1: dimethyl malonic acid

Residue Definition – TTC consideration and further discussion on ,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1)

The information on the toxicology of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in this section and also in section 2.6.9, indicates that the structures are very different to parent, they are simpler molecules, and they are not considered as covered by parent. No toxicological data have been provided to help inform on reference values suitable for dietary intake.

Bixlozone (F9600)	
M118/1 (2,2-dimethyl-3-hydroxy propionic acid)	
M132/1 (Dimethyl-malonic acid)	

The proposed toxicological reference values for dietary risk assessment of bixlozone are 0.3 mg/kg bw/day (ADI) and 0.75 mg/kg bw (ARfD).

There is an entry for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in the REACH dossier database as it has been assessed under Articles 17 and 18 as an 'intermediate product' REACH dossier database entry for 3-hydroxypivalic acid (EC name) or 3-hydroxy-2,2-dimethylpropanoic acid (IUPAC name) (https://echa.europa.eu/registration-dossier/-/registered-dossier/22481/1). This REACH assessment has not considered any toxicological data of relevance to the dietary exposure assessment of pesticides. The use of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) as an 'intermediate' product is also referenced in a commercial chemicals catalogue where it is stated that 3-Hydroxy-2,2-dimethylpropionic acid is used as pharmaceutical intermediate" (4835-90-9 - 3-Hydroxy-2,2-dimethylpropionic acid, 97+% - 2,2-Dimethyl-3-hydroxypropionic acid - Hydroxypivalic acid - L12858 - Alfa Aesar). Also, the published paper (submitted by the applicant) Rezanka (2012) Metabolic Pathways, Appl. Micr. Biotech. 95(6), 1371-1376, 2012, refers to pivalic acid (M118/1 is also termed 3-hydroxypivalic acid) potentially present in the environment as a result of man-made activities (as pro-drug). Please see section 2.7.4 (Volume 1) where the information from this paper (Rezanka, 2012), and the applicant's proposal that residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) might be 'natural' are further discussed.

In section B.6.8.1, it is noted that different Threshold of Toxicological Concern (TTC) models yielded different outcomes for the TTC Cramer Class (CC) classification for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1). Toxtree online indicated CCI for both 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) (presumption of low toxicity) and OECD Toolbox indicated CCIII (presumption of some serious toxicity). This discrepancy was raised with the applicant, and section B.6.8.1.2 explains the rationale for the proposal that the TTC exposure estimation approach should apply the classification of Cramer Class I for each of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) and noting that a combined assessment would be needed (M118/1 and M132/1) due to similarity in structure.

As such, HSE has used TTC CCI as the screening approach to the initial consideration of the risk assessment for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in plants, since no toxicological data are available for these components. Toxicological advice (section 2.6.9) states that the CCI values of 30 µg/kg bw/day (0.03 mg/kg bw) can be used for TTC consideration (for both chronic and acute exposure consideration). Based on their structural similarity a combined assessment for these components should be performed. See section 2.6.9 of Vol 1 to refer to the toxicological information.

HSE took the following approach (results are tabulated below):

- Use of STMR values from field trials for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) for chronic and acute exposure assessment.

- Information of co-presence of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) taken from both the rice and wheat metabolism studies. Equal proportions/presence of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) assumed in cereal grain. (see the discussions above, complex due to the contributions of a single peak of both 2,2-dimethyl-3-hydroxy propionic acid (M118/1) conjugate and dimethyl malonic acid (M132/1) conjugate in the rice metabolism). Residues of dimethyl malonic acid (M132/1) were not included in the TTC consideration for oilseed rape seed; all individual residues are expected to be very low in oilseed rape (confirmed by metabolism and field study data). In the field trials residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) were <LOQ (<0.05 mg/kg). 2,2-dimethyl-3-hydroxy propionic acid (M118/1) was not sought in the metabolism *seed* data as the residues (TRR) in seed for the carbonyl label were too low. In the TTC consideration, it was consider too worst case to also add in a proposed co-exposure of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) for oilseed rape.

- Residue values were summed in the combined assessment without making any adjustment for molecular weight conversion. The molecular weights are 118.1 g/mol (2,2-dimethyl-3-hydroxy propionic acid, M118/1) and 132.1 g/mol (dimethyl malonic acid, M132/1). As the exposure assessments are anyhow uncertain, it was considered reasonable to double the residue levels (STMR and HRs) of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) for cereal uses in order to do a combined assessment for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1).

- Processing data are available for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in bran indicating a concentration (PF=1.6). The processing factor is based on data for fine bran/coarse bran/total bran and is considered 'indicative' as only one of the trials had determinable residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (see section 2.7.6 for further information). For the assessment of chronic total dietary intakes, the assessment has not included a concentration factor (processing factor) for bran, as the consumption data for wheat includes all contributions of wheat consumption, and it would be too worst case to apply the processing factor (PF) for bran to all of the wheat consumed. A TTC consideration was done for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in bran in the form of an individual commodity (bran) NEDI as well as a bran NESTI (based on 97.5th %le chronic and 97.5th %le acute consumption values for bran). Processing data were not available for dimethyl malonic acid (M132/1) so a TTC consideration for bran for this metabolite has not been performed. The assessment for bran is uncertain but regarded as worst case for 2,2-dimethyl-3-hydroxy propionic acid (M118/1). The consumption data estimate for bran covers all forms of bran. The consumption of bran by infants and toddlers is considered to be virtually all in the form of processed breakfast cereal. For infants, the critical consumer group, 97.6% of the consumption of bran, was as bran based processed breakfast cereals. Processing (residues) data are not available for bran based processed breakfast cereal. As such, the current TTC consideration for bran (infants) is considered worst case for 2,2-dimethyl-3-hydroxy propionic acid (M118/1).

- Use of field trial residues data analysing for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in GAP compliant trials was used to inform on prevalence of residues following the intended uses, so to inform for both 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1). HSE did not conduct a TTC consideration for rotational crops, as all residues of metabolites are not likely to be found in rotational crops following the intended uses following the assessment of the rotational crop field trials.

The applicant provided a different exposure TTC consideration for metabolites (position paper FMC-55114). The applicant assessment differed in the following respects:

- The applicant used residue input values from the plant metabolism study (wheat) and scaled to the N rate, instead of using the quantitative trial values for 2,2-dimethyl-3-hydroxy propionic acid (M118/1). The position paper stated:

"Metabolite M118/1 was detected in both control and treated crop samples from the field trials due to its natural occurrence. The field residues did not represent true residues of M118/1 from the application of bixlozone, and therefore, normalized metabolism data of M118/1 were used for exposure assessment".

However HSE considers that it is highly likely that the residues arose in the field trials from direct pesticide treatment. The applicant has not provided any specific information on the occurrence of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) as a natural component in food. The results found in the metabolism studies for grain and straw (2,2-dimethyl-3-hydroxy propionic acid, M118/1) are broadly in accordance with the range of field trial values obtained for 2,2-dimethyl-3-hydroxy propionic acid (M118/1). The field trial values represent 13 data points (wheat and barley), 4 data points (maize), whereas the wheat metabolism study represents one value only for each of grain and straw. A further discussion about the proposal that residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) might be natural and a discussion regarding the finding of residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) in untreated controls is provided in section 2.7.4 (Vol 1).

- The applicant did not include dimethyl malonic acid (M132/1) in the assessment for primary crops (since dimethyl malonic acid (M132/1) was only reported in rice and not wheat metabolism). Due to extrapolation within the metabolism cereal crop group, HSE considers that, in the absence of field trials data analysing for dimethyl malonic acid (M132/1), dimethyl malonic acid should be considered as potentially present in all cereals, and with reference to Section 2.6.9 (toxicological assessment) a combined assessment for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) should be performed. The applicant did consider the combined assessment of various metabolites, however this did not include dimethyl malonic acid (M132/1) as a potential primary crop metabolite, as they had used the wheat metabolism information only and not the information from rice and wheat metabolism studies taken together.

- In their TTC consideration, the applicant addressed metabolites in rotational crops, as they were using metabolism data from the confined radiolabel metabolism data rather than the follow on rotational crop field trial data, which tested a large range of metabolites and showed absence of residues of metabolites in all rotational crops. [HSE did not include rotational crops in the TTC consideration as stated above].

The results of HSE's TTC consideration for metabolites 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) are presented in Table 2.7.3.5, Table 2.7.3.6 and Table 2.7.3.7.

Table 2.7.3.5 TTC chronic exposure assessment - total dietary exposure assessment for items directly consumed by humans: grain and oilseed rape seed

Input values (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) combined assessment assuming equal proportions of these metabolites in cereals (2,2-dimethyl-3-hydroxy propionic acid (M118/1) only considered for oilseed rape)): no account made for processing (see the individual NEDIs and NESTIs performed for bran, where in processing trials a concentration of residues in bran was observed, in the tables 2.7.3.6 and 2.7.3.7 below).

	STMR (mg/kg)	UK total chronic exposure (for critical consumer group) mg/kg bw/day	EU total chronic exposure (PRIMo v 3.1) exposure (for critical consumer group) mg/kg bw/day	CCI TTC of 0.03 mg/kg bw/day Exceeded ?
2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure (oilseed rape no input for dimethyl malonic acid (M132/1))[&]	Wheat 0.18 Barley 0.18 Maize 0.24 OSR 0.05[&]	0.00196 (4-6 year old)	0.00248 (NL toddler)	No

[&] 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure is not being considered for the intended oilseed rape use (see HSE's explanation of approach above the tables).

Table 2.7.3.6 TTC chronic exposure assessment -Individual crop NEDIs assessment (in view of the total chronic intakes [Table 2.7.3.5] being estimated as <10% of the TTC CCI, the detail in this table isn't strictly needed, however it shows the assessment for individual commodities and bran (where a concentration in residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) has been observed).

Input values (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) combined assessment assuming equal proportions of these metabolites in cereals (2,2-dimethyl-3-hydroxy propionic acid (M118/1) only considered for oilseed rape)).

	residue level (mg/kg)	highest individual crop NEDI (UK)	highest individual crop NEDI (EU PRIMO 3.1)	CCI TTC of 0.03 mg/kg bw/day Exceeded ?
Wheat 2,2-dimethyl-3-hydroxy propionic acid (M118/1) – no PF	0.09	UK 4-6 year old child 0.00080	Gems/Food G06 0.00065	No
Wheat 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure – no PF	0.18	0.0016	0.0013	No
Wheat bran 2,2-dimethyl-3-hydroxy propionic acid (M118/1) (PF of 1.6) [£]	0.144	UK infant 0.00079 UK 0.00040 (toddler) 0.00023 (4-6 year old) Results for the three highest intakes for the various consumer groups are given, as estimates for infants are especially considered to be worst case- see bullet point text explaining about the consumption data for bran and the approach on the previous pages.	N/A (no specific bran consumption data in PRIMo v 3.1)	No
Barley 2,2-dimethyl-3-hydroxy propionic acid (M118/1)	0.09	UK Toddler 0.00039	Gems/Food G08 0.000080	No
Barley 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure	0.18	0.00078	0.00016	No
Maize 2,2-dimethyl-3-hydroxy propionic acid (M118/1)	0.12	UK Infant 0.00055	NL Toddler 0.00085	No
Maize 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure	0.24	0.0011	0.0017	No
Oilseed rape	0.05	UK Toddler	NL Toddler	No

	residue level (mg/kg)	highest individual crop NEDI (UK)	highest individual crop NEDI (EU PRIMO 3.1)	CCI TTC of 0.03 mg/kg bw/day Exceeded ?
2,2-dimethyl-3-hydroxy propionic acid (M118/1)[&]		0.00036	0.000048	

& 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure is not being considered for the intended oilseed rape use (see HSE's explanation of approach above the tables).
[£] Processing data is not available for dimethyl malonic acid (M132/1) in bran, so the assessment for bran is for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) only.

Table 2.7.3. 7 TTC acute exposure assessment (for items directly consumed by humans: grain and oilseed rape seed)

Input values (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) combined assessment assuming equal proportions of these metabolites in cereals (2,2-dimethyl-3-hydroxy propionic acid (M118/1) only considered for oilseed rape)).

	STMR	UK acute NESTI exposure (for critical consumer group) mg/kg bw/day	EU acute (PRIMo v 3.1) IESTI exposure (for critical consumer group) mg/kg bw/day	CCI TTC of 0.03 mg/kg bw/day Exceeded ?
Wheat grain 2,2-dimethyl-3-hydroxy propionic acid (M118/1)	0.09	0.0013 (4-6 year old)	0.0013 (UK 4-6 year old)	No
Wheat grain (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure)		*0.0026 (4-6 year old)*	*0.0026 (UK 4-6 year old)*	No
Wheat bran 2,2-dimethyl-3-hydroxy propionic acid (PF of 1.6) [£]	0.144	0.00070 (UK infant) 0.00067 (4-6 year old, next highest intake after infants the critical consumer group). See bullet point text explaining about the consumption data for bran and the approach on the previous pages.	N/A (no specific bran consumption data in PRIMo v 3.1)	No
Barley grain 2,2-dimethyl-3-hydroxy propionic acid (M118/1)	0.09	0.00051 (7-10 year old)	0.00051 (UK 7-10 year old)	No
Barley grain (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl		*0.0010 (7-10 year old)*	*0.0010 (UK 7-10 year old)*	No

	STMR	UK acute NESTI exposure (for critical consumer group) mg/kg bw/day	EU acute (PRIMo v 3.1) IESTI exposure (for critical consumer group) mg/kg bw/day	CCI TTC of 0.03 mg/kg bw/day Exceeded ?
malonic acid (M132/1) co-exposure)				
Maize grain 2,2-dimethyl-3-hydroxy propionic acid (M118/1)	0.12	0.00081 (infant)	0.00081 (UK infant)	No
Maize grain (2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure)		*0.0016 (infant)*	*0.0016 (UK infant)*	No
Oilseed rape seed 2,2-dimethyl-3-hydroxy propionic acid (M118/1)[&]	<0.05	0.00072 (4-6 year old)	0.00007 (DE child)	No

[&] 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) co-exposure is not being considered for the intended oilseed rape use (see HSE's explanation of approach above the tables).
[£] Processing data is not available for dimethyl malonic acid (M132/1) in bran, so the assessment for bran is for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) only.
Toxicological advice (section 2.9.6) is to apply the TTC CCI threshold to 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1), and for co-exposure to both to be considered when doing the TTC consideration.

The co-exposure of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) total chronic dietary exposure estimates and acute exposure estimates do not exceed the TTC CCI value. The highest estimated total chronic dietary intake and the highest estimated acute exposure intake (acute- for wheat grain), considering co-exposure of M118/1 and M132/1, are each less than 10% of the TTC value. Therefore, it proposed that these metabolites are excluded from residue definition for dietary risk assessment at the current time, based on the currently intended uses.

A brief assessment has been undertaken for honey (TTC screen for 2,2-dimethyl-3-hydroxy propionic acid (M118/1)):

> Separate to the exposure consideration specifically for the requested uses assessed above a separate exposure assessment is conducted below, to consider the 'estimated potentially worst case' residue in honey of 0.06 mg/kg for 2,2-dimethyl-3-hydroxy propionic acid (M118/1). 0.06 mg/kg was the highest level of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) observed in oilseed rape flowers (Vol 3, section B.7.3).
>
> Highest estimated dietary intakes for the critical consumer groups are presented below for honey:
>
> > Highest individual NEDI for honey in EU PRIMO (v 3.1) for DE child = 0.000006 mg/kg bw/day, well below the TTC CCI value (chronic) of 0.03 mg/kg bw/day
> >
> > Highest individual NESTI for honey in EU PRIMO (v 3.1) for NL toddler = 0.00021 mg/kg bw/day, well below the TTC CCI value (acute) of 0.03 mg/kg bw/day

Highest individual UK NEDI for honey for UK infant = 0.000048 mg/kg bw/day, well below the TTC CCI value (chronic) of 0.03 mg/kg bw/day

Highest individual UK NESTI for honey for UK infant = 0.00011 mg/kg bw/day, well below the TTC CCI value (acute) of 0.03 mg/kg bw/day

No estimates for dimethyl malonic acid (M132/1) in oilseed rape flowers have been made (see Vol 3 B.7.2 on plant metabolism, it is not fully clear how the presence of dimethyl malonic acid (M132/1) in canola/oilseed rape was investigated). If it is assumed that it can be found in equal proportions to 2,2-dimethyl-3-hydroxy propionic acid (M118/1) then co-exposure estimates for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in honey would be well below the respective TTC values, as outlined above.

Human dietary exposure considerations:

The TTC CCI screen for the requested uses show that the estimated chronic and acute dietary intakes, covering all off the intended uses, are well below the threshold when co-exposure to 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) is considered. The estimated exposures were all below 10% of the TTC for CCI. As such, based on the currently intended uses, residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) do not need to be included in the residue definition for dietary risk assessment. For future extensions of uses, the exposure estimations for M118/1 and M132/1, combined, would need to be revisited to consider whether the intakes remain below the TTC.

Proposal for residue definition for risk assessment (relevant to primary crops):	**Sum of residues of bixlozone and 2 x 2,4-dichlorobenzoic acid expressed as bixlozone**
Note: Any extensions to the intended uses (increased application rates, change of application timing or range of crop uses), will require a reconsideration of the residue definition (and recalculation of the exposure assessment versus the TTC for co-exposure of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) which are considered to be present in significant amounts in cereal matrices)	Intended early application use on oilseed rape, wheat, barley and maize only [the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid].

Rotational crops:

The available rotational crop metabolism data are summarised in section 2.7.7 and written up in full in section B.7.6.1. The study (phenyl and carbonyl labelled residues investigated) involved treatment to bare soil at a rate of 270 to 288 g as/ha. The plantings were done in a confined rotational crop system where wooden boxes were lined with plastic (as a contained system). The times of the replanting intervals and respective total radioactive residue ranges were 30-63 (0.02 to 0.59 mg/kg), 120-153 (0.02 to 0.34 mg/kg), and 310 days after treatment, DAT (0.005 to 0.11 mg/kg). In the rotational crops (wheat, lettuce, and radish), the total radioactive residues were markedly lower in the last replanting interval, and the %TRRs of metabolites seen in the last timing were broadly in accordance with the % in the earlier replanting intervals (PBI = plant back interval). Therefore, the below consideration and discussion of key metabolites found in rotational crops is from the earlier replant times of 30 to 153 DAT. Full details of the amounts of all the metabolites for all the replant timings are presented in the overview of metabolism table presented below (Table 2.7.3.8).

Table 2.7.3. 8 Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871 (Desai, 2019)															
Year	2015															
Rate	300 g as/ha															
Crop part	lettuce (immature)				lettuce (mature)				radish top				radish root			
PBI	63								30							
Label	P		C		P		C		P		C		P		C	
%TRR / mg parent eq. /kg	100	0.01	100	0.045	100	0.018	100	0.017	100	0.09	100	0.208	100	0.049	100	0.055
bixlozone	2.3	<0.001	2.9	0.002					24.4	0.022	25.9	0.054	75.7	0.037	40.3	0.022
M190/1	*1.4*	*<0.001*			*1.8*	*<0.001*										
M467/1	**16.6**	**0.002**	**7.9**	**0.004**	**11.4**	**0.002**	**7.8**	**0.001**								
M289/2	27.1	0.003	17.5	0.008	30.7	0.006	11.8	0.002	6.4	0.006	2.9	0.006				
M451/2	2.8	<0.001														
M261/1									19.1	0.017	26.6	0.055			4.5	0.003
M289/4	2.5	<0.001			3.2	0.001			4.9	0.004	6.5	0.013			2.4	0.001
M289/3									4.2	0.004	3.1	0.006			2.4	0.001
M132/1			*26.9*	*0.012*			*39.8*	*0.007*							*14.2*	*0.008*
Total Identified	52.7	0.009	55.2	0.012	47.1	0.007	59.4	0.01	65.8	0.059	65	0.134	75.7	0.037	63.8	0.035
extracted and unassigned	30.5	0.011	28.4	0.012	42.2	0.014	21.4	0.004	34.6	0.032	33.1	0.068	14.6	0.007	23.3	0.013

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M467/1: Dihydroxy-bixlozone conjugate; M289/2: bixlozone-dimethyl-malonamide; M451/2: a hydroxy glucoside conjugate of bixlozone; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone; M289/3: 5'-hydroxy-bixlozone; M132/1: dimethyl malonic acid

Table 2.7.3. 8 continued Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871															
Year	2015															
Rate	300 g as/ha															
Crop part	lettuce (immature)				lettuce (mature)				radish top				radish root			
PBI	153								120							
Label	P		C		P		C		P		C		P		C	
%TRR / mg eq./kg	100	0.034	100	0.082	100	0.026	100	0.042	100	0.107	100	0.057	100	0.021	100	0.034
bixlozone							2.2	0.001			17.1	0.01	33.2	0.007	35.6	0.012
M190/1					*4.9*	*0.001*			10.5	0.011			22.5	0.005		
M467/1			**2.5**	**0.002**	**22.7**	**0.006**	**10.0**	**0.004**								
M289/2	45.6	0.015	9.0	0.007	31	0.008	16.0	0.007	11.8	0.013	8.5	0.005	14.3	0.003		
M261/1									37.3	0.04	12.0	0.007				
M289/4	6.8	0.002			3.2	0.001	3.4	0.001	10.7	0.012	4.6	0.003				
M289/3											2.8	0.002				
M132/1			*46.3*	*0.038*			*46.7*	*0.02*			*29.7*	*0.017*			*20.3*	*0.007*
Total Identified	52.4	0.017	57.8	0.047	61.8	0.016	78.3	0.033	70.3	0.076	74.7	0.044	70	0.015	55.9	0.019
extracted and unassigned	31.5	0.01	29.6	0.024	21	0.005	6.5	0.003	23	0.025	16.7	0.011	22.8	0.005	32.8	0.012

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M467/1: Dihydroxy-bixlozone conjugate; M289/2: bixlozone-dimethyl-malonamide; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone; M289/3: 5'-hydroxy-bixlozone; M132/1: dimethyl malonic acid

Table 2.7.3. 8 continued Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871 (Desai, 2019)															
Year	2015															
Rate	300 g as/ha															
Crop part	lettuce (immature)				lettuce (mature)				radish top				radish root			
PBI	310															
Label	P		C		P		C		P		C		P		C	
%TRR / mg eq./kg	100	0.005	100	0.005	100	0.008	100	0.004	100	0.017	100	0.005	100	0.012	100	0.005
bixlozone									19.3	0.003			27.1	0.003		
M190/1									4.6	0.001			23.8	0.003		
M289/2									2.3	0.001			4.9	0.001		
M261/1									23.5	0.004						
M289/4									7.4	0.001						
Total Identified									68.6	0.011			55.8	0.007		
extracted and unassigned									35.4	0.006			25.7	0.005		

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone

Table 2.7.3. 8 continued Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871															
Year	2015															
Rate	300 g as/ha															
Crop part	wheat forage				wheat hay				wheat straw				wheat grain			
PBI	30															
Label	P		C		P		C		P		C		P		C	
%TRR / mg eq./kg	100	0.207	100	0.163	100	0.59	100	0.345	100	0.227	100	0.359	100	0.037	100	0.019
M190/1	14.2	0.029							8.0	0.018			29.6	0.011		
M289/2					6.4	0.047			3.3	0.008			11.5	0.004		
M261/1			5.6	0.009	6.4	0.038	1.2	0.004	6.2	0.014	6.5	0.023				
M289/4									4.1	0.009	4.5	0.016				
M289/3	34.5	0.072	21.9	0.036	23.5	0.139	6.2	0.021	31.7	0.072	36.6	0.132				
M132/1			*18.2*	*0.03*			*44.4*	*0.153*			*5.4*	*0.019*				
M289/6									3.2	0.007						
Total Identified	48.7	0.101	45.7	0.075	37.8	0.224	51.8	0.178	56.5	0.128	53	0.19	41.1	0.015		
extracted and unassigned	38.5	0.081	42.1	0.069	41.8	0.248	13.4	0.046	22.9	0.051	32.8	0.118	14.5	0.006		

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone; M289/3: 5'-hydroxy-bixlozone; M132/1: dimethyl malonic acid; M289/6: 3'-hydroxy-bixlozone

Table 2.7.3. 8 continued Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871 (Desai, 2019)															
Year	2015															
Rate	300 g as/ha															
Crop part	wheat forage				wheat hay				wheat straw				wheat grain			
PBI	30															
Label	P		C		P		C		P		C		P		C	
%TRR / mg eq./kg	100	0.207	100	0.163	100	0.59	100	0.345	100	0.227	100	0.359	100	0.037	100	0.019
M190/1	14.2	0.029							8.0	0.018			29.6	0.011		
M289/2					6.4	0.047			3.3	0.008			11.5	0.004		
M261/1			5.6	0.009	6.4	0.038	1.2	0.004	6.2	0.014	6.5	0.023				
M289/4									4.1	0.009	4.5	0.016				
M289/3	34.5	0.072	21.9	0.036	23.5	0.139	6.2	0.021	31.7	0.072	36.6	0.132				
M132/1			*18.2*	*0.03*			*44.4*	*0.153*			*5.4*	*0.019*				
M289/6									3.2	0.007						
Total Identified	48.7	0.101	45.7	0.075	37.8	0.224	51.8	0.178	56.5	0.128	53	0.19	41.1	0.015		
extracted and unassigned	38.5	0.081	42.1	0.069	41.8	0.248	13.4	0.046	22.9	0.051	32.8	0.118	14.5	0.006		

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone; M289/3: 5'-hydroxy-bixlozone; M132/1: dimethyl malonic acid; M289/6: 3'-hydroxy-bixlozone

Table 2.7.3. 8 continued Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871 (Desai, 2019)															
Year	2015															
Rate	300 g as/ha															
Crop part	wheat forage				wheat hay				wheat straw				wheat grain			
PBI	120															
Label	P		C		P		C		P		C		P		C	
%TRR / mg eq./kg	100	0.13	100	0.084	100	0.325	100	0.2	100	0.23	100	0.339	100	0.033	100	0.016
M190/1					8.9	0.029			7.3	0.017			24.2	0.008		
M289/2													9.4	0.003		
M261/1	6.9	0.009	9.5	0.008	7.0	0.023	6.3	0.013	4.0	0.009	9.7	0.023				
M289/4									2.9	0.007	2.5	0.009				
M289/3	33.6	0.044	33	0.028	39.1	0.128	25.4	0.051	41.4	0.095	33.1	0.112				
M132/1			*4.5*	*0.004*			*13.6*	*0.027*			*33.7*	*0.114*				
M289/6									6.6	0.015						
Total Identified	40.6	0.053	47	0.04	55	0.18	45.3	0.091	62.2	0.143	79	0.268	33.6	0.011		
extracted and unassigned	44.4	0.058	39.9	0.034	23.7	0.077	25	0.05	12.9	0.029	7.3	0.014	18.8	0.007		

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone; M289/3: 5'-hydroxy-bixlozone; M132/1: dimethyl malonic acid; M289/6: 3'-hydroxy-bixlozone

Table 2.7.3. 8 continued Summary table of the overview of metabolism of bixlozone in rotational crops

Study reference	2015/1001871 (Desai, 2019)															
Year	2015															
Rate	300 g as/ha															
Crop part	wheat forage				wheat hay				wheat straw				wheat grain			
PBI	310															
Label	P		C		P		C		P		C		P		C	
%TRR / mg eq./kg	100	0.028	100	0.031	100	0.086	100	0.082	100	0.032	100	0.107	100	0.009	100	0.007
M190/1	7.6	0.002			5.1	0.004			11.6	0.004						
M289/2	22.9	0.006							13.9	0.004						
M261/1	5.6	0.002	4.2	0.001	2.6	0.002	5.2	0.004	3.9	0.001	7.5	0.008				
M289/4											7.5	0.008				
M289/3	18.4	0.005	16.6	0.005	42.3	0.036	35.8	0.029	19.4	0.006	30.8	0.033				
M132/1			*18.4*	*0.006*			*33*	*0.027*			*27.6*	*0.029*				
Total Identified	54.5	0.015	39.2	0.012	50	0.042	74	0.06	48.8	0.015	73.4	0.078				
extracted and unassigned	37.8	0.01	48.7	0.016	26.9	0.024	10.3	0.008	13.2	0.003	4.1	0.004				

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

Identification not robust according to the chromatograms

M190/1: 2,4-dichlorobenzoic acid; M289/2: bixlozone-dimethyl-malonamide; M261/1: bixlozone-hydroxy-isobutyramide; M289/4: 4-hydroxy-methyl-bixlozone; M289/3: 5'-hydroxy-bixlozone; M132/1: dimethyl malonic acid

Whilst the level of metabolites varied according to matrix (and label being studied), when looking at the highest amounts of metabolites found, the most prevalent metabolites (>10%TRR and >0.01 mg/kg) found were as follows in Table 2.7.3.9.

Table 2.7.3. 9 Summary of levels of metabolites found

Residues	Metabolite Code	Highest level found (%TRR and mg/kg (mg parent eq. /kg)	Crop matrix (and PBI in DAT) in which this highest amount was observed
Contrary to primary crop metabolism parent bixlozone was found in rotational crops at all replant intervals: Bixlozone	F9600	 76%TRR and 0.034 mg/kg 26%TRR and 0.054 mg/kg	 Radish root at PBI 53 DAT Radish top at PBI of 53 DAT
2,4-dichlorobenzoic acid	M190/1	30%TRR and 0.011 mg/kg 14%TRR and 0.029 mg/kg	Wheat grain at PBI of 30 DAT Wheat forage at PBI of 30 DAT
Bixlozone-dimethyl-malonamide	M289/2	46%TRR and 0.015 mg/kg	Immature lettuce at PBI of 153 DAT
Bixlozone-hydroxy-isobutyramide	M261/1	27%TRR and 0.055 mg/kg 37%TRR and 0.040 mg/kg	Radish top at PBI of 53 DAT Radish top at PBI of 53 DAT
4-hydroxymethyl-bixlozone	M289/4	11%TRR and 0.012 mg/kg	Radish top at PBI of 153 DAT
5'-hydroxy-bixlozone	M289/3	37%TRR and 0.132 mg/kg 42%TRR and 0.095 mg/kg	Wheat straw at PBI of 30 DAT Wheat straw at PBI of 120 DAT
Dimethyl malonic acid	M132/1	44%TRR and 0.153 mg/kg 46%TRR and 0.038 mg/kg	Wheat hay at PBI of 153 DAT Immature lettuce at PBI of 153 DAT

All of the above metabolites, except for dimethyl malonic acid (M132/1), and bixlozone were analysed for in the follow-on rotational crop field trials. In these trials, a single application was made to a primary crop of maize (treated at early post-emergence stage) at a rate that is at the expected soil exposure level for the proposed wheat and barley GAPs, if account is made for the potential soil accumulation of bixlozone following year to year use, as well as the maximum seasonal application rate. The trial rates are however underdosed for the intended maize and oilseed rape GAPs (0.51 'N' maize use and 0.64 'N' oilseed rape use) when taking account of such potential soil accumulation, with the maximum seasonal use rate.

See Vol 3, section B7.6.2, for a full summary of the results of the rotational crop field trials. Residues of only bixlozone were found in all the rotational crops samples, in only two samples: 229 day PBI samples for radish tops and immature lettuce leaves (0.013 and 0.011 mg/kg respectively). In all samples, including wheat forage, hay and straw, no residues of any of the metabolites were found above the LOQ (0.01 mg/kg).

Therefore, following the study of rotational crops in the field under realistic GAP conditions, in two different trials, and the absence of all metabolites confirmed, it is concluded that the metabolism study whilst useful for characterising the potential nature of residues, is probably worst case compared to the expectation of rotational crop residues of bixlozone arising under more realistic field conditions.

It is noted that dimethyl malonic acid (M132/1) was not sought in the field trials, which would have been useful. However, the low levels of parent bixlozone found, the absence of all other metabolite residues found in broadly similar proportions to dimethyl malonic acid (M132/1) would suggest that dimethyl malonic acid (M132/1) would not be found following applications to crops under the current GAP (requested rates and timings) conditions. It is also noted that dimethyl malonic acid (M132/1) has been assessed in an exposure consideration (for primary crops) using the TTC for CCI (presumption of low toxicity). As such any (low) findings of dimethyl malonic acid (M132/1) in rotational crops will not be expected to be of any consumer exposure concern.

Other rotational crop metabolites that were not sought in trials were found at lower levels in the rotational crop metabolism study: dihydroxy-bixlozone conjugate (M467/1) (23%TRR but low 0.006 mg/kg level), a hydroxy glucoside conjugate of bixlozone (M451/2) (3%TRR and low <0.001 mg/kg level), and 3'-hydroxy-bixlozone (M289/6) (7%TRR and 0.015 mg/kg).

Based on prevalence of residues, aside for the non-inclusion of dimethyl malonic acid (M132/1) in the follow on field trials, the metabolites selected represent a good choice of marker metabolites to test for to consider the potential for

rotational crop residues. Where data is available, the toxicological significance of these rotational crop metabolites is covered in section 2.6.9. For some metabolites, where specific toxicological information or data is not available, it is proposed (in section 2.6.9) that a CCI or CCIII consideration could be made where needed. Following the generation of the metabolism studies and the following rotational crop field studies using the 'marker compounds' (section B.7.6.2), it is considered that based on the expectation that according to GAP, no metabolites would be expected to be found at significant levels in rotational crops, no metabolites need to be further considered for the residue definition for rotational crops when considering the currently requested uses. In consideration of any future extensions of uses with increased application rates, it would be desirable for new trials to be generated. In these trials bixlozone must be included (as the main marker compound for rotational crops), however we suggest that the trials should also analyse for the main rotational crop metabolites.

As such, HSE propose that **only parent bixlozone** needs to currently be included in the proposal for residue definitions to cover **rotational crops**.

Livestock:

The available livestock metabolism data are summarised in section 2.7.2 and written up in full in section B.7.7.2 (poultry- hens) and B.7.7.3 (ruminant-goat). The studies involved dosing with parent bixlozone only (phenyl and carbonyl labelled residues investigated).

Consideration of main plant metabolites:

The current assessment proposes that residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) do not need to be included in the residue definition for dietary risk assessment (human dietary exposure considerations, see above) and also that these residue components, for the current time, do not need to be included in the assessment of livestock dietary burden.

This is due to the conclusion that the human dietary exposure estimates are calculated to be well below the TTC (Cramer Class I) when residues of residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) are combined in the exposure estimate (similarity in structure). The estimated exposures for the currently intended uses were all below 10% of the TTC for CCI. HSE considers that in the current case (low exposures versus the TTC in the human dietary assessment), that any residues arising from livestock exposures to residues of M118/1 and M132/1 in products of animal origin are not expected to be of concern. HSE proposes that, for the currently intended uses, these metabolites can be excluded from the livestock dietary intake assessment. This proposal should be revisited (to decide if the proposal still holds) in cases of future extensions of uses.

The livestock metabolism studies involved dosing with parent bixlozone (as per the rat metabolism studies). Overall, based on the identified metabolites and the applicant's proposed metabolic pathways for each species, it is generally concluded that the rat and livestock metabolism studies show comparable metabolism of parent bixlozone. The goat and poultry metabolism appear to be 'subsets' of metabolism in the rat, and no unique metabolite paths have been identified in the goat or poultry compared to the rat. It can be concluded that livestock and rat metabolism of parent bixlozone are similar. It is possible that there were low level residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) in the rat, which were not identified in the rat metabolism. They were not actively sought in the rat metabolism work (Section B.6.1.4), but there were some low level unidentified components.

When found in livestock species, these residues of 2,2-dimethyl-3-hydroxypropionic acid (M118/1) and dimethyl malonic acid (M132/1) were variable across matrices -some matrices where these residues were not found - see 'overview of metabolism' Table 2.7.3.11 (poultry) and Table 2.7.3.12 (ruminant) – highest levels were as follows: 2,2-dimethyl-3-hydroxy propionic acid (M118/1) up to 10%TRR, 0.011 mg/kg in eggs and dimethyl malonic acid (M132/1) up to 59%TRR, 0.034 mg/kg in poultry muscle, and up to 40%TRR, 0.2 mg/kg in (microwave extract) poultry liver, 2,2-dimethyl-3-hydroxy propionic acid (M118/1) up to 17%TRR 0.064 mg/kg in goat kidney and up to 29% 0.003 mg/kg in goat muscle, and dimethyl malonic acid (M132/1) not found in the goat metabolism. As per wheat metabolism studies, dimethyl malonic acid (M132/1) was included in some initial HPLC scoping work in the goat metabolism study and it is not clear in the goat metabolism study report how comprehensively this reference standard was used to check against metabolite fractions, since this component (dimethyl malonic acid, M132/1) was not designated as an identified residue, and this metabolite (and its identification) was not discussed in the goat metabolism study report). Based on

the CCI assignation (presumption of low toxicity) of the metabolites 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) it is not expected, based on the currently intended uses, that these residues will be of potential concern in products of animal origin.

HSE considers that the animal dietary burden should, for the currently intended uses, address sum of residues of bixlozone and 2,4-dichlorobenzoic acid (M190/1). See section 2.7.5 for HSE's calculations of animal dietary burden, which includes the possible occurrence of only low residues of parent bixlozone in rotational crops, where the animal dietary burden 'trigger' of 0.004 mg/kg bw/day is not exceeded. Furthermore, a label replant restriction is proposed with the aim of maintaining potential rotational crop residues of bixlozone below 0.01 mg/kg; therefore the current estimate is considered a worst case, based on the proposed uses.

As such, the currently estimated livestock exposures to all relevant bixlozone metabolites in feed in this assessment is not expected to result in significant residues (≥0.01 mg/kg) in animal commodities.

Therefore, for the currently intended oilseed rape and cereal uses a residue definition (RD-RA) is not currently proposed for products of animal origin.

Based on the existing livestock metabolism data, **dosed with parent bixlozone**, the following section considers the range of metabolites found in the goat and livestock data in order to consider a possible future residue definition for livestock, if anticipated exposures increased such that livestock exposures to bixlozone residues needed to be addressed.

Full details of the amounts of all the metabolites in poultry and goat are presented in the overview of metabolism tables presented below (Table 2.7.3. 11 and Table 2.7.3.12).

As in commonly the case in livestock metabolism studies, the nature and levels of residues varied considerably across different matrices. The **main** metabolite components in livestock (across different matrices) arising from the studies dosed with parent bixlozone are summarised below in Table 2.7.3. 10.

(X) denotes 10-20%TRR.
X denotes >20%TRR and < 30%
X (emboldened) represents > 30%

This comparative table only summarises **main metabolite** components. Parent bixlozone (F9600) was only found in poultry- hens at <10%TRR and was not found in ruminants - goat). See metabolism study evaluation in section B.7.2.2 and B.7.2.3 for full details, as well as the 'summary of overview of metabolism' tables (Tables 2-14 and 2-15) presented in this section. Although %TRR of metabolites might be high, corresponding mg/kg amounts in matrices might be low. Please see overview of metabolism tables (see Table 2.7.3.12 (ruminants) and Table 2.7.3. 11 (poultry)) for amounts, and where >10% represents < 0.01 mg/kg.

Table 2.7.3. 10 Summary of main metabolite components in livestock

Mcodes→	118/1	190/1	175/1	465/2	451/3	275/1	465/1 Conj of 289/1	369/1 Sulfate deriv' of 289/1	289/1	189/1	132/1	289/2	355/1
Poultry													
Muscle				(X)				(X)			**X**	X	
Fat									X	(X)	**X**		
Liver			(X)							(X)	**X**		
Eggs	(X)		(X)		(X)				**X**		X		
Ruminant													
Muscle	X					(X)	**X**	(X)	**X**			X	
Fat													
Liver	(X)					(X)	**X**	(X)	**X**			X	
Kidney	(X)	(X)				X	**X**	(X)	**X**			X	(X)
Milk						X		**X**	**X**				X

Table key:

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M190/1: 2,4-dichlorobenzoic acid;
M175/1: 2,4-dichlorobenzamine; M465/2: 4-hydroxyl-bixlozone-glucuronide;
M451/3: bixlozone-3-hydroxy-propanamide-glucuronide; M275/1: bixlozone-3-hydroxy-propanamide;
M465/1: 5-hydroxyl-bixlozone-glucuronide; M369/1: 5-hydroxy-bixlozone-sulfate;
M289/1: 5-hydroxy-bixlozone; M189/1: 2,4-dichlorobenzaloxime; M132/1: dimethyl malonic acid;
M289/2: bixlozone-dimethyl-malonamide; M355/1: bixlozone-3-hydroxy-propanamide-sulfate

It has been noted in this residues evaluation that dimethyl malonic acid (M132/1) (which is especially prevalent) in the poultry metabolism was included as a reference standard in the goat metabolism. However, whilst dimethyl malonic acid (M132/1) was included in some initial HPLC scoping work in the goat metabolism study it is not clear in the goat metabolism study report how comprehensively this reference standard was used to check against metabolite fractions, since this component (dimethyl malonic acid, M132/1) was not designated as an identified residue, and this metabolite (and its identification) was not discussed in the goat metabolism study report. If the goat metabolism study is to be relied upon in the future, this should be further explained by the applicant with reference to the raw analytical data (including chromatograms). HSE notes that based on the human dietary exposure consideration versus the TTC CCI (presumption of low toxicity) for this metabolite (dimethyl malonic acid, M132/1), it is proposed that for the currently intended uses, this metabolite is not of toxicological significance.

In consideration of a future residues definition for dietary risk assessment, the toxicological information provided (for most of these metabolites) in section 2.6.9 could be considered. It is noted that 2,4-dichlorobenzoic acid (M190/1) is concluded as potentially 2 x more toxic than parent. However, its presence in livestock metabolism samples was low:

2,4-dichlorobenzoic acid (M190/1) in poultry - muscle at up to 0.002 mg/kg and up to 0.004 mg/kg in eggs, 0.033 mg/kg in poultry liver
2,4-dichlorobenzoic acid (M190/1) in ruminants – up to 0.042 mg/kg in kidney, up to 0.005 mg/kg in liver and up to 0.001 mg/kg in muscle

In terms of the most prevalent metabolites in livestock, these include dimethyl malonic acid (M132/1) in poultry and 5-hydroxy-bixlozone (M289/1) including its conjugates 5-hydroxy-bixlozone glucuronide conjugate (M465/1) and 5-hydroxy-bixlozone sulfate conjugate (M369/1). In section 2.6.9, the toxicological assessment concludes that for 5-hydroxy-bixlozone (M289/1) the residues can be assessed using the dietary intake values for parent bixlozone.

Please refer to section 2.7.2 where similarity of metabolic pathway between livestock and rats is discussed. Broad comparability is concluded, and the residues in livestock are mainly considered as not fat-soluble.

Considering the sum of dimethyl malonic acid (M132/1), 5-hydroxy-bixlozone (M289/1) and conjugates of 5-hydroxy-bixlozone (M289/1), the following %TRR are accounted for (up to this %TRR level across the different samples analysed, e.g. phenyl or carbonyl label samples):

Poultry- fat – 46% TRR
Poultry- muscle– 62% TRR
Poultry- liver – 69% TRR
Poultry- egg– 33% TRR

Ruminant - fat – not analysed for individual residues
Ruminant - muscle– 52% TRR
Ruminant - liver – 42% TRR
Ruminant - milk– 94% TRR
Ruminant – kidney – 56% TRR

With reference to the above discussed main metabolites, toxicology advisers have made the following conclusions (section 2.6.9 of Vol 1):

Metabolite	Covered by parent	Tox. compared to parent	Ref. value	Tox. relevant
M289/1 (5-hydroxy bixlozone)	'covered'	Can be considered as equivalent to parent	Parent	Y
M132/1 (dimethyl malonic acid)	N	Not known	None (Cramer class I TTC value of 30 µg/kg bw/day can be used to assess the chronic and acute exposure assessments) A combined assessment with 2,2-dimethyl-3-hydroxy propionic acid (M118/1) is needed (structural similarity)	N (not for the currently assessed uses, as discussed in this section).

Furthermore (section 2.6.9) bixlozone-3-hydroxy-propanamide (M275/1), sulfate conjugate of bixlozone-3-hydroxy-propanamide (M355/1) and bixlozone-dimethyl-malonamide (M289/2) could be (initially) considered if required using Cramer Class III TTC, and that given their close structural similarity, a combined (exposure) risk assessment of these three metabolites against the TTC values could be performed, if required.

Table 2.7.3. 11 Summary table of the overview of the metabolism of bixlozone in poultry

Animal	Poultry													
Study reference	15171-RPT04349													
Year	([redacted], 2019)													
Rate	15 mg/kg feed DM													
Label	Phenyl													
Number of animals	10													
Duration	13 Days													
Matrix	Pooled fat		Thigh muscle		Breast Muscle		Liver				Eggs			
Extraction	Organic		Organic		Organic		Organic		Microwave		DCM		Acid Hydrolysis	
%TRR / mg eq./kg	74.9	0.197	73.5	0.02	71.1	0.014	44.4	0.27	51.1	0.311	45.9	0.041	44.7	0.039
bixlozone	7.2	0.019												
M190/1			**6.1**	**0.002**	**4.2**	**0.001**	**5.4**	**0.033**			**0.5**	**<0.001**	**4.7**	**0.004**
M175/1			**0.9**	**<0.001**	**2.4**	**<0.001**	**1.5**	**0.009**	**12.2**	**0.074**			**11.0**	**0.01**
M465/1					**1.4**	**<0.001**	**9.5**	**0.058**	**2.1**	**0.074**				
M465/2			**14.4**	**0.004**	**3.6**	**0.001**	**3.6**	**0.022**	**0.9**	**0.005**	**4.4**	**0.004**	**4.7**	**0.004**
M451/3									**1.1**	**0.007**	**1.4**	**0.001**	**10.5**	**0.009**
M369/1	**1.5**	**0.004**	**11.3**	**0.003**	**6.7**	**0.001**	**2.7**	**0.016**	**1.9**	**0.012**	**0.4**	**<0.001**	**2.1**	**0.002**
M275/1									**0.5**	**0.003**	**1.5**	**0.001**		
M289/4											2.5	0.002		
M289/1	29.3	0.077	2.0	0.001	0.7	<0.001			0.3	0.002	30.9	0.028		
M189/1	12.4	0.033					10.4	0.063			0.3	<0.001		
M289/2	1.2	0.003	26.5	0.007	22.3	0.004	3.9	0.024						
Total identified	51.6	0.136	61.2	0.018	41.3	0.01	37	0.225	19	0.116	41.9	0.039	33	0.029

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M190/1: 2,4-dichlorobenzoic acid; M175/1: 2,4-dichlorobenzamine; M465/1: 5-hydroxyl-bixlozone-glucuronide; M465/2: 4-hydroxyl-bixlozone-glucuronide; M451/3: bixlozone-3-hydroxy-propanamide-glucuronide; M369/1: 5-hydroxy-bixlozone-sulfate; M275/1: bixlozone-3-hydroxy-propanamide; M289/4: 4-hydroxy-methyl-bixlozone; M289/1: 5-hydroxy-bixlozone; M189/1: 2,4-dichlorobenzaloxime; M289/2: bixlozone-dimethyl-malonamide.

Table 2.7.3. 11 continued Summary table of the overview of the metabolism of bixlozone in poultry

Animal	Poultry													
Study reference	15171-RPT04349													
Year	([redacted], 2019)													
Rate	15 mg/kg feed DM													
Label	Carbonyl													
Number of animals	10													
Duration	13 Days													
Matrix	Pooled fat		Thigh muscle		Breast Muscle		Liver				Eggs			
Extraction	Organic		Organic		Organic		Organic		Microwave		DCM		Acid Hydrolysis	
%TRR / mg eq./kg	74.6	0.043	76.2	0.044	74.6	0.043	40.4	0.198	49.2	0.242	5.4	0.006	91.5	0.094
bixlozone											0.1	<0.001		
M118/1	**0.6**	**<0.001**	**3.1**	**0.002**	**2.6**	**0.001**			**9.3**	**0.046**			**10.3**	**0.011**
M465/1					**2.1**	**0.001**	**6.4**	**0.031**						
M369/1	**0.6**	**<0.001**	**1.0**	**0.001**	**0.3**	**0.001**	**1.1**	**0.006**			**0.6**	**0.001**		
M289/4											3.8	0.004		
M132/1	45.0	0.026	58.4	0.034	59.2	0.034	21.2	0.104	39.9	0.196			25.8	0.026
M289/2	2.2	0.001	1.8	0.001	0.6	<0.001	2.1	0.01						
Total identified	48.4	0.027	64.3	0.038	64.8	0.038	30.8	0.241	49.2	0.242	4.5	0.006	36.1	0.037

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M465/1: 5-hydroxyl-bixlozone-glucuronide; M369/1: 5-hydroxy-bixlozone-sulfate; M289/4: 4-hydroxy-methyl-bixlozone; M132/1: dimethyl malonic acid; M289/2: bixlozone-dimethyl-malonamide.

Table 2.7.3.12 Summary table of the overview of the metabolism of bixlozone in ruminants

Animal	Ruminants																					
Study reference	15086-RPT04035																					
Year	([redacted], 2019)																					
Rate	15 mg/kg feed DM																					
Label	Phenyl (organic extraction)								Phenyl (Acid hydrolysis)								Phenyl (Enzyme hydrolysis)					
Number of animals	1								1								1					
Duration	7 days								7 days								7 days					
Matrix	Kidney		Liver		Milk		Muscle		Kidney		Liver		Milk		Muscle		Kidney		Liver		Milk	
Extraction	Organic extraction								Acid hydrolysis								Enzyme hydrolysis					
%TRR / mg eq./kg	95.5	0.328	90.6	0.122	95.3	0.069	96.6	0.01	95.5	0.328	90.6	0.122	95.3	0.069	96.6	0.01	95.5	0.328	90.6	0.122	95.3	0.069
M190/1															9.0	0.001	12.3	0.042	3.6	0.005		
M465/1	**30.4**	**0.104**	**20.3**	**0.027**			**22.9**	**0.002**	**4.3**	**0.015**					**8.2**	**0.001**						
M465/2	**1.5**	**0.005**	**2.4**	**0.003**			**1.7**	**<0.001**											**3.8**	**0.005**		
M451/3	**7.1**	**0.024**	**5.5**	**0.007**			**5.2**	**0.001**														
M369/1	**13.2**	**0.045**	**10.2**	**0.014**	81.8	0.059	**13.6**	**0.001**											**9.0**	**0.012**		
M275/1									29.7	0.102	16.0	0.022	11.6	0.008	15.0	0.002	9.1	0.031	6.2	0.008	0.5	<0.001
M289/4									4.1	0.014	4.1	0.006	0.7	0.001	4.0	<0.001			4.9	0.007		
M289/1									46.0	0.158	19.0	0.026	73.0	0.053	43.5	0.004	49.5	0.17	33.4	0.045	79.5	0.057
M289/2	24.3	0.083	27.6	0.037			24.0	0.002	9.7	0.033	21.4	0.029			9.5	0.001	13.8	0.047	14.4	0.019		
M355/1	13.2	0.045	7.4	0.01	13.6	0.01	6.3	0.001									10.8	0.037	9.4	0.013	16.0	0.012
Total identified	89.7	0.306	73.4	0.098	95.4	0.069	73.7	0.007	93.8	0.322	60.5	0.083	85.3	0.062	89.2	0.009	95.5	0.327	84.7	0.114	96	0.069

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M190/1: 2,4-dichlorobenzoic acid; M465/1: 5-hydroxyl-bixlozone-glucuronide; M465/2: 4-hydroxyl-bixlozone-glucuronide; M451/3: bixlozone-3-hydroxy-propanamide-glucuronide; M369/1: 5-hydroxy-bixlozone-sulfate; M275/1: bixlozone-3-hydroxy-propanamide; M289/4: 4-hydroxy-methyl-bixlozone; M289/1: 5-hydroxy-bixlozone; M289/2: bixlozone-dimethyl-malonamide; M355/1: bixlozone-3-hydroxy-propanamide-sulfate

Table 2.7.3.12 continued Summary table of the overview of the metabolism of bixlozone in ruminants

Animal	Ruminants																					
Study reference	15086-RPT04035																					
Year	([redacted], 2019)																					
Rate	15 mg/kg feed DM																					
Label Number of animals Duration	Carbonyl (organic extraction) 1 7 days								Carbonyl (Acid hydrolysis) 1 7 days								Carbonyl (Enzyme hydrolysis) 1 7 days					
Matrix	Kidney		Liver		Milk		Muscle		Kidney		Liver		Milk		Muscle		Kidney		Liver		Milk	
Extraction	Organic extraction								Acid hydrolysis								Enzyme hydrolysis					
%TRR / mg eq./kg	94.1	0.347	92.6	0.111	95.3	0.069	96.3	0.011	94.1	0.347	92.9	0.111	95.3	0.069	96.3	0.011	94.1	0.347	92.9	0.111	95.3	0.069
M118/1									17.4	0.064	17.2	0.021			29.3	0.003						
M465/1	**50.1**	**0.185**	**33.2**	**0.04**			**35.3**	**0.004**	**1.9**	**0.007**	**9.5**	**0.011**										
M465/2	**3.1**	**0.012**	**7.2**	**0.009**															**4.2**	**0.005**		
M451/3	**5.2**	**0.019**	**6.9**	**0.008**			**3.0**	**<0.001**														
M369/1	**6.0**	**0.022**	**5.2**	**0.006**	72.8	0.05											**2.7**	**0.01**	**3.7**	**0.004**		
M275/1									19.1	0.07	9.2	0.011	26.5	0.018	7.0	0.001	5.0	0.018	6.9	0.008		
M289/4									2.8	0.01	3.9	0.005			3.0	<0.001	2.4	0.009	3.9	0.005	1.6	0.001
M289/1									31.2	0.115	17.7	0.021	63.6	0.044	12.1	0.001	53.1	0.196	32.2	0.039	93.6	0.068
M289/2	19.7	0.073	24.7	0.03			17.4	0.002	15.8	0.058	8.1	0.01			5.9	0.001	16.5	0.061	17.9	0.021		
M355/1	10.0	0.037	4.9	0.006	22.5	0.016											12.6	0.047	4.0	0.005		
Total identified	94.1	0.348	82.1	0.099	93.1	0.068	55.7	0.006	88.2	0.324	65.6	0.079	90.1	0.062	57.3	0.006	92.3	0.341	72.8	0.087	95.2	0.069

Table key:

> 10 % TRR < 0.01 mg/kg

> 10 % TRR > 0.01 mg/kg

Tentatively characterised or conjugates characterised by hydrolysis behaviour only

M118/1: 2,2-dimethyl-3-hydroxy propionic acid; M465/1: 5-hydroxyl-bixlozone-glucuronide; M465/2: 4-hydroxyl-bixlozone-glucuronide; M451/3: bixlozone-3-hydroxy-propanamide-glucuronide; M369/1: 5-hydroxy-bixlozone-sulfate; M275/1: bixlozone-3-hydroxy-propanamide; M289/4: 4-hydroxy-methyl-bixlozone; M289/1: 5-hydroxy-bixlozone; M289/2: bixlozone-dimethyl-malonamide; M355/1: bixlozone-3-hydroxy-propanamide-sulfate

Conclusions: Proposals for residue definition

Plants (products of plant origin):

RD-RA: Residue definition for dietary risk assessment

For the intended (early application) uses on oilseed rape, wheat, barley and maize:

RD-RA (plants): Sum of residues of bixlozone and **2 x** 2,4-dichlorobenzoic acid expressed as bixlozone

[the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid].

For the currently intended uses, the exposures to residues of M118/1 and M132/1 are below the TTC (for CCI). Therefore, these components are not included in the proposal for the residue definition.

Parent bixlozone (F9600) is the only component needing to be considered for rotational crops currently arising from the intended GAPs. Furthermore, a label replant restriction is proposed with the aim of maintaining potential rotational crop residues of bixlozone below 0.01 mg/kg.

For other crops and use patterns, no conclusion can be currently reached on a suitable residue definition.

Any extensions to the intended uses (increased application rates, change of application timing or range of crop uses), will require a reconsideration of the residue definition (and recalculation of the exposure assessment versus the TTC for co-exposure of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) which are considered to be present in significant amounts in cereal matrices).

RD-Enf: Residue definition for enforcement and monitoring of residues

RD-Enf (plants): Bixlozone

A conversion factor (CF) for converting residues measured as per RD-Enf → RD-RA cannot be proposed as virtually all of the trials (oilseed rape and cereals) contained residues of parent and 2,4-dichlorobenzoic acid at a level of <LOQ of <0.01 mg/kg. OECD (2016) Guidance on Crop Field trials states that for the calculation of CFs residue trials resulting in residue levels below the LOQ should not be taken into account.

It is noted in other evaluations that 2,4-dichlorobenzoic acid (M190/1) is in some common moiety analytical methods for propiconazole (e.g. JMPR, 2007). In the EFSA Conclusion (2017) for propiconazole, this issue was highlighted in the write up of the residue definitions regarding an option considered (for both RD-RA and RD-Enf), but not favoured by the majority of experts, as 'the total propiconazole, including all compounds convertible to the 2,4-dichlorobenzoic acid moiety and expressed as propiconazole equivalents". In propiconazole EFSA (2015), EU MRL Review it was noted how "metabolites containing the dichlorophenyl-moiety and convertible to the 2,4-dichlorobenzoic acid (2,4-DBA) (CGA 91304 (ketone), CGA 91305 (alkanol), CGA 118244 (β- hydroxy alcohol isomers) and CGA-118245 (γ-hydroxy alcohol), free and/or conjugated) contributed altogether to a significant part of the radioactivity". In the EU Renewal Assessment Report (2016) for propiconazole (https://www.efsa.europa.eu/en/consultations/call/160804) it states that "DCBA (2,4-dichloro benzoic acid), a derivative of a common moiety, which is as well, likely formed from a number of active substances".

The metabolism data suggests 2,4-dichlorobenzoic acid is a better 'marker' in oilseed or cereal grain than parent (residues of 2,4-dichlorobenzoic acid were found at 35%TRR in OSR seed and up to 26% TRR in wheat grain and 38% TRR in rice grain, whilst parent was not detected). However, despite this, in view of all residues sought (including parent and 2-4, dichlorobenzoic acid) being <LOQ in the OSR field trials (oilseed samples) and very low in cereal grain (wheat and barley trials, parent all residues were <LOQ, and 2,4-dichlorobenzoic acid were 12 x <0.01

and 0.01), and since there is a potential lack of specificity of this compound to bixlozone, it is proposed that the RD-Enf should include parent only. In terms of future considerations, if 2,4-dichlorobenzoic acid (M190/1) were to be included in the RD-Enf with parent bixlozone, even if 2,4-dichlorobenzoic acid (M190/1) were unusually found in monitoring, then it might also be likely that parent bixlozone itself would not be found (bixlozone is only currently anticipated as a rotational crop finding, however a restriction is proposed with the aim of maintaining residues of bixlozone below 0.01 mg/kg), and the source of these 2,4-dichlorobenzoic acid (M190/1) residues potentially arising from bixlozone would not be known. Taking account of these complexities, it is suggested that the RD-Enf should be parent (bixlozone) only.

Livestock (products of animal origin):

RD-RA: Residue definition for dietary risk assessment

For the intended early application uses on oilseed rape, wheat, barley and maize:

RD-RA (products of animal origin): Currently not needed for the intended uses (animal dietary intakes are not significant), so a RD-RA is not currently proposed for products of animal origin.

For other crops and use patterns, no conclusion can be currently reached on a suitable RD-RA.

A further consideration would be needed, if residues become more prominent in animal feed items for any additional/new uses, including cereals and oilseed rape.

RD-Enf: Residue definition for enforcement and monitoring of residues

RD-Enf (products of animal origin): Bixlozone

Bixlozone is proposed by default. This residue definition will need to be reconsidered if there are extensions of use beyond those considered currently in this assessment.

A conversion factor (CF) for converting residues measured as per RD-Enf → RD-RA is not needed at this time.

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

The proposed use of 'F9600-4 SC' in GB is on winter wheat, winter barley, winter oilseed rape and maize. The representative formulation 'F9600-4 SC' is a suspension concentrate (SC) containing 400 g/L of the active substance. The proposed GAPs are shown in Table 2.7.4.1.

Table 2.7.4. 1 Summary of GAPs proposed for the representative product

Crop	Outdoor/protected	Growth stage		Number of applications	Application rate (g a.s./ha)	Water volume (L/ha)	PHI (days)
Winter wheat and Winter barley	Outdoor	BBCH 00-09	Sowing to emergence (pre-emergence)	1	200	150-400	N/A
Winter wheat	Outdoor	BBCH 11-13	First leaf unfolded to three leaves unfolded (post-emergence)	1	200	150-400	N/A

Crop	Outdoor/protected	Growth stage		Number of applications	Application rate (g a.s./ha)	Water volume (L/ha)	PHI (days)
Winter oilseed rape	Outdoor	BBCH 00-09	Sowing to emergence (pre-emergence)	1	200-300	150-400	N/A
Maize	Outdoor	BBCH 00-09	Sowing to emergence (pre-emergence)	1	250-375	150-400	N/A

Oilseed rape

The residue field trials were performed in various European Member States in both European regions (NEU and SEU). The trials performed in the NEU are directly relevant to the GB climate and therefore can be used in support of the GB GAPs. The SEU trials have been reported for completeness and to give an understanding of residue behaviour, however, the SEU trials have not been relied upon in support of the GB GAP. Trials were performed with the representative product, 'F9600-4-SC'.

Results from data considered relevant to the pre-emergence (BBCH 00-09) GAP have been summarised in Table 2.7.4.2.

Note: Data from the SEU are not being used in the risk assessment therefore HR, STMR and MRL values have not been determined using the SEU data. Results from these trials have been summarised in Table 2.7.4.2 for information only. The SEU data are similar to the NEU data, showing similar trends in residue levels.

These trials were all performed using application rates within ±25% of the proposed GAP (300 g a.s./ha). However, all of the application rates were below the proposed maximum application rate (NEU trials: 247 – 273 g a.s./ha, SEU trials: 244 – 260 g a.s./ha). As positive residues were not observed in the relevant crop fractions in these trials, it is not possible to apply the proportionality principle to estimate the possible residues at a slightly higher application rate. This is not an ideal data set; however, the application rates are within ±25% of the proposed GAP, showing results <LOQ, therefore, the trials data are considered sufficient for setting MRLs and risk assessment in this case.

The trials were performed with applications made at BBCH 00, 03, 05 and 08 (PHI >100 days), with all residues in oilseed rape seed being <LOQ. This is considered sufficient to support the proposed pre-emergence GAP (application at BBCH 00-09).

As oilseed rape is a major crop, 8 trials are required that reflect the agronomic and climatic conditions in the UK. However, as residues determined are <LOQ, a reduced data set can be accepted. Therefore, in accordance with Reg. (EU) 283/2013, a minimum of 4 trials are required to support the proposed use on oilseed rape (major crop). There are a total of 7 trials in the NEU zone (and 5 trials in the SEU zone) showing residues in seeds are <LOQ; this is sufficient to support the proposed use in GB. It should be noted that there is 1 trial performed in the NEU zone and 3 trials from the SEU zone reported in the processing studies which show the same pattern of residues and can be considered supportive information.

Residues above the respective LOQs were not found in the untreated control samples of oilseed rape fractions (whole plant, flowers, plants without pods, pods and seeds), including 2,2-dimethyl-3-hydroxy propionic acid. It is noted that positive residues of this metabolite are consistently found in untreated samples of wheat, barley and maize, see section 2.7.3 for further discussion.

Residues in seeds at harvest were <LOQ for all of the chemical components which were analysed for. Some positive residues of bixlozone and 2,2-dimethyl-3-hydroxy propionic acid (M118/1) were observed in samples of whole plant, pods, plants without pods and flowers. However, in this case, given the proposed GAP, which is a non-forage use on oilseed rape, residues in these matrices are not relevant for MRL setting, animal dietary burden or consumer risk assessment, therefore these positive residues have not been considered further.

There are sufficient data to support the proposed use in GB (7 NEU trials representative of the proposed GAP). This is presented in Table 2.7.4.2.

Table 2.7.4. 2 Summary of supporting field trials data for oilseed rape

Crop	Analyte	Range	STMR (mg/kg)	HR (mg/kg)	MRL (OECD Calculator) (mg/kg)
Oilseed rape seed	Bixlozone	NEU: 7 x <0.01 *SEU 5 x <0.01*	<0.01	<0.01	-
	5'-hydroxy bixlozone (M289/3)	NEU: 7 x <0.01 *SEU 5 x <0.01*	<0.01	<0.01	-
	2,4-dichlorobenzoic acid (M190/1)	NEU: 7 x <0.01 *SEU 5 x <0.01*	<0.01	<0.01	-
	2,2-dimethyl-3-hydroxy propionic acid (M118/1)	NEU: 7 x <0.05 *SEU 5 x <0.05*	<0.05	<0.05	-
	RD-RA: Sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone [£]	7 x <0.039 (human exposure assessment) [7 x <0.024 (livestock dietary intake assessment)]	<0.039[£]	<0.039[£]	-
	RD-Enf: bixlozone	7 x <0.01	<0.01	<0.01	0.01*

[£] the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid. It should be noted that this 2 x factor is only required in assessments comparing to the toxicological endpoints for bixlozone, i.e., this additional 2 x factor has not been used in the animal dietary burden estimate of exposure as this is an estimate of livestock dietary intakes, rather than comparison with a toxicological endpoint. Hence, results of <0.024 mg/kg have been taken forward into the animal dietary burden calculation (0.01 + 0.01 x 1.435 MW conversion).

Wheat and Barley

The residue field trials were performed in various European Member States in both European regions (NEU and SEU). The trials performed in the NEU are directly relevant to the GB climate and therefore can be used in support of the GB GAPs. The SEU trials have been reported for completeness and to give an understanding of residue behaviour, however, the SEU trials have not been relied upon in support of the GB GAPs. Trials were performed with the representative product, 'F9600-4-SC'.

Results from data considered relevant to the pre-emergence (BBCH 00-09) and post-emergence (BBCH 11-13) GAPs have been summarised separately in Table 2.7.4.3. Where positive results have been reported in the untreated control samples these have been reported also, denoted as 'UTC'. The occurrence of positive residues in untreated control samples is discussed in Volume 1, section 2.7.3. In addition to the positive residues of 2,2-dimethyl-3-hydroxy propionic acid and 2,4-dichlorobenzoic acid given in Table 2.7.4.3, a single positive residue of bixlozone was found in a barley whole plant (BBCH 51) untreated sample; this crop fraction is not relevant in this case with non-forage GAPs. A single positive residue of 5'-hydroxy bixlozone was found in wheat hay (BBCH 75-77) in a SEU trial; this crop fraction and geographical location are not relevant in this case.

As discussed in Volume 3 B7, section 7.3.1, extrapolation between wheat and barley is acceptable in this case where application is made before forming the edible part of the crop. A comparison of the pre- and post- emergence data sets has been made following the consideration of the proportionality principle, discussed in the next section.

Note: Data from the SEU are not being used in the risk assessment therefore HR, STMR and MRL values have not been determined. Results from these trials have been summarised in Table 2.7.4.3 and Table 2.7.4.4 for information only. The SEU data are similar to the NEU data, showing similar trends in residue levels.

Proportionality principle

For the post-emergence GAP there are a total of 6 trials (wheat and barley). For the pre-emergence GAP there are a total of 7 trials (wheat and barley). Where positive residues are found, at least 8 trials are required in support of a

GAP on a major crop. Combining the pre-emergence and post-emergence trials together, would give a total of thirteen trials on wheat and barley conducted in NEU.

Nine of the field trials on wheat and barley were performed using overdosed (>25%, 26 to 33% overdosed) application rates; the remaining 4 trials were performed within ± 25% of the proposed application rate. It is considered appropriate to apply the proportionality principle to these results. In accordance with the OECD guidance on crop field trials, the trials performed with application rates within ± 25% of the proposed rate were also scaled in line with the proposed GAP (200 g a.s./ha) to prevent bias. The results reported in Table 2.7.4.4 and Table 2.7.4.5 are those determined following this scaling. The scaled results shown in Table 2.7.4.4 show the same similarity between the pre- and post-emergence GAPs. The varying LOQ for 2,2-dimethyl-3-hydroxy propionic acid across the trials (in grain the LOQs vary from 0.05 to 0.2 mg/kg; see next section for full discussion), has likely had an impact on the derived STMR levels for grain. The STMR and HR in straw and grain for the residues of 2,2-dimethyl-3-hydroxy propionic acid determined were higher in the (scaled) post-emergence data sets compared to the respective pre-emergence data sets. However, for these GAPs with long PHIs, it seems reasonable to group the pre-emergence and post-emergence data together. In the field trials, applications were made at a range of growth stages, some of which were close to the border between pre- and post-emergence, making categorising the trials as being representative of either GAP more difficult. Therefore, given the similarity of results across all trials it was considered appropriate to combine the data and consider that a sufficient number of trials have been submitted in support of these GAPs for the intended uses on wheat and barley. Full details of the scaling factors used are given in the field trial summaries in Volume 3 B7, section 7.3.1. Although not recommended for the comparison of different GAPs, the Mann-Whitney U test was used to indicate if combining these data was reasonable. The scaled data for both grain and straw (separately) for both GAPs (pre- and post- emergence) were considered similar populations.

LOQ for method 'CAM 0180' used in field trials

The analytical method 'CAM-0180/002' was used in these field trials to determine the content of bixlozone, 5'-hydroxy-bixlozone, 2,4-dichlorobenzoic acid and 2,2-dimethyl-3-hydroxy propionic acid in wheat and barley crop fractions. This method has been fully validated in Volume 3, B5, section B.5.1.2.5. The validated LOQs were 0.01 mg/kg for bixlozone, 5'-hydroxy-bixlozone and 2,4-dichlorobenzoic acid. In the method validation studies, the validated LOQ for 2,2-dimethyl-3-hydroxy propionic acid was 0.05 mg/kg. However, in some of the field trials, interference above 30% of the LOQ (0.05 mg/kg) was observed in the 'blank' samples. Matrix matched standards were used in each set of analysis and the LOQ adjusted accordingly to account for the increased interference seen in some cases (LOQ either 0.05 or 0.2 mg/kg in grain). Hence, the results given in Table 2.7.4.3, Table 2.7.4.4 and Table 2.7.4.5 appear to show different LOQs for the same analyte in the same matrix. However, in each set of analysis, the LOQ stated in that report was fully supported at that time, for those specific samples.

When the available trials data on both pre- and post- emergence GAPs, on both barley and wheat are combined, there are a sufficient data to support the proposed use in the UK (13 NEU trials representative of the proposed GAPs). This is presented in Table 2.7.4.5. It should be noted that there is some uncertainty regarding the storage stability of samples, given the long storage periods observed in the trials (370 – 661 days) and lack of storage stability data for the relevant analytes in grain. This has been considered in full in Volume 1, section 2.7.1; no further data were considered necessary at this time.

Overall conclusion

There are sufficient residue field trials considered relevant to the proposed GAPs for use of 'F9600-4 SC' on wheat and barley. This is summarised in Table 2.7.4.5. It should be noted that there is 1 trial performed in the NEU zone and 1 trial from the SEU zone for both wheat and barley, with a pre-emergence application, reported in the processing studies which show the same pattern of residues in grain and can be considered supportive information.

Table 2.7.4. 3 Summary of data supporting pre- and post- emergence GAPs on wheat and barley (results not scaled, some data from overdosed trials [up to +33%])

GAP	Crop	Range (mg/kg)			
		Bixlozone	5'-hydroxy bixlozone	2,4-dichlorobenzoic acid	2,2-dimethyl-3-hydroxy propionic acid
Pre-emergence BBCH 00-09	Barley and wheat **grain** (N EU)	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	0.081 [UTC 0.176], 0.088 [UTC 0.106], 0.093 [UTC 0.096], 0.097 [UTC 0.092], 0.11 [UTC 0.093], 2 x <0.2 [UTC 0.23] **STMR: 0.097** **HR: <0.2**
Post emergence BBCH 11-13		6 x <0.01 **STMR: <0.01** **HR: <0.01**	6 x <0.01 **STMR: <0.01** **HR: <0.01**	5 x <0.01, 0.013 [UTC 0.012] **STMR: <0.01** **HR: 0.013**	<0.05, 0.10 [UTC 0.084], 0.123 [UTC 0.09], 3 x <0.2 **STMR: 0.16** **HR: <0.2**
Pre-emergence *BBCH 00-09*	*Barley and wheat grain (SEU)*	*4 x <0.01, 0.01*	*5 x <0.01*	*5 x <0.01*	*0.083 [UTC 0.080], 0.117 [UTC 0.084], 0.13 [UTC 0.14], 0.16 [UTC 0.16], <0.2*
Post emergence *BBCH 11-13*		*5 x <0.01*	*5 x <0.01*	*5 x <0.01*	*0.060 [UTC 0.111], 0.098 [UTC 0.081], 3 x <0.2 [UTC 0.22]*
Pre-emergence BBCH 00-09	Barley and wheat **straw** (N EU)	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	3 x <0.05, 0.073 [UTC 0.063], 0.091 [UTC 0.10], 0.17 [UTC 0.14], 0.241 [UTC 0.183] **STMR: 0.073** **HR: 0.241**
Post emergence BBCH 11-13		6 x <0.01 **STMR: <0.01** **HR: <0.01**	5 x <0.01, 0.02 **STMR: <0.01** **HR: 0.02**	4 x <0.01, 0.012, 0.036 [UTC 0.033] **STMR: <0.01** **HR: 0.036**	<0.05, 0.08 [UTC 0.08], 0.089 [UTC 0.083], 0.10 [UTC 0.081],0.13 [UTC 0.17], 0.334 [UTC 0.294] **STMR: 0.095** **HR: 0.334**
Pre-emergence *BBCH 00-09*	*Barley and wheat straw (SEU)*	*5 x <0.01*	*4 x <0.01, 0.02*	*4 x <0.01, 0.022 [UTC 0.023]*	*0.105 [UTC 0.113], 0.119 [UTC 0.136], 0.19 [UTC 0.22], 0.20 [UTC 0.18], 0.27 [UTC 0.17]*
Post emergence *BBCH 11-13*		*4 x <0.01, 0.014*	*2 x <0.01, 0.018, 2 x 0.02*	*5 x <0.01*	*0.12, 0.13 [UTC 0.06], 0.21 [UTC 0.20], 0.247 [UTC 0.270], 0.31 [UTC 0.38]*

Table 2.7.4. 4 Summary of data supporting pre- and post- emergence GAPs on wheat and barley (Proportionality principle applied)

GAP	Crop	Range (mg/kg)			
		Bixlozone	5'-hydroxy bixlozone	2,4-dichlorobenzoic acid	2,2-dimethyl-3-hydroxy propionic acid
Pre-emergence BBCH 00-09	Barley and wheat **grain** (N EU)	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	0.064 [UTC 0.139], 0.072 [UTC 0.087], 0.075 [UTC 0.078], 0.076 [UTC 0.072], 0.09 [UTC 0.073], 2 x <0.2 [UTC <0.2] **STMR: 0.076** **HR: <0.2**
Post emergence BBCH 11-13		6 x <0.01 **STMR: <0.01** **HR: <0.01**	6 x <0.01 **STMR: <0.01** **HR: <0.01**	5 x <0.01, 0.01 **STMR: <0.01** **HR: 0.01**	<0.05, 0.077 [UTC 0.065], 0.096 [UTC 0.07], 3 x <0.2 [UTC <0.2] **STMR: 0.148** **HR: <0.2**
Pre-emergence *BBCH 00-09*	*Barley and wheat grain (SEU)*	*5 x <0.01*	*5 x <0.01*	*5 x <0.01*	*0.066 [UTC 0.063], 0.10 [UTC 0.074], 0.11 [UTC 0.11], 0.14 [UTC 0.15], <0.2 [UTC <0.2]*
Post emergence *BBCH 11-13*		*5 x <0.01*	*5 x <0.01*	*5 x <0.01*	*<0.05 [UTC 0.087], 0.072 [UTC 0.059], 3 x <0.2 [UTC <0.2]*
Pre-emergence BBCH 00-09	Barley and wheat **straw** (N EU)	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	7 x <0.01 **STMR: <0.01** **HR: <0.01**	3 x <0.05, 0.058 [UTC 0.05], 0.072 [UTC 0.079], 0.14 [UTC 0.11], 0.198 [UTC 0.15] **STMR: 0.058** **HR: 0.198**
Post emergence BBCH 11-13		6 x <0.01 **STMR: <0.01** **HR: <0.01**	5 x <0.01, 0.015 **STMR: <0.01** **HR: 0.015**	5 x <0.01, 0.028 [UTC 0.026] **STMR: <0.01** **HR: 0.028**	<0.05, 0.064 [UTC 0.064], 0.069 [UTC 0.064], 0.077 [UTC 0.062], 0.10 [UTC 0.13], 0.261 [UTC 0.229] **STMR: 0.146** **HR: 0.261**
Pre-emergence *BBCH 00-09*	*Barley and wheat straw (SEU)*	*5 x <0.01*	*4 x <0.01, 0.016*	*4 x <0.01, 0.018 [UTC 0.019]*	*0.086 [UTC 0.092], 0.094 [UTC 0.107], 0.15 [UTC 0.17], 0.16 [UTC 0.13], 0.22 [UTC 0.17]*
Post emergence *BBCH 11-13*		*4 x <0.01, 0.011*	*2 x <0.01, 0.013, 2 x 0.015*	*5 x <0.01*	*0.088, 0.098 [UTC <0.05], 0.16 [UTC 0.15], 0.193 [UTC 0.211], 0.23 [UTC 0.29]*

Table 2.7.4. 5 Summary of supporting field trials data for wheat and barley (after proportionality principle applied and combining trials from pre-emergence and post-emergence, NEU data only)

Crop	Analyte	Range	STMR (mg/kg)	HR (mg/kg)	MRL (OECD Calculator) (mg/kg)
Barley and wheat **grain**	Bixlozone	13 x <0.01	<0.01	<0.01	-
	5'-hydroxy bixlozone	13 x <0.01	<0.01	<0.01	-
	2,4-dichlorobenzoic acid	12 x <0.01, 0.01	<0.01	0.01	-
	2,2-dimethyl-3-hydroxy propionic acid	<0.05, 0.064, 0.072, 0.075, 0.076, 0.077, 0.09, 0.096, 5 x <0.2	0.09	<0.2	-
	RD-RA: Sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone £	12 x <0.039, 0.039 (human exposure assessment) [12 x <0.024, 0.024 (livestock dietary intake assessment)]	<0.039£	0.039£	-
	RD-Enf: bixlozone	13 x <0.01	<0.01	<0.01	0.01*
Barley and wheat **straw**	Bixlozone	13 x <0.01	<0.01	<0.01	-
	5'-hydroxy bixlozone	12 x <0.01, 0.015	<0.01	0.015	-
	2,4-dichlorobenzoic acid	12 x <0.01, 0.028	<0.01	0.028	-
	2,2-dimethyl-3-hydroxy propionic acid	4 x <0.05, 0.058, 0.064, 0.069, 0.072, 0.077, 0.10, 0.14, 0.198, 0.261	0.069	0.261	-
	RD-RA: Sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone £	RA: 12 x <0.024, 0.05 (livestock dietary intake assessment)	<0.024£	0.05£	-
	RD-Enf: bixlozone	13 x <0.01	<0.01	<0.01	MRLs not currently set for animal feed items

£ the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid.
It should be noted that this 2 x factor is only required in assessments comparing to the toxicological endpoints for bixlozone, i.e., this additional 2 x factor has not been used in the animal dietary burden estimate of exposure as this is an estimate of livestock dietary intakes, rather than comparison with a toxicological endpoint. Hence, results of <0.024 mg/kg (grain and STMR for straw) and 0.05 mg/kg (HR for straw) have been taken forward into the animal dietary burden calculation (0.01 + 0.01 x 1.435 MW conversion).

Maize

The residue field trials were performed in various European Member States in both European regions (NEU and SEU). The trials performed in the NEU are directly relevant to the GB climate and therefore can be used in support of the GB GAPs. The SEU trials have been reported for completeness and to give an understanding of residue behaviour, however, the SEU trials have not been relied upon in support of the GB GAP.

Suspension Concentrate vs Capsule Suspension formulations

Trials were performed with either the representative product, 'F9600-4-SC' or 'F9600-28 CS', a capsule suspension formulation containing 34.3% active substance. These two formulations were applied to different plots within the same trial sites. A summary of the results using both formulations is presented in Table 2.7.4.6. Where positive results have been reported in the untreated control samples these have been reported also, denoted as 'UTC'. The occurrence of positive residues in untreated control samples is discussed later in Volume 1. The results presented in Table 2.7.4.6**Error! Reference source not found.** were generated using application rates within ± 25% of the proposed application rate (375 g a.s./ha). Some additional trials performed using a significantly underdosed application rate (124 – 128 g a.s./ha) were also presented in Volume 3, B7, section 7.3.3. These trials were also conducted with the 'SC' and 'CS' formulations at the same trial sites. For the purposes of comparison of results generated using the 'SC' and 'CS' formulations, a summary of these data has been presented in Table 2.7.4.7. None of the trials data has been scaled. Both these data sets (Table 2.7.4.6 and Table 2.7.4.7) show there is not a significant difference between the results generated using either the 'SC' or 'CS' formulations. As these trials were performed at the same time, at the same location, the trials cannot be considered independent. The comparison of the data shows that the results are similar, therefore neither data set appears to be more markedly critical. The OECD guidance on crop field trials (2016) and the EFSA 2015 guidance on residues trials and MRL calculations are open to some varying interpretations as to whether highest residue or mean residue (from the CS and SC treatments) from each trial site should be used. According to both guidance documents, the results are not to be regarded as independent from one another, and only one value from each trial should be used. As the data comparisons show the values are not distinctly different according to which formulation has been used (CS versus SC), the approach taken should not have a large influence on the assessment outcomes. OECD 2016 indicates for acute assessment the highest residues should be considered (and this seems to tally with the suggestion from EFSA 2015 that using different formulations would represent a different trial design, and as such the highest value should be used. Therefore, the highest result from each replicate trial ('SC' or 'CS') has been selected as the representative result from each trial.

Table 2.7.4. 6 Summary of trials data generated using 'SC' and 'CS' formulations (within ± 25% of the GAP in terms of application rate only; further discussion on timing follows)

Formulation	**Crop**	**Range (mg/kg)**				
		Bixlozone	5'-hydroxy bixlozone	2,4-dichloroben zoic acid	5-hydroxy-bixlozone	2,2-dimethyl-3-hydroxy propionic acid
SC	Maize **grain** (NEU)	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	0.10 [UTC 0.12], 0.12 [UTC 0.09]
CS		2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	0.13 [UTC 0.12], 0.17 [UTC 0.09]
SC	*Maize grain (SEU)*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*0.19 [UTC 0.2], 0.33 [UTC 0.29]*
CS		*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*0.25 [UTC 0.20], 0.33 [UTC 0.29]*
SC	Maize **straw** (NEU)	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.05
CS		2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.05
SC	*Maize straw (SEU)*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*<0.05, 0.05*
CS		*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.05*

Table 2.7.4. 7 Comparison of 'SC' and 'CS' formulations from trials using underdosed application rate (66% underdosed, presented for comparison of results from the different formulations only)

Formulation	**Crop**	**Range (mg/kg)**				
		bixlozone	5'-hydroxy bixlozone	2,4-dichlorobenzoic acid	5-hydroxy-bixlozone	2,2-dimethyl-3-hydroxy propionic acid
SC	Maize **grain** (N EU)	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	0.091 [UTC 0.157], 0.30 [UTC 0.349]
CS		2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	0.168 [UTC 0.157], 0.396 [UTC 0.349]
SC	*Maize grain (SEU)*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*0.259 [UTC 0.384], 0.599 [UTC <0.05]*
CS		*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*0.232 [UTC <0.05], 0.336 [UTC 0.384]*
SC	Maize **straw** (N EU)	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	<0.05, 0.055 [UTC 0.059]
CS		2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.01	<0.05, 0.059 [UTC 0.059]
SC	*Maize straw (SEU)*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*<0.05 [UTC 0.055], 0.072 [UTC 0.075]*
CS		*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*2 x <0.05 [UTC 0.055, 0.075]*

Note: Data from the SEU are not being used in the risk assessment therefore HR, STMR and MRL values have not been determined. Results from these trials have been summarised in Table 2.7.4.6 and Table 2.7.4.7 for information only. The SEU data are similar to the NEU data, showing similar trends in residue levels.

Trials using Suspension Concentrate formulation only

A further set of trials data generated using only the 'SC' formulation have been presented in Volume 3. These trials were performed within ± 25% of the proposed GAP but were all underdosed (293 – 325 g a.s./ha). A summary of the relevant results is given in Table 2.7.4.8.

Table 2.7.4. 8 Summary of supporting field trials data for maize (within ± 25% of the GAP in terms of application rate only; further discussion on timing follows)

Crop	**Range (mg/kg)**			
	bixlozone	5'-hydroxy bixlozone	2,4-dichlorobenzoic acid	2,2-dimethyl-3-hydroxy propionic acid
Maize **grain** (N EU)	4 x <0.01	4 x <0.01	4 x <0.01	<0.05, 0.057 [UTC 0.055], 0.11 [UTC 0.15], 0.32 [UTC 0.22]
Maize grain (SEU)	*3 x <0.01, 0.035*	*4 x <0.01*	*4 x <0.01*	*0.22 [UTC 0.31], 0.24 [UTC 0.31], 2 x 0.27 [UTC 0.31, 0.28]*
Maize **straw** (N EU)	4 x <0.01	4 x <0.01	4 x <0.01	4 x <0.05
Maize straw (SEU)	*3 x <0.01, 0.013*	*4 x <0.01*	*4 x <0.01*	*4 x <0.05 [UTC 0.051, 0.064]*

Application timing

Several of the above reported trials were performed with the application made post-emergence (BBCH 11-13), whereas the proposed GAP details pre-emergence (BBCH 00-09) applications only. The results reported in the post-emergence trials show higher positive residues of the metabolite 2,2-dimethyl-3-hydroxy propionic acid. Additionally, the positive results of bixlozone reported in grain and straw in a SEU trial (see Table 2.7.4.9 and Table 2.7.4.10) were from a post-emergence application. It could be argued that considering the long PHI after application in both instances (pre- or post-emergence), the trials should not be markedly different in their outcomes. However, there does appear to be a difference in results (more critical results in post-emergence trials). Given the difference in results (more critical results in post-emergence trials), and clear difference in crop parts being present or not across the two application timings, the trials performed at a later growth stage (post-

emergence) have not been considered relevant to the proposed GAP in this case, and not considered further for risk assessment or MRL setting. Although not recommended for the comparison of different GAPs, the Mann-Whitney U test was used to indicate if combining these data was reasonable. The test, comparing the pre- and post-emergence data sets for grain and straw separately did not result in a conclusive relationship; there are too few data points. When the NEU and SEU data are combined, and the trials data scaled considering the under-dosing observed in some field trials, the test is still inconclusive (changing a single value in the dataset changes the conclusion). Therefore, it is not possible to conclude that the data sets belong to the same population. The test is supportive of the difference in residues observed in trials performed pre- and post-emergence.

It should be noted that the results from the NEU trials for bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid were all <0.01 mg/kg in both the pre- and post-emergence trials. Only positive residues of 2,2-dimethyl-3-hydroxy propionic acid were observed. A summary is presented in Table 2.7.4.9. For maize straw, the results are broadly similar across pre- and post- emergence applications, except for the positive residues of bixlozone found in post-emergence trials. For maize grain, considering the positive results of 2,2-dimethyl-3-hydroxy propionic acid, the results are higher in the post-emergence trials, regardless of the application rate used in these trials; the significantly under dosed trials also show higher residues when this post-emergence application is made.

Table 2.7.4. 9 Summary of results from field trials with application pre- or post-emergence

Crop	**Application timing**	**Application rate**	**Geographical zone**	**Range (mg/kg)**			
				bixlozone	5'-hydroxy bixlozone	2,4-dichlorobenzoic acid	2,2-dimethyl-3-hydroxy propionic acid
Maize grain	Pre-emergence (BBCH 00-08)	Within ± 25% of GAP	NEU	4 x <0.01	4 x <0.01	4 x <0.01	<0.05, 0.11 [UTC 0.15], 0.13 [UTC 0.12], 0.17 [UTC 0.09]
			SEU	*4 x <0.01*	*4 x <0.01*	*4 x <0.01*	*0.24 [UTC 0.31], 0.25 [UTC 0.20], 0.27 [UTC 0.31], 0.33 [UTC 0.29]*
	Post-emergence (BBCH 11-13)	Within ± 25% of GAP	NEU	2 x <0.01	2 x <0.01	2 x <0.01	0.057 [UTC 0.055], 0.32 [UTC 0.22]
		Under dosed		2 x <0.01	2 x <0.01	2 x <0.01	0.168 [UTC 0.157], 0.396 [UTC 0.349]
		Within ± 25% of GAP	*SEU*	*<0.01, 0.035*	*2 x <0.01*	*2 x <0.01*	*0.22 [UTC 0.31], 0.27 [UTC 0.28]*
		Under dosed		*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*0.336 [UTC 0.384], 0.599 [UTC <0.05]*
Maize straw	Pre-emergence (BBCH 00-08)	Within ± 25% of GAP	NEU	4 x <0.01	4 x <0.01	4 x <0.01	4 x <0.05
			SEU	*4 x <0.01*	*4 x <0.01*	*4 x <0.01*	*3 x <0.05, 0.05*
	Post-emergence (BBCH 11-13)	Within ± 25% of GAP	NEU	2 x <0.01	2 x <0.01	2 x <0.01	2 x <0.05
		Under dosed		2 x <0.01	2 x <0.01	2 x <0.01	<0.05, 0.059 [UTC 0.059]
		Within ± 25% of GAP	*SEU*	*<0.01, 0.013*	*2 x <0.01*	*2 x <0.01*	*2 x <0.05 [UTC 0.051, 0.064]*
		Under dosed		*2 x <0.01*	*2 x <0.01*	*2 x <0.01*	*<0.05 [UTC 0.055], 0.072 [UTC 0.075]*

Note: Data from the SEU are not being used in the risk assessment therefore HR, STMR and MRL values have not been determined. Results from these trials have been summarised in Table 2.7.4.9 for information only. The SEU data are similar to the NEU data, showing similar trends in residue levels.

Proportionality principle

Considering the pre-emergence trials (with the removal of replicates from the 'SC'/'CS' trials), all trials were performed within ±25% (293 – 384 g a.s./ha) of the proposed application rate (375 g a.s./ha).

If the post-emergence trials were to be considered relevant to the proposed GAP, several of these trials were underdosed (126 – 128 g a.s./ha) and led to high residues of 2,2-dimethyl-3-hydroxy-propionic acid. As mentioned previously, the Mann Whitney U test (used as supportive information), was performed comparing the scaled results from the pre- and post-emergence trials. However, considering the results for the other analytes (bixlozone, 5'-hydroxy bixlozone and 2,4-dichlorobenzoic acid) were all <LOQ, it was not possible to scale these results up. This also applies to the pre-emergence trials where these analytes were also <LOQ. Therefore, the comparison was made using the results for 2,2-dimethyl-3-hydroxy-propionic acid only. A summary of the scaled results is shown in Table 2.7.4.10. Full details of the scaling factors used are given in the field trial summaries in Volume 3 B7, section 7.3.1.

Table 2.7.4. 10 Summary of results from field trials with application pre- or post-emergence (scaled)

Crop	**Application timing**	**Application rate**	**Geographical zone**	**2,2-dimethyl-3-hydroxy propionic acid (mg/kg)**	
				Results from trials	Scaled to GAP application rate (375 g a.s./ha)
Maize grain	Pre-emergence (BBCH 00-08)	Within ± 25% of GAP	NEU	<0.05, 0.11 [UTC 0.15], 0.13 [UTC 0.12], 0.17 [UTC 0.09]	-[$], 0.13 [UTC 0.17], 0.13 [UTC 0.12], 0.17 [UTC 0.09]
			SEU	*0.24 [UTC 0.31], 0.25 [UTC 0.20], 0.27 [UTC 0.31], 0.33 [UTC 0.29]*	*0.30 [UTC 0.38], 0.24 [UTC 0.19], 0.31 [UTC 0.36], 0.32 [UTC 0.28]*
	Post-emergence (BBCH 11-13)	Within ± 25% of GAP	NEU	0.057 [UTC 0.055], 0.32 [UTC 0.22]	0.069 [UTC 0.067], 0.40 [UTC 0.28]
		Under dosed		0.168 [UTC 0.157], 0.396 [UTC 0.349]	0.49 [UTC 0.46], 1.18 [UTC 1.04]
		Within ± 25% of GAP	*SEU*	*0.22 [UTC 0.31], 0.27 [UTC 0.28]*	*0.28 [UTC 0.39], 0.32 [UTC 0.33]*
		Under dosed		*0.336 [UTC 0.384], 0.599 [UTC <0.05]*	*1.06 [UTC 1.21], 1.81 [UTC -[$]]*
Maize straw	Pre-emergence (BBCH 00-08)	Within ± 25% of GAP	NEU	4 x <0.05	2 x <0.05, 2 x -[$]
			SEU	*3 x <0.05, 0.05*	2 x <0.05, 2 x -[$]
	Post-emergence (BBCH 11-13)	Within ± 25% of GAP	NEU	2 x <0.05	2 x -[$]
		Under dosed		<0.05, 0.059 [UTC 0.059]	-[$], 0.18 [UTC 0.18]
		Within ± 25% of GAP	*SEU*	*2 x <0.05 [UTC 0.051, 0.064]*	*2 x -[$] [UTC 0.060, 0.08]*
		Under dosed		*<0.05 [UTC 0.055], 0.072 [UTC 0.075]*	*-[$] [UTC 0.17], 0.23 [UTC 0.24]*

[$] Result <LOQ therefore not possible to scale up.

Overall conclusion

There are 4 residue field trials performed in the NEU zone considered relevant to support the proposed GB GAP. The results are summarised in Table 2.7.4.11. As maize is a major crop in Northern Europe, 8 trials are required to support this use. However, considering the bixlozone and 2,4-dichlorobenzoic acid components of the residue definition for risk assessment, residues are <LOQ. Therefore 4 trials are considered sufficient to support the use on this major crop. It should be noted that there is 1 trial performed in the NEU zone and 1 trial from the SEU zone reported in the processing studies which show the same pattern of residues in grain and can be considered supportive information.

Table 2.7.4. 11 Summary of supporting field trials data for maize (NEU)

Crop	Analyte	Range	STMR (mg/kg)	HR (mg/kg)	MRL (OECD Calculator) (mg/kg)
Maize **grain**	Bixlozone	4 x <0.01	<0.01	<0.01	-
	5'-hydroxy bixlozone	4 x <0.01	<0.01	<0.01	-
	2,4-dichlorobenzoic acid	4 x <0.01	<0.01	<0.01	-
	2,2-dimethyl-3-hydroxy propionic acid	<0.05, 0.11, 0.13, 0.17	0.12	0.17	-
	RD-RA: Sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone £	4 x <0.039 (human exposure assessment) [4 x <0.024 (livestock dietary intake assessment)]	<0.039£	<0.039£	-
	RD-Enf: bixlozone	<0.01	<0.01	<0.01	0.01*
Maize **straw**	bixlozone	4 x <0.01	<0.01	<0.01	-
	5'-hydroxy bixlozone	4 x <0.01	<0.01	<0.01	-
	2,4-dichlorobenzoic acid	4 x <0.01	<0.01	<0.01	-
	2,2-dimethyl-3-hydroxy propionic acid	4 x <0.05	<0.05	<0.05	-
	RD-RA: Sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone £	4 x <0.024 (livestock dietary intake assessment)	<0.024£	<0.024£	-
	RD-Enf: bixlozone	<0.01	<0.01	<0.01	MRLs not currently set for animal feed items

£ the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid.
It should be noted that this 2 x factor is only required in assessments comparing to the toxicological endpoints for bixlozone, i.e., this additional 2 x factor has not been used in the animal dietary burden estimate of exposure as this is an estimate of livestock dietary intakes, rather than comparison with a toxicological endpoint. Hence, results of <0.024 mg/kg have been taken forward into the animal dietary burden calculation (0.01 + 0.01 x 1.435 MW conversion).

Positive residues in untreated control samples and applicant proposal regarding natural occurrence of residues

Positive residues of the metabolite 2,2-dimethyl-3-hydroxy propionic acid (M118/1) are frequently observed in untreated control samples of wheat, barley and maize fractions. Positive residues were not observed in untreated samples of oilseed rape.

Positive residues of other analytes are found in some samples, but these are generally much lower levels and significantly less frequently occurring than the residues of 2,2-dimethyl-3-hydroxy propionic acid. A single positive residue of bixlozone was found in a barley whole plant untreated sample; this crop fraction is not relevant in this case. A single positive residue of 5'-hydroxy bixlozone was found in wheat hay in a SEU trial; this crop fraction and geographical location are not relevant in this case. Positive residues of 2,4-dichlorobenzoic acid were found in a barley straw sample from a SEU trial (geographical location not relevant in this case) and in wheat grain, straw and whole plant untreated samples in a NEU trial (up to 0.026 mg/kg in wheat straw and hay). Positive residues of 2,4-dichlorobenzoic acid were also reported in the corresponding treated samples in this NEU trial (up to 0.035 mg/kg in wheat hay). The conduct of this trial ('HU02', 15SGS109') is in line with all other field trials; there is no clear reason for these positive results in the untreated control samples. Given the small number of samples with positive residues of these other analytes in the untreated control samples which are considered relevant to the proposed GAPs, these results have not been considered further and are not expected to have impacted upon the corresponding treated results significantly. It should be noted that positive residues of any

analyte were not observed in the rotational crop field trials. A similar pattern of positive residues was observed in the processing studies; a summary is presented with the evaluation of the processing studies.

Considering the corresponding treated samples, there is no clear relationship between the positive residues of 2,2-dimethyl-3-hydroxy propionic acid found in treated and untreated samples. They are frequently found in the untreated control samples from cereal trials and tend to be found at a broadly similar levels to those found in the corresponding treated sample for that trial. There is no mention of possible cross contamination being a reason for this observation. This seems a less likely explanation as there is not a consistent picture across all analytes and the positive results are found across numerous samples from several different field trials.

In most residues field trials, the 'SC' formulation was used. In only maize the 'CS' (encapsulated) and the 'SC' formulations were assessed at the same field site, at the same time, so controls could have been impacted by the nearby treatment of either formulation.

Residues were not found in the untreated controls in the radiolabelled primary or rotational crop metabolism studies (TRRs in controls were all ≤0.001 mg/kg). The control plots were set aside from the treated plots in these studies (primary crops and rotational crops) and the treated and untreated controls were far apart from one another (≥60 m (except for canola metabolism where distance between controls and treated plots were > 22m [controls were 'downwind from the treated plots']). In the primary crop and rotational crop metabolism studies, some high plastic sheeting was placed around the treated plots around the time of application as a barrier to prevent contamination of spray outside of the application area (the treated plots).

The presence of 2,2-dimethyl-3-hydroxy propionic acid in the untreated control samples has had an impact on the analytical method validation and supported LOQs. As discussed previously, a range of LOQs have been supported for 2,2-dimethyl-3-hydroxy propionic acid given the range of interference observed in each analytical set. The amount of 2,2-dimethyl-3-hydroxy propionic acid in the untreated control samples has varied for the same analyte, in the same matrix, between studies to the extent that different LOQs have been supported (0.05 – 0.2 mg/kg). Although residues were not noted in untreated control samples for oilseed rape, in some field trials, interference above 30% of the LOQ (0.05 mg/kg, 2,2-dimethyl-3-hydroxy-propionic acid) was observed in the 'blank'samples of oilseed rape.

Noting the above, positive residues of 2,2-dimethyl-3-hydroxy propionic acid (above 0.05 mg/kg) were not found in untreated samples of oilseed rape. An explanation for this difference in magnitude of interference/residue in untreated controls across species is not available.

The applicant provided a case to address the frequent positive residues of 2,2-dimethyl-3-hydroxy propionic acid in untreated samples of cereals:

'This metabolite residues have been found in almost all untreated and treated crop matrices in the field residue studies, these residue results have sufficiently demonstrated this metabolite is a natural product in various crops, the same conclusion also has been found in the literature search. [Rezanka T, Kolouchova I, Cejkova A and Sigler K. Biosynthesis and metabolic pathways of pivalic acid. Appl. Micobiol. Biotechnol. 95, 1371-1376 (2012).] Reviewer's questions on method recoveries and LOQ levels have been expected; on the other hand, we would like to seek reviewer's understanding that this metabolite can't be evaluated as other normal analytes. Further, it's a reasonable request that this natural product metabolite should be excluded from the residue definition for bixlozone, so that future analysis of this metabolite at any private and country analytical labs, which may result in confusions from data interpretation, can be avoided.'

In considering a proposal for the residue definition, the applicant stated that they consider that the residue 2,2-dimethyl-3-hydroxy propionic acid (M118/1) should not need to be included in the residue definition on the basis that it is mostly likely a natural residue that has been found in both controls and treated plots. The applicant made reference to a published paper (*Rezanka T, Kolouchova I, Cejkova A and Sigler K.* Biosynthesis and metabolic pathways of pivalic acid. Appl. Micobiol. Biotechnol. 95, 1371-1376 (2012)) (in a letter response to HSE 20th August 2019) and proposed that 2,2-dimethyl-3-hydroxypropionic acid with other smaller molecules (e.g. dimethyl malonic acid) were polar, natural products and likely benign in nature. An alternative name for 2,2-dimethyl-3-hydroxypropionic acid is hydroxypivalic acid. This paper (*Rezanka et al., 2012*), suggests there is evidence for pivalic acid (and some derivatives) to be found in nature, as part of fatty acid biosynthesis in some bacteria and also some pivalic acid is expected to be in the environment as a result of man-made activities (as pro-drug). There is no statement in this paper that pivalic acid (and derivatives) would be found naturally in plants.

In a letter to the applicant, HSE made the point that the metabolites that were detected in untreated controls in field trials were found in radiolabelled metabolism studies, as radiolabelled residues (2,2-dimethyl-3-hydroxy propionic acid (M118/1) observed in the acid hydrolysed extracts of cereals (wheat and rice metabolism) and oilseed rape forage and straw). Additionally, broadly the same levels of residues of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) arise in the radiolabelled treated samples, as arise in the treated crop field samples (also the level found in the treated field sample is often accompanied by a similar level in the corresponding trial untreated control sample). As such, presence of the residues as a result of application of the pesticide seems a reasonable interpretation. It should be noted that the metabolism data on rice found both M118/1 and M132/1 occurring together at broadly similar levels. Quantitative trials are not available to confirm the levels of M132/1 that would be expected following the intended uses, hence, there is uncertainty relating to the levels of M132/1 and its natural occurrence also.

HSE raised in a letter with the applicant the possibility of residues of bixlozone arising in untreated controls as a result of overspray or possible deposits on untreated crops from volatile residues. Overspray (and affecting so many of the trials) should not normally be a concern if trials are sprayed without undue winds, and since control and treated plots were located (variously, e.g. up to 45 m apart) at least 10 m apart from one another on the same trial site/crop. The applicant responded that volatility of bixlozone has been investigated in a study (Staffa, 2016, "Study No. AS442, FMC Tracking no. 2016EFT-ISX2732"). This is evaluated in the fate and behaviour section (section CA B8). The aim of this study was to determine realistic worst-case aqueous deposition values of volatilised bixlozone. The fate evaluation (see section CA B.8.3.2) has concluded that the deposition of bixlozone took place at a relatively low level. Highest deposition was measured at the 48 h and 72 h sampling at the 1 m distance and corresponded to 0.42% of applied or about 135 $\mu g/m^2$. At a 10m distance (the minimum distance between the controls and treated plots in the residue field trials, section B.7.3) the level of deposition at the 48/72/96 hr timepoint was at 0.07% or 0.08% of applied level (this was double the level seen at the 24 hr timepoint). As such volatilisation of bixlozone might be expected to a fairly limited extent.

The applicant did not respond on the volatilisation potential of metabolites. It is not known whether 2,2-dimethyl-3-hydroxypropionic acid (M118/1) is forming on the surface of the treated crops or the soil surface from which volatility could possibly occur. The following vapour pressure information is noted for bixlozone and the metabolite 2,2-dimethyl-3-hydroxy propionic acid (M118/1), which may give a very basic indication of whether a substance might have potential to volatilise:

	vapour pressure information	section CA B.2.2	EFSA (2014)-£
Bixlozone	Data in the DAR section CA B.2.2 vapour pressure at 25ºC = 2.5 x 10^{-3} Pa vapour pressure at 20ºC = 1.1 x 10^{-3} Pa	"Slightly volatile"	Low volatility
M118/1 (2,2-dimethyl-3-hydroxypropionic acid)	REACH dossier vapour pressure at 25ºC = 0.446 Pa (EPISUITE estimate)		This value seems to represent a fairly high volatility as it is around 90 x times higher than the EFSA (2014) prompt to consider as for 'moderately volatile' (above 5 mPa at 25°C)

£- Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (EFSA, 2014) states:

> -Substances with low volatility having a vapour pressure of < 5 × 10–3 Pa (the default average concentration in air in the 24 hours after application is 1 $\mu g/m^3$).
>
> -Moderately volatile substances with a vapour pressure between 5 × 10–3 Pa and 10–2 Pa (the default average concentration in air in the 24 hours after application is 15 $\mu g/m^3$).

If residues of either the active substance or metabolites were volatile, this may have enabled transfer of residues from the treated plots to the untreated plots during these field trials. Whilst the vapour pressure of bixlozone is regarded as low volatility (EFSA 2014 Guidance on the assessment of exposure of operators, workers, residents

and bystanders in the risk assessment of plant protection products), the value is not far away from being considered 'moderately volatile' (above 5 mPa at 25°C). Most plant protection product active substances have much lower volatility. The conclusion of this volatility study evaluation is that whilst the active substance has some volatilisation, it is not considered high. However, it should be noted that this volatility investigation study only considered bixlozone and not any metabolites. The above (REACH dossier) value for vapour pressure, estimated for M118/1 (2,2-dimethyl-3-hydroxypropionic acid) suggests fairly high volatility.

The reasoning for these frequent positive residues of 2,2-dimethyl-3-hydroxy propionic acid in untreated control samples is uncertain. HSE considers that whilst derivatives of pivalic acid may be found in nature, the currently available evidence does not support the proposal that these residues determined in the field trials are normally natural components of crops. A key reason is that the level of residue of 2,2-dimethyl-3-hydroxy propionic acid, as aradiolabelled residue in the treated metabolism samples. Therefore, HSE considers it a likelihood that it occurs in treated crops as a direct consequence of application of bixlozone to crops.

With regards to the possible inclusion of 2,2-dimethyl-3-hydroxy propionic acid and dimethyl malonic acid in the residue definition, the applicant provided information to address the toxicological properties of both 2,2-dimethyl-3-hydroxy propionic acid and dimethyl malonic acid. HSE noted that both metabolites were assigned TTC Cramer Class I in the current online version of Toxtree (https://apps.ideaconsult.net/data/ui/toxtree). However, in a report provided by the applicant (Wijeyesakere S.J. et al., 2020 Report number FMC-55114) it was stated that both metabolites should be assigned TTC Cramer Class III according to the extended Cramer classification profiler implemented within the OECD QSAR Toolbox (version 4.4.1) and based on both compounds having a complex chemical structure and not being a normal component of food.

In order to address the inconsistencies described above, HSE requested the applicant to present any further data or justification to consider the Cramer Class assignation further. The applicant reviewed the rule interpretation of the Cramer decision tree from Toxtree (ver. 3.1.0) and the OECD QSAR Toolbox (ver. 4.4) and, while both models are useful tools to implement the Cramer decision tree, following expert judgment for rule interpretation the applicant concluded that the OECD QSAR Toolbox classification was incorrect and that Cramer Class I should be assigned to both metabolites 2,2-dimethyl-3-hydroxy propionic acid and dimethyl-malonic acid. HSE reviewed the case provided by the applicant and considered it acceptable (please refer to Volume 3 CA B6, section 6.8.1).

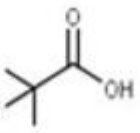
In addition to the further toxicology information, the applicant also submitted a position paper (Position paper on Residue Definition, FMC 60629, 21 April 2022) providing some additional information regarding the natural occurrence of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl-malonic acid (M132/1). Having reviewed this paper, HSE remains of the view that there is insufficient evidence to conclude that these are present due to natural occurrence rather than due to use of bixlozone as a pesticide:

> The applicant refers to the challenges with method development. Efforts explained in the method validation and field trials studies to work with plant material noted the use of control sample material for cereals and oilseed rape (and findings of residues in these samples), and there was then a switch to using quinoa (that HSE anticipates was not obtained from field trials where bixlozone had been used). This is backed up by the applicant letter (response to HSE) dated June 2020 which discusses the way different untreated control material was worked on as field trials on cereals progressed (it was found that some barley untreated samples were obtained that did not contain residues of M118/1 but these were found later than when the samples of maize, wheat and quinoa worked on). If other organic material purchased from local markets was used, then this has not been explained in the study reports, as there are no analyses presented or explanations of residues found in either wheat/barley/OSR or maize from field studies unrelated to bixlozone or organic samples. Additionally, the rotational crop field trials did not involve analysing residues of either M118/1 or M132/1 in any of the field samples and residues of M118/1 were not found in samples of potato and grape analysed as part of the monitoring method development. It is considered likely that the 'blank' material in the method validation work for potato and grape would not be associated with applications of bixlozone.
>
> The applicant has referred to Australian field trials not included in the data submission where the same issue of residues in untreated control samples was observed and has made an observation on the levels found in treated samples, a lack of dose dependent response. If the residues were arising in the untreated control plot material for these trials, potentially due to volatile transfer of residues, then it might be difficult to draw firm quantitative conclusions in relation to this. HSE cannot comment further on the Australian trials.

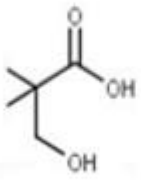
The applicant notes that the source of M118/1 and M132/1 as natural products in plants is not clear. The applicant discussed that M118/1 is also called 3-hydroxypivalic acid and M132/1 (dimethyl malonic acid) is proposed to be formed by oxidation of M118/1. In the position paper, the applicant again referred to the paper submitted with the main data submission (Rezanka, 2012). which indicates that pivalic acid (and some derivatives) are found in nature (in some bacteria) and in the environment as a result of man made activities (pro-drug), and as a breakdown product of isooctane in gasoline. This does not explain either these very widespread findings in the untreated controls of cereals (and OSR) or the lack of residues in the blank material used in the method validation work for the other species (potato and grape).

The applicant provided a reference for a paper - Dembitsky, V. 2006 "Natural Neo Acids and neo Alkanes: Their Analogs and Derivatives. Lipids, 41 (4) 309-240. HSE obtained this paper; this reports the following with regard to presence of pivalic acid in foods: pivalic acid has been detected in peaches and melons, in phenolic fractions of grapes, in fermented soybean curd, in tobacco, and in the volatile flavour of a wheat flour and butter mixture and roux cooked to 100°C. It was also detected in fish sauce and volatile components in products when crab was cooked. These observations do not report background levels and are in relation to pivalic acid not M118/1 (3-hydroxypivalic acid or 2,2-dimethyl-3-hydroxy propionic acid) specifically.

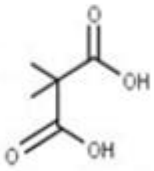
HSE notes that pivalic acid is a smaller molecule than both M118/1 and M132/1 and it is not clear that residues of M118/1 or M132/1 would necessarily be formed as breakdown products resulting from biodegradation of pivalic acid.



Pivalic Acid



2,2-dimethyl-3-hydroxy propionic acid



Dimethyl Malonic Acid

Overall Conclusion:

Following consideration of the additional position paper and all information submitted during the assessment, HSE has concluded that the residues of M118/1 and M132/1 should not be excluded from the risk assessment considerations, based on the proposal that these might be residues of natural provenance. The reasoning for the presence of residues of M118/1 in untreated control sample material from field trials is uncertain, but volatile transfer of residues (e.g. from application to soil or young plants in neighbouring plots) might be a plausible explanation. Residues of M118/1 were the main residues found in field trials on cereals, and residues of M132/1 were not sought in quantitative trials, but the possible co-presence of M132/1 with M118/1 is suggested from the plant (rice) metabolism data. Currently the residues of M118/1 and M132/1 have been screened as part of an exposure assessment of these metabolites in a TTC consideration. For all future uses a TTC exposure assessment approach or further toxicological assessment should be used to ensure assessment of these residues.

If the applicant would wish to improve the case for these residues being natural, this could also be considered as part of a future submission. Any new analyses provided in regulatory context should be in GLP studies that determine the levels of the residues of M118/1 and M132/1 in plant material, that has had no association with any prior or current use with bixlozone, in order to be able to quantitatively compare estimated background levels (and corresponding exposure estimates) from non-pesticide food exposures compared to the exposure from use of bixlozone as a pesticide. Data on background levels in the literature may have already been searched exhaustively, but additional evidence could be provided (on detection and levels of M118/1 or M132/1 naturally found in foods) if there was further evidence available from the literature.

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

Animal dietary burden

The dietary burden has been performed according to the approach presented in the OECD Guidance document on residues in livestock, series on pesticides No 73 for a total of 9 animal species. All feed items which might be treated with the active substance under evaluation have been considered (wheat, barley, maize and oilseed rape as primary crops, and leafy crops including above ground vegetables as rotational crops). Calculations are performed using the Excel calculator proposed by EFSA (pesticides_mrl_guidelines_animal_model_2017). The following assumptions have been made:

1) The highest likely inclusion rate of all crops which may have been treated has been used with the proviso that the aggregate does not exceed 100% diet;
2) All produce eaten which may have been treated, has been treated and contains residues as summarized below;
3) There is no loss of residue during transport, storage, preparation of feed or processing prior to consumption.

The inputs for the dietary burden are presented in Table 2.7.5.1. The following estimate includes residues of bixlozone only in leafy vegetables (and above ground vegetables) which may be grown in rotation, residues of bixlozone and 2,4-dichlorobenzoic acid in oilseed rape, maize, wheat and barley grown as primary crops. Several inputs (all above ground vegetables and possible 'leafy' crops) have been included to give a worst case indicative estimate, given the possibility of positive residues in leafy crops grown in rotation (see section 2.7.7 for further details). It is noted that with the proposed plant back restriction (see section 2.7.7) that residues above the LOQ would not be expected in crops grown in rotation. Furthermore the calculation includes the contribution of residues estimated to be <LOQ and the residue level at the LOQ is included in the calculation; as such the estimation is 'worst case'.

It should be noted the default processing factors have been used as the processing factors derived from the submitted processing studies specific to bixlozone are only considered indicative and have some uncertainties.

Table 2.7.5. 1 Inputs for animal dietary burden

Feed commodity	**Median dietary burden**		**Maximum dietary burden**	
	(mg/kg)	Comment	(mg/kg)	Comment
Primary crop oilseed rape, maize, wheat and barley: Sum of bixlozone and 2,4-dichlorobenzoic acid, expressed as bixlozone.[$] Rotational crops (*RC*): bixlozone.				
Alfalfa forage, hay, meal, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Barley forage, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Barley straw	0.024	STMR	0.05	HR
Bean vines	0.01	STMR *(RC)*	0.028	HR *(RC)*
Beet, mangel fodder	0.01	STMR *(RC)*	0.028	HR *(RC)*
Beet, sugar	0.01	STMR *(RC)*	0.028	HR *(RC)*
Cabbage heads, leaves	0.01	STMR *(RC)*	0.028	HR *(RC)*
Clover forage, hay, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Corn, field, forage/silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Corn, field (maize), pop, stover	0.024	STMR	0.024	HR
Cowpea, forage, hay	0.01	STMR *(RC)*	0.028	HR *(RC)*
Grass, forage (fresh), hay, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*

Feed commodity	**Median dietary burden**		**Maximum dietary burden**	
	(mg/kg)	Comment	(mg/kg)	Comment
Kale, leaves	0.01	STMR *(RC)*	0.028	HR *(RC)*
Lespedeza, forage, hay	0.01	STMR *(RC)*	0.028	HR *(RC)*
Millet, forage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Oat forage, hay	0.01	STMR *(RC)*	0.028	HR *(RC)*
Pea vines, hay, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Rape forage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Rye forage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Sorghum forage, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Soybean forage, hay, silage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Trefoil forage	0.01	STMR *(RC)*	0.028	HR *(RC)*
Triticale forage, hay	0.01	STMR *(RC)*	0.028	HR *(RC)*
Turnip tops, leaves	0.01	STMR *(RC)*	0.028	HR *(RC)*
Vetch forage, hay	0.01	STMR *(RC)*	0.028	HR *(RC)*
Wheat forage, hay	0.01	STMR *(RC)*	0.028	HR *(RC)*
Wheat straw	0.024	STMR	0.05	HR
Barley grain	0.024	STMR	-	-
Corn, field (maize), pop, grain	0.024	STMR	-	-
Wheat grain	0.024	STMR	-	-
Brewer's grain (dried)	0.024	STMR (barley grain) x PF†	-	-
Canola (rape seed) meal	0.024	STMR (rape meal) x PF†	-	-
Corn, field, milled by-products, hominy meal, gluten feed, gluten meal	0.024	STMR (maize grain) x PF†	-	-
Distiller's grain (dried)	0.024	STMR (wheat grain) x PF†	-	-
Rape meal	0.024	STMR (rape seed) x PF†	-	-
Wheat gluten (meal)	0.024	STMR (wheat grain) x PF†	-	-
Wheat (milled by-products)	0.024	STMR (wheat grain) x PF†	-	-

† PF = 1; waiving the use of default processing factors (PF) as residues in the RAC are < LOQ. It is noted that the processing factors derived as part of the evaluation are tentative only and do not show significant concentration for oilseed rape fractions. There is an indication of concentration in wheat bran and barley malt sprouts but these are not animal feed items.

[$] the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid.
It should be noted that this 2 x factor is only required in assessments comparing to the toxicological endpoints for bixlozone, i.e., this additional 2 x factor has not been used in the animal dietary burden estimate of exposure as this is an estimate of livestock dietary intakes, rather than comparison with a toxicological endpoint.

The maximum and median calculated animal intakes are reported in Table 2.7.5.2.

Table 2.7.5. 2 Median and Maximum dietary burden of bixlozone by domestic animals

New data requirements	Regulation (EU) No 283/2013)							
Relevant groups	**Dietary burden expressed in**				**Most critical diet (a)**	**Most critical commodity (b)**		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0.002	0.003	0.05	0.09	Dairy cattle	Grass	silage	No
Cattle (dairy only)	0.002	0.003	0.04	0.09	Dairy cattle	Grass	silage	No
Sheep (all diets)	0.002	0.004	0.05	0.11	Ram/Ewe	Grass	forage (fresh)	No
Sheep (ewe only)	0.002	0.004	0.05	0.11	Ram/Ewe	Grass	forage (fresh)	No
Swine (all diets)	0.001	0.001	0.03	0.05	Swine (breeding)	Beet, mangel	fodder	No
Poultry (all diets)	0.002	0.002	0.03	0.04	Poultry layer	Pea	vines	No
Poultry (layer only)	0.002	0.002	0.03	0.04	Poultry layer	Pea	vines	No
(a): When several diets are relevant (e.g. cattle sheep and poultry "all diets") the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"								
(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".								

Both the estimated (worst case) animal dietary burden - median and maximum animal dietary intakes do not exceed the animal dietary burden trigger of 0.004 mg/kg bw/day.

Please see section 2.7.3 which discusses the non-inclusion of 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) in the livestock dietary intake calculation.

Feeding studies

No feeding study is required for ruminants, poultry, pigs and or fish; no feeding studies have been submitted.

The requirements for feeding studies are set out according to Commission Regulation (EU) No 283/2013 with data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market as well as and in OECD guidelines.

Feeding studies are required:

(1) If metabolism studies indicate that significant residues (above 0.01 mg/kg for each analyte) may occur in any edible animal tissue, considering the residue levels in potential feeding stuff obtained at the 1x dose rate.

(2) However, feeding studies shall not be required where intake is below 0.004 mg/kg bw/d, except in cases where the residue, namely the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

In the context of this document, feed burden calculations were performed using the EU Animal Model 2017 considering only the representative uses on primary crops (wheat, barley, maize and oilseed rape) and the possible residues resulting in rotational crops following these primary uses. The calculated dietary burden is considered worst case. The resulting maximum dietary burden is 0.004 mg/kg bw/d for ruminants and 0.002 mg/kg bw/d for poultry, which does not exceed the trigger of 0.004 mg/kg bw/d. The animal feed burden may require further consideration if additional uses or uses leading to possible higher residues in rotational crops are proposed in the future.

Comparing the feed burdens with the metabolism studies on hens and goats overdosing factors of 540-575 N for poultry and 103 - 123 N for ruminants have been derived. When the over-dosing factors are applied to the TRR measured in animal commodities in the metabolism studies, it shows the residues in products of animal origin are expected to be < 0.01 mg/kg at the maximum reasonable worst-case feed burden. The animal feed burden and subsequent possible residues in products of animal origin may require further consideration if additional extensions of uses are proposed.

See the end of section 2.7.2 for a consideration of fish data requirements (metabolism and feeding studies); these requirements do not need to be addressed in the current evaluation. The applicant proposed that dietary intakes for fish would be very low. Depending on the residues in crops, further information to address this data requirement (residues in fish (nature [metabolism], and if needed magnitude of the residues [feeding studies]) will be required when guidance becomes available.

2.7.6. Summary of effects of processing

Nature of the residue

The nature of residues of bixlozone upon processing was investigated in a standard hydrolysis study conducted with two labels of bixlozone (phenyl label and carbonyl label) simulating pasteurisation, baking/boiling/brewing and sterilisation conditions. Bixlozone was observed to be stable upon processing under all 3 representative conditions.

The residue definition for risk assessment also includes the metabolite 2,4-dichlorobenzoic acid (M190/1) (for a full discussion of the residue definition please see section 2.7.3 of this document). The effect of processing upon the nature of metabolites 2,4-dichlorobenzoic acid has not been investigated under standard hydrolysis conditions. However, given that residues of 2,4-dichlorobenzoic acid are <0.01 mg/kg in all wheat, barley and maize grain and rape seeds in the residues trials, with the exception of one wheat grain sample which shows a positive residues of 0.01 mg/kg, and considering that 2,4-dichlorobenzoic acid is a relatively small structure, the lack of a hydrolysis study covering 2,4-dichlorobenzoic acid is considered a minor data gap.

Whilst not a major data gap, further data on the nature of residues of 2,4-dichlorobenzoic acid under standard hydrolysis conditions are needed and should be provided to support a product authorisation for cereal crops. It may be possible for an improved case for non-submission of such radiolabelled hydrolysis data for 2,4-dichlorobenzoic acid to be provided, however such a case would need to be detailed and sufficient to address the potential fate of the molecule 2,4-dichlorobenzoic acid. New data should be generated in accordance with OECD test guideline 507.

For the current time, based on the available data, the residue definition for processed commodities is proposed to be the same as for the raw agricultural commodity (RAC).

Magnitude of the residue

Based on the RD-RA, the residue levels in the RACs are below 0.1 mg/kg, the theoretical maximum daily intake is <10% of the ADI and the estimated daily intake is <10% of the ARfD, and therefore magnitude of residues studies are not strictly required.

However, as studies were submitted on the magnitude of residues of parent bixlozone, and other analytes (2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid), over processing in wheat (*production of various processed fractions, including flour (white and wholemeal), bread, (white and wholemeal) germ, bran, starch/gluten*), barley (*pearl barley, malt and beer*), oilseed rape (*crude oil and refined oil, and press cake used as animal feed*), and maize (*bran, flour, starch, protein, meal and oil*) and have been presented below for completeness. The studies used field trial derived residues (field incurred residues) and the processing operations followed detailed simulated industrial practices conducted in the laboratory. Full details of the processes were provided.

Data are available for the following number of trials: wheat (2), barley (1), oilseed rape (2) and maize (1).

For all analytes tested, <LOQ residues were observed in the RAC and/or processed fractions in at least one of the analysed trials and therefore, the derivation of processing factors is complicated and uncertain in view of the frequent finding of results below the LOQ. Where possible an indicative processing factor was proposed. In accordance with OECD 508, the application conditions used in the trails should aim to allow for quantifiable residues to allow the study of processing and impact of processing on residues to be observed. This is why the applicant designed the trials (plot 3) with a shortened harvest interval (of around 30 days for the cereal studies and around 45 days for oilseed rape) to increase the chances of observing positive residues. An increased application rate was not used to prevent potential phytotoxicity effects and therefore the shortened harvest interval was deemed an acceptable measure made by the applicant to increase the chances of positive residues in the RAC. Where positive residues were not observed (especially in RAC prior to processing) it was not possible to derive a reliable processing factor. Also, the low reliability of processing factors that could be proposed was also due to the low number of trials (only 1) for each of barley and maize. A summary of the processing factors proposed for bixlozone is presented in the tables in this section.

In the wheat, barley and maize studies, residues of 2,2-dimethyl-3-hydroxy propionic acid were found in the control samples for some processed fractions. In the oilseed rape study, the control samples did not contain any residues above the LOQ. This is consistent with the findings in the primary crop field trials where residues of 2,2-dimethyl-3-hydroxy propionic acid were commonly observed in untreated controls in the wheat, barley and maize trials. The hydrolysis study carried out on parent bixlozone concluded that 2,2-dimethyl-3-hydroxy propionic acid was not formed on processing from bixlozone. The applicant has proposed that the metabolite 2,2-dimethyl-3-hydroxy propionic acid is a natural residue and HSE has raised the possibility of residues of bixlozone arising in untreated controls as a result of overspray/drift or possible deposits on untreated crops from volatile residues. For a full discussion of the residues found in untreated controls and consideration of the residue definition please see section 2.7.4 of this document.

Where possible processing factors were calculated, this is defined as the residue in the processed product divided by the residue in the RAC.

An example calculation of a processing factor (for RD-RA) is given below:

e.g. processing factor for milling wheat into flour type 550 (trial S16-05487-01)

$$= \frac{\text{residue level in flour type 550}}{\text{residue level in wheat (RAC)}} = \frac{0.024}{0.118} = 0.2$$

The derivation of processing factors is complex for a number of reasons:

- Only one processing trial is available for each of barley and maize
- Even where two trials available residues in grain (RAC) were <LOQ in for at least one of the analytes (bixlozone or 2,4-dichlorobenzoic acid which are included in the RD-RA).
- Residues were generally low and so there were a number of <LOQ residues in grain (RAC) and processed fractions. This was the case for the all of the analyses (grain and all processed fractions for maize) for maize involving bixlozone and 2,4-dichlorobenzoic acid, and so no processing factors can be proposed for maize.
- The residues of 2,4-dichlorobenzoic acid are proposed as twice as toxic as residues of parent bixlozone and therefore deriving an overall processing factor to apply to the 'sum of residues' in the risk assessment is complicated. As such, processing factors have been calculated, where possible, for parent only and 2,4-dichlorobenzoic acid only, as well as for the 'sum' (RD-RA) - sum of residues of bixlozone and 2 x 2,4-dichlorobenzoic acid expressed as bixlozone (see section 2.7.3 regarding the proposal for residue definition for dietary intake (RD-RA)). Where estimated processing factors are uncertain, this is noted in the below tables and the reasons explained.
- Residues are low affecting the possibility of deriving a complete set of processing factors; however as the overall residues are low (residues in the RAC based on the RD-RA are <0.1 mg/kg) the data on magnitude of the residues over processing do not need to be relied upon currently.
- A Cramer class I TTC consideration is required for 2,2-dimethyl-3-hydroxy propionic acid (M118/1, it is not currently included in the proposed residue definition for risk assessment). As such individual PFs have also been derived for 2,2-dimethyl-3-hydroxy propionic acid and are presented below. These processing factors are all uncertain but at least give an indication of the fractions in which concentration of residues might be expected to occur.

- The indicative PFs for bixlozone and 2,4-dichlorobenzic acid, as individual residues, and for the RD-RA (sum of residues of bixlozone and 2 x 2,4-dichlorobenzoic acid expressed as bixlozone) are stated in the LoEPs.
- It is noted that where concentration is observed and a processing factor is observed with a '>' based on some <LOQ findings in the RAC, these processing factors are more limited and especially uncertain as they may underrepresent the concentration in the matrices.

Due to low residues in primary crops (oilseed rape, barley, wheat and maize) following the intended uses, (sum of bixlozone and 2,4-dichlorobenzoic acid were <0.1 mg/kg), the processing studies provided on magnitude of residues over processing are not a regulatory requirement at this time. The indicative processing factors from all studies (e.g. for 2,2-dimethyl-3-hydroxy propionic acid in cereals) are used in the risk assessment (in the TTC consideration in section 2.7.3).

From the data available, no accumulation was observed for the majority of processed commodities analysed, with the exception of wheat course bran (indicative PF RD-RA = 1.58), barley malt sprouts (indicative PF RD-RA = 1.49) and rape crude oil (indicative PF RD-RA = 1.28), oilseed rape presscake (indicative PF RD-RA = 1.42) and oilseed rape refined oil (indicative PF RD-RA = 1.19). For these commodities a concentration is predicted, however accurate processing factors could not be derived due to <LOQ residues in the RAC for one or both of the analytes tested, in one or both of the trials.

Table 2.7.6. 1 Bixlozone results from processing trials on wheat and derivation of individual processing factor for bixlozone (on its own)

Product	Trial S16-05487-01 NEU Residue in mg/kg	Individual Trial PF (NEU)$	Trial S16-05487-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$	Overall processing factor (PF)	Remark
Grain (RAC)	<0.01		0.017			
Fine bran	<0.01		nd			
Coarse bran	0.012	>1.20	nd		>1.20	Indicative only (n=1*)
Total bran	<0.01		0.014	0.82	0.82	Indicative only (n=1*)
Toppings	<0.01		nd			
Flour type 550	<0.01		<0.01	<0.59	<0.59	Indicative only (n=1*)
White dough	<0.01		nd			
White bread	<0.01		<0.01	<0.59	<0.59	Indicative only (n=1*)
Wholemeal flour	<0.01		0.013	0.75	0.75	Indicative only (n=1*)
Wholemeal dough	<0.01		nd			
Wholemeal bread	<0.01		0.010	0.59	0.59	Indicative only (n=1*)
Wet gluten	<0.01		nd			
Dried gluten	<0.01		<0.01	<0.59	<0.59	Indicative only (n=1*)
Dried starch	<0.01		<0.01	<0.59	<0.59	Indicative only (n=1*)
Gluten feed meal	<0.01		<0.01	<0.59	<0.59	Indicative only (n=1*)
Wheat germs	<0.01		<0.01	<0.59	<0.59	Indicative only (n=1*)

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in Germany compared to the trial conducted in Spain).

*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, for some fractions, an indicative PF could only be suggested from the available data.

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 2 2,4-dichlorobenzoic acid results from processing trials on wheat and derivation of individual processing factor for 2,4-dichlorobenzoic acid (on its own)

Product	Trial S16-05487-01 NEU Residue in mg/kg	Individual Trial PF (NEU) $	Trial S16-05487-02 SEU Residue in mg/kg	Individual Trial PF (SEU) $	Overall processing factor (PF)	Remark
Grain (RAC)	0.075		<0.01			
Fine bran	<0.01	<0.13	nd		<0.13	Indicative only (n=1*)
Coarse bran	0.120	1.60	nd		1.60	Indicative only (n=1*)
Total bran	0.085	1.13	<0.01		1.13	Indicative only (n=1*)
Toppings	0.017	0.23	nd		0.23	Indicative only (n=1*)
Flour type 550	0.010	0.13	<0.01		0.13	Indicative only (n=1*)
White dough	<0.01	<0.13	nd		<0.13	Indicative only (n=1*)
White bread	<0.01	<0.13	<0.01		<0.13	Indicative only (n=1*)
Wholemeal flour	0.068	0.91	<0.01		0.91	Indicative only (n=1*)
Wholemeal dough	0.050	0.67	nd		0.67	Indicative only (n=1*)
Wholemeal bread	0.064	0.85	<0.01		0.85	Indicative only (n=1*)
Wet gluten	<0.01	<0.13	nd		<0.13	Indicative only (n=1*)
Dried gluten	0.012	0.16	<0.01		0.16	Indicative only (n=1*)
Dried starch	0.012	0.16	<0.01		0.16	Indicative only (n=1*)
Gluten feed meal	0.010	0.13	<0.01		0.13	Indicative only (n=1*)
Wheat germs	0.030	0.40	<0.01		0.40	Indicative only (n=1*)

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in Germany compared to the trial conducted in Spain).

*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, an indicative PF could only be suggested from the available data.

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 3 Sum of bixlozone and **2 x** 2,4-dichlorobenzoic acid (expressed as parent) results from processing trials on wheat and derivation of a processing factor for sum of residues

Product	Trial S16-05487-01 NEU Residue in mg/kg	Individual Trial PF (NEU)$ & UC=uncertain	Trial S16-05487-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$ UC=uncertain	Overall processing factor (PF) UC=uncertain	Remark
Grain (RAC)	<0.225 (&)		<0.046 (&)			
Fine bran	<0.039	0.17 UC	nd	nd	0.17 (n=1) UC	**Indicative only.** **All estimated PF are uncertain (UC) (since each estimated individual trial PF involves use of trials results with at least one of the analytes being <LOQ in grain (RAC) prior to processing).**
Coarse bran	**0.356** (&)	1.58 UC	nd	nd	1.58 (n=1) UC	
Total bran	<0.254	1.13 UC	<0.043	0.93 UC	1.03 (n=2) UC	
Toppings	<0.059	0.26 UC	nd	nd	0.26 (n=1) UC	
Flour type 550	<0.039	0.17 UC	<0.039	0.85 UC	0.51 (n=2) UC	
White dough	<0.039	0.17 UC	nd	nd	0.17 (n=1) UC	
White bread	<0.039	0.17 UC	<0.039	0.85 UC	0.51 (n=2) UC	
Wholemeal flour	<0.205	0.91 UC	<0.042	0.91 UC	0.91 (n=2) UC	
Wholemeal dough	<0.154	0.68 UC	nd	nd	0.68 (n=1) UC	
Wholemeal bread	<0.194	0.86 UC	<0.039	0.85 UC	0.86 (n=2) UC	
Wet gluten	<0.039	0.17 UC	nd	nd	0.17 (n=1) UC	
Dried gluten	<0.044	0.20 UC	<0.039	0.85 UC	0.53 (n=2) UC	
Dried starch	<0.044	0.20 UC	<0.039	0.85 UC	0.53 (n=2) UC	
Gluten feed meal	<0.039	0.17 UC	<0.039	0.85 UC	0.51 (n=2) UC	
Wheat germs	<0.096	0.43 UC	<0.039	0.85 UC	0.64 (n=2) UC	

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in Germany compared to the trial conducted in Spain).

*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, for some fractions an indicative PF could only be suggested from the available data.

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

& Most of the results determined involve at least one < LOQ determination of residues. Only the emboldened values are based on positive residue determinations only. This makes estimation of the PFs highly uncertain, as the grain (RAC) results (for grain prior to processing) included at least one of the analytes with <LOQ residues.

example calculation of sum of residues (bixlozone <0.01 mg/kg) + ([1.435 (M Wt conversion to express as parent) x 0.075] x 2) = <0.225.

{Molecular mass of 2,4-dichlorobenzoic acid is 191.01 g/mol and the molecular mass of parent bixlozone is 274.17 g/mol}

Table 2.7.6. 4 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on wheat and derivation of individual processing factor for 2,2-dimethyl-3-hydroxy propionic acid (on its own)

Product	Trial S16-05487-01 NEU Residue in mg/kg	Individual Trial PF (NEU) $	Trial S16-05487-02 SEU Residue in mg/kg	Individual Trial PF (SEU) $	Overall processing factor (PF)	Remark
Grain (RAC)	0.22		<0.20			
Fine bran	<0.20	<0.91	nd		<0.91	Indicative only (n=1*)
Coarse bran	0.36	1.64	nd		1.64	Indicative only (n=1*)
Total bran	0.25	1.36	<0.20		1.36	Indicative only (n=1*)
Toppings	<0.20	<0.91	nd		<0.91	Indicative only (n=1*)
Flour type 550	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)
White dough	<0.20	<0.91	nd		<0.91	Indicative only (n=1*)
White bread	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)
Wholemeal flour	0.22	1.00	<0.20		1.00	Indicative only (n=1*)
Wholemeal dough	<0.20	<0.91	nd		<0.91	Indicative only (n=1*)
Wholemeal bread	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)
Wet gluten	<0.20	<0.91	nd		<0.91	Indicative only (n=1*)
Dried gluten	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)
Dried starch	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)
Gluten feed meal	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)
Wheat germs	<0.20	<0.91	<0.20		<0.91	Indicative only (n=1*)

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in Germany compared to the trial conducted in Spain).
*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, an indicative PF could only be suggested from the available data.
$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 5 Bixlozone results from processing trials on barley and derivation of individual processing factor for bixlozone (on its own)

Product	Trial S16-05488-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$	Overall processing factor (PF)	Remark
Grain (RAC)	<0.01			
Malt sprouts	0.027	>2.70	>2.70	Indicative only (n=1*)
Malt	<0.01			
Spent grain	<0.01			
Flocs	<0.01			
Brewer's yeast	<0.01			
Beer	<0.01			
Pearl barley	<0.01			

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 6 2,4-dichlorobenzoic acid results from processing trials on barley and derivation of individual processing factor for 2,4-dichlorobenzoic acid (on its own)

Product	Trial S16-05488-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$	Overall processing factor (PF)	Remark
Grain (RAC)	0.033			
Malt sprouts	0.045	1.36	1.36	Indicative only (n=1*)
Malt	0.031	0.94	0.94	Indicative only (n=1*)
Spent grain	0.016	0.48	0.48	Indicative only (n=1*)
Flocs	<0.01	<0.30	<0.30	Indicative only (n=1*)
Brewer's yeast	<0.01	<0.30	<0.30	Indicative only (n=1*)
Beer	<0.01	<0.30	<0.30	Indicative only (n=1*)
Pearl barley	0.019	0.58	0.58	Indicative only (n=1*)

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 7 Sum of bixlozone and **2 x** 2,4-dichlorobenzoic acid (expressed as parent) results from processing trials on barley and derivation of a processing factor for sum of residues

Product	Trial S16-05488-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$ & UC=uncertain	Overall processing factor (PF) UC=uncertain	Remark
Grain (RAC)	<0.105			
Malt sprouts	**0.156 (&)**	1.49 UC	1.49 UC	**Indicative only.** **All estimated PF are uncertain (UC) (since each estimated individual trial PF involves use of trials results with at least one of the analytes being <LOQ in grain (RAC) prior to processing).**
Malt	<0.099	0.95 UC	0.95 UC	
Spent grain	<0.056	0.53 UC	0.53 UC	
Flocs	<0.039	0.37 UC	0.37 UC	
Brewer's yeast	<0.039	0.37 UC	0.37 UC	
Beer	<0.039	0.37 UC	0.37 UC	
Pearl barley	<0.065	0.62 UC	0.62 UC	

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

& Most of the results determined involve at least one < LOQ determination of residues. Only the emboldened values are based on positive residue determinations only. This makes estimation of the PFs highly uncertain, as the grain (RAC) results (for grain prior to processing) included at least one of the analytes with <LOQ residues.

example calculation of sum of residues (bixlozone <0.01 mg/kg) + ([1.435 (M Wt conversion to express as parent) x 0.033] x 2) = <0.105.

{Molecular mass of 2,4-dichlorobenzoic acid is 191.01 g/mol and the molecular mass of parent bixlozone is 274.17 g/mol}

Table 2.7.6. 8 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on barley and derivation of individual processing factor for 2,2-dimethyl-3-hydroxy propionic acid (on its own)

Product	Trial S16-05488-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$	Overall processing factor (PF)	Remark
Grain (RAC)	<0.20			
Malt sprouts	0.42	>2.10	>2.10	Indicative only (n=1*)
Malt	0.23	>1.15	>1.15	Indicative only (n=1*)
Spent grain	<0.20			
Flocs	<0.20			
Brewer's yeast	<0.20			
Beer	<0.20			
Pearl barley	<0.20			

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 9 Bixlozone results from processing trials on oilseed rape and derivation of individual processing factor for bixlozone (on its own)

Product	Trial S16-05489-01 NEU Residue in mg/kg	Individual Trial PF (NEU)$	Trial S16-05489-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$	Overall processing factor (PF)	Remark
Seeds (RAC)	<0.01		0.027			
Raw oil	<0.01		nd			
Crude oil	<0.01		0.044	1.63	1.63	Indicative only (n=1*)
Press cake	<0.01		0.038	1.41	1.41	Indicative only (n=1*)
Refined Oil	<0.01		0.039	1.44	1.44	Indicative only (n=1*)

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in NEU compared to the trial conducted in SEU).
*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, for some fractions an indicative PF could only be suggested from the available data.
$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 10 2,4-dichlorobenzoic acid results from processing trials on oilseed rape and derivation of individual processing factor for 2,4-dichlorobenzoic acid (on its own)

Product	Trial S16-05489-01 NEU Residue in mg/kg	Individual Trial PF (NEU)$	Trial S16-05489-02 SEU Residue in mg/kg	Individual Trial PF (SEU) $	Overall processing factor (PF)	Remark
Seeds (RAC)	<0.01		<0.01			
Raw oil	<0.01		nd			
Crude oil	<0.01		<0.01			
Press cake	<0.01		0.015	>1.50	>1.50	Indicative only (n=1*)
Refined Oil	<0.01		<0.01			

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in NEU compared to the trial conducted in SEU).
*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, an indicative PF could only be suggested from the available data.
$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

Table 2.7.6. 11 Sum of bixlozone and **2 x** 2,4-dichlorobenzoic acid (expressed as parent) results from processing trials on oilseed rape and derivation of a processing factor for sum of residues

Product	Trial S16-05489-01 NEU Residue in mg/kg	Individual Trial PF (NEU)$ & UC= uncertain	Trial S16-05489-02 SEU Residue in mg/kg	Individual Trial PF (SEU)$ & UC= uncertain	Overall processing factor (PF) UC= uncertain	Remark
Seeds (RAC)	<0.039		<0.056			
Raw oil	<0.039					**Indicative only. All estimated PF are uncertain (UC) (since each estimated individual trial PF involves use of trials results with at least one of the analytes being <LOQ in seed (RAC) prior to processing).**
Crude oil	<0.039		<0.073	1.28 UC	1.28 UC	
Press cake	<0.039		**0.081 (&)**	1.42 UC	1.42 UC	
Refined Oil	<0.039		<0.068	1.19 UC	1.19 UC	

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in NEU compared to the trial conducted in SEU).

*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore, for some fractions an indicative PF could only be suggested from the available data.

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

& Most of the results determined involve at least one < LOQ determination of residues. Only the emboldened values are based on positive residue determinations only. This makes estimation of the PFs highly uncertain, as the seed (RAC) results (for seed prior to processing) included at least one of the analytes with <LOQ residues.

example calculation of sum of residues (bixlozone 0.027 mg/kg) + ([1.435 (M Wt conversion to express as parent) x <0.01] x 2) = <0.056.

{Molecular mass of 2,4-dichlorobenzoic acid is 191.01 g/mol and the molecular mass of parent bixlozone is 274.17 g/mol}

Table 2.7.6. 12 2,2-dimethyl-3-hydroxy propionic acid results from processing trials on oilseed rape and derivation of individual processing factor for 2,2-dimethyl-3-hydroxy propionic acid (on its own)

Product	Trial S16-05489-01 NEU Residue in mg/kg	Individual Trial PF (NEU)$	Trial S16-05489-02 SEU Residue in mg/kg	Individual Trial PF (SEU) $	Overall processing factor (PF) $	Remark
Seeds (RAC)	<0.05		<0.05			Indicative PFs not possible*
Raw oil	<0.05		nd			Indicative PFs not possible*
Crude oil	<0.05		<0.05			Indicative PFs not possible*
Press cake	<0.05		<0.05			Indicative PFs not possible*
Refined Oil	<0.05		<0.05			Indicative PFs not possible*

nd = not determined (= not sought – more fractions were analysed for residues from trial conducted in NEU compared to the trial conducted in SEU).

*Whilst two trials were conducted, it is not possible to derive any estimated processing factor if residues in the RAC and the processed fraction are <LOQ. Therefore an indicative PF could only be suggested from the available data.

$ if a cell in the table is left blank, it means a processing factor cannot be derived for this trial from the available data.

The submitted processing studies attempted to assess the impact of processing on the magnitude of residue levels of bixlozone, and other analytes (2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone and 2,2-dimethyl-3-hydroxy propionic acid) over processing of wheat and relevant fractions. For all analytes tested, <LOQ residues were observed in the RAC in at least one of the two analysed trials. The applicant aimed to increase chances of getting

positive residues by carrying out the application with a shortened PHI (28-33 days in cereals, around 45 days in oilseed rape). However, there was still a high frequency of <LOQ results observed throughout the trials. The applicant provided acceptable method validation work for the analytes in some processed fractions. The submitted trials used field trials to generate incurred residues for the processing study, which was then assessed by following a scheme of practice simulating industrial practice at a laboratory scale. Full details of each of the simulated processes were provided. The derivation of processing factors is complicated in view of the frequent finding of results below the LOQ (which was seen in both RAC and processed fraction results).

The available data on the magnitude of processing data (studies on wheat, barley, maize and oilseed rape) seem acceptable for the analytes of bixlozone, 2,4-dichlorobenzoic acid (M190/1), 2,2-dimethyl-3-hydroxy-propionic acid (M118/1) and 5'hydroxy bixlozone (M289/3) based on the levels of residues that are expected to arise following the currently proposed uses in cereals and oilseed rape (noting also that the provision of these data is not strictly needed at the current time due to low residues in the RACs). Data might be needed to support more robust processing factors covering all possible analytes of the RD-RA if proposed future uses were to lead to higher levels of residues. Any new data should be generated in accordance with OECD test guideline 508, using suitable components of the residue definition and suitably validated analytical methods, and should be supported by adequate freezer storage stability of residues data.

2.7.7. Summary of residues in rotational crops

Representative uses on wheat, barley, maize and oilseed rape can be grown in rotation and field soil degradation studies indicate the DT_{90} value for bixlozone is a maximum of 819 days, based on a DT_{50} of 247 days (Volume 3, section CP.B.8.2.2). Therefore, a consideration of residues in rotational crops is required, and there is the potential for accumulation over multiple years of use. The plateau concentrations for bixlozone are given in Table 2.7.7.1. There are no major soil metabolites for bixlozone and therefore no potential for accumulation of soil metabolites over multiple years of use.

Table 2.7.7. 1 Maximum seasonal application rates and soil plateau concentrations for the uses of 'F9600-4 SC'

Crop	Proposed maximum seasonal application rate (g a.s./ha)	Application rate required to achieve: (g a.s./ha)	
		Soil plateau concentration $A_{plateau-£}$	Soil plateau plus maximum seasonal application rate $A_{total-£}$
Wheat	200	112.04	312.04
Barley	200	112.04	312.04
Maize	375	210.07	585.07
Oilseed rape	300	168.06	468.06

£- The fate and behaviour evaluation derivations of $A_{plateau}$ and A_{total} are provided in section CP.B.8.2.2

Nature of the residue

Some remarks made on how the plant metabolism studies have been carried out are also relevant to the rotational crop metabolism study. Please therefore refer to the remarks made on plant metabolism in section 2.7.1 (introductory remarks on 'sample storage periods', including regarding additional uncertainty relating to long periods of freezer storage of samples, including extracts in the rotational crop metabolism study, and also 'general remarks' at the start of this section).

The full evaluation of the rotational crop metabolism study is provided in full in Vol 3, Section B.7.6.1. Some residue evaluation remarks are made at the end of the detailed evaluation of the study just prior to the Figure that provides the applicant's proposed metabolic pathway for rotational crops.

In the nature of residues study, bixlozone, either phenyl labelled or carbonyl labelled, was applied at around 300 g as/ha to bare soil in a confined rotational crop setting. Rotational crops of lettuce (representative of leafy crops), radish (representative of root and tuber vegetable crops), and wheat (representative of cereal crops), were grown in plastic lined wooden boxes containing soil into which the rotational crops were planted at different replant

intervals. The replant intervals were 30 DAT (days after treatment) or 63 DAT, 120 DAT or 153 DAT, and 310 DAT for each crop.

Comparisons to the GAP 'N' rates for the achieved application rates were as follows:

> Considering Maximum seasonal application rates only: around 1.5N with regard to the wheat and barley GAP, 1N with regard to oilseed rape GAP and around 0.8N with regard to the maize GAP.
>
> Considering Maximum seasonal application rates and the potential soil plateau contribution from year on year use: around 0.9N with regard to the wheat and barley GAP, 0.6N with regard to oilseed rape GAP and around 0.5N with regard to the maize GAP.

The overall residue levels (TRR) were broadly comparable across the different labels (results for phenyl label versus results for carbonyl label). TRRs in lettuce were 0.02 to 0.09 mg/kg (63 DAT), 0.03 to 0.08 mg/kg (153 DAT) and 0.004 to 0.008 mg/kg (310 DAT). The pattern across all crops was that TRRs were markedly lower in the final timepoint (310 DAT). TRRs in radish tops were 0.09 to 0.21 mg/kg (30 DAT), 0.06 to 0.11 mg/kg (120 DAT) and 0.005 to 0.017 mg/kg (310 DAT). TRRs in radish roots were around 0.05 mg/kg (30 DAT), around 0.03 (120 DAT) and 0.005 to 0.012 mg/kg (310 DAT). TRRs in wheat grain were 0.02 to 0.04 mg/kg (30 DAT), 0.02/0.03 mg/kg (120 DAT) and around 0.008 mg/kg (310 DAT). TRRs in wheat forage/hay or straw were 0.16 to 0.59 mg/kg (30 DAT), 0.13 to 0.34 mg/kg (120 DAT) and 0.03 to 0.11 mg/kg (310 DAT).

Solvent extraction of the samples involved thrice extraction with acetonitrile/water (3x, 80:20) and in the case of wheat followed by (2x) extraction with methanol: water (50:50). Extracts were subject to acid hydrolysis (1N HCL) under reflux and then analysed. Results were presented in detail for extracts 'post acid hydrolysis'. For both labels, solvent extractability was

For both labels, solvent extractability was fairly high for all samples (at least 67% TRR, with much higher levels of extractability for many of the commodity matrices) with the exception of wheat grain where extractability was ≈45-55% TRR (0.008-0.021 mg/kg).

Enzyme hydrolysis using various enzymes sequentially of the PES was carried on some of the wheat, lettuce and radish samples to varying degrees. Also, acid and base treatments were used in wheat samples, and base hydrolysis was found to be the most successful method of releasing radioactivity from the PES, 1N and 6N NaOH treatments.

Despite efforts to release and identify radioactive residues, a large number of extracted residues remained as unknowns especially in commodities such as wheat hay and straw (that are not consumed directly by humans). Taken together they could represent appreciable amounts of radioactivity. The applicant has indicated the levels of maximum individual unknowns, and some of these did represent >10%TRR (please see the breakdown for 'unknowns' below the metabolite distribution tables for further details). For crop items that can be consumed directly by humans the maximum unidentified levels were very low and all were <0.01 mg/kg and for crop items that are animal feed items the maximum individual levels were generally low, and at a maximum level of 0.04 mg/kg.

Residues of a similar nature were found at the different PBIs (plant back intervals). As residues were markedly lower at the longest replant interval (310 DAT), the consideration of key metabolites below (table as presented in section 2.7.3 on residue definition) found in rotational crops is from the earlier replant times of 30 to 153 DAT.

Whilst the level of metabolites in the rotational crop metabolism samples varied according to matrix (and label being studied), when looking at the highest amounts of metabolites found, the most prevalent metabolites (>10%TRR and >0.01 mg/kg) found were:

Residues	Metabolite Code	Highest level found	Crop matrix (and PBI in DAT) in which this highest amount was observed
Contrary to primary crop metabolism parent bixlozone was found in rotational crops at all replant intervals: Bixlozone (F9600)		 76%TRR and 0.034 mg/kg 26%TRR and 0.054 mg/kg	 Radish root at PBI 53 DAT Radish top at PBI of 53 DAT
2,4-dichlorobenzoic acid	M190/1	30%TRR and 0.011 mg/kg 14%TRR and 0.029 mg/kg	Wheat grain at PBI of 30 DAT Wheat forage at PBI of 30 DAT
Bixlozone-dimethyl-malonamide	M289/2	46%TRR and 0.015 mg/kg	Immature lettuce at PBI of 153 DAT
Bixlozone-hydroxy-isobutyramide	M261/1	27%TRR and 0.055 mg/kg 37%TRR and 0.040 mg/kg	Radish top at PBI of 53 DAT Radish top at PBI of 53 DAT
4-hydroxymethyl- bixlozone	M289/4	11%TRR and 0.012 mg/kg	Radish top at PBI of 153 DAT
5'-hydroxy-bixlozone	M289/3	37%TRR and 0.132 mg/kg 42%TRR and 0.095 mg/kg	Wheat straw at PBI of 30 DAT Wheat straw at PBI of 120 DAT
Dimethyl malonic acid	M132/1	44%TRR and 0.153 mg/kg 46%TRR and 0.038 mg/kg	Wheat hay at PBI of 153 DAT Immature lettuce at PBI of 153 DAT

All of the above (tabulated) six 'major' metabolites, except for dimethyl malonic acid (M132/1), and bixlozone were analysed for in the follow-on rotational crop field trials.

Other rotational crop metabolites that were not sought in the follow on rotational crop field trials were found at lower levels in the rotational crop metabolism study: dihydroxy-bixlozone conjugate (M467/1) (23%TRR but low 0.006 mg/kg level), a hydroxy glucoside conjugate of bixlozone (M451/2) (3%TRR and low <0.001 mg/kg level), and 3'-hydroxy-bixlozone (M289/6) (7%TRR, and 0.015 mg/kg).

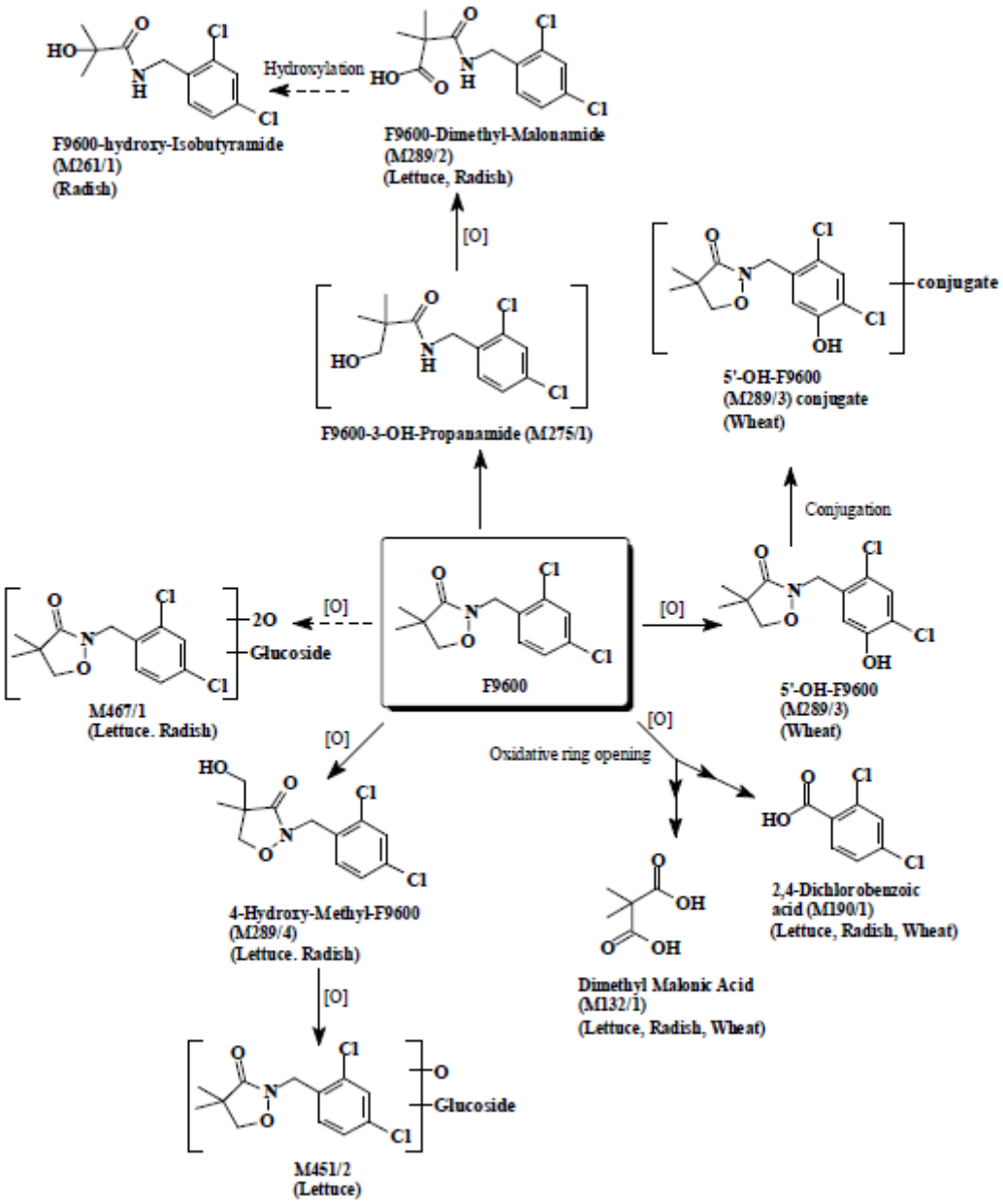
Metabolism of bixlozone in rotational crops includes primarily, oxidative ring opening. Hydroxylation and conjugation were also observed. Unchanged parent bixlozone was detected in radish roots and tops, accounting for the largest proportion of TRR in most samples (~17-76% TRR). In lettuce samples bixlozone-dimethyl-malonamide (M289/2) accounted for the highest proportion of the radioactive residue in phenyl labelled samples (accounting for ~27-46% TRR) whereas in carbonyl labelled samples the highest proportion of the residue was accounted for by dimethyl malonic acid (M132/1) (~27-47% TRR). A very low amount of unchanged parent bixlozone was found in lettuce (0.002 mg/kg). Similar to the primary crop metabolism, parent was not found in wheat. Also, as per the primary crop situation, wheat forage, hay and straw, contained 5'-hydroxy-bixlozone (M289/3) which accounts for the main proportion of the radioactivity (~16-43% TRR). Dimethyl malonic acid (M132/1) was also detected in high proportions (~5-44% TRR). In wheat grain 5'-hydroxy-bixlozone (M289/3) was not detected, and the major metabolite detected was 2,4-dichlorobenzoic acid (M190/1) (accounting for 25-30% TRR).

The work on the nature of residues in rotational crops has enabled a profiling of an overall metabolic pathway suitable for rotational crops. Based on the applicant's proposed metabolic pathway, whilst some differences are observed the main patterns of metabolism are the same as those observed in the primary crop metabolism. Oxidative ring opening was a prime metabolic route in rotational crops, leading to formation of dimethyl malonic acid (M132/1) in rotational crops, In the rotational crop metabolism, parent bixlozone was also found (this was not the case in the primary crop metabolism) and hydroxylation and conjugation was also observed. Main rotational crop residues observed in the metabolism study also included 2,4-dichlorobenzoic acid (M190/1), 5'-hydroxy-bixlozone (M289/3), bixlozone-dimethyl-malonamide (M289/2) and bixlozone-dimethyl-isobutyramide (M261/1, also termed bixlozone hydroxy isobutyramide).

The summary of metabolism tables in section 'definition of residue' in section 2.7.3 provide a full overview of metabolites found and their levels in the rotational crop metabolism studies, representing the distribution of metabolites in the acid hydrolysed extract (acid hydrolysed following initial solvent extraction).

The applicant's proposed metabolic pathways for rotational crops is presented below in Figure 2.7.2.7 below.

Figure 2.7.2.7: Applicant's proposed metabolic pathway of bixlozone in rotational crops



Magnitude of the residue

Two outdoor field trials on three rotational crops (wheat, radish and lettuce) were conducted. Maize was grown as a primary crop and the formulation 'F9600-4 SC' applied at growth stage BBCH 11-13, at a rate of 300 g a.s./ha. Using the standard FOCUS crop interception values, as suggested in the OECD guidance on residues in rotational crops, for application to maize at BBCH 10-19, 25% crop interception should be considered when considering the amount of active substance reaching the soil. In this case, the application was made to maize at BBCH 11-13, therefore an interception value of 10% is considered a more realistic estimate. BBCH 19 represent nine or more leaves unfolded and 11-13 BBCH represents one to three leaves unfolded, both in the 'leaf development stage' principal growth stage 1 which follows 'germination stage' principal growth stage 0, which ends at BBCH 09 ('emergence' when coleoptile penetrates soil surface). A comparison of the application rate in this study to the proposed maximum seasonal rate in cereals and oilseed rape is shown in Table 2.7.7.2.

Table 2.7.7. 2 Maximum seasonal application rates and plateau concentrations compared to the application rate used in the rotational crop field trials

Proposed crop (GAP intended use)	Proposed maximum seasonal application rate (g a.s./ha)	Application rate required to achieve: (g a.s./ha)		Application rate in rotational field trial (g a.s/ha)	Application rate in rotational field trial considering 10% crop interception (g a.s/ha)	N rate (application rate in trial compared to maximum seasonal application rate)	N rate (application rate in trial compared to soil plateau plus maximum seasonal application rate)
		Soil plateau concentration $A_{plateau}$ - £	Soil plateau plus maximum seasonal application rate A_{total} - £				
Wheat	200	112.04	312.04	300	270	1.35	0.87
Barley	200	112.04	312.04	300	270	1.35	0.87
Maize	375	210.07	585.07	300	270	0.72	0.46
Oilseed rape	300	168.06	468.06	300	270	0.9	0.58

£- The fate and behaviour evaluation derivations of $A_{plateau}$ and A_{total} are provided in section CP.B.8.2.2

Wheat, radish and lettuce were planted approximately 30, 60 or 220 days after foliar spray to the primary crop maize. A plant back interval that covers crops rotated the following year (270-365 days) was not investigated as part of these trials. However, the rotational crop metabolism study showed that residues of metabolites and parent bixlozone would be lower at a later timing. The rotational crop metabolism study showed that the nature of the residues was similar at the different replant intervals and all residues (total radioactive residues, and levels of individual metabolites) were found at markedly lower levels at the 310 day plant back interval compared to the earlier plant back intervals (30 to 153 days). Therefore, additional field trial data reflecting a later plant back interval data is not considered necessary to address this.

For all crop groups, cereals, root/tuber and leafy vegetables, residue levels of bixlozone metabolites: 2,4-dichlorobenzoic acid, 5'-hydroxy-bixlozone, bixlozone-dimethyl-malonamide, bixlozone-hydroxy-isobutyramide and 4-hydroxymethyl-bixlozone, in samples from the treated plots were below the LOQ of 0.01 mg/kg at all plant back intervals. Residue levels of bixlozone were also below the LOQ of 0.01 mg/kg, with the exception of two low positive residues of bixlozone detected in the 229 day PBI samples for radish tops and immature lettuce leaves (0.013 and 0.011 mg/kg respectively) from the NEU trial.

A good range of potential metabolites (as informed by the rotational crop metabolism study) have been studied in addition to parent bixlozone. It should be noted that the primary crop metabolite 2,2-dimethyl-3-hydroxy propionic acid was not studied in these field trials (nor the rotational crop metabolism study). Given the positive residues of bixlozone observed in these field trials, for any future rotational crop field trials, parent bixlozone appears to be the most suitable indicator analyte to explore the potential for soil residues to be taken up into crops grown in rotation. However, if significant residues of bixlozone are found, it may also be necessary to consider the potential for some metabolite residues to be also found.

In the magnitude of residues study, bixlozone was applied at 300 g a.s./ha to the primary crop maize. Considering 10% crop interception from this primary crop, the amount of active substance estimated to reach the soil is 270 g a.s./ha. The application rate, whilst covering the maximum seasonal rate for the intended uses of wheat and barley (200 g a.s./ha), does not cover the intended primary crop application rate for the oilseed rape (300 g a.s./ha) or maize GAPs (375 g a.s./ha). The application rate used is also underdosed with regard to the soil plateau concentration plus the maximum seasonal application rate (0.46 – 0.87N). Given the majority of results are <LOQ it is not possible to scale up the results to estimate the expected residue levels following an application intended to represent the soil plateau concentration plus the maximum seasonal application rate for each proposed use, although it is noted that a good range of metabolites were selected based on their prevalence in the rotational crop metabolism study, and in the field trials all the metabolites sought were not found. The two positive results of bixlozone in representative crops of leafy vegetables are indicative of infrequent occurrence of residues of parent in rotational crops, and can be scaled up. Given there are only two positive residue data points, and as bixlozone is acutely toxic, it is reasonable to scale up the higher of these two data points, giving an estimated highest residue of 0.028 mg/kg, which may be expected in leafy crops (and other above ground vegetables in an overall worst case rotational crops assessment, in the absence of further rotational crops data) following the maximum seasonal application rate for maize (highest application rate in the proposed GAPs) and accumulation in the soil following multiple years of use. Considering an estimated median residue, as positive results of bixlozone were found in one trial (0.013 and 0.011 mg/kg in radish tops and immature lettuce leaves respectively) and residues of bixlozone were <0.01 in the other field trial, it seems reasonable to estimate a median residue of 0.01 mg/kg expected in leafy crops (and other above ground vegetables).

As positive residues may be found in leafy crops grown in rotation, further consideration of either MRLs to accommodate these possible residues or a plant back restriction to mitigate any possible positive residues has been made. For both of these options, further data (additional rotational crop field trials) are required to either support the proposed MRLs or remove the proposed plant back restriction.

If a plant back restriction is recommended, significant residues would not be expected in crops grown in rotation (see discussion below). However, an indicative risk assessment considering the possible positive residues that may be found in leafy crops grown in rotation has been performed. In the case that MRLs were set, this consideration of the associated risk may be useful. It is noted that the available data set is limited therefore this risk assessment should only be considered indicative and if further data on residues in crops grown in rotation becomes available this will require further consideration. As discussed above, a HR of 0.028 mg/kg and an STMR of 0.01 mg/kg are considered reasonable estimates of the expected residues, based on the currently available information. It is noted that higher residues were observed in the rotational crop metabolism study (up to 0.054 mg/kg of bixlozone in radish leaves at the 30 day plant back interval). However, the confined rotational crop metabolism study appears more critical, possibly due to the conduct of the study (radiolabelled metabolism study with limited area of application). Therefore, the field trial results may be more representative and are supported by validated analytical methods. Additionally, data at the 'short' plant back interval from two independent field trials shows residues of bixlozone are <0.01 mg/kg. Therefore, values from these field trials have been taken forward into the dietary burden estimates and consumer risk assessments. It should be noted that these estimates are indicative with regards to expected residues in rotational crops; as discussed below if a plant back restriction is recommended, residues above the LOQ would not be expected in crops grown in rotation. Similarly, additional data is required to support MRLs in leafy crops and above ground vegetables grown in rotation and the risk assessments would be re-visited in light of these data.

Using these HR and STMR inputs for leafy crops and other above ground vegetables in the animal dietary burden estimations (see section 2.7.5) and in the acute and chronic consumer risk assessments (see section 2.7.9) the dietary burden is not triggered (based on the contribution from possible residues in rotational crops) and there are no consumer risk concerns.

<u>Option 1 - MRLs for leafy crops grown in rotation</u>

Setting MRLs to accommodate these possible positive residues, which may exceed the default MRLs at the LOQ (0.01 mg/kg), may be the least restrictive option (no restrictions on which crops can be grown in rotation). However, the number of available rotational crop field trials is limited (2 trials) with insufficient data points to set a robust MRL, using the OECD calculator. Additionally, the available field trials tested a small range of leafy crops (lettuce and radish (tops)), whereas the MRLs required to accommodate these possible residues will apply to a much wider range of edible crops. Further data showing possible residues on a wider range of leafy crops and above ground vegetables which may be grown in rotation would be required to support any proposed MRLs.

It is noted (see section 2.7.4) that residues of parent bixlozone are not expected to be found in primary crops considering the proposed intended uses and so a consideration of an MRL level is only needed in regard of

potential residues of bixlozone that might be found in rotational crops. Further rotational crop field trials data would be required to ensure these MRLs are fully supported. These rotational crop field trials should investigate a sufficiently high application rate and analyse for key marker residues to consider possible residues resulting from the maximum seasonal application rate plus accumulation in soil, and range of crops to enable robust MRL setting.

Considering the available data, MRLs of 0.05 mg/kg appear reasonable to accommodate potential residues in leafy vegetables and above ground vegetables grown as rotational crops. However, setting MRLs when data is limited is not ideal. If MRLs were proposed for rotational crops, these would be temporary MRLs with an associated data requirement and data submission deadline to support permanent MRLs. If this data requirement was not addressed before the deadline, plant back restrictions would be required. Hence, it may be more appropriate to recommend plant back restrictions in the first instance.

Option 2 - Possible plant back restrictions

To avoid any positive residues above the default MRL at the LOQ (0.01 mg/kg) for leafy vegetables grown in rotation, the crops which can be grown following use of bixlozone could be restricted. However, the available data do not clearly support an obvious plant back interval after which residues are expected to be <LOQ. A summary of the residues of parent bixlozone found in both the rotational crop metabolism and field studies after each plant back interval tested are given in Table 2.7.7.3.

Table 2.7.7. 3 Summary of residues of bixlozone (mg/kg) found in leafy crops grown in rotation

		Plant back interval (days)										
Study	**Sample**	**Short**			**Medium**							**Long**
		27	30	40	56	63	69	120	153	229	230	310
Field trial 1 (0.46 N[$])	Radish (leaves)	<0.01	-	-	<0.01	-	-	-	-	**0.013**	-	-
	Lettuce (immature)	<0.01	-	-	<0.01	-	-	-	-	**0.011**	-	-
Field trial 2 (0.46N[$])	Radish (leaves)	-	-	<0.01	-	-	<0.01	-	-	-	<0.01	-
	Lettuce (immature)	-	-	<0.01	-	-	<0.01	-	-	-	<0.01	-
Metabolism study (0.51N[$])	Lettuce (immature and mature, both labels)	-	-	-	-	<0.01 *(0.002)*	-	-	<0.01 *(0.001)*	-	-	<0.01 *(TRR 0.008)*
	Radish (leaves, phenyl label)	-	**0.022**	-	-	-	-	<0.01 *(<0.001)*	-	-	-	<0.01 *(0.003)*
	Radish (leaves, carbonyl label)	-	**0.054**	-	-	-	-	**0.01**	-	-	-	<0.01 *(TRR 0.005)*

[$] 0.46 N or 0.51 N relate to considering the application rate in these studies compared to the most critical GAP in terms of application rate and associated accumulation (maize).

Positive residues above the LOQ are indicated in bold text. Where samples were not taken at the plant back interval stated, this is indicated by a dash. Where residues <0.01 mg/kg of bixlozone were determined in the metabolism study, the estimate of the exact residue level is presented in brackets and italicised text also. In the case of lettuce and radish leaves (carbonyl label) grown after the 310 day plant back interval, in the metabolism study, further analytical work was not done given the low absolute residue level of the TRR for these samples. Therefore, these TRR values are presented in the table.

Table 2.7.7.3 shows that positive residues are observed in crops representative of leafy crops after 'short' and 'medium' term plant back intervals (residues up to 0.054 mg/kg observed at 'short' 30 day PBI and up to 0.013 mg/kg observed at 'medium' 229 day PBI). It should be noted that these results were determined using under-dosed application rates considering the possible contribution from year on year use. There is a general pattern of residues decreasing with increased plant back interval time.

Although there is no quantitative data from a rotational crop field trial, the available metabolism data indicate that residues are likely to be <LOQ in leafy crops grown in the following year. Therefore, a plant back restriction preventing the planting of leafy crops and above ground vegetables up to 310 days following application of bixlozone is likely to avoid significant (>0.01 mg/kg) residues in these crops.

The restriction of possible uses of bixlozone when there are no consumer risk concerns may seem overly restrictive. It should be noted that due to potential phytotoxicity and the persistence of bixlozone in soil, some of these leafy crops may not be realistically grown as following crops after use of this active substance, especially in the event of crop failure. (It is noted that in the rotational crop metabolism study, some leafy crops did not grow following a short plant back interval and a lower dose was used to generate some 30 DAT results, demonstrating the potential issues growing these crops in rotation, especially at early plant back intervals).

Conclusion

Given the consideration of option 1, setting MRLs, and option 2, recommending a plant back restriction above, recommending a plant back restriction seems the most reasonable option. Therefore, the following should be included in the approval of bixlozone: **leafy crops and above ground vegetables must not be planted until at least 10 months after application of bixlozone.**

It should be noted that this recommendation is based upon the consideration of the representative uses of 'F9600-4-SC' proposed as part of this new active substance evaluation; any future GAPs may require further consideration. Additionally, for future products containing bixlozone, the product labels and conditions of authorisation should reflect this plant back restriction, where required.

To address this concern with positive residues in leafy crops grown in rotation, and refine or remove this plant back restriction, or, to support MRLs for leafy crops and above ground vegetables grown in rotation, further data are required.

Additional rotational crop field trials are required in accordance with OECD guidance 2018*, either to remove or refine the plant back restriction or to support MRL setting. Further trials could also be needed to support overall increases in dose rates for future uses. These rotational crop field trials should investigate a sufficiently high application rate and analyse for key marker residues to consider possible residues resulting from the maximum seasonal application rate plus accumulation in soil, and a range of crops representative of 'leafy' crops, including above ground vegetables (note: additional data on above ground vegetables and leafy crops which are animal feed items may be beneficial to demonstrate the expected levels in these crops also). All new rotational crop field trials generated should be conducted using suitable components of the residue definition, using suitably validated analytical methods, and should be supported by adequate freezer storage stability of residues data, where needed.

*https://www.oecd.org/chemicalsafety/guidance-document-on-residues-in-rotational-crops-99457f3f-en.htm

2.7.8. Summary of other studies

Literature studies:

HSE concludes that regarding the literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to this residues risk assessment.

Effect on the residue level in pollen and bee products:

At the date of submission (29/6/2018) there were no agreed EU guidance documents or test methods to address these data requirements. Since submission the Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey **(**SANTE/11956/2016 rev. 9) have been noted with an agreed implementation date of 1st January 2020.

The applicant has submitted the information in Volume 3, section 7.7.1 based on a draft version of the guidelines. The applicant's case was based upon the proposed applications to primary crops being made pre- and early post-emergence, i.e. prior to flowering.

HSE further considered the potential risk to consumers if residues that have been observed in flowers transfer into honey. Wheat, barley and maize are not considered melliferous crops, whereas oilseed rape is a melliferous crop. Data from the oilseed rape primary crop field trials confirms that residues of bixlozone and 2,4-dichlorobenzoic acid in aerial parts of crops (results for flowers) are likely to be low (2,4-dichlorobenzoic acid residues <0.01 mg/kg, bixlozone residues up to 0.012 mg/kg in NEU field trials; up to 0.048 mg/kg in SEU field trials). Using the NEU data only (STMR & HR of 0.04 mg/kg, based on residues as sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone (see section 2.7.3), the acute and chronic intakes based on these residues are expected to contribute <1% of the ADI and 0.02% of the ARfD. Residues of 2,2-dimethyl-3-hydroxy propionic acid were found at up to 0.06 mg/kg in flowers. As there are no agreed toxicological reference values for this substance, an exposure assessment for residues of 2,2-dimethyl-3-hydroxy propionic acid was performed, indicating that estimated exposures did not exceed the threshold of toxicological concern (TTC CCI). See section 2.7.3 above for further details. There is no significant risk to consumers with regards to honey, based on proposed uses.

The MRL for bixlozone in honey is proposed at approval at 0.05 mg/kg (default MRL for honey). It should be noted that a monitoring method for residues in honey is not currently available and not required at this time.

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

Two sets of consumer risk assessment have been conducted to predict the chronic exposure scenarios for consumers, based on the predicted levels as sum of bixlozone and 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone (see section 2.7.3), for primary crops and bixlozone for rotational crops, within food items.

The first of these approaches utilises the UK national calculator and considers a diverse range of consumer groups relevant to the UK. The second uses the EFSA PRIMo version 3.1 calculator to predict the dietary intakes for consumer groups across the EU. An assessment has been performed for the primary crop uses and considering the possible residues in rotational crops.

The following toxicological reference values have been used in the consumer risk assessments:

ADI (mg/kg bw/day)	0.3
ARfD (mg/kg bw)	0.75

For a full consideration of the metabolites 2,2-dimethyl-3-hydroxy propionic acid and dimethyl malonic acid and the TTC approach, please refer to section 2.7.3.

The TMDI has been calculated using the RD-Enf (bixlozone). The NEDI/IEDI and NESTI/IESTI have been calculated using the RD-RA (bixlozone + 2 x 2,4-dichlorobenzoic acid, expressed as bixlozone for primary crops, bixlozone for rotational crops).

The following estimates consider the proposed uses on oilseed rape, wheat, barley and maize. The risk assessment for rotational crops is indicative only, demonstrating that there are no consumer risk concerns with the possible residues in crops grown in rotation, but either a plant back restriction (or new MRLs) are required from an MRLs perspective.

Chronic (long term) UK dietary intake estimates – UK NTMDI and NEDIs

The UK NEDIs for the active and commodities listed below have been calculated for ten consumer groups as detailed in the Regulatory Update 21/2005. The following assumptions have been made:

1) Upper range of normal (97.5th percentile) consumption of each individual crop which may have been treated.

2) All produce eaten which may have been treated has been treated and contains residues at the MRL (NTMDI) or median residue (STMR) (NEDI) found in the trials to support the GAP, as given below

Commodity	***NTMDI***		***NEDI***	
	MRL (mg/kg)		**STMR (mg/kg)**	
Fruiting vegetables (RC)	0.05	Indicative MRL level based on HR of 0.028 from rotational crops field trial data	0.01	Indicative STMR based on rotational crop field trials data (See Vol 1 section 2.7.7)
Brassica vegetables (RC)	0.05		0.01	
Leaf vegetables, herbs and edible flowers (RC)	0.05		0.01	
Legume vegetables (RC)	0.05		0.01	
Stem vegetables (RC)	0.05		0.01	
Pulses (RC)	0.05		0.01	
Oilseed rape seed	0.01	Proposed MRL	0.039§	STMR from field trials data
Barley grain	0.01	Indicative MRL	0.039§	
Wheat grain	0.01		0.039§	
Maize grain	0.01		0.039§	
Honey$	-	-	-	-

$ Honey is not a commodity that can be input into the UK consumer risk assessment models.
§ Residues of 2,4-dichlorobenzoic acid doubled to account for this substance being twice as toxic as parent bixlozone. By doubling the residue levels for this metabolite, a risk assessment can be performed using the toxicological endpoints for parent bixlozone. (e.g. bixlozone residue 0.01 mg/kg + 2,4-dichlorobenzoic acid residue 0.01 mg/kg x 1.435 MW conversion x 2 to account for toxicity = 0.039 mg/kg)

3) There is no loss of residue during transport or storage, or processing of foods prior to consumption.

The relevant intakes are presented in Table 2.7.9.7 and Table 2.7.9.8.

For the NTMDI, chronic intakes for all consumer groups are below the ADI of 0.3 mg/kg bw/day, UK intakes estimated as <1 % of the ADI (critical consumer group toddlers). For the NEDI, chronic intakes for all consumer groups are below the ADI of 0.3 mg/kg; all consumer groups have intakes of <1 % ADI (critical consumer group 4-6 year olds). Therefore, no chronic health effects are expected.

Table 2.7.9. 1 UK NTMDI for 10 consumer groups (calculated using chronic consumer version 1.1) for bixlozone

Active substance: Bixlozone **ADI:** 0.3 mg/kg bw/day **Source:** dDAR

		TOTAL INTAKE based on 97.5th percentile									
		ADULT	**INFANT**	**TODDLER**	**4-6 YEARS**	**7-10 YEARS**	**11-14 YEARS**	**15-18 YEARS**	**VEGETARIAN**	**ELDERLY (OWN HOME)**	**ELDERLY (RESIDENTIAL)**
mg/kg bw/day		0.00029	0.00057	0.00065	0.00050	0.00042	0.00031	0.00033	0.00033	0.00029	0.00019
% of ADI		<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%

Commodity	**STMR (mg/kg)**	**P**	**COMMODITY INTAKES (mg/kg bw/day)**									
Tomatoes	0.05		0.00007	0.00009	0.00013	0.00010	0.00009	0.00005	0.00007	0.00009	0.00007	0.00007
Peppers	0.05		0.00002	L/C	0.00004	0.00002	0.00003	0.00002	0.00001	0.00003	0.00003	0.00001
Aubergines	0.05		0.00002	L/C	0.00008	0.00004	0.00001	0.00003	0.00002	0.00003	0.00002	L/C
Marrows	0.05		0.00003	L/C	0.00008	0.00002	0.00003	0.00003	0.00001	0.00003	0.00007	0.00003
Cucumbers	0.05		0.00002	0.00001	0.00012	0.00008	0.00005	0.00003	0.00002	0.00003	0.00002	0.00001
Gourd	0.05		0.00003	L/C	L/C	L/C	L/C	0.00001	L/C	0.00001	L/C	L/C
Courgettes	0.05		0.00002	0.00007	0.00012	0.00006	0.00004	0.00002	0.00002	0.00003	0.00003	0.00002
Melons	0.05		0.00012	0.00015	0.00026	0.00018	0.00015	0.00011	0.00014	0.00013	0.00015	0.00005
Sweet corn	0.05		0.00003	0.00005	0.00011	0.00005	0.00006	0.00002	0.00003	0.00003	0.00004	0.00002
Broccoli	0.05		0.00003	0.00006	0.00009	0.00006	0.00005	0.00004	0.00003	0.00003	0.00005	0.00002
Cauliflower	0.05		0.00004	0.00016	0.00011	0.00008	0.00004	0.00004	0.00004	0.00006	0.00006	0.00003
Brussels sprouts	0.05		0.00003	0.00012	0.00009	0.00007	0.00004	0.00005	0.00003	0.00004	0.00005	0.00002
Head cabbage	0.05		0.00003	0.00009	0.00009	0.00006	0.00004	0.00004	0.00003	0.00004	0.00006	0.00004
Chinese cabbage	0.05		0.00002	L/C	L/C	L/C	L/C	L/C	L/C	0.00003	0.00002	L/C
Kohl Rabi	0.05		L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Cress	0.05		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Lettuce	0.05		0.00003	0.00002	0.00004	0.00003	0.00004	0.00002	0.00002	0.00004	0.00003	0.00001
Spinach	0.05		0.00003	0.00005	0.00008	0.00004	0.00004	0.00003	0.00002	0.00003	0.00003	0.00002
Watercress	0.05		0.00001	L/C	L/C	0.00000	0.00000	0.00001	L/C	0.00001	0.00002	L/C
Chicory	0.05		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C	L/C
Parsley	0.05		0.00001	L/C	0.00001	L/C	0.00001	0.00000	0.00000	0.00001	0.00001	0.00002
Beans with pods	0.05		0.00003	0.00006	0.00010	0.00007	0.00003	0.00002	0.00004	0.00002	0.00004	0.00002
Runner Beans	0.05		0.00003	L/C	0.00007	0.00002	0.00004	0.00003	0.00002	0.00008	0.00005	0.00003
Beans without pods	0.05		0.00002	0.00003	0.00012	0.00002	0.00005	0.00002	0.00002	0.00003	0.00004	0.00003
Peas with pods	0.05		0.00001	L/C	0.00002	0.00006	0.00001	0.00001	0.00001	0.00001	0.00003	L/C
Peas without pods	0.05		0.00004	0.00012	0.00010	0.00007	0.00005	0.00004	0.00005	0.00004	0.00005	0.00004
Beansprouts	0.05		0.00002	L/C	0.00004	0.00003	0.00003	0.00002	0.00001	0.00002	0.00003	0.00001
Asparagus	0.05		0.00002	L/C	L/C	L/C	L/C	L/C	0.00001	0.00004	0.00002	L/C
Bamboo shoots	0.05		0.00001	L/C	0.00001	L/C	0.00000	0.00001	0.00000	0.00001	0.00000	L/C
Celery	0.05		0.00002	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00002	0.00002	0.00001
Fennel	0.05		0.00002	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Globe artichokes	0.05		0.00002	L/C	L/C	L/C	L/C	L/C	L/C	0.00001	L/C	L/C
Leeks	0.05		0.00002	L/C	0.00003	0.00002	0.00002	0.00002	0.00001	0.00002	0.00003	0.00002
Rhubarb	0.05		0.00002	0.00004	0.00006	0.00001	0.00003	0.00001	0.00001	0.00002	0.00003	0.00002
Beans	0.05		0.00008	0.00030	0.00024	0.00017	0.00014	0.00010	0.00011	0.00009	0.00007	0.00005
Lentils	0.05		0.00004	0.00007	0.00009	0.00010	0.00003	0.00006	0.00003	0.00004	0.00003	0.00001
dried Peas	0.05		0.00003	L/C	0.00009	0.00002	0.00004	0.00006	0.00003	0.00003	0.00005	0.00004
Oilseeds	0.01		0.00003	0.00006	0.00007	0.00007	0.00006	0.00004	0.00004	0.00005	0.00003	0.00004
Barley	0.01		0.00000	L/C	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000
Maize	0.01		0.00000	0.00005	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Wheat	0.01		0.00004	0.00003	0.00008	0.00009	0.00007	0.00005	0.00004	0.00004	0.00003	0.00003

* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

L/C Low consumption (<0.1 g/day) or low number of consumers (<4)

Body weights of the 10 consumer groups are as detailed in the regulatory update 21/2005.
Residues below the LOQ have been assumed to be at the LOQ for the purpose of this calculation

Table 2.7.9. 2 UK NEDI for 10 consumer groups (calculated using chronic consumer version 1.1) for bixlozone

Active substance: Bixlozone **ADI:** 0.3 mg/kg bw/day **Source:** dDAR

			TOTAL INTAKE based on 97.5th percentile									
			ADULT	INFANT	TODDLER	4-6 YEARS	7-10 YEARS	11-14 YEARS	15-18 YEARS	VEGETARIAN	ELDERLY (OWN HOME)	ELDERLY (RESIDENTIAL)
	mg/kg bw/day		0.00028	0.00055	0.00064	0.00065	0.00050	0.00037	0.00031	0.00037	0.00027	0.00030
	% of ADI		<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
	STMR	P	COMMODITY INTAKES									
Commodity	(mg/kg)		(mg/kg bw/day)									
Tomatoes	0.01		0.00001	0.00002	0.00003	0.00002	0.00002	0.00001	0.00001	0.00002	0.00001	0.00001
Peppers	0.01		0.00000	L/C	0.00001	0.00000	0.00001	0.00000	0.00000	0.00001	0.00001	0.00000
Aubergines	0.01		0.00000	L/C	0.00002	0.00001	0.00000	0.00001	0.00000	0.00001	0.00000	L/C
Marrows	0.01		0.00001	L/C	0.00002	0.00000	0.00001	0.00001	0.00000	0.00001	0.00001	0.00001
Cucumbers	0.01		0.00000	0.00000	0.00002	0.00002	0.00001	0.00001	0.00000	0.00001	0.00000	0.00000
Gourd	0.01		0.00001	L/C	L/C	L/C	L/C	0.00000	L/C	0.00000	L/C	L/C
Courgettes	0.01		0.00000	0.00001	0.00002	0.00001	0.00001	0.00000	0.00000	0.00001	0.00001	0.00000
Melons	0.01		0.00002	0.00003	0.00005	0.00004	0.00003	0.00002	0.00003	0.00003	0.00003	0.00001
Sweet corn	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00000	0.00001	0.00001	0.00001	0.00000
Broccoli	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
Cauliflower	0.01		0.00001	0.00003	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Brussels sprouts	0.01		0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
Head cabbage	0.01		0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Chinese cabbage	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00001	0.00000	L/C
Kohl Rabi	0.01		L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Cress	0.01		0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

Lettuce	0.01		0.00001	0.00000	0.00001	0.00001	0.00001	0.00000	0.00000	0.00001	0.00001	0.00000
Spinach	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00001	0.00000	0.00001	0.00001	0.00000
Watercress	0.01		0.00000	L/C	L/C	0.00000	0.00000	0.00000	L/C	0.00000	0.00000	L/C
Chicory	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C	L/C
Parsley	0.01		0.00000	L/C	0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Beans with pods	0.01		0.00001	0.00001	0.00002	0.00001	0.00001	0.00000	0.00001	0.00000	0.00001	0.00000
Runner Beans	0.01		0.00001	L/C	0.00001	0.00000	0.00001	0.00001	0.00000	0.00002	0.00001	0.00001
Beans without pods	0.01		0.00000	0.00001	0.00002	0.00000	0.00001	0.00000	0.00000	0.00001	0.00001	0.00001
Peas with pods	0.01		0.00000	L/C	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00001	L/C
Peas without pods	0.01		0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Beansprouts	0.01		0.00000	L/C	0.00001	0.00001	0.00001	0.00000	0.00000	0.00000	0.00001	0.00000
Asparagus	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	0.00000	0.00001	0.00000	L/C
Bamboo shoots	0.01		0.00000	L/C	0.00000	L/C	0.00000	0.00000	0.00000	0.00000	0.00000	L/C
Celery	0.01		0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Fennel	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C	L/C
Globe artichokes	0.01		0.00000	L/C	L/C	L/C	L/C	L/C	L/C	0.00000	L/C	L/C
Leeks	0.01		0.00000	L/C	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000
Rhubarb	0.01		0.00000	0.00001	0.00001	0.00000	0.00001	0.00000	0.00000	0.00000	0.00001	0.00000
Beans	0.01		0.00002	0.00006	0.00005	0.00003	0.00003	0.00002	0.00002	0.00002	0.00001	0.00001
Lentils	0.01		0.00001	0.00001	0.00002	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
dried Peas	0.01		0.00001	L/C	0.00002	0.00000	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
Oilseeds	0.039		0.00012	0.00025	0.00028	0.00028	0.00022	0.00016	0.00014	0.00018	0.00012	0.00015
Barley	0.039		0.00001	L/C	0.00001	0.00001	0.00003	0.00001	0.00001	0.00001	0.00001	0.00001
Maize	0.039		0.00000	0.00018	0.00003	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00000
Wheat	0.039		0.00014	0.00011	0.00033	0.00035	0.00026	0.00019	0.00016	0.00017	0.00013	0.00013

* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

L/C Low consumption (<0.1 g/day) or low number of consumers (<4)

Body weights of the 10 consumer groups are as detailed in the regulatory update 21/2005.
Residues below the LOQ have been assumed to be at the LOQ for the purpose of this calculation

Acute (short term) UK dietary intake estimates – UK NESTIs

The UK NESTIs for the active and commodities listed below have been calculated for ten consumer groups as detailed in the Regulatory Update 21/2005. The following assumptions have been made:

1) Upper range of normal (97.5th percentile) consumption of each individual crop which may have been treated.
2) All produce eaten which may have been treated has been treated and contains residues at the highest residue found in the trials considered to support GAP, as given below.

Commodity	***NESTI***	
	HR (STMR for grain, and oilseeds) (mg/kg)	
Fruiting vegetables (RC)	0.028	Indicative HR based on rotational crop field trials data (See Vol 1 section 2.7.7)
Brassica vegetables (RC)	0.028	
Leaf vegetables, herbs and edible flowers (RC)	0.028	
Legume vegetables (RC)	0.028	
Stem vegetables (RC)	0.028	
Pulses (RC)	0.028	
Oilseed rape seed	0.039§	STMR from field trials data
Barley grain	0.039§	
Wheat grain	0.039§	
Maize grain	0.039§	
Honey$	-	-

$ Honey is not a commodity that can be input into the UK consumer risk assessment models.
§ Residues of 2,4-dichlorobenzoic acid doubled to account for this substance being twice as toxic as parent bixlozone. By doubling the residue levels for this metabolite, a risk assessment can be performed using the toxicological endpoints for parent bixlozone. (e.g. bixlozone residue 0.01 mg/kg + 2,4-dichlorobenzoic acid residue 0.01 mg/kg x 1.435 MW conversion x 2 to account for toxicity = 0.039 mg/kg)

3) There is no loss of residue during transport or storage, or processing of foods prior to consumption.

The relevant intake assessment is presented in Table 2.7.9.9.

Acute intakes for all consumer groups are below the ARfD of 0.75 mg/kg bw. The most critical group are 4-6 year old children consuming melon with an estimated consumption of 0.3 % ARfD. Therefore no acute health effects are expected.

Table 2.7.9. 3 UK NESTIs for 10 consumer groups (calculated using acute consumer version 1.2) for bixlozone

Acute Intakes (97.5th percentiles)

			adult		infant		toddler		4-6 year old child		7-10 year old child	
commodity	HR	P	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD
Oilseeds	0 039		0.00023	0.0	0.00045	0.1	0.00053	0.1	0.00056	0.1	0.00043	0.1
Tomatoes	0 028		0.00029	0.0	0.00135	0.2	0.00116	0.2	0.00087	0.1	0.00062	0.1
Peppers	0 028		0.00037	0.0	0.00000	0.0	0.00046	0.1	0.00033	0.0	0.00046	0.1
Aubergines	0 028		0.00027	0.0	0.00000	0.0	0.00060	0.1	0.00070	0.1	0.00026	0.0
Marrows	0 028		0.00036	0.0	0.00000	0.0	0.00060	0.1	0.00030	0.0	0.00040	0.1
Cucumbers	0 028		0.00020	0.0	0.00021	0.0	0.00083	0.1	0.00066	0.1	0.00050	0.1
Gourd	0 028		0.00041	0.1	0.00000	0.0	0.00000	0.0	0.00036	0.0	0.00000	0.0
Courgettes	0 028		0.00031	0.0	0.00089	0.1	0.00130	0.2	0.00112	0.1	0.00072	0.1
Melons	0 028		0.00103	0.1	0.00204	0.3	0.00201	0.3	0.00233	0.3	0.00205	0.3
Sweet corn	0 028		0.00050	0.1	0.00084	0.1	0.00122	0.2	0.00098	0.1	0.00118	0.2
Broccoli	0 028		0.00036	0.0	0.00058	0.1	0.00059	0.1	0.00069	0.1	0.00063	0.1
Cauliflower	0 028		0.00043	0.1	0.00162	0.2	0.00093	0.1	0.00097	0.1	0.00055	0.1
Brussels sprouts	0 028		0.00007	0.0	0.00020	0.0	0.00013	0.0	0.00020	0.0	0.00010	0.0
Head cabbage	0 028		0.00034	0.0	0.00121	0.2	0.00071	0.1	0.00090	0.1	0.00049	0.1
Chinese cabbage	0 028		0.00043	0.1	0.00000	0.0	0.00030	0.0	0.00000	0.0	0.00062	0.1
Kohl Rabi	0 028		0.00000	0.0	0.00000	0.0	0.00000	0.0	0.00000	0.0	0.00000	0.0
Cress	0 028		0.00000	0.0	0.00000	0.0	0.00001	0.0	0.00001	0.0	0.00000	0.0
Lettuce	0 028		0.00028	0.0	0.00035	0.0	0.00034	0.0	0.00050	0.1	0.00038	0.1
Spinach	0 028		0.00007	0.0	0.00015	0.0	0.00011	0.0	0.00016	0.0	0.00009	0.0
Watercress	0 028		0.00001	0.0	0.00000	0.0	0.00001	0.0	0.00001	0.0	0.00001	0.0
Chicory	0 028		0.00010	0.0	0.00000	0.0	0.00000	0.0	0.00000	0.0	0.00027	0.0
Parsley	0 028		0.00002	0.0	0.00000	0.0	0.00001	0.0	0.00001	0.0	0.00003	0.0
Beans with pods	0 028		0.00006	0.0	0.00014	0.0	0.00014	0.0	0.00010	0.0	0.00006	0.0
Runner Beans	0 028		0.00006	0.0	0.00000	0.0	0.00012	0.0	0.00010	0.0	0.00009	0.0
Peas with pods	0 028		0.00005	0.0	0.00000	0.0	0.00006	0.0	0.00010	0.0	0.00004	0.0
Beansprouts	0 028		0.00006	0.0	0.00003	0.0	0.00010	0.0	0.00011	0.0	0.00011	0.0
Peas without pods	0 028		0.00007	0.0	0.00023	0.0	0.00015	0.0	0.00016	0.0	0.00011	0.0
Beans without pods	0 028		0.00005	0.0	0.00011	0.0	0.00019	0.0	0.00007	0.0	0.00021	0.0
Asparagus	0 028		0.00007	0.0	0.00000	0.0	0.00013	0.0	0.00005	0.0	0.00002	0.0
Bamboo shoots	0 028		0.00002	0.0	0.00000	0.0	0.00002	0.0	0.00000	0.0	0.00001	0.0
Celery	0 028		0.00016	0.0	0.00019	0.0	0.00017	0.0	0.00015	0.0	0.00012	0.0
Fennel	0 028		0.00040	0.1	0.00000	0.0	0.00000	0.0	0.00000	0.0	0.00000	0.0
Globe artichokes	0 028		0.00024	0.0	0.00000	0.0	0.00000	0.0	0.00000	0.0	0.00011	0.0
Leeks	0 028		0.00036	0.0	0.00000	0.0	0.00056	0.1	0.00044	0.1	0.00031	0.0
Rhubarb	0 028		0.00022	0.0	0.00095	0.1	0.00104	0.1	0.00033	0.0	0.00048	0.1
Beans	0 028		0.00015	0.0	0.00051	0.1	0.00035	0.0	0.00033	0.0	0.00023	0.0
Lentils	0 028		0.00007	0.0	0.00017	0.0	0.00014	0.0	0.00017	0.0	0.00012	0.0
dried Peas	0 028		0.00008	0.0	0.00000	0.0	0.00012	0.0	0.00009	0.0	0.00009	0.0
Barley	0 039		0.00003	0.0	0.00000	0.0	0.00003	0.0	0.00007	0.0	0.00022	0.0
Maize	0 039		0.00002	0.0	0.00026	0.0	0.00015	0.0	0.00006	0.0	0.00003	0.0
Wheat	0 039		0.00024	0.0	0.00050	0.1	0.00051	0.1	0.00056	0.1	0.00043	0.1

			11-14 year old child	15-18 year old child	vegetarian	Elderly - own home	Elderly - residential

commodity	HR	P	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD	NESTI	%ARfD
Oilseeds	0 039		0.00032	0.0	0.00027	0.0	0.00037	0.0	0.00018	0.0	0.00021	0.0
Tomatoes	0 028		0.00040	0.1	0.00033	0.0	0.00038	0.1	0.00028	0.0	0.00033	0.0
Peppers	0 028		0.00029	0.0	0.00028	0.0	0.00046	0.1	0.00027	0.0	0.00015	0.0
Aubergines	0 028		0.00035	0.0	0.00022	0.0	0.00050	0.1	0.00019	0.0	0.00000	0.0
Marrows	0 028		0.00049	0.1	0.00021	0.0	0.00051	0.1	0.00045	0.1	0.00017	0.0
Cucumbers	0 028		0.00027	0.0	0.00026	0.0	0.00024	0.0	0.00018	0.0	0.00008	0.0
Gourd	0 028		0.00023	0.0	0.00012	0.0	0.00017	0.0	0.00020	0.0	0.00000	0.0
Courgettes	0 028		0.00032	0.0	0.00027	0.0	0.00038	0.1	0.00032	0.0	0.00035	0.0
Melons	0 028		0.00136	0.2	0.00110	0.1	0.00084	0.1	0.00074	0.1	0.00055	0.1
Sweet corn	0 028		0.00051	0.1	0.00068	0.1	0.00061	0.1	0.00040	0.1	0.00027	0.0
Broccoli	0 028		0.00044	0.1	0.00039	0.1	0.00047	0.1	0.00036	0.0	0.00019	0.0
Cauliflower	0 028		0.00047	0.1	0.00043	0.1	0.00065	0.1	0.00042	0.1	0.00028	0.0
Brussels sprouts	0 028		0.00008	0.0	0.00009	0.0	0.00011	0.0	0.00007	0.0	0.00005	0.0
Head cabbage	0 028		0.00047	0.1	0.00033	0.0	0.00048	0.1	0.00037	0.0	0.00028	0.0
Chinese cabbage	0 028		0.00006	0.0	0.00071	0.1	0.00025	0.0	0.00011	0.0	0.00000	0.0
Kohl Rabi	0 028		0.00038	0.1	0.00000	0.0	0.00000	0.0	0.00000	0.0	0.00000	0.0
Cress	0 028		0.00000	0.0	0.00000	0.0	0.00001	0.0	0.00001	0.0	0.00000	0.0
Lettuce	0 028		0.00023	0.0	0.00022	0.0	0.00031	0.0	0.00020	0.0	0.00011	0.0
Spinach	0 028		0.00009	0.0	0.00005	0.0	0.00010	0.0	0.00006	0.0	0.00004	0.0
Watercress	0 028		0.00001	0.0	0.00001	0.0	0.00003	0.0	0.00002	0.0	0.00000	0.0
Chicory	0 028		0.00000	0.0	0.00039	0.1	0.00006	0.0	0.00000	0.0	0.00000	0.0
Parsley	0 028		0.00001	0.0	0.00000	0.0	0.00003	0.0	0.00001	0.0	0.00001	0.0
Beans with pods	0 028		0.00005	0.0	0.00008	0.0	0.00008	0.0	0.00006	0.0	0.00003	0.0
Runner Beans	0 028		0.00008	0.0	0.00009	0.0	0.00011	0.0	0.00007	0.0	0.00005	0.0
Peas with pods	0 028		0.00004	0.0	0.00003	0.0	0.00004	0.0	0.00003	0.0	0.00000	0.0
Beansprouts	0 028		0.00006	0.0	0.00005	0.0	0.00007	0.0	0.00006	0.0	0.00003	0.0
Peas without pods	0 028		0.00009	0.0	0.00007	0.0	0.00009	0.0	0.00006	0.0	0.00006	0.0
Beans without pods	0 028		0.00004	0.0	0.00008	0.0	0.00011	0.0	0.00007	0.0	0.00005	0.0
Asparagus	0 028		0.00001	0.0	0.00004	0.0	0.00010	0.0	0.00005	0.0	0.00003	0.0
Bamboo shoots	0 028		0.00005	0.0	0.00001	0.0	0.00004	0.0	0.00001	0.0	0.00000	0.0
Celery	0 028		0.00016	0.0	0.00012	0.0	0.00023	0.0	0.00018	0.0	0.00006	0.0
Fennel	0 028		0.00000	0.0	0.00000	0.0	0.00052	0.1	0.00029	0.0	0.00000	0.0
Globe artichokes	0 028		0.00000	0.0	0.00000	0.0	0.00018	0.0	0.00000	0.0	0.00000	0.0
Leeks	0 028		0.00038	0.1	0.00030	0.0	0.00043	0.1	0.00039	0.1	0.00021	0.0
Rhubarb	0 028		0.00017	0.0	0.00022	0.0	0.00026	0.0	0.00022	0.0	0.00025	0.0
Beans	0 028		0.00021	0.0	0.00018	0.0	0.00018	0.0	0.00009	0.0	0.00008	0.0
Lentils	0 028		0.00019	0.0	0.00007	0.0	0.00009	0.0	0.00006	0.0	0.00002	0.0
dried Peas	0 028		0.00018	0.0	0.00006	0.0	0.00009	0.0	0.00007	0.0	0.00004	0.0
Barley	0 039		0.00002	0.0	0.00002	0.0	0.00003	0.0	0.00002	0.0	0.00001	0.0
Maize	0 039		0.00003	0.0	0.00004	0.0	0.00008	0.0	0.00002	0.0	0.00001	0.0
Wheat	0 039		0.00035	0.0	0.00033	0.0	0.00031	0.0	0.00018	0.0	0.00018	0.0

Pesticide Bixlozone
ARfD 0.750 mg/Kg bw/day
Source dDAR
* 0.00000 corresponds to <0.000005 mg/kg bw/day (any value ≥0.000005 is rounded to 0.00001

Body weights of the 10 consumer groups are as detailed in the regulatory update 21/2005.
Residues below the LOQ have been assumed to be at the LOQ for the purpose of this calculation

Acute and chronic EU dietary intake estimates

The EU MS national TMDIs, IEDIs and IESTIs for the active and commodities listed below have been calculated using PRIMo - Pesticide Residues Intake Model (revision 3.1).

The following assumptions have been made:

1) All produce eaten which may have been treated, has been treated and contains residues at the proposed MRL (TMDI) or STMR (IEDI) or HR (IESTI), as given below:

Commodity	***TMDI***		***IEDI***		***IESTI***	
	MRL (mg/kg)		**STMR (mg/kg)**		**HR (STMR for grain and oilseeds) (mg/kg)**	
Fruiting vegetables (RC)	0.05	Indicative MRL level based on HR of 0.028 from rotational crops field trial data	0.01	Indicative STMR based on rotational crop field trials data (See Vol 1 section 2.7.7)	0.028	Indicative HR based on rotational crop field trials data (See Vol 1 section 2.7.7)
Brassica vegetables (RC)	0.05		0.01		0.028	
Leaf vegetables, herbs and edible flowers (RC)	0.05		0.01		0.028	
Legume vegetables (RC)	0.05		0.01		0.028	
Stem vegetables (RC)	0.05		0.01		0.028	
Pulses (RC)	0.05		0.01		0.028	
Herbal infusions (dried) (RC)	0.05		0.01		0.028	
Oilseed rape seed	0.01	Proposed MRL	0.039§	STMR from field trials data	0.039§	STMR from field trials data
Barley grain	0.01	Indicative MRL	0.039§		0.039§	
Wheat grain	0.01		0.039§		0.039§	
Maize grain	0.01		0.039§		0.039§	
Honey	0.05	Proposed MRL	0.04§	STMR from oilseed rape field trials data (see Vol 1 section 2.7.8)	0.04§	HR from oilseed rape field trials data (see Vol 1 section 2.7.8)

§ Residues of 2,4-dichlorobenzoic acid doubled to account for this substance being twice as toxic as parent bixlozone. By doubling the residue levels for this metabolite, a risk assessment can be performed using the toxicological endpoints for parent bixlozone. (e.g. bixlozone residue 0.01 mg/kg + 2,4-dichlorobenzoic acid residue 0.01 mg/kg x 1.435 MW conversion x 2 to account for toxicity = 0.039 mg/kg)

2) There is no loss of residue during transport or storage, or processing of foods prior to consumption.

A full description of PRIMo and the underlying assumptions is in the document: 'Use of EFSA pesticide residues intake model (EFSA PRIMo revision 3.1)' available at the following link:
http://www.efsa.europa.eu/en/applications/pesticides/tools

Information is also included in the PRIMo model in the tab 'Background information'.

The relevant intake estimates for the TMDI are presented in Table 2.7.9.10 the IEDI in Table 2.7.9.11 and the IESTI in Table 2.7.9.12.

For the TMDI, chronic intakes for all consumer groups are below the ADI of 0.3 mg/kg bw/day, the critical consumer group is 'GEMS Food G06' with intakes estimated as up to 0.2 % of the ADI. For the IEDI, chronic intakes for all consumer groups are below the ADI of 0.3 mg/kg bw/day, the critical consumer group are NL toddlers with intakes estimated as up to 0.2 % of the ADI. Therefore, no chronic health effects are expected. Acute intakes for all consumer groups are below the ARfD of 0.75 mg/kg bw. The most critical group are children consuming melons with an estimated consumption of 0.6 % ARfD. Therefore, no acute health effects are expected.

Table 2.7.9. 4 EFSA model (PRIMo) TMDI for chronic risk assessment – rev. 3.1 for bixlozone

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Bixlozone

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	0.3	ARfD (mg/kg bw):	0.75
Source of ADI:	dDAR	Source of ARfD:	dDAR
Year of evaluation:	2022	Year of evaluation:	2022

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 : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Expsoure (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)
0.2%	GEMS/Food G06	0.50	0.1%	Tomatoes	0.0%	Wheat	0.0%	Watermelons		
0.1%	NL toddler	0.40	0.0%	Maize/corn	0.0%	Tomatoes	0.0%	Beans (with pods)		
0.1%	RO general	0.31	0.0%	Tomatoes	0.0%	Head cabbages	0.0%	Wheat		
0.1%	IE adult	0.28	0.0%	Melons	0.0%	Wheat	0.0%	Tomatoes		
0.1%	GEMS/Food G15	0.28	0.0%	Tomatoes	0.0%	Wheat	0.0%	Head cabbages		
0.1%	GEMS/Food G10	0.27	0.0%	Tomatoes	0.0%	Wheat	0.0%	Head cabbages		
0.1%	GEMS/Food G08	0.25	0.0%	Tomatoes	0.0%	Wheat	0.0%	Head cabbages		
0.1%	FR child 3 15 yr	0.24	0.0%	Wheat	0.0%	Tomatoes	0.0%	Beans (with pods)		
0.1%	SE general	0.24	0.0%	Tomatoes	0.0%	Wheat	0.0%	Head cabbages		
0.1%	DE child	0.23	0.0%	Tomatoes	0.0%	Wheat	0.0%	Cucumbers		
0.1%	IT toddler	0.23	0.0%	Tomatoes	0.0%	Wheat	0.0%	Lettuces		
0.1%	GEMS/Food G07	0.22	0.0%	Tomatoes	0.0%	Wheat	0.0%	Lettuces		
0.1%	GEMS/Food G11	0.21	0.0%	Tomatoes	0.0%	Wheat	0.0%	Beans (without pods)		
0.1%	IT adult	0.21	0.0%	Tomatoes	0.0%	Wheat	0.0%	Lettuces		
0.1%	ES child	0.20	0.0%	Tomatoes	0.0%	Wheat	0.0%	Lettuces		
0.1%	DK child	0.20	0.0%	Cucumbers	0.0%	Wheat	0.0%	Tomatoes		
0.1%	NL child	0.18	0.0%	Wheat	0.0%	Tomatoes	0.0%	Beans (with pods)		
0.1%	FR toddler 2 3 yr	0.18	0.0%	Beans (with pods)	0.0%	Wheat	0.0%	Tomatoes		
0.1%	ES adult	0.17	0.0%	Tomatoes	0.0%	Lettuces	0.0%	Wheat		
0.1%	UK toddler	0.16	0.0%	Wheat	0.0%	Beans	0.0%	Tomatoes		
0.1%	PT general	0.15	0.0%	Tomatoes	0.0%	Wheat	0.0%	Beans (without pods)		
0.0%	UK infant	0.15	0.0%	Peas (without pods)	0.0%	Wheat	0.0%	Beans		
0.0%	FI 3 yr	0.14	0.0%	Cucumbers	0.0%	Tomatoes	0.0%	Watermelons		
0.0%	NL general	0.14	0.0%	Tomatoes	0.0%	Wheat	0.0%	Beans (with pods)		
0.0%	DE women 14-50 yr	0.13	0.0%	Tomatoes	0.0%	Wheat	0.0%	Cucumbers		
0.0%	UK vegetarian	0.13	0.0%	Tomatoes	0.0%	Wheat	0.0%	Beans		
0.0%	FR adult	0.13	0.0%	Tomatoes	0.0%	Wheat	0.0%	Beans (with pods)		
0.0%	FR infant	0.13	0.0%	Beans (with pods)	0.0%	Courgettes	0.0%	Spinaches		
0.0%	DE general	0.13	0.0%	Tomatoes	0.0%	Wheat	0.0%	Head cabbages		
0.0%	FI 6 yr	0.12	0.0%	Cucumbers	0.0%	Tomatoes	0.0%	Watermelons		
0.0%	LT adult	0.09	0.0%	Tomatoes	0.0%	Head cabbages	0.0%	Cucumbers		
0.0%	UK adult	0.09	0.0%	Tomatoes	0.0%	Wheat	0.0%	Beans		
0.0%	PL general	0.09	0.0%	Tomatoes	0.0%	Head cabbages	0.0%	Cauliflowers		
0.0%	DK adult	0.09	0.0%	Tomatoes	0.0%	Wheat	0.0%	Cucumbers		
0.0%	FI adult	0.08	0.0%	Tomatoes	0.0%	Cucumbers	0.0%	Lettuces		
0.0%	IE child	0.03	0.0%	Wheat	0.0%	Beans (without pods)	0.0%	Broccoli		

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Bixlozone is unlikely to present a public health concern.

Table 2.7.9. 5 EFSA model (PRIMo) IEDI for chronic risk assessment – rev. 3.1 for bixlozone

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Bixlozone

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	0.3	ARfD (mg/kg bw):	0.75
Source of ADI:	dDAR	Source of ARfD:	dDAR
Year of evaluation:	2022	Year of evaluation:	2022

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 : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Expsoure (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)
0.2%	NL toddler	0.53	0.1%	Maize/corn	0.1%	Wheat	0.0%	Rapeseeds/canola seeds		
0.1%	GEMS/Food G06	0.42	0.1%	Wheat	0.0%	Maize/corn	0.0%	Tomatoes		
0.1%	IT toddler	0.29	0.1%	Wheat	0.0%	Tomatoes	0.0%	Lettuces		
0.1%	RO general	0.29	0.1%	Wheat	0.0%	Maize/corn	0.0%	Tomatoes		
0.1%	GEMS/Food G15	0.28	0.1%	Wheat	0.0%	Barley	0.0%	Maize/corn		
0.1%	GEMS/Food G08	0.26	0.1%	Wheat	0.0%	Barley	0.0%	Maize/corn		
0.1%	GEMS/Food G10	0.25	0.1%	Wheat	0.0%	Maize/corn	0.0%	Barley		
0.1%	GEMS/Food G07	0.25	0.1%	Wheat	0.0%	Barley	0.0%	Rapeseeds/canola seeds		
0.1%	FR child 3 15 yr	0.23	0.1%	Wheat	0.0%	Maize/corn	0.0%	Tomatoes		
0.1%	ES child	0.22	0.1%	Wheat	0.0%	Maize/corn	0.0%	Tomatoes		
0.1%	NL child	0.22	0.1%	Wheat	0.0%	Rapeseeds/canola seeds	0.0%	Maize/corn		
0.1%	DE child	0.21	0.1%	Wheat	0.0%	Tomatoes	0.0%	Cucumbers		
0.1%	GEMS/Food G11	0.21	0.0%	Wheat	0.0%	Barley	0.0%	Tomatoes		
0.1%	DK child	0.20	0.1%	Wheat	0.0%	Cucumbers	0.0%	Tomatoes		
0.1%	IT adult	0.20	0.1%	Wheat	0.0%	Tomatoes	0.0%	Lettuces		
0.1%	PT general	0.19	0.1%	Wheat	0.0%	Maize/corn	0.0%	Tomatoes		
0.1%	UK toddler	0.18	0.1%	Wheat	0.0%	Beans	0.0%	Tomatoes		
0.1%	SE general	0.17	0.0%	Wheat	0.0%	Tomatoes	0.0%	Head cabbages		
0.1%	UK infant	0.17	0.0%	Wheat	0.0%	Maize/corn	0.0%	Peas (without pods)		
0.1%	FR toddler 2 3 yr	0.15	0.0%	Wheat	0.0%	Beans (with pods)	0.0%	Tomatoes		
0.1%	IE adult	0.15	0.0%	Wheat	0.0%	Maize/corn	0.0%	Melons		
0.0%	ES adult	0.14	0.0%	Wheat	0.0%	Barley	0.0%	Tomatoes		
0.0%	NL general	0.12	0.0%	Wheat	0.0%	Barley	0.0%	Rapeseeds/canola seeds		
0.0%	DE general	0.12	0.0%	Wheat	0.0%	Barley	0.0%	Tomatoes		
0.0%	DE women 14-50 yr	0.12	0.0%	Wheat	0.0%	Tomatoes	0.0%	Barley		
0.0%	FR adult	0.11	0.0%	Wheat	0.0%	Tomatoes	0.0%	Maize/corn		
0.0%	UK vegetarian	0.10	0.0%	Wheat	0.0%	Tomatoes	0.0%	Beans		
0.0%	UK adult	0.08	0.0%	Wheat	0.0%	Tomatoes	0.0%	Beans		
0.0%	FI 3 yr	0.08	0.0%	Wheat	0.0%	Cucumbers	0.0%	Tomatoes		
0.0%	FI 6 yr	0.06	0.0%	Wheat	0.0%	Cucumbers	0.0%	Tomatoes		
0.0%	LT adult	0.06	0.0%	Wheat	0.0%	Tomatoes	0.0%	Head cabbages		
0.0%	DK adult	0.06	0.0%	Wheat	0.0%	Tomatoes	0.0%	Cucumbers		
0.0%	FR infant	0.06	0.0%	Wheat	0.0%	Beans (with pods)	0.0%	Courgettes		
0.0%	IE child	0.05	0.0%	Wheat	0.0%	Beans (without pods)	0.0%	Broccoli		
0.0%	FI adult	0.03	0.0%	Wheat	0.0%	Tomatoes	0.0%	Cucumbers		
0.0%	PL general	0.02	0.0%	Tomatoes	0.0%	Head cabbages	0.0%	Cauliflowers		

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of Bixlozone is unlikely to present a public health concern.

Table 2.7.9. 6 EFSA model (PRIMo) IESTI for acute risk assessment – rev. 3.1 for bixlozone

Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults

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

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)
0.6%	Melons	0 / 0.03	4.2
0.5%	Watermelons	0 / 0.03	3.4
0.2%	Cucumbers	0 / 0.03	1.8
0.2%	Sweet peppers/bell	0 / 0.03	1.7
0.2%	Leeks	0 / 0.03	1.7
0.2%	Tomatoes	0 / 0.03	1.6
0.2%	Cauliflowers	0 / 0.03	1.6
0.2%	Kohlrabies	0 / 0.03	1.5
0.2%	Courgettes	0 / 0.03	1.3
0.2%	Head cabbages	0 / 0.03	1.2
0.2%	Kales	0 / 0.03	1.2
0.2%	Sweet corn	0 / 0.03	1.2
0.2%	Broccoli	0 / 0.03	1.2
0.1%	Escaroles/broad-leaved	0 / 0.03	1.1
0.1%	Witloofs/Belgian endives	0 / 0.03	1.1

Expand/collapse list

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)
0.2%	Head cabbages	0 / 0.03	1.2
0.2%	Watermelons	0 / 0.03	1.1
0.1%	Melons	0 / 0.03	1.1
0.1%	Cucumbers	0 / 0.03	0.78
0.1%	Aubergines/egg plants	0 / 0.03	0.76
0.09%	Chinese cabbages/pe-tsai	0 / 0.03	0.71
0.09%	Broccoli	0 / 0.03	0.67
0.09%	Courgettes	0 / 0.03	0.65
0.09%	Cauliflowers	0 / 0.03	0.65
0.08%	Escaroles/broad-leaved	0 / 0.03	0.56
0.07%	Kales	0 / 0.03	0.54
0.07%	Chards/beet leaves	0 / 0.03	0.53
0.07%	Florence fennels	0 / 0.03	0.52
0.07%	Witloofs/Belgian endives	0 / 0.03	0.52
0.06%	Sweet peppers/bell	0 / 0.03	0.46

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

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)
0.3%	Pumpkins / boiled	0 / 0.03	2.5
0.3%	Witloofs / boiled	0 / 0.03	2.5
0.3%	Broccoli / boiled	0 / 0.03	2.2
0.3%	Cauliflowers / boiled	0 / 0.03	1.9
0.2%	Escaroles/broad-leaved er	0 / 0.03	1.9
0.2%	Leeks / boiled	0 / 0.03	1.6
0.2%	Florence fennels / boiled	0 / 0.03	1.3
0.1%	Rhubarbs / sauce/puree	0 / 0.03	1.0
0.1%	Courgettes / boiled	0 / 0.03	0.99
0.1%	Maize / oil	0 / 0.98	0.91
0.1%	Chards/beet leaves / boile	0 / 0.03	0.87
0.1%	Kales / boiled	0 / 0.03	0.77
0.1%	Gherkins / pickled	0 / 0.03	0.64
0.1%	Wheat / milling (flour)	0 / 0.04	0.47
0.1%	Spinaches / frozen; boiled	0 / 0.03	0.39

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)
0.2%	Pumpkins / boiled	0 / 0.03	1.5
0.2%	Cauliflowers / boiled	0 / 0.03	1.2
0.1%	Celeries / boiled	0 / 0.03	0.95
0.09%	Broccoli / boiled	0 / 0.03	0.67
0.09%	Courgettes / boiled	0 / 0.03	0.64
0.08%	Kohlrabies / boiled	0 / 0.03	0.60
0.08%	Escaroles/broad-leaved	0 / 0.03	0.57
0.07%	Florence fennels / boiled	0 / 0.03	0.54
0.07%	Witloofs / boiled	0 / 0.03	0.52
0.07%	Maize / oil	0 / 0.98	0.50
0.07%	Leeks / boiled	0 / 0.03	0.49
0.05%	Rhubarbs / sauce/puree	0 / 0.03	0.41
0.05%	Chards/beet leaves /	0 / 0.03	0.35
0.05%	Cardoons / boiled	0 / 0.03	0.34
0.04%	Barley / beer	0 / 0.01	0.28

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity.
A short term intake of residues of Bixlozone is unlikely to present a public health risk.
For processed commodities, no exceedance of the ARfD/ADI was identified.

Drinking water: Assessment of dietary intakes of 2,4-dichlorobenzoic acid:

The assessment of 2,4-dichlorobenzoic acid as a potential metabolite in drinking water is presented below in Table 2.7.9.13 and in section 2.11.5 (STEP 5).

Table 2.7.9. 7 Dietary intake estimate of 2,4-dichlorobanzoic acid in drinking water

Consumer group	**Water consumption (litres/kg bw/day)**	**Basis for the estimated intake**	**Estimated dietary intake of** 2,4-dichlorobenzoic acid arising from potential presence in drinking water at up to 11.62 µg/L[$] (mg/kg bw/day)
Adult (WHO)	0.033	2 litres water/day; 60 kg bw	0.00039 (0.1% of the ADI of 0.3 mg/kg bw/day)
Child (WHO)	0.100	1 litre water/day; 10 kg bw	0.00116 (0.4% of the ADI of 0.3 mg/kg bw/day)
Infant WHO)	0.150	0.75 litre water/day; 5 kg bw	0.00174 (0.6% of the ADI of 0.3 mg/kg bw/day)
Infant (EFSA, 2018 and used for UK assessments)	0.227	260 g/kg bw/day formula based on 33 g/kg bw powder and 227 ml water/kg bw/day	0.00264 (0.9% of the ADI of 0.3 mg/kg bw/day)

[$] Residues of 2,4-dichlorobenzoic acid doubled to account for this substance being twice as toxic as parent bixlozone. By doubling the residue levels for this metabolite, a risk assessment can be performed using the toxicological endpoints for parent bixlozone. (2,4-dichlorobenzoic acid residue 4.048 µg/L x 1.435 MW conversion x 2 to account for relative toxicological potency compared to parent bixlozone = 11.62 µg/L) It should be noted that although this value is >10 µg/L, this is due to the exposure being doubled to account for higher toxicity and enabling comparison to the parent toxicological end point. Additionally, this is due to the application of a MW conversion factor. The actual level of 2,4-dichlorobenzoic acid expected in ground water is 4.048 µg/L which is below the limit of 10 µg/L outlined in SANCO/221/2000 –rev.10.

Taking account of the possible presence of metabolite 2,4-dichlorobenzoic acid in food and drinking water, the co-exposures are expected to be low.

Overall, this is based on the low individual exposures as follows:

Estimation of long term (chronic) dietary exposures arising from foods (section 2.7.9) – total residues and associated intake across all consumer groups <1% of the ADI for parent of 0.3 mg/kg bw/day (this assessment accounts for the higher proposed toxicity of metabolite 2,4-dichlorobenzoic acid compared to parent).

Estimation of long term (chronic) dietary exposures arising from drinking water– metabolite 2,4-dichlorobenzoic acid <1% of the ADI for parent bixlozone of 0.3 mg/kg bw/day (for the critical consumer group infants). It should be noted that this estimation accounts for the higher toxicity of 2,4-dichlorobenzoic acid, considering twice the exposure in order to compare to the toxicological reference value for parent bixlozone.

Taken together these exposures are low.

Conclusions:

The concentrations of the metabolite 2,4-dichlorobenzoic acid are predicted to occur in groundwater at concentrations above 0.1 µg/L. The assessment of the relevance of this metabolite was performed according to the stepwise procedure of the EC guidance document SANCO/221/2000 –rev.10 (see section 2.11). In terms of the risk assessment, this residue of 4.048 µg/L (2,4-dichlorobenzoic acid) has been assessed on the basis of 'parent bixlozone equivalents' as 11.62 µg/L. This takes account of the proposed two fold toxicological potency of residues of 2,4-dichlorobenzoic acid compared to parent bixlozone, and also includes an adjustment due to molecular weight (x 1.435).

The refined risk assessment above (at step 5 of the assessment) concludes that overall chronic dietary intakes from food and drinking water sources are low: metabolite 2,4-dichlorobenzoic acid from both drinking water (<1% of the ADI of 0.3 mg/kg bw/day for bixlozone); food sources ('total residues' dietary intakes assessed taking account of the higher toxicity of 2,4-dichlorobenzoic acid, all < 1% of the ADI of 0.3 mg/kg bw/day for bixlozone). Taken together these exposures are low.

2.7.10. Proposed MRLs and compliance with existing MRLs

To support the GB representative uses of bixlozone on wheat, barley, maize and oilseed rape, and the subsequent possible residues in rotational crops and honey, the MRLs in Table 2.7.10.1**Error! Reference source not found.** are proposed. The residue definition for enforcement is proposed as bixlozone.

Table 2.7.10. 1 Proposed MRLs

Code number	Commodity	Proposed MRL (mg/kg)
401060	Oilseed rape seed	0.01*
500010	Barley	0.01*
500030	Maize/corn	0.01*
500090	Wheat	0.01*
1040000	Honey	0.05*

** denotes MRL at the LOQ*

2.7.11. Proposed import tolerances and compliance with existing import tolerances

No import tolerances are proposed and there are no existing import tolerances.

2.8. FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1. Summary of fate and behaviour in soil

Parent dosed studies

A laboratory aerobic degradation study was submitted in which bixlozone degradation was investigated in four European soils and three US soils (pH range 5.4 to 8.0). At study end (120 d), 24.54-75.83 % AR of bixlozone was remaining. Mineralisation resulted in CO_2 steadily increasing over the duration of the study, reaching 10.40-47.41 % AR (Phenyl-U-^{14}C] label) and 11.64-54.36 % AR [carbonyl-^{14}C] label after 120 days. Unextracted residues ranged between 3.30-11.64 % AR (Phenyl-U-^{14}C] label) and 21.8-28.48 % AR [carbonyl-^{14}C] label after 120 days. There was no significant difference between the results from the two radiolabel positions. [^{14}C]-bixlozone degraded with best-fit DT_{50} values in the range 64.1 days to >1000 days and normalised DT_{50} values for use in exposure modelling in the range 52.5 to 330 days (geomean value of 134 days).

No metabolites were observed >5 % of applied radioactivity. Metabolite 2,4-dichlorobenzoic acid peaked at day 30 reaching a mean maximum of 4.9 % AR before declining to <LOQ by study end, and 2,4-dichlorobenzyl alcohol reached a mean maximum of 2.8 % of applied radioactivity. 2,4-dichlorobenzaldehyde did not exceed 1% of applied radioactivity in any soil at any timepoint. All unknown metabolites individually accounted for less than 3.6 % of applied radioactivity.

An anaerobic degradation study was also submitted for bixlozone in two European soils and two US soils. In the aerobic phase, no metabolites were observed at concentrations >5 %AR. In the anaerobic phase, the metabolite bixlozone-3-hydroxy-propanamide was detected at >10% AR (maximum mean of 14.76 % AR, 120 d sample), and 2,4-dichlorobenzoic acid was present at ≥5% AR at a single time-point (maximum mean of 5.80 % AR at day 120 and increasing). 2,4-dichlorobenzaldehyde and 2,4-dichlorobenzyl alcohol were observed at mean maximum concentrations of 2.4 and 2.16% AR, respectively. All unknown metabolites individually accounted for less than 3.6% AR. Bixlozone degraded in soils incubated under anaerobic conditions with a DT50 values ranging from 206

to 871 days (geomean = 470 days). See metabolite summary section below for justification regarding the exclusion of the anaerobic metabolite results from the terrestrial exposure assessment.

The applicant submitted a soil photolysis study for bixlozone in which the degradation rate was assessed under irradiated and dark conditions in each of 2 soils and with 2 radiolabels. The treated soils were continuously irradiated for up to 15 days alongside dark control samples. The irradiation intensity to the soil surface per day by artificial sunlight was approximately equivalent to 34 days of natural summer sunlight at latitude 30-50°N. [Carbonyl-^{14}C]- and [phenyl-U-^{14}C]-bixlozone degraded slowly on soil surface under irradiated conditions (geomean DT_{50} = 100 days, converted to natural summer light), with no degradates > 5% AR being observed in either irradiated or dark control samples. The largest degradate reached a maximum of 3.85 % AR in irradiated samples after 15 days continuous irradiation and was tentatively identified as 2, 4-dichlorobenzoic acid. A number of other minor degradates were also observed, none exceeding 3.57% of applied radioactivity. Degradation in the dark controls was slower over the incubation period, except for carbonyl labelled Leimersheim soil where it was almost identical.

Three field soil dissipation studies were submitted, covering 7 test sites in Europe. Generally, at each site, studies were conducted using two formulation types and encompassed both soil incorporation and bare soil treatment. Two metabolites were detected in the field studies. Metabolite 3-OH-propanamide (3-OH) was detected at a maximum of 6.95% (on a mass basis; 6.90% on a molar basis) in one study at one time point (but was not increasing at study termination); due to the very limited evidence of 3-OH formation under aerobic soil conditions, the CA does not consider it necessary to consider 3-OH in the terrestrial exposure assessment. Metabolite 2,4-dichlorobenzoic acid (2,4-DBA) was detected at a maximum of 69.4% (on a mass basis; 99.53% on a molar basis) and so the CA does consider it necessary to include 2,4-DBA in the terrestrial exposure assessment. It is noted the applicant considers a worst-case 2,4-DBA formation of 100% in the PECsoil calculations which is accepted by the CA.

A kinetic assessment was undertaken on the soil dissipation studies to determine triggering, PEC_{soil}, Persistence and modelling endpoints. The outcome of the triggering endpoint assessment was that the potential for bixlozone accumulation in soil is to be assessed as part of the PECsoil assessment. Due to the short 2,4-DBA laboratory DT_{50} values, accumulation of metabolite 2,4-DBA does not need to be considered and so only PECsoil,initial values need to be determined. The longest non-normalised bixlozone DT_{50} value was 300 d (from the CS formulation at site GE01). The longest non-normalised SC formulation bixlozone DT_{50} value was 247 d (site IT01) and is to be used in the bixlozone PECsoil calculations for the representative SC product.

For all soil dissipation trial sites, SFO fits were considered good enough to determine modelling endpoints. Based on the results of the EFSA DegT50 tool and independent statistical advice, the SC formulation endpoints were considered most appropriate for consideration with the laboratory data. The EFSA DegT50 calculator indicated the SC field soil dissipation endpoints were shorter than the laboratory values and so it is not appropriate to combine the data. The geomean DT_{50} of the SC formulation field data, to be used in the exposure models, is ~~54.4~~ 48.0 days. Modelling endpoints for 2,4-DBA could not be obtained from the field due to insufficient data. Therefore, the CA considers the modelling endpoints from the laboratory study to be appropriate for use in the exposure calculations, with a formation fraction of 1 as a worst case.

Bixlozone persistence endpoints greater than the 120 d trigger were calculated at 10 trial sites. Furthermore, persistence endpoints greater than the 180 d ‘very Persistent’ trigger were calculated for 6 trial sites. Therefore, the CA considers it appropriate to consider bixlozone as very persistent in soil.

The adsorption and desorption behaviour of [^{14}C]-bixlozone was studied in five European and three US soils (pH 5.4 to 8.0). Adsorption K_{Foc} values for [^{14}C]-bixlozone were 334 – 465 mL/g (geometric mean 381.5 mL/g, arithmetic mean 1/n = 0.874) and desorption $K_{Foc\text{-}des}$ values were 481 – 754 mL/g (geometric mean 564 mL/g, arithmetic mean 1/n = 0.876), indicating that there is a degree of irreversibility to [^{14}C]-bixlozone adsorption. There was no evidence of any pH dependence.

Metabolite dosed studies

As indicated above, metabolite 2,4-DBA was concluded as being a major soil metabolite under both aerobic and anaerobic soil conditions. Metabolite 3-OH was concluded as being a major anaerobic soil metabolite only. Nevertheless, the applicant submitted aerobic degradation studies for both metabolites. The aerobic degradation

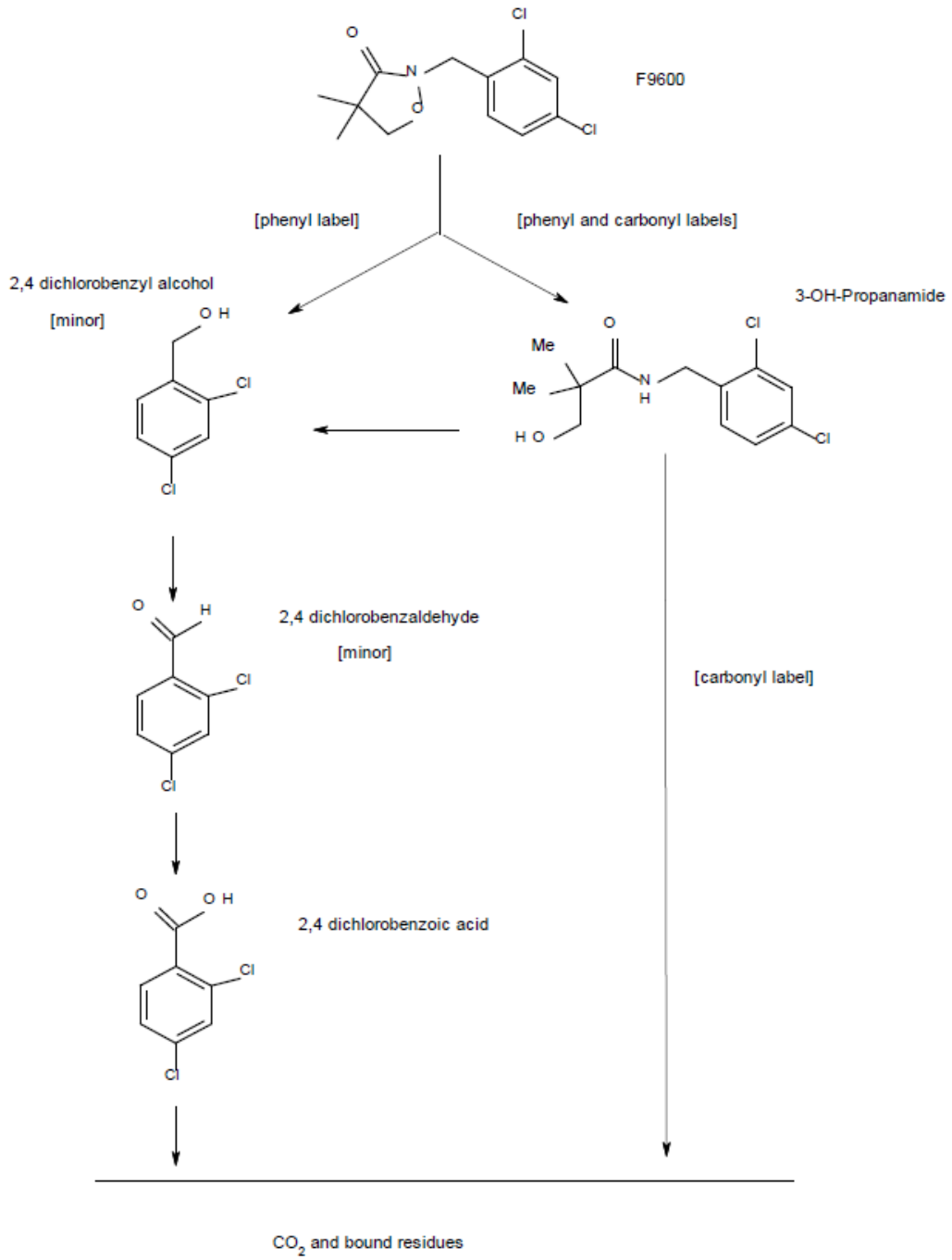
studies used three European soils (pH 4.84 to 7.53) and were treated with non-labelled test substances. The specimens were incubated in the dark at 20 °C. 3-OH degraded with normalised (20 °C, pF2) SFO DT_{50} values in the range 6.8 to 12.0 hours (geomean value = 9.1 hours). 2,4-DBA degraded with normalised (20 °C, pF2) SFO DT_{50} values in the range 3.5 to 8.9 days (geomean value = 5.4 days). The 2,4-DBA geomean value of 5.4 days is appropriate for use in the exposure calculations.

Anaerobic degradation studies for 3-OH and 2,4-DBA were carried out on one European soil (pH 7.3). For 2,4-DBA, the specimens were incubated in the dark at 20 ± 2°C prior to flooding. Due to the rapid 3-OH aerobic degradation, no incubation prior to flooding was performed in the 3-OH study. The soils were flooded with nitrogen purged de-ionised water to an approximate depth of 2 cm above the soil surface to establish anaerobic conditions which were maintained by a flow of nitrogen through the flasks for *ca* 120 days. Anaerobic modelling DT_{50} values for 3-OH and 2,4-DBA were 66.1 days and 275 days respectively.

The adsorption/desorption properties of 3-OH and 2,4-DBA were determined in four different soils of European origin (pH ($CaCl_2$) 4.84-7.53, %OC 0.68-2.62) . Adsorption K_{FOC} values for 3-OH-propanamide were 65-107 mL/g (geometric mean 81.7 mL/g, arithmetic mean 1/n = 0.925) and desorption K_{FOC}-des values were 71-136 mL/g (geometric mean 93.9 mL/g, arithmetic mean 1/n = 0.924). There was no evidence of a relationship between sorption and soil pH. However, the CA rejects the 2,4-DBA results from all four soils used within the study for use in the exposure assessment. An insufficient centrifuge speed and/or time was used to remove the aqueous solution from the soil pellet resulting in errors in the soil concentration. For two soils, because of low recoveries for the soil samples (*i.e.* negative values) and broken samples, only two or three concentrations were analysed in duplicate. Accurate and robust K_{FOC} and 1/n parameters could not be derived for these soils. Therefore, default sorption parameters (Koc = 0 mL/g, 1/n = 1) are to be used in the exposure calculations.

The metabolic pathway of bixlozone in soil is presented in Figure 2.8.1-1.

Figure 2.8.1-1: Bixlozone metabolic pathway in soil



2.8.2. Summary of fate and behaviour in water and sediment

The applicant submitted an aqueous hydrolysis study for bixlozone. In a preliminary test [phenyl-U-^{14}C]-bixlozone and [carbonyl-^{14}C]-bixlozone were added to sterile buffer solutions (7.5 mL) at pH 4, 7 and 9. Bixlozone was shown to be hydrolytically stable at pH 4 and 7 over 5 days at 50°C. Since both labels of bixlozone degraded only at pH 9 (>10% AR), a definitive study was conducted at 25, 40, and 50°C for 30 days at pH 9. Bixlozone did not hydrolyse at pH 9 over 30 days at the environmentally relevant temperature of 25°C with expected DT_{50}-values > 1 year. Therefore, no metabolic pathway has been proposed by the applicant. The rate and extent of degradation, however, increased with increasing temperature and pH. Unidentified metabolites were formed at >10% at pH 9 and 40-50˚C, but the CA considers that these metabolites will be unlikely to form at significant levels under

environmentally relevant temperature and pH conditions at which hydrolysis is unlikely to be a major route of degradation for bixlozone.

A direct photolysis study was submitted by the applicant using [carbonyl-^{14}C]- and [phenyl-U-^{14}C]-bixlozone. Bixlozone was slowly degraded to multiple minor photoproducts after 13 days continuous irradiation. All degradation products were < 5% AR at each sampling point. The first-order DT_{50} values were 44.0 and 54.4 days for [carbonyl-^{14}C]- and [phenyl-U-^{14}C]-bixlozone, respectively, under natural summer sunlight at latitude 30-50°N. It was not possible to determine the quantum yield for bixlozone due to the very low UV absorption at wavelengths > 290 nm.

The applicant submitted a ready biodegradability study in accordance with OECD Guideline 301B (CO_2 Evolution (Modified Sturm Test)). The study was undertaken on non-radiolabelled bixlozone and sodium benzoate was used as a reference substance. Bixlozone showed limited biodegradation with a maximum replicate biodegradation of 13% during the study. Therefore, bixlozone cannot be considered readily biodegradable.

A study of aerobic mineralisation in surface water was carried out. A single water sample was collected from Carsington Reservoir UK and treated with [phenyl-U-^{14}C]-bixlozone at nominal application rates of 10 and 100 µg/L and incubated at 20 ± 2°C, in the dark. After 62 days, >90 % of the test substance was recovered in both the 10 µg/L and 100 µg/L test systems. Only one sample recorded an unknown degradation product at a concentration >5 % AR and so no major metabolites were detected in the study.

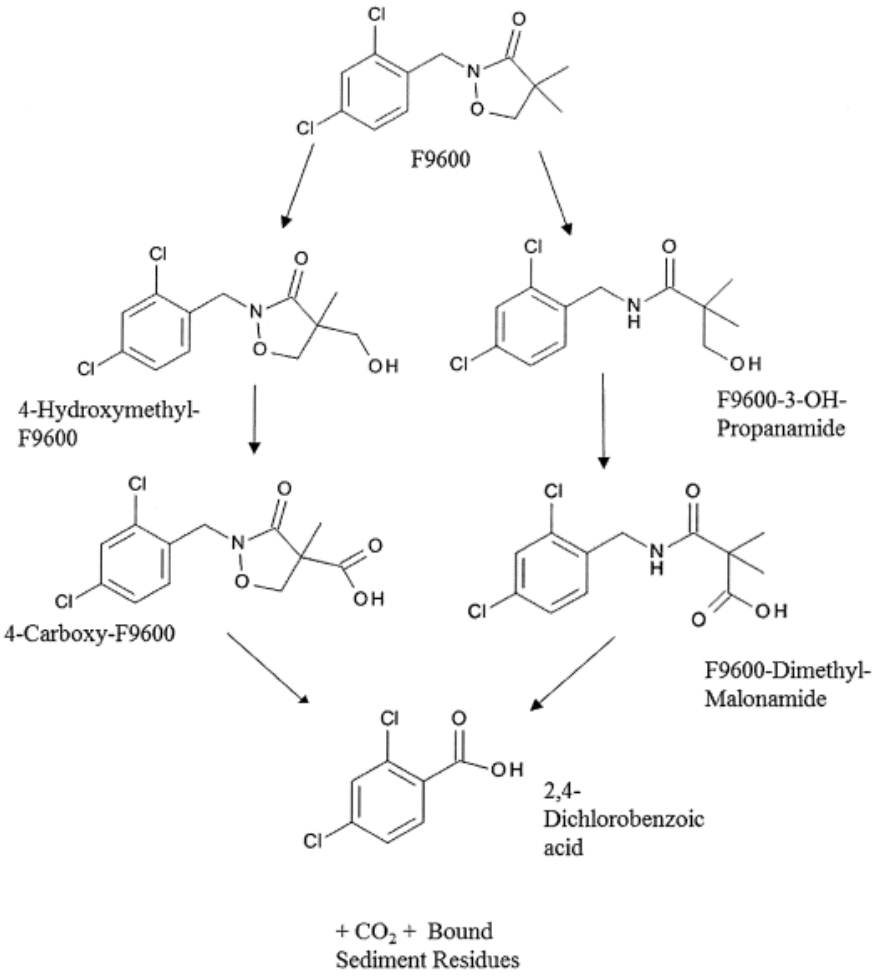
A study of aerobic aquatic metabolism in two UK water/sediment systems was carried out. The water-sediment systems were incubated at 20 ± 2°C in the dark until there was complete phase separation and to allow the oxygen levels, pH and redox potentials to establish. The samples were treated with [carbonyl-^{14}C]- and [phenyl-U-^{14}C]-bixlozone and were maintained at 20 ± 2°C throughout the course of the study. Bixlozone (mean of both labels) declined to 5.0% AR and 20.6% AR in the total system, in the Calwich Abbey and Swiss Lake systems, respectively, after 100 days. Bixlozone was observed in sediment at mean maxima of 20.99% AR (phenyl label, mean day 30) and 23.07% AR (carbonyl label, mean day 30) in the Calwich Abbey and Swiss Lake systems, respectively. The longest non-normalised water DissT50 value to be used in the spray drift exposure assessment was 16 days, derived from Swiss Lake system. The longest non-normalised sediment DissT50, to be used in the UK spray drift calculations, is 35.2 days, derived from the Calwich Abbey test system.

Four major metabolites were observed in the water/sediment study: 2,4-dichlorbenzoic acid (max mean total system formation = 40.9% AR), 3-OH-propanamide (max mean total system formation = 10.3% AR), dimethyl malonamide (max mean total system formation = 16.7% AR) and 4-carboxy-bixlozone (max mean total system formation = 24.5% AR). These metabolites are therefore to be considered in the exposure assessment. No kinetic analysis has been performed on the metabolites by the applicant and so default water DT50 values of 1000 days are appropriate for use in the exposure assessment.

Due to total system DegT50 values being <40 days for both test systems, bixlozone was concluded as not being persistent in water/sediment. However due to the lack of degradation observed in the surface water aerobic mineralisation study bixlozone could be considered persistent in water.

The metabolic pathway of bixlozone in water/sediment is summarised in Figure 2.8.2-1.

Figure 2.8.2-1: Bixlozone metabolic pathway in water/sediment



2.8.3. Summary of fate and behaviour in air

The degradation rates for reactions of bixlozone with OH radicals and ozone in the atmosphere were calculated by the applicant using the AOPWIN program based on ATKINSON's increment method. A rate constant of 21.4854 x 10^{-12}/ cm³/molecule/s was calculated for reaction with OH radicals. The atmospheric degradation half life of bixlozone was calculated to be 0.498 d (12 hour days) based on an OH radical concentration of 1.5 x 10^6 cm^3 on a 12-hour day basis. Due to its degradation in air and chemical structure, it was considered to have a low risk of long-range transport and, therefore, no hazard to the ozone layer.

The vapour pressure of bixlozone is 1.1 x 10^{-3} (20 °C) and so meets the FOCUSair trigger of 10^{-4} for the potential of short range transport from application to soil. The Henry's Law constant is 7.2 x 10^{-3} (20 °C). The potential for transport of bixlozone in air was therefore investigated in a wind tunnel study. The amount of deposition of bixlozone was measured at varying distances from the area of application and following set time intervals after the application event. Highest aqueous deposition occurred at 48 h and 72 h at 1m distance from application and represented 0.42% of applied amount. First bleaching of the indicator plants was observed 7 days after treatment and accounted for 7% of total leaf surface area at 1 m distance form application, and 4% of total leaf surface area at 5 m. Bleaching increased over time. At the last assessment on day 21 after exposure, bleaching of 13%, 7% and 1% of the total leaf surface was observed for the 1 m, 5 m and 10 m indicator plants, respectively.

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

As this is a new active substance, no monitoring data is available.

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

Soil: Bixlozone, 2,4-DBA

Surface water: Bixlozone, 2,4-DBA, 3-OH, bixlozone-DMM, 4-COOH-bixlozone

Sediment: Bixlozone, 2,4-DBA, 3-OH, bixlozone-DMM, 4-COOH-bixlozone

Groundwater: Bixlozone, 2,4-DBA

Air: Bixlozone

2.8.6. Summary of exposure calculations and product assessment

Environmental exposure assessments were conducted for the representative formulation Bixlozone-4 SC based on the intended use pattern: maize (BBCH 00-09, 1 x 375 g a.s./ha, 0% crop interception), winter oilseed rape (BBCH 00-09, 1 x 300 g a.s./ha, 0% crop interception) and winter cereals (BBCH 00-09 and 11-13, 1 x 200 g a.s./ha, 0% crop interception).

Differences in the degradation of bixlozone in the field soil dissipation studies between the SC formulation and a CS formulation were observed. The representative product under consideration is an SC formulation. As such, a soil DT50 from the SC formulation trials only has been considered further in the Vol 3 CP. See Vol 3 CA, section CA.B.8.1.2.3 for further discussion on the appropriate endpoint for a CS formulation.

Soil

Standard PEC_{soil} calculations were undertaken for bixlozone and two-tiers of calculations for 2,4-DBA. For bixlozone, the longest non-normaliaed DT_{50} value from the SC formulation soil dissipation studies (247 d) was considered in the calculations. As the bixlozone DT_{90} is >365 days, soil accumulation was also considered. For metabolite 2,4-DBA, only $PEC_{soil,initial}$ values were calculated and so no DT_{50} was used in the calculations. At tier 1, calculations were based on correcting the bixlozone $PEC_{soil,accumulation}$ values and at tier 2, calculations were based on correcting the bixlozone $PEC_{soil,initial}$ values. This second tier approach is considered justifiable because directly converting the parent accumulated load is an absolute worst-case and, because 2,4-DBA is much less persistent than the parent (laboratory geomean DT_{50} = 5.4d), it would be expected to decline during the time it takes for bixlozone to reach the accumulated plateau. The metabolite is not at risk of accumulation as determined in the laboratory aerobic degradation studies.

For all PEC_{soil} calculations, a soil bulk density of 1.5g/cm^3 and a soil depth of 5cm were also considered.

The maize GAP provided the worst-case PEC_{soil} values, resulting in a bixlozone $PEC_{soil,initial}$ value of 0.500 mg/kg and a $PEC_{soil,accumulation}$ value of 0.780 mg/kg. For 2,4-DBA, the tier 1 $PEC_{soil,initial}$ was 0.544 mg/kg and the tier 2 $PEC_{soil,initial}$ was 0.349 mg/kg.

Furthermore, formulation PEC_{soil} calculations were undertaken resulting in a worst-case initial concentration, for maize, of 1.402 mg/kg.

Further details are provided in section CP.B.8.2 of Volume 3CP of the DAR.

Groundwater

Standard PEC_{GW} calculations for bixlozone and 2,4-DBA were undertaken using PEARL 4.4.4, PELMO 5.5.3 and MACRO 5.5.4. No PEC_{GW} >0.001 µg/L were calculated for bixlozone. For 2,4-DBA, the maximum PEC_{GW} was 4.048 µg/L (PELMO, Hamburg, wOSR). As metabolite PEC_{GW} >0.1 µg/L were identified, a toxicological

relevance assessment is required for 2,4-DBA. A metabolite toxicological relevance assessment concluded 2,4-DBA as being non-relevant, see section 2.11.

In the CA's original representative product exposure calculations, a DT50 of 54.4 days was used in the groundwater calculations. For future product submissions based on SC formulations (or other formulation types not expected to influence the environmental fate and behaviour of the active substance), it is considered a DT50 of 48.0 days is most appropriate for use in the groundwater and higher tier drainflow exposure calculations. This updated DT50 (48.0 d) is considered to be sufficiently similar to the original DT50 (54.4 d) used in the exposure calculations to not warrant re-performing the exposure calculations as any change is expected to be insignificant. Should authorisation for a CS formulated product be sought in the future, detailed consideration and justification should be provided at that point to determine the appropriate DT50 value for use in the exposure calculations.

Further details are provided in section CP.B.8.3 of Volume 3CP of the DAR.

Surface water/sediment – spray drift

Standard spray drift calculations (default 1 m buffer, 2.77% drift) were undertaken for bixlozone and water/sediment metabolites 2,4-DBA, 3-OH, DMM and 4-carboxy-bixlozone. The worst-case initial PEC values, for the maize application, are summarised in Table 2.8.6-1. 5 m buffer (0.71% drift – calculated as 0.57% drift plus 0.14% deposition following volatilisation), spray drift calculations were also undertaken for the maize GAP, bixlozone only.

Additionally, formulation PEC_{SW} values were calculated and the worst-case maize value is also summarised in Table 2.8.6-1. Further details are provided in section CP.B.8.5.1 of Volume 3CP of the DAR.

Table 2.8.6-1: Summary of maize spray drift $PEC_{SW/sed}$

Compound	**Buffer zone (m)**	**PEC_{SW} (µg/L)**	**PEC_{sed} (µg/kg)**
Bixlozone	1	3.463	3.687
	5	0.888	0.945
2,4-DBA	1	0.732	0.355
3-OH	1	0.126	0.057
DMM	1	0.453	0.119
4-carboxy-bixlozone	1	0.676	0.214
Formulation bixlozone-4 SC	1	9.704	n/a

Surface water/sediment – drainflow

Tier 1 drainflow calculations were undertaken for the compounds detailed in the spray drift section above. For the metabolites formed in water/sediment, the parent PEC_{SW} was converted to metabolite $PEC_{SW/sed}$ based on molecular weight and maximum occurrence in water.

The worst-case (from maize) tier 1 drainflow $PEC_{SW/sed}$ values are summarised in Table 2.8.6-2.

Table 2.8.6-2: Summary of maize tier 1 drainflow $PEC_{SW/sed}$

	$PEC_{SW/sed}$ from soil		**$PEC_{SW/sed}$ for metabolites formed in water/sediment**	
	PEC_{SW} (µg/L)	PEC_{sed} (µg/kg)	PEC_{SW} (µg/L)	PEC_{sed} (µg/kg)
Bixlozone	20.192	21.500	-	-
2,4-DBA	38.146	176.06	4.271	6.824
3-OH	n/a	n/a	0.730	9.312
DMM	n/a	n/a	2.641	5.622
4-carboxy-bixlozone	n/a	n/a	3.942	7.081

Additional Higher Tier Drainflow (HTDF) assessments were performed for bixlozone and 2,4-DBA (with a subsequent combined assessment). As the bixlozone and 2,4-DBA RACs come from different aquatic groups (i.e.

bixlozone from aquatic plants and 2,4-DBA from aquatic invertebrates), the CA has undertaken separate combined risk assessments considering the relevant RAC in each group. For aquatic plants, this corresponds to 3.3 µg/L for bixlozone and 2400 µg/L for 2,4-DBA. For aquatic invertebrates, the relevant RAC values are 6.69 µg/L for bixlozone and 12 µg/L for 2,4-DBA. The combined assessments were performed using the Finney equation and annual max PEC_{SW}. For the wOSR GAP, it was necessary to also consider the daily PEC_{SW} in the combined assessment. Acceptable HTDF assessments were obtained for all proposed GAPs. Further details are provided in section CP.B.8.5.2 of Volume 3CP of the DAR.

Table 2.8.6-3: Summary of maize HTDF, approach 1, number of ~~PEC_{SW}~~ RAC exceedances (percentage in brackets)

Soil	Aquatic plants RACs					
	Bixlozone (RAC: 3.3 µg/L)			2,4-DBA (RAC: 2400 µg/L)		
	Dry Climate	Medium Climate	Wet Climate	Dry Climate	Medium Climate	Wet Climate
Denchworth	1 (3.3)	2 (6.7)	1 (3.3)	0	0	0
Hanslope	0	0	0	0	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Aquatic invertebrate RACs					
-	Bixlozone (RAC: 6.69 µg/L)			2,4-DBA (RAC: 12 µg/L)		
Denchworth	0	0	0	1 (3.3)	0	0
Hanslope	0	0	0	1 (3.3)	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Combined assessments (Finney equation with annual max PECsw)					
-	Aquatic plants			Aquatic invertebrates		
Denchworth	1 (3.3)	2 (6.7)	1 (3.3)	3 (10)	0	3 (10)
Hanslope	0	0	0	2 (6.7)	0	1 (3.3)
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0

Table 2.8.6-4: Summary of maize HTDF, approach 2, weighted level of exceedance

HTDF – Maize – Approach 2 (weighted level of exceedances)				
Soil drainage status	Bixlozone (aquatic plant RAC)	2,4-DBA (aquatic invertebrate RAC)	Combined annual max PECsw (aquatic plant RACs)	Combined annual max PECsw (aquatic invertebrate RACs)
Not drained	50.01	50.01	50.01	50.01
Peat	1.56	1.56	1.56	1.56
Drained but ‘safe’	48.05	48.36	48.05	47.64
Drained and not ‘safe’	0.38	0.07	0.38	0.79
Total ‘safe’ years	99.62	99.93	99.62	99.21

Table 2.8.6-5: Summary of wOSR HTDF, approach 1, number of RAC exceedances (percentage in brackets)

Soil	Aquatic plants RACs					
	Bixlozone (RAC: 3.3 µg/L)			2,4-DBA (RAC: 2400 µg/L)		
	Dry Climate	Medium Climate	Wet Climate	Dry Climate	Medium Climate	Wet Climate
Denchworth	0	0	0	0	0	0
Hanslope	0	0	0	0	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Aquatic invertebrate RACs					
-	Bixlozone (RAC: 6.69 µg/L)			2,4-DBA (RAC: 12 µg/L)		
Denchworth	0	0	0	0	0	0
Hanslope	0	0	0	0	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Combined assessments (Finney equation with annual max PECsw)					
-	Aquatic plants			Aquatic invertebrates		
Denchworth	0	4 (13)	3 (10)	0	**4 (13)**	2 (6.7)
Hanslope	0	4 (13)	0	0	3 (10)	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Combined assessments (Finney equation with daily PECsw)					
-	Aquatic plants			Aquatic invertebrates		
Denchworth	Not required			0	3 (10)	1 (3.3)
Hanslope				0	2 (6.7)	0
Brockhurst				0	0	0
Clifton				0	0	0

Table 2.8.6-6: Summary of wOSR HTDF, approach 2, weighted level of exceedance

Soil drainage status	Bixlozone (aquatic plant RAC)	2,4-DBA aquatic invertebrate RAC)	Combined annual max PECsw (aquatic plant RACs)	Combined annual max PECsw (aquatic invertebrate RACs)	Combined daily PECsw (aquatic invertebrate RACs)
Not drained	44.80	44.80	44.80	44.80	44.80
Peat	1.54	1.54	1.54	1.54	1.54
Drained but ‘safe’	53.66	53.66	52.07	52.35	52.76
Drained and not ‘safe’	0.00	0.00	1.59	1.31	0.90
Total ‘safe’ years	100.00	100.00	98.41	98.69	99.10

Table 2.8.6-7: Summary of winter cereals HTDF, approach 1, number of RAC exceedances (percentage in brackets)

Soil	Aquatic plants RACs					
	Bixlozone (RAC: 3.3 µg/L)			2,4-DBA (RAC: 2400 µg/L)		
	Dry Climate	Medium Climate	Wet Climate	Dry Climate	Medium Climate	Wet Climate
Denchworth	0	0	0	0	0	0
Hanslope	0	0	0	0	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Aquatic invertebrate RACs					
-	Bixlozone (RAC: 6.69 µg/L)			2,4-DBA (RAC: 12 µg/L)		
Denchworth	0	0	0	0	0	0
Hanslope	0	0	0	0	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0
-	Combined assessments (Finney equation with annual max PECsw)					
-	Aquatic plants			Aquatic invertebrates		
Denchworth	0	0	0	0	0	0
Hanslope	0	0	0	0	0	0
Brockhurst	0	0	0	0	0	0
Clifton	0	0	0	0	0	0

Table 2.8.6-8: Summary of winter cereals HTDF, approach 2, weighted level of exceedance

Soil drainage status	Bixlozone (aquatic plant RAC)	2,4-DBA (aquatic invertebrate RAC)	Combined annual max PECsw (aquatic plant RACs)	Combined annual max PECsw (aquatic invertebrate RACs)
Not drained	49.67	49.67	49.67	49.67
Peat	2.87	2.87	2.87	2.87
Drained but ‘safe’	47.46	47.46	47.46	47.46
Drained and not ‘safe’	0.00	0.00	0.00	0.00
Total ‘safe’ years	100.00	100.00	100.00	100.00

Air

The results of the deposition following volatilisation of bixlozone study were used to determine a drift + deposition percentage of 3.19% (2.77% + 0.42%), which is to be used in the ecotoxicology risk assessment for the non-target plant assessment. Similarly, as detailed in the spray drift section above, deposition following volatilisation was also considered in the PEC_{SW} (spray drift) calculations for the maize GAP with a 5 m buffer (0.71% (0.57% drift plus 0.14% deposition)).

Further details are provided in section CP.B.8.6.2 of Volume 3CP of the DAR.

2.9. EFFECTS ON NON-TARGET SPECIES

2.9.1. Summary of effects on birds and other terrestrial vertebrates

Birds

- **Acute oral toxicity data –** An acute study with the active substance was conducted and considered valid for regulatory purposes resulting in an endpoint of >**2000 mg a.s./kg bw** for use in the risk assessment.

- **Short-term toxicity data –** Three dietary toxicity studies were conducted with the active substance. EU retain regulation 283/2013, states that the avian dietary study is only required

  > "where the mode of action or results from mammalian studies indicate a potential for the dietary LD 50 measured by the short-term dietary toxicity study to be lower than the LD 50 based on an acute oral study. The short-term dietary toxicity test shall not be conducted for any other purpose than to determine intrinsic toxicity through dietary exposure, unless a justification of the need to do so is supplied."

  It is noted that three studies have been submitted, one using the Bobwhite Quail, one using the Mallard Duck and one the Zebra Finch. It was noted that the endpoint from the study using the zebra finch is less than the LD50 from the standard species, noting that they are differ in terms of conduct one being a gavage dose and the other a dietary study, i.e., one is an LD50 whereas the other is an LDD50. However, given the TERa (see CP B.9.1) and the degree to which it passes the risk assessment, and the fact that bixlozone does not match any of the criteria highlighted in the regulation indicates that these studies were gratuitous and as a result have not been evaluated or used in the risk assessment.

- **Long-term toxicity –** A total of 4 long-term/reproductive studies were conducted with the active substance, however it should be noted that 2 of these studies were repeat studies as no NOEC could be set as there were adverse effects at all tested concentrations. Therefore the studies were repeated over a lower concentration range and a NOEC was set. However, they have been considered as additional information in the context of the ED assessment. From the two studies where a NOEC could be set, there were several issues with the mallard duck study; in particular several of the control birds were not in mature reproductive physiology, raising concerns regarding the batch of birds used in the test and the conduct of the study. Regarding the study conducted with the bobwhite quail, several issues were noted, including some effects on reproductive parameters and female bodyweight; however none of these effects were statistically significant and no clear dose respose was evident, with larger effects noted at the lower test concentrations. As such the endpoint from this study is considered valid for use in the risk assessment **77.7 mg a.s./kg bw/d.**

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: 34 mg a.s./kg bw/day.** Discussion about how this endpoint was chosen is found in Section B.9.1.2. (PPP: 'F9600-4 SC').

Endocrine disruption assessment for birds and mammals:

Birds

For birds when considering reproductive toxicity the NOAEL values were the highest test concentration of 77.7 mg a.s./kg bw/d. However, in a couple of available studies ([redacted], 2016a & [redacted], 2016b) some birds were observed to be regressing from or not in mature reproductive physiology at the two top doses. It was deemed that limited details in the reports means that no clear conclusion could be drawn. Currently there are no further tests available for assessing endocrine activity in birds hence HSE agrees with the applicant that further testing is not required at this stage.

Overall, HSE concludes that based on current EFSA/ECHA 2018 guidance it is not possible to reach a conclusion for birds or reptiles when considering endocrine disruption, it is stated in the guidance that 'for birds, only a limited number of standardised in vivo methods are available, and little information can be gained from those guidelines concerning potential ED-related effects. In general, little is known of the impact of endocrine disruptors in birds compared to other species, and more research is needed to develop responsive parameters and *in vitro* and *in vivo* protocols to specifically address the differences between birds and other vertebrate taxa'. (please refer to the Section B.9.1.5 of CA dossier, Volume 3 for further consideration).

Wild mammals

For wild mammals the toxicology data and conclusions for endocrine disruption have been summarised and considered from an ecotoxicology perspective below:

- In all species investigated (rat, mouse, dog) there were no specific adverse effects on reproductive organs and other endocrine organs related to EAS modalities (e.g. adrenal, pituitary, mammary – please refer to Section B.6.3) following repeated exposure to bixlozone. In addition, there were no specific adverse effects on reproduction in the rat and on development in the rat and the rabbit. Overall, there was no clear pattern of adversity for the EAS modalities. Scenario 1a was applied i.e. no EAS-mediated adversity.
- Overall, it was shown that repeated exposure to bixlozone in Level 5 and Level 4 studies in rats, mice and dogs was not associated with any clear or specific effects on the thyroid gland, with only isolated incidences of thyroid weight changes reported in the 90-day dog study or histopathology described in the 90-day rat study. Therefore there is no evidence of a clear pattern of adversity for the T modality.
- In relation to the observed 'isolated effects' the toxicology specialist has confirmed: *'The thyroid weight changes reported in the 90-day dog study and the histopathology finding (mild follicular cell hypertrophy noted at the top dose in males) described in the 90-day rat study' are both considered to be isolated incidences because there were no other occurrences observed for these effects in any of the other studies conducted in the rat, in the mouse, the dog and the rabbit, including in studies where comparable/higher dose levels of bixlozone were tested. So there were no consistent findings for organ weight changes or histopath between the studies and the species tested. Thus, overall they do not describe a pattern of adverse effect.*

  Moreover, the histopathology finding occurred concomitantly with systemic toxicity in excess of the maximum tolerated dose (at that dose you had one death, statistically significantly lower body weights and body weight gains and clear adverse effects in the liver) . Thus, the thyroid effect (histopath) is not considered to be a specific effect of bixlozone but rather to be secondary to general toxicity'.
- In addition, there is no indication of adverse pre- and post-natal neurological development of the offspring in the available Level 5 2-generation reproduction toxicity study in the rat ([redacted], 2016c; Study ID Matrix: 16) and the Level 4 developmental toxicity studies in the rat and the rabbit ([redacted], 2016e; Study ID Matrix: 12 & [redacted], 2015; Study ID Matrix: 14). Therefore a potential concern for neurodevelopment is considered unlikely for bixlozone. Overall bixlozone does not present a clear pattern of adversity for the T modality in relation to effects on the thyroid gland and/or neurodevelopment effects. Scenario 1a was applied i.e. no T-mediated adversity.

Overall, HSE (ecotoxicology) considers that based on the toxicology conclusion the ED criteria are not met for mammals as non-target organisms when considering EAS and T modalities and that these modalities have been sufficiently investigated. Further consideration of EAS and T modalities for wild mammals is not required (please refer to the Section B.9.1.5 of CA dossier, Volume 3 for further consideration).

Overall conclusion:

Based on current guidance (EFSA/ECHA 2018) and available test methods it is not possible to reach a conclusion for birds or reptiles. Currently there are no further tests available for assessing endocrine activity in birds hence HSE agrees with the applicant that further testing is not required at this stage

For wild mammals bixlozone is not considered an endocrine disruptor following EFSA/ECHA guidance 2018 and agreed regulatory criteria based on available data/information.

Literature review

The literature review was conducted for the active substance bixlozone and relevant metabolites in accordance with Article 8(5) of Regulation (EC) No. 1107/2009 and based on the EFSA guidance (EFSA Journal 2011; 9(2):2092) and is described in detail in Section B.9.10 of CA dossier, Volume 3. HSE considers the literature review acceptable for the endocrine disruption ecotoxicology assessment. The search results showed that a total of 37 records were retrieved as part of the literature review, for all aspects of the literature review. All these studies were excluded after the rapid assessment of relevance. Based on the information in the literature review HSE agrees with the exclusion of these studies as not relevant (the issues identified above regarding the relevance criteria do not affect this conclusion). Therefore, no further consideration is required.

2.9.2. Summary of effects on aquatic organisms

Toxicity data to address the risk from bixlozone, the representative 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 Volume 3 CA Section B.9.2. Formulation toxicity data have also been submitted and evaluated in the Volume 3 CP B.9.

Table B2.9.2-1: Tier 1 and tier 2a toxicity data relevant to the active substance bixlozone, its metabolites and representative formulation F9600-4 SC

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
Acute toxicity to fish					
Bixlozone (active substance)	*Oncorhynchus mykiss*	Static, 96-hours	LC_{50}	**9.8 m m.**	[redacted] (2015)
'F9600 4SC'	*Oncorhychus mykiss*	Static, 96-hours	LC_{50}	**32 nom.**	[redacted] (2016a)
Long-term toxicity to fish					
Bixlozone (active substance)	*Pimephales promelas*	Flow through, ELS (early life-stage test), 32-days	NOEC (total length)	**0.38 m m.**	[redacted] (2016)
Biconcentration in fish					
Bixlozone (active substance)	*Lepomis macrochirus*	Flow-through, bioconcentration study	($BCF_{steady\ state}$, lipid normalised	**77.5**	[redacted] (2016)
Acute toxicity to invertebrates					
Bixlozone (active substance)	*Daphnia magna*	Static, 48-hours	EC_{50}	>2.6 m m.	Shaw (2015c)
	Americamysis bahia	Static, 96-hours	LC_{50}	**0.14 m m.**	Shaw (2016a)
	Caecidotea communis	Static, 48-hours	EC_{50}	>1.6 m m.	Mason (2017a)
	Chironomus riparius	Static, 48-hours	EC_{50}	1.9 m.m.	Mason (2017d)
	Pycnopsyche gentilis	Static, 48-hours	EC_{50}	0.33 m.m.	Mason (2018a)
	Hexagenia limbata	Static, 48-hours	EC_{50}	1.5 m.m.	Mason (2018b)
	Thamnocephalus platyurus	Static, 48-hours	EC_{50}	0.11 m.m.	Mason (2018c)
Bixlozone (active substance)	Aquatic invertebrate, acute	Geometric mean	EC_{50}	**0.669 m m.**	Geometric mean (EFSA Journal 2013;11(7):3290)
'F9600 4SC'	*Americamysis bahia*	Static, 96-hours	LC_{50}	**3.9 nom.**	Mason (2017d)
2,4-dichlorobenzoic acid	*Americamysis bahia*	Static renewal, 96-hours	LC_{50}	**> 100 nom.**	Mason (2018c)
4-Carboxyl-F9600	*Americamysis bahia*	Static, 96-hours	LC_{50}	**> 100 nom.**	Mason (2018d)
F9600-dimethyl-malonamide	*Americamysis bahia*	Static, 96-hours	LC_{50}	**100 nom.**	Mason (2018e)
F9600-3-OH-propanamide	*Americamysis bahia*	Static, 96-hours	LC_{50}	**22 m.m.**	Mason (2017a)
Long-term toxicity to invertebrates					
Bixlozone (active substance)	*Americamysis bahia*	Flow through, 28-days	NOEC	**0.12 m m.**	Marini (2017)
Toxicity to sediment dwelling invertebrates					
Bixlozone (active substance)	*Chironomus riparius*	Static, water-sediment system (dosed via sediment), 28-days	EC_{10}, development rate	**69 (mg/ kg sed. dw)** **m m.** **(3.0 mg/L)**	Snow (2019)

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
2,4-dichlorobenzoic acid	*Chironomus riparius*	Static, water-sediment system (dosed via sediment), 28-days	EC_{10} / EC_{20}	**≥ 104.88 mg /kg sed. dw m m. (93.26 mg/L)**	Dabrunz (2018a)
4-Carboxyl-F9600	*Chironomus riparius*	Static, water-sediment system (dosed via sediment), 28-days	EC_{10} / EC_{20}	**≥ 494.54 mg /kg sed. dw m m. (42.75 mg/L)**	Dabrunz (2018b)
F9600-dimethyl-malonamide	*Chironomus riparius*	F9600-dimethyl-malonamide	EC_{10} / EC_{20}	**≥ 502 mg /kg sed. dw ini. (89.5 mg/L)**	Dabrunz (2018c)
Toxicity to algae					
Bixlozone (active substance)	*Raphidocelis subcapitata* (formerly known as *Pseudo -kirchneriella subcapitata* or *Selenastrum capricornutum*)	Static, 96-hours	72 h E_rC_{50}	**14.0 m m.**	Softcheck (2015a)
'F9600 4SC'	*Raphidocelis subcapitata*	Static, 96-hours	72 h E_rC_{50}	**17.0 m m.**	Softcheck (2017a)
2,4-dichlorobenzoic acid	*Raphidocelis subcapitata*	Static, 96-hours	72 h E_rC_{50}	**90.1 m m.**	Obert -Rauser (2018a)
4-Carboxyl-F9600	*Raphidocelis subcapitata*	Static, 96-hours	96 h E_rC_{50}	**77 m.m.**	Softcheck (2018a)
F9600-dimethyl-malonamide	*Raphidocelis subcapitata*	Static, 96-hours	96 h E_rC_{50}	**71 m.m.**	Softcheck (2018b)
F9600-3-OH-propanamide	*Raphidocelis subcapitata*	Static, 96-hours	96 h E_rC_{50}	**>84 m m.**	Softcheck (2017a)
Toxicity to aquatic macrophytes					
Bixlozone (active substance)	*Myriophyllum spicatum*	Static renewal, water-sediment system (dosed via water), 14-days	$E_rC_{20, \text{shoot length}}$	**0.033 i.m.**	Kirkwood (2015b)
'F9600 4SC'	*Myriophyllum spicatum*	Static renewal, water-sediment system (dose via water), 14-days	E_rC_{20}	**0.29 i.m.**	Kirkwood (2017)
2,4-dichlorobenzoic acid	*Myriophyllum spicatum*	Static renewal, water-sediment system (dose via water), 14-days	$E_rC_{50, \text{shoot length}}$	**24 m.m.**	Kirkwood (2018)
4-Carboxyl-F9600	*Myriophyllum spicatum*	Static renewal, water-sediment system (dose via water), 14-days	$E_rC_{50, \text{shoot length}}$	**>1.3 m.m. ***	Dill (2018a)
F9600-dimethyl-malonamide	*Myriophyllum spicatum*	Static renewal, water-sediment system (dose via water), 14-days	$E_rC_{50, \text{shoot wet weight}}$	**>100 nom.**	Dill (2018b)

n.r. = not reported; nom. = nominal concentration; m.m. = mean measured concentration; i.m. = initial measured concentration

**corrected endpoint – highest endpoint with < 50% effects and without the presence of foaming/precipitate/turbidity in the test solutions. Given precipitate was noted in the stock and 31.3 mg/L solution, and turbidity at 9.77 mg/L, it is deemed more appropriate to derive an E_rC_{50} of >1.3 mg/L (mean measured).*

Metabolite endpoints

The risk from the metabolites 2,4-dichlorobenzoic acid, 4-Carboxyl-F9600, F9600-dimethyl-malonamide and F9600-3-OH-propanamide is considered below following the EFSA AGD (2013) stepwise approach:

- Aquatic invertebrates are the most sensitive taxonomic group at tier 1, with an acute toxicity endpoint of 0.14 mg a.s/L from the study with *A.bahia.*
- An acute metabolite toxicity study has been conducted with *A.bahia* for all of the relevant metabolites above.
- Consideration of the chronic risk is necessary as exposure of surface water for all metabolites is likely and
- The endpoint for all relevant aquatic metabolites is >10 times less toxic than that for the active substance on a molar basis where based on:
  LC_{50met} >10 x $M_{met}/M_{a.s.}$ x $LC_{50a.s.}$

where LC_{50met} and $LC_{50a.s.}$ are mass concentrations (mg/L) of metabolite and a.s. at 50 % mortality and M_{met} and $M_{a.s.}$ are the molar masses (g/mol) of the metabolite and a.s.

According to the AGD stepwise approach, the parent acute and chronic endpoints can be used in the metabolite risk assessment as surrogate values for all Tier 1 taxonomic groups where data are not available. Thus the chronic risk assessment for all metabolites is based on parent endpoints and the acute risk assessment for fish is based on acute parent endpoints (see Section 9.4 of Volume 3 – B.9 (PPP) for further details).

Endocrine disruption assessment for aquatic organisms:

For the endocrine disruption assessment two studies testing aquatic organisms and measuring endocrine parameters were conducted: A Fish Short Term Reproduction Assay (FSTRA) with the Fathead minnow ([redacted], 2021a) and an Amphibian Metamorphosis Assay (AMA) with the African clawed frog ([redacted], 2021b). A fish early life stage (ELS) study testing the Fathead minnow ([redacted], 2016) was also considered as it included parameters that are sensitive to but not diagnostic of Estrogen, Androgen, Thyroid and Steriodogenisis modalities (EATS). Please refer to the Section B.9.2.2 of CA dossier, Volume 3 for further consideration.

In the FSTRA study, there were no indications of endocrine effects related to E, A or S modalities following exposure to bixlozone. Gonad histopathology revealed slight increases in occyte atresia at the highest treatment level (1.1 mg a.s./L), however, the causes of oocyte atresia are not limited to endocrine modes of action. A reduction in fecundity was observed at the highest treatment level, along with a transitory reduction in feeding in one replicate. Taken together these results are thought to be indicative of systemic toxicity, rather than endocrine-mediated. In the ELS study there was evidence of systemic toxicity at the highest test concentration, where a 33.3 % reduction in larval survival was observed. There were effects on body length and weight: a significant reduction in weight was observed at the highest test concentration and length was significantly reduced at concentrations above 0.38 mg a.s./L. Please refer to the Section B.9.2.2 of CA dossier, Volume 3 for further consideration.

Based on the available evidence HSE concluded that, in line with EFSA/ECHA 2018 guidance, the results of the FSTRA and ELS studies with bixlozone do not indicate activity in the EAS modalities.

In the AMA study, there were no indications of endocrine effects (T modality) following exposure to bixlozone at any of the concentrations tested. It is noted that whilst not statistically significant, there was an 8.38% decrease in hind limb length normalized by snout-vent length (SVL) in comparison to the control at the highest test concentration of 2.0 mg/L in tadpoles NF>60. However, it was concluded that this delay is likely to be indicative of systemic toxicity. In addition, there was statistically significant effects on wet body weight at day 7 and day 21 (NF<60) and whilst there were no statistically significant effects at day 21 (NF>60), a dose response relationship was present, showing a similar trend to the previous stages. It was concluded that the reduced growth observed in this study is also likely to be indicative of systemic toxicity. Please refer to the Section B.9.2.2 of CA dossier, Volume 3 for further consideration.

Based on the available evidence, HSE concluded that in-line with EFSA/ECHA 2018 guidance the results in submitted AMA testing bixlozone do not indicate anti-thyroidal activity

Overall conclusion:

For aquatic organisms HSE concluded that in-line with EFSA/ECHA 2018 guidance the results in submitted AMA testing bixlozone do not indicate anti-thyroidal activity. The results of the ELS and FSTRA study do not indicate activity in the EAS modalities.

For full details of the endocrine disruption assessment of aquatic organisms see Section B.9.2.2 of CA dossier, Volume 3.

2.9.3. Summary of effects on arthropods

Bees

Toxicity data to address F9600 has been provided. The first tier toxicity data used in the risk assessment is summarised here (Table 2.9.3-1). For full details of all the available toxicity data see the list of endpoints and Section B.9 (CA and CP).

Table 2.9.3-1: First tier toxicity data relevant to the active substance, F9600, for use in the risk assessment

Test organism	Test system	Endpoint (µg a.s./bee)		Reference
Acute toxicity to bees				
Apis mellifera	Acute oral toxicity, 48-hours	$LD_{50, oral}$	> 100	Noél (2014a)
	Acute contact toxicity, 48-hours	$LD_{50, contact}$	> 100	

Toxicity data for the representative formulation, 'F9600 4SC', has also been submitted and evaluated in the relevant CP document. The first tier toxicity data used in the risk assessment is summarised here (Table 2.9.3-2).

Table 2.9.3-2: First tier toxicity data relevant to the representative formulation, 'F9600 4SC', for use in the risk assessment

Test organism	Test system	Endpoint (µg a.s./bee)		Reference
Acute toxicity to bees				
Apis mellifera	Acute oral toxicity, 48-hours	$LD_{50, oral}$	> 111.1 (> 275 µg test item/bee)	Schmitzer (2017)
	Acute contact toxicity, 48-hours	$LD_{50, contact}$	> 100 (> 305 µg test item/bee)	

Non-target arthropods

Toxicity data for the representative formulation, 'F9600 4SC', has been submitted and evaluated in the relevant CP document. The toxicity data used in the risk assessment is summarised here (Table 2.9.3-3).

Table 2.9.3-3: Toxicity data relevant to the representative formulation, 'F9600 4SC', for use in the risk assessment

Test organism	Test system	Endpoint (g a.s./ha)		Reference
First-tier toxicity studies				
Aphidius rhopalosiphi	Glass plate, 48-hours	LR_{50}	≥ 344	Moll (2016a)
Typhlodromus pyri	Glass plate, 7-days	LR_{50}	97.4	Moll (2016b)
Extended laboratory toxicity studies				

Test organism	Test system	Endpoint (g a.s./ha)		Reference
Typhlodromus pyri	Vine leaves	Mortality (< 50 % effects)	473	Moll (2016c)
		Reproduction (< 50 % effects)	367	
Chrysoperla carnea	Vine leaves	Mortality (< 50 % effects)	> 489	Moll (2016d)
		Reproduction (< 50 % effects)	489	

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

The first-tier toxicity data used in the risk assessment is summarised here (Table 2.9.4-1). For full details of all the available toxicity data see the list of endpoints and Section B.9 (CA and CP).

Table 2.9.4-1: Summary of earthworm and soil macro-organism toxicity endpoints

Test Item	Timescale	Species	Endpoint	Results	References[f]
Earthworm Toxicity Endpoints					
Bixlozone (active substance)[a]	56 d, chronic	*Eisenia fetida*	NOEC(mortality) NOEC(mortality)$_{corr}$[b] NOEC(body weight) NOEC(body weight)$_{corr}$[b] NOEC(reproduction) NOEC(reproduction)$_{corr}$[b]	200 mg a.s./kg soil dw 100 mg a.s./kg soil dw 400 mg a.s./kg soil dw 200 mg a.s./kg soil dw 100 mg a.s./kg soil dw **50 mg/kg soil dw**	CA 8.4.1/01 Patnaude, MR., 2015
F9600-4 SC[a,c]	56 d, chronic	*Eisenia fetida*	NOEC(mortality) NOEC(mortality)$_{corr}$[b] NOEC(body weight) NOEC(body weight)$_{corr}$[b] NOEC(reproduction) NOEC(reproduction)$_{corr}$[b]	160mg F9600-4/kg soil dw (58.2 mg a.s./kg soil dw) 80 mg F9600-4/kg soil dw (29.1 mg a.s./kg soil dw) 80 mg F9600-4/kg soil dw (29.1 mg a.s./kg soil dw) 40 mg F9600-4/kg soil dw (14.55 mg a.s./kg soil dw) 80 mg F9600-4/kg soil dw (29.1 mg a.s./kg soil dw) **40 mg F9600-**	CP 10.4.1.1/01 Pavič, B., 2017

Test Item	Timescale	Species	Endpoint	Results	References[f]
				4/kg soil dw (14.55 mg a.s./kg soil dw)	
2,4-dichlorobenzoic acid	56 d, chronic	*Eisenia fetida*	NOEC(mortality) NOEC(mortality)$_{corr}$[b] NOEC(body weight) NOEC(body weight)$_{corr}$[b] NOEC(reproduction) NOEC(reproduction)$_{corr}$[b] EC_{50} EC_{50corr}[b] EC_{20} EC_{20corr}[b] EC_{10} EC_{10corr}[b]	340 mg/kg soil dw 170 mg/kg soil dw 612 mg/kg soil dw 306 mg/kg soil dw 58.3 mg/kg soil dw_d **29.15 mg/kg soil dw** 112 mg/kg soil dw (95% confidence limits: 89.2 – 136 mg/kg soil dw) 106 mg/kg soil dw 76.9 mg/kg soil dw (95% confidence limits: 32.7 – 98.4 mg/kg soil dw) 38.45 mg/kg soil dw 61.6 mg/kg soil dw **(**95% confidence limits: 16.3 – 84.9 mg/kg soil dw**)** 30.8 mg/kg soil dw	CA 8.4.1/02 Wagenhoff, E., 2018
Soil Macro-organism Endpoints					
F9600-4 SC[a,c]	Chronic, 28 d	*Folsomia candida*	NOEC(mortality) NOEC(mortality)corr[b] NOEC(reproduction) NOEC(reproduction)corr[b]	250 mg F9600-4/kg soil dw (90 mg a.s./kg soil dw) 125 mg F9600-4 SC/kg soil dw (45 mg a.s./kg soil dw) 62.5 mg F9600-4	CP 10.4.2.1/01 Pavić B. (2017a)

Test Item	Timescale	Species	Endpoint	Results	References[f]
				SC/kg soil dw (22.5 mg a.s./kg soil dw)[d] **31.25 mg F9600-4 SC/kg soil dw (11.25 mg a.s./kg soil dw)**[e]	
F9600-4 SC[a,c]	Chronic, 14 d	*Hypoaspis aculeifer*	NOEC(mortality) NOEC(mortality)$_{corr}$[b] NOEC(reproduction) NOEC(reproduction)$_{corr}$[b]	1000 mg F9600-4/kg soil dw (360 mg a.s./kg soil dw) 500 mg F9600-4/kg soil dw (180 mg a.s./kg soil dw) 250 mg F9600-4/kg soil dw (90 mg a.s./kg soil dw) **125 mg F9600-4 SC/kg soil dw (45 mg a.s./kg soil dw)**	CP 10.4.2.1/02 Pavić B. (2017b)

Endpoints highlighted in bold used in the risk assessment

In accordance with the outcome of the EFSA (2015) pesticides peer review meeting on general recurring issues in ecotoxicology, the lower between the median EC_{10} and the NOEC will be used in the risk assessment, when reliable.

[a] It was not possible to calculate meaningful EC_{10}, EC_{20} and EC_{50} values for reproduction due to the distribution of the data and the number of concentrations used. Therefore, the NOEC will be used in the risk assessment.

[b] Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

[c] Formulation contained 36.4% w/w active substance, corresponding to 400 g/L; density 1.2 g/mL

[d] It is noted that the study authors proposed a NOEC$_{(reproduction)}$ of 105 mg/kg soil dw for 2,4-dichlorobenzoic acid, however, at this concentration a 34.3% reduction in juvenile number was observed. As such, HSE considers that the NOEC should be set at the lower test concentration of 58.3 mg/kg soil dw (29.15 mg/kg soil dw$_{corr}$) at which there was no reduction in reproductive output (-4% in comparison to the control group).

[e] The study authors have proposed a NOEC for reproduction of 125 mg F9600-4 SC/kg soil dw, however, at this concentration there was a 15% reduction in comparison to the control, in addition this appears to be part of a dose-response relationship. As such, HSE considers that the NOEC should be set at the lower concentration of 62.5 mg/kg soil dw (31.25 mg/kg soil dw$_{corr}$) at which there was a 7% reduction.

It is noted that although acute earthworm studies with the technical active substance (Définod, C., 2014a) and the formulated product (Pavić, B., 2016) are available, acute studies are no longer necessary under current data requirements (Commission Regulation (EU) No. 283/2013 and 284/2014), therefore they have not been evaluated by HSE or considered further in the risk assessment.

The active substance, bixlozone, exceeds the relevant trigger values for volatilisation for Environmental Fate and as detailed in the chemistry dossier (CA B.2.) the vapour pressure of bixlozone is 1.1 × 10-3 Pa at 20 °C and classified as 'slightly volatile'. Following a request for additional information, the applicant referred to the available soil metabolism study by Simmonds, R., (2015a) (CA B.8.). This was considered in consultation with the Environmental Fate evaluator and it was agreed that bixlozone is not volatile when incorporated into soil.

2.9.5. Summary of effects on soil nitrogen transformation

The first-tier toxicity data used in the risk assessment is summarised here (Table 2.9.5-1). For full details of all the available toxicity data see the list of endpoints and Volume 3 CA Section B.9.5 and Volume 3 CP Section B9.9.

Table 2.9.5-1: Summary of effects on soil micro-organisms

Test Item	Exposure system	Results	References[b]
Bixlozone (active substance)	28 d	No effect >25% at **1000mg/kg soil dw**	CA 8.5/01 Deslandes, L., 2014a
F9600-4SC[a]	28 d	No effect at 1.51 mg F9600-4/kg soil dw (375 g a.s./ha) and **7.55 mg F9600-4/kg soil dw (1875 g a.s./ha)**	CP 10.5/01 Hammesfahr, U., 2016
2,4-Dichlorobenzoic acid	28 d	No effect >25% at .357 mg/kg soil dw and **1.79 mg/kg soil dw**	CA 8.5/02 Häuser, R., 2018

[a] Formulation contained 36.4% w/w active substance, corresponding to 400 g/L
Endpoints highlighted in bold used in the risk assessment
[b] A carbon transformation study was also submitted (Deslandes, L., 2014b), however, as this endpoint is no longer necessary under current data requirements (Commission Regulation (EU) No. 283/2013 and 284/2014), it has not been evaluated by HSE.

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

Toxicity data for the representative formulation, 'F9600 4SC', has been submitted and evaluated in the relevant CP document. The toxicity data used in the risk assessment is summarised here (Table 2.9.6-1).

Table 2.9.6-1: Toxicity data relevant to the representative formulation, 'F9600 4SC', for use in the risk assessment

Test organism	Test system	Endpoint (g a.s./ha)		Reference
Toxicity to non-target higher plants				
Allium cepa	Vegetative vigour, 21-days	ER_{50}	99.8 [a]	Kirkwood (2017b)
Lycopersicon esculentum	Seedling emergence, 21-days	ER_{50}	19 [a]	Kirkwood (2017a)

[a] *Note: The endpoints quoted here only address toxicity in terms of survival, emergence, shoot length and shoot dry weight (depending on the study type). These endpoints do not address observation of phytotoxicity in the study. Further consideration of phytotoxicity is required during risk assessment (see the discussion below).*

Phytotoxicity: The endpoints quoted in Table 2.9.6-1 only account for effects on survival, emergence, shoot length and shoot dry weight. In both studies (Kirkwood, 2017a and Kirkwood, 2017b) other morphological/phytotoxic effects were observed as a result of the treatments (i.e. chlorosis, necrosis, leaf curling and leaf blotching). The impact of these symptoms in plants growing within a non-laboratory system cannot be established.

In the absence of a modelled ER_{50} for these effects an alternative conservative screening approach has been taken. Specifically, the treatment concentration below the lowest treatment in which < 50 % of surviving plants were observed as healthy has been considered as an endpoint for these effects. This approach results in no further action being required for seedling emergence as the endpoint quoted in Table 2.9.6-1 (19 g a.s./ha) addresses the observed phytotoxic effects. However, for vegetative vigour the endpoint in Table 2.9.6-1 (99.8 g a.s./ha) does not addresses the observed phytotoxic effects so **an endpoint of 1.5 g a.s./ha has been considered to account for phytotoxic effects**.

Volatilisation: The active substance, bixlozone, is known to volatilise resulting in the potential for aerial transport and deposition (Vp = 10^{-5} Pa (plant) or 10^{-4} Pa (soil) at 20℃ as outlined in Reg. 283/2013). A wind tunnel study, Staffa (2016), has been submitted to consider this potential route of exposure. A risk assessment has been conducted based on the exposure established in Staffa (2016) and the lower-tier phytotoxicity estimate (1.5 g a.s./ha).

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

No further data was submitted.

2.9.8. Summary of effects on biological methods for sewage treatment

The first-tier toxicity data used in the risk assessment is summarised here (Table 2.9.8-1). For full details of all the available toxicity data see the list of endpoints and Volume 3 CA Section B.9.8.

Table 2.9.8: Table of endpoints

Organism	Test substance	Test type	Endpoint	Reference
Activated sludge microorganisms	Bixlozone	OECD 209 (2010)	NOEC (Total respiration) = **100 mg/L** NOEC (Heterotrophic Respiration) = 1000 mg/L NOEC (Nitrification Respiration) = 100 mg/L EC_{10} (Total respiration) = 291 (104 – 820) mg/L EC_{10} (Heterotrophic Respiration) = n.d. (considered to be > 1000 mg/L) EC_{10} (Nitrification Respiration) = 140 (51-382) mg/L	Hammesfahr, U., (2016)

2.9.9. Summary of product exposure and risk assessment

2.9.9.1. Risk assessment for birds

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to Guidance of EFSA : Risk Assessment for Birds and Mammals: EFSA Journal 2009; 7(12):1438

Acute and long-term/reproductive risk to birds

The acute and long-term/reproductive risk assessment for birds is summarised in Table 2.9.9.1-1 for exposure to the active substance. Risk assessment is only presented for use on winter wheat (BBCH 11 – 13) at 200 g a.s./ha as this risk assessment addresses all the proposed uses. **An acceptable acute and long-term/reproductive risk to birds has been demonstrated. Therefore, no further consideration is required for any of the proposed uses.**

Table 2.9.9.1-1: Screening assessment of the acute and long-term/reproductive risk to birds due to the uses of F9600-4SC on Winter wheat at BBCH 11 - 13

Active substance			F9600			
Acute toxicity (mg/kg bw)			>2000			
Short-term dietary toxicity (mg/kg bw/d)			679			
TER criterion			10			
Crop scenario	**Application rate (kg a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**[1]TER_a**
Winter wheat BBCH 11 - 13	0.200	Small omnivorous bird	158.8	1.0	31.76	>63.0
Long-term/reproductive toxicity (mg/kg bw/d)			77.7			
TER criterion			5			
Crop scenario	**Application rate (kg a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m x TWA**	**DDD_m (mg/kg bw/d)**	**TER_{LT}**
Winter wheat BBCH 11 - 13	0.200	Small omnivorous bird	64.8	1.0 x 0.53	6.87	11.3

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; n.a.: not applicable ; TWA: time-weighted average factor. TER values shown in **bold** fall below the relevant trigger.
[1]Considering the acute toxicity endpoint

Risk assessment for birds drinking contaminated water

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

F9600 has a K(f)oc value of 381.5 mL/g, therefore, the trigger value is 50. At the maximum application rate (375 g a.s./ha in maize) the resulting value is below 50 and therefore no TER calculation is necessary and the risk to birds from contaminated drinking water is acceptable.

Risk assessment for secondary poisoning

Bixlozone has a $logP_{OW}$ of 3.3, 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 29.2 (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 353 (trigger value of 5) which **indicates an acceptable risk to fish-eating birds.**

Metabolites of bixlozone

Numerous metabolites were detected in a plant metabolism study, however under field conditions their occurrence was considered to be negligible in foliage. As regards residues of metabolites in seeds and grains, the risk assessment was conducted assuming that the toxicity of the metabolites was ten times that of the parent, whilst the exposure was based on the highest residue found. The outcome of the risk assessment was that both the acute and long-term/reproductive risk assessment was acceptable.

2.9.9.2. Risk assessment for mammals

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to Guidance of EFSA : Risk Assessment for Birds and Mammals: EFSA Journal 2009; 7(12):1438

The acute and long-term risk assessment for mammals is summarised in Table 2.9.9.2-1 for exposure to the active substance. Risk assessment is only presented for use on winter wheat (BBCH 11 – 13) at 200 g a.s./ha as this risk assessment addresses all the proposed uses. **An acceptable acute and long-term/reproductive risk to mammals has been demonstrated. Therefore, no further consideration is required for any of the proposed uses**.

Table 2.9.9.2-1: Screening assessment of the acute and long-term risk to mammals due to the uses of F9600-4SC on Winter wheat at BBCH 11 - 13

Active substance			F9600			
Acute toxicity (mg/kg bw)			>2000			
TER criterion			10			
Crop scenario	**Application rate (kg a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Winter wheat BBCH 11 - 13	0.200	Small herbivorous mammal	118.4	1.0	23.68	>84.5
Long-term/reproductive toxicity (mg/kg bw/d)			34			
TER criterion			5			
Crop scenario	**Application rate (kg a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m x TWA**	**DDD_m (mg/kg bw/d)**	**TER_{LT}**
Winter wheat BBCH 11 - 13	0.200	Small herbivorous mammal	48.3	1.0 x 0.53	5.12	6.6

SV: shortcut value; MAF: multiple application factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; n.a.: not applicable ; TWA: time-weighted average factor. TER values shown in **bold** fall below the relevant trigger.

Risk assessment for mammals drinking contaminated water

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

F9600 has a K(f)oc value of 381.5 mL/g, therefore, the trigger value is 50. At the maximum application rate (375 g a.s./ha in maize) the resulting value is below 50 and therefore no TER calculation is necessary and the risk to mammals from contaminated drinking water is acceptable.

Risk assessment for secondary poisoning

Bixlozone has a $logP_{OW}$ of 3.3, 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 10.5 (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 138 (trigger value of 5) which **indicates an acceptable risk to fish-eating mammals.**

Metabolites of bixlozone

Numerous metabolites were detected in a plant metabolism study, however under field conditions their occurrence was considered to be negligible in foliage. As regards residues of metabolites in seeds and grains, the risk assessment was conducted assuming that the toxicity of the metabolites was ten times that of the parent, whilst the exposure was based on the highest residue found. The outcome of the risk assessment was that both the acute and long-term/reproductive risk assessment was acceptable.

2.9.9.3. Risk assessment for aquatic organisms

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters: EFSA Journal 2013;11(7):3290.

Active substance

Tier 1 aquatic risk assessment for Bixlozone

Table B2.9.9.3-1 shows the aquatic risk assessment for surface water and sediment for the proposed uses of 'F9600-4SC'on maize (BBCH 00 - 09) at 375 g a.s./ha ; this use gives the highest PEC values for surface water and sediment and therefore all other uses are covered by the below assessment.

Table B2.9.9.3-1 Tier 1 PEC/RAC ratios for Bixlozone in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Fish long-term	Aquatic invertebrates	Aquatic invertebrates long-term	Algae	Higher plant	Sediment dwelling invertebrate
		O. mykiss	*P. promelas*	*A. bahia*	*A. bahia*	*R. subcapitata*	*M. spicatum*	*C. riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (LC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (E_rC_{20})	RAC (NOEC)
		98 µg/L	38 µg/L	1.4 µg/L	12 µg/L	1400 µg/L	3.3 µg/L	6900 µg/kg sed. dw
Spraydrift (1 m)	3.463 (3.687)[a]	0.035	0.09	**2.474**	0.289	0.002	**1.05**	0.0005
Drainflow	20.192 (21.500)	0.206	0.53	**14.423**	**1.683**	0.014	**6.12**	0.003

[a] PEC values in parentheses are sediment exposure concentrations expressed as µg/kg sed. dw; they have been used for risk assessment of the sediment dwelling invertebrates
Values in **bold** are above the trigger of 1

Conclusion: For the proposed use in maize at 375 g a.s./ha, there is an unacceptable acute and chronic risk to aquatic invertebrates via drainflow and an unacceptable acute risk to aquatic invertebrates and risk to aquatic plants via spraydrift. Therefore further consideration is required at tier 2 ; this is detailed below.

Tier 2 aquatic risk assessment for Bixlozone

Table B2.9.9.3-2 Tier 2 PEC/RAC ratios for Bixlozone in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Aquatic invertebrates acute	Aquatic invertebrates long-term	Aquatic plants
		7 aquatic invertebrate endpoints (without B. calyciflorus and G. fasciatus)	*A. bahia*	*M. spicatum*
		Geomean RAC	RAC (NOEC)	RAC
		6.69 µg/L	12 µg/L	3.3 µg/L
Spraydrift (1 m)	3.463	0.512	0.289	**1.049**
Spraydrift (5 m)	0.888	0.133	0.074	0.269
Drainflow	20.192	**3.02**	**1.683**	**6.12**

Values in **bold** are above the trigger of 1

Conclusion: For the proposed use in maize at 375 g a.s./ha, the acute risk to aquatic invertebrates from spray drift is resolved however the acute and chronic risk to aquatic invertebrates and risk to aquatic plants from drainflow is unacceptable and further consideration is required. A 5 m buffer zone is required to address the risk to aquatic plants from spray drift.

Higher tier drainflow modelling - Bixlozone

Conclusion: Higher tier drainflow modelling (HTDF) was carried out by the Environmental Fate and Behaviour specialist in volume 3CP B8, section B8.5.2.2. The modelling uses the RAC of 3.3 µg/L based on aquatic plants for bixlozone.

As the total number of years with bixlozone RAC exceedances were ≤18 (the threshold for acceptability for aquatic plants) and the overall weighted level of exceedance 'safe years' were ≥90%, an acceptable HTDF was obtained for the maize, winter oilseed rape and winter cereals GAP for bixlozone.

Metabolites of Bixlozone

Tier 1 aquatic risk assessment for 2,4-dichlorobenzoic acid

Table B2.9.9.3-3 shows the aquatic risk assessment for surface water and sediment for the proposed uses of 'F9600-4SC'on maize (BBCH 00 - 09) at 375 g a.s./ha ; this use gives the highest PEC values for surface water and sediment and therefore all other uses are covered by the below assessment.

Table B2.9.9.3-3 Tier 1 PEC/RAC ratios for 2,4-dichlorobenzoic acid in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Fish long-term	Aquatic invertebrates	Aquatic invertebrates long-term	Algae	Higher plant	Sediment dwelling invertebrate
		Parental toxicity	*Parental toxicity*	*A. bahia*	*Parental toxicity*	*R. subcapitata*	*M. spicatum*	*C. riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (LC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (E_rC_{50})	RAC (NOEC)
		98 µg/L	38 µg/L	> 10000 µg/L	12 µg/L	9010 µg/L	2400 µg/L	10488 µg/kg sed. dw
Spraydrift (1 m)	0.732 (0.355)[a]	0.007	0.019	<0.001	0.061	<0.001	<0.001	<0.001
Drainflow	38.146 (176.06)	0.389	**1.0**	0.004	**3.179**	0.004	0.016	0.004
Ground water	2.787	0.028	0.073	0.000	0.232	<0.001	0.001	-

[a] PEC values in parentheses are sediment exposure concentrations expressed as µg/kg sed. dw; they have been used for risk assessment of the sediment dwelling invertebrates
Values in **bold** are above the trigger of 1

Conclusion: For the proposed use in maize at 375 g a.s./ha, there is an unacceptable chronic risk to aquatic invertebrates via drainflow and a marginal failure for the chronic risk to fish when based on parental NOEC. Therefore further refinement is necessary; this is detailed below.

Higher tier drainflow modelling - 2,4-dichlorobenzoic acid

Higher tier drainflow modelling was carried out by HSE Environmental Fate and Behaviour in volume 3CP section B8.5.2.2. In summary, as the total number of years with 2,4-DBA RAC exceedances were ≤3 (the threshold for acceptability for aquatic invertebrates) and the overall weighted level of exceedance 'safe years' were ≥90%, an acceptable HTDF was also obtained for the maize, winter oilseed rape and winter cereals GAP for 2,4-DBA.

HSE has also assessed the combined HTDF risk of bixlozone and 2,4-DBA using the Finney equation (based on annual maximum PEC_{SW} and, where necessary, daily PEC_{SW}). As the bixlozone and 2,4-DBA RACs come from different aquatic groups (i.e. bixlozone from aquatic plants and 2,4-DBA from aquatic invertebrates), the CA has undertaken separate combined risk assessments considering the relevant RAC in each group. For aquatic plants, this corresponds to 3.3 µg/L for bixlozone and 2400 µg/L for 2,4-DBA. For aquatic invertebrates, the relevant RAC values are 6.69 µg/L for bixlozone and 12 µg/L for 2,4-DBA. As the total number of years where the aquatic plant RACs were exceeded were ≤18 and the aquatic invertebrate RACs were ≤3, and in both instances the weighted level of exceedances 'safe years' were ≥90%, acceptable combined HTDF assessments were obtained for the maize, winter oilseed rape and winter cereals GAP**.** For all three GAPs, both HTDF acceptability criteria (the number of exceedances and the overall scenario year assessment) were acceptable.

Tier 1 aquatic risk assessment for 4-Carboxyl-F9600

Table B2.9.9.3-3 shows the aquatic risk assessment for surface water and sediment for the proposed uses of 'F9600-4SC'on maize (BBCH 00 - 09) at 375 g a.s./ha ; this use gives the highest PEC values for surface water and sediment and therefore all other uses are covered by the below assessment.

Table B2.9.9.3-3 Tier 1 PEC/RAC ratios for 4-Carboxyl-F9600 in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Fish long-term	Aquatic invertebrates	Aquatic invertebrates long-term	Algae	Higher plant	Sediment dwelling invertebrate
		Parental toxicity	*Parental toxicity*	*A. bahia*	*Parental toxicity*	*R. subcapitata*	*M. spicatum*	*C. riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (LC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (E_rC_{50})	RAC (NOEC)
		98 µg/L	38 µg/L	>10000 µg/L	12 µg/L	7100 µg/L	130 µg/L	49454 µg/kg sed. d w
Spraydrift (1 m)	0.676 (0.214)	0.007	0.018	<0.001	0.056	<0.001	0.005	<0.001
Drainflow	3.942 (7.081)	0.040	0.104	<0.001	0.329	0.001	0.030	<0.001

[a] PEC values in parentheses are sediment exposure concentrations expressed as µg/kg sed. dw; they have been used for risk assessment of the sediment dwelling invertebrates

Tier 1 aquatic risk assessment for F9600-dimethyl-malonamide

Table B2.9.9.3-4 shows the aquatic risk assessment for surface water and sediment for the proposed uses of 'F9600-4SC'on maize (BBCH 00 - 09) at 375 g a.s./ha ; this use gives the highest PEC values for surface water and sediment and therefore all other uses are covered by the below assessment.

Table B2.9.9.3-4 Tier 1 PEC/RAC ratios for F9600-dimethyl-malonamide in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Fish long-term	Aquatic invertebrates	Aquatic invertebrates long-term	Algae	Higher plant	Sediment dwelling invertebrate
		Parental toxicity	*Parental toxicity*	*A. bahia*	*Parental toxicity*	*R. subcapitata*	*M. spicatum*	*C. riparius*
		RAC (LC_{50})	RAC (NOEC)	RAC (LC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (E_rC_{50})	RAC (NOEC)
		98 µg/L	38 µg/L	>10000 µg/L	12 µg/L	7100 µg/L	>10000 µg/L	50200 µg/kg sed. dw
Spraydrift (1 m)	0.453 (0.119)	0.005	0.012	<0.001	0.038	<0.001	<0.001	<0.001
Drainflow	2.641 (5.622)	0.027	0.070	<0.001	0.220	<0.001	<0.001	<0.001

[a] PEC values in parentheses are sediment exposure concentrations expressed as µg/kg sed. dw; they have been used for risk assessment

Tier 1 aquatic risk assessment for F9600-3-OH-propanamide

Table B2.9.9.3-5 shows the aquatic risk assessment for surface water and sediment for the proposed uses of 'F9600-4SC'on maize (BBCH 00 - 09) at 375 g a.s./ha ; this use gives the highest PEC values for surface water and sediment and therefore all other uses are covered by the below assessment.

Table B2.9.9.3-5 Tier 1 PEC/RAC ratios for F9600-3-OH-propanamide in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Fish long-term	Aquatic invertebrates	Aquatic invertebrates long-term	Algae	Higher plant	Sediment dwelling invertebrate
		Parental toxicity	*Parental toxicity*	*A. bahia*	*Parental toxicity*	*R. subcapitata*	*Equal to a.s. toxicity*	*Equal to a.s. toxicity*
		RAC (LC_{50})	RAC (NOEC)	RAC (LC_{50})	RAC (NOEC)	RAC (E_rC_{50})	RAC (E_rC_{50})	RAC (NOEC)
		98 µg/L	38 µg/L	2200 µg/L	12 µg/L	> 8400 µg/L	320 µg/L	6900 µg/kg sed. Dw
Spraydrift (1 m)	0.126 (0.057)	0.001	0.003	<0.001	0.011	<0.001	<0.001	<0.001
Drainflow	0.730 (9.312)	0.007	0.019	<0.001	0.061	<0.001	0.002	<0.001

[a] PEC values in parentheses are sediment exposure concentrations expressed as µg/kg sed. Dw; they have been used for risk assessment of the sediment dwelling invertebrates

Conclusion for metabolites: Acceptable risks to aquatic organisms have been demonstrated for all proposed uses for all metabolites with the exception of the chronic risk to aquatic invertebrates from 2,4-DBA via drainflow where the trigger value was exceeded for all proposed GAPs. As such, higher tier drainflow modelling was considered and an acceptable risk to aquatic organisms concluded.

Formulation

Tier 1 aquatic risk assessment for F9600-4SC'

Table B2.9.9.3-6 shows the aquatic risk assessment for surface water for the proposed uses of 'F9600-4SC'on maize (BBCH 00 - 09) at 375 g a.s./ha ; this use gives the highest PEC values for surface water and therefore all other uses are covered by the below assessment.

Table B2.9.9.3-6 Tier 1 PEC/RAC ratios for 'F9600-4SC'on in maize at 1 x 375 g a.s./ha

Scenario	PEC (µg/L)	Fish acute	Aquatic invertebrates		Algae	Higher plant
		O. mykiss	*D. magna*	*A. bahia*	*S. costatum*	*M. spicatum*
		RAC (LC_{50})	RAC (LC_{50})	RAC (LC_{50})	RAC	RAC (E_rC_{20})
		320 µg/L	610 µg/L	39 µg/L	1700	290 µg/L
Spraydrift (1 m)	9.704	0.030	0.016	0.249	0.006	0.033

Conclusion for representative formulation: Acceptable risks to aquatic organisms have been demonstrated for all proposed uses for the formulated product without risk mitigation.

Overall conclusion for aquatic organisms: An acceptable risk to aquatic organisms from the active substance, all metabolites and the representative formulation of Bixlozone can be concluded for the proposed use on winter oilseed rape at 300 g a.s./ha and winter cereals at 200 g a.s./ha without the need for risk mitigation. A 5 m buffer zone is required to address the risk to aquatic plants from spray drift for the proposed use on maize at 375 g a.s./ha.

2.9.9.4. Risk assessment for bees

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to the 'Guidance Document on Terrestrial Ecotoxicology' (SANCO/10329/2002).

The risk assessment for bees is summarised in Table 2.9.9.4-1, for exposure to both the active substance and representative formulation. Risk assessment is only presented for use on maize at 375 g a.s./ha as this risk assessment addresses all the proposed uses. An acceptable risk has been demonstrated. Therefore, no further consideration is required for any of the proposed uses.

Table 2.9.9.4-1: Summary of the first-tier risk assessment for bees due to the use of 'F9600-4SC' on maize

Active substance	F9600		
Application rate (g a.s./ha)	1 × 375		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	375	< 3.75
Contact toxicity	> 100		< 3.75
Product	'F9600-4 SC'		
Application rate (g a.s./ha)	1 × 375		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	375	< 3.75
Contact toxicity	> 111.1		< 3.38

*Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.*

2.9.9.5. Risk assessment for non-target arthropods

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to the 'ESCORT 2' guidance (Candolfi *et al.*, 2001).

The first-tier risk assessment for non-target arthropods is summarised in Table 2.9.9.5-1, for exposure to the representative formulation. An acceptable risk has been demonstrated in all cases for the off-field scenario, no further consideration was required. Acceptable risks were not demonstrated for the in-field scenario for any use; further consideration was therefore required, summarised in Table 2.9.9.5-2. Acceptable risks were concluded for the proposed uses on winter cereals and winter oilseed rape. Further consideration is required for the proposed use on maize (see below).

Table 2.9.9.5-1: Summary of the first-tier risk assessment for non-target arthropods, due to the proposed uses of 'F9600 4SC'

Test species Tier-1	**LR_{50} (lab.) (g a.s./ha)**	**$PER_{in\text{-}field}$ (g a.s./ha)**	**$HQ_{in\text{-}field}$ criterion: HQ < 2**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**$HQ_{off\text{-}field}$ criterion: HQ < 2**
Use on winter cereals at 200 g a.s./ha (1 x application; MAF = 1)					
Typhlodromus pyri	97.4	200	**2.05**	- [a]	- [a]
Aphidius rhopalosiphi	≥ 344		≤ 0.58		- [a]
Use on winter oilseed rape at 300 g a.s./ha (1 x application; MAF = 1)					

Typhlodromus pyri	97.4	300	**3.08**	- [a]	- [a]
Aphidius rhopalosiphi	≥ 344		≤ 0.87		- [a]
Use on maize at 375 g a.s./ha (1 x application; MAF = 1)					
Typhlodromus pyri	97.4	375	**3.85**	1.04	0.11
Aphidius rhopalosiphi	≥ 344		≤ 1.09		≤ 0.03

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient.
*HQ values shown in **bold** breach the relevant trigger.*
[a] *Risk assessment addressed by assessment for the use on maize, same conclusion applies*

Table 2.9.9.5-2: Summary of the higher risk assessment for non-target arthropods, due to the proposed uses of 'F9600 4SC'

Test species Higher Tier	**Rate with ≤ 50 % effect (g a.s./ha)**	**$PER_{in\text{-}field}$ (g a.s./ha)**	**$PER_{in\text{-}field}$ below rate with < 50 % effect? [a]**
Use on winter cereals at 200 g a.s./ha (1 x application; MAF = 1)			
Typhlodromus pyri	LR_{50} = 473 g a.s./ha	200	Yes
	Reproduction = 367 g a.s./ha		Yes
Chrysoperla carnea	LR_{50} > 489 g a.s./ha		Yes
	Reproduction = 489 g a.s./ha		Yes
Use on oilseed rape at 300 g a.s./ha (1 x application; MAF = 1)			
Typhlodromus pyri	LR_{50} = 473 g a.s./ha	300	Yes
	Reproduction = 367 g a.s./ha		Yes
Chrysoperla carnea	LR_{50} > 489 g a.s./ha		Yes
	Reproduction = 489 g a.s./ha		Yes
Use on maize at 375 g a.s./ha (1 x application; MAF = 1)			
Typhlodromus pyri	LR_{50} = 473 g a.s./ha	375	Yes
	Reproduction = 367 g a.s./ha		**No**
Chrysoperla carnea	LR_{50} > 489 g a.s./ha		Yes
	Reproduction = 489 g a.s./ha		Yes

[a] *Where $PER_{in\text{-}field}$ is below the rate with ≤ 50 % effect an acceptable risk can be concluded*

Further consideration of in-field risk for use on maize

It is noted that a range of application rates are proposed for use on maize (250-375 g a.s./ha). An acceptable in-field risk could be concluded for an application rate to maize of ≤ 367 g a.s./ha (as < 50% effects were reported at this concentration).

A further consideration is the potential for re-colonisation. The risk assessment for off-field exposure (presented above) indicates an acceptable risk when considering the maximum application rate proposed of 375 g a.s./ha. Assuming a default foliar DT_{50} value of 10 days, the residues in-field would be anticipated to fall below the rate resulting in < 50% effects (367 g a.s./ha), after only 1 day. Therefore, there appears to be the potential for recolonisation in-field from the off-field area within a short duration.

As a consequence it is deemed reasonable to conclude an acceptable risk to non-target arthropods, when considering an application rate to maize of ≤ 367 g a.s./ha.

2.9.9.6. Risk assessment for soil meso- and macro-fauna

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to the 'Guidance Document on Terrestrial Ecotoxicology' (SANCO/10329/2002).

The risk assessment for earthworms and (other) soil meso- and macro-fauna is summarised in Tables 2.9.9.6- 1-3, for exposure to both the active substance, metabolites and representative formulation. Overall an acceptable risk was concluded for soil organisms.

Risk assessment for earthworms

The potential long-term risk to earthworms has been determined by calculating long-term TER (TER_{LT}) values by comparing the $NOEC/EC_{10}$ resulting from the chronic earthworm studies with the maximum PEC_{soil}

Table 2.9.9.6-1: First-tier assessment of the chronic risk for earthworms due to the use of F9600-4 SC

Use pattern	Test item	$NOEC/EC_{10}$ Corr (mg a.s./kg dw)	PEC_{soil} (mg/kg dw)[b]	TER_{LT} (criterion TER ≥ 5)
Maize 1 x 375 g a.s./ha	Bixlozone (active substance)	50	0.780	64.1
	2,4-dichlorobenzoic acid	29.15	0.544	53.6
	F9600-4 SC	40[c]	1.402[c]	28.5
Winter oilseed rape 1 x 300 g a.s./ha	Bixlozone (active substance)	50	0.624	80.1
	2,4-dichlorobenzoic acid	29.15	0.435	67.0
	F9600-4 SC	40[c]	1.121[c]	35.7
Winter cereals 1 x 200 g a.s./ha	Bixlozone (active substance)	50	0.416	120
	2,4-dichlorobenzoic acid	29.15	0.290	100
	F9600-4 SC	40[c]	0.748[c]	53.5

[a] The $logK_{ow}$ values of bixlozone (3.3) and 2,4-dichlorobenzoic acid (2.82) are both greater than 2 (Section 3CA B2), therefore the $NOEC/EC_{10}$ values have been corrected by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
[b] The maximum $PEC_{accumulation}$ value for bixlozone and maximum $PEC_{initial}$ values for 2,4-dichlorobenzoic acid and bixlozone-4 SC (Section 3CP B.8), have been used in the risk assessment.
c mg formulation/kg dw

All TER values are above the relevant regulatory trigger value of 5 for chronic effects on earthworms.

It is noted that in the studies with F9600-4 SC (Pavič, B., 2017) and the metabolite 2,4-dichlorobenzoic acid (Wagenhoff, E., 2018), it was not clear if behavioural and morphological observations were made throughout the study period, although it was noted that no adverse effects were apparent on Day 28. It is considered by HSE that the large margins of safety in the risk assessment are protective of any uncertainty in this case. Therefore, an acceptable chronic risk from the intended uses of F9600-4 SC is concluded for earthworms. No further assessment is required.

Risk assessment for other soil macro-organisms

The potential risk to soil macro-organisms has been determined by calculating long-term TER (TER_{LT}) values by comparing the NOEC/EC_{10} resulting from the chronic toxicity studies with the maximum PEC_{soil}

Table 2.9.9.6-2: First-tier assessment of the chronic risk for *Folsomia candida* due to the use of F9600-4 SC

Use pattern	Test item	NOEC/EC_{10} Corr (mg/kg dw)[a]	PEC_{soil} (mg/kg dw)[b]	TER_{LT} (criterion TER ≥ 5)
Maize 1 x 375 g a.s./ha	Bixlozone (active substance)	11.25[c]	0.780	14.4
	2,4-dichlorobenzoic acid	1.125[d]	0.544	**2.1**
	2,4-dichlorobenzoic acid	1.125[d]	0.349[f]	**3.2**
	F9600-4 SC	31.25[e]	1.402[e]	22.3
Winter oilseed rape 1 x 300 g a.s./ha	Bixlozone (active substance)	11.25[c]	0.624	18.0
	2,4-dichlorobenzoic acid	1.125[d]	0.435	**2.6**
	2,4-dichlorobenzoic acid	1.125[d]	0.279[f]	**4.0**
	F9600-4 SC	31.25[e]	1.121[e]	27.9
Winter cereals 1 x 200 g a.s./ha	Bixlozone (active substance)	11.25[c]	0.416	27.0
	2,4-dichlorobenzoic acid	1.125[d]	0.290	**3.9**
	2,4-dichlorobenzoic acid	1.125[d]	0.186[f]	6.0
	F9600-4 SC	31.25[e]	0.748[e]	41.8

Values in bold are below the regulatory trigger value of 5

[a] The $logK_{ow}$ values of bixlozone (3.3) and 2,4-dichlorobenzoic acid (2.82) are both greater than 2 (Section 3CA B2), therefore the NOEC/EC_{10} values have been corrected by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

[b] The maximum $PEC_{accumulation}$ value for bixlozone and maximum $PEC_{initial}$ values for 2,4-dichlorobenzoic acid and bixlozone-4 SC (Section 3CP B.8), have been used in the risk assessment.

[c] In the absence of toxicity data with the technical a.s., the formulation data have been expressed in terms of the a.s. content and used alongside the a.s. PEC_{soil} in the risk assessment.

[d] In the absence of toxicity data with the metabolite 2,4-dichlorobenzoic acid, it has been assumed in the risk assessment that the metabolite is 10x more toxic than the parent.

[e] mg formulation/kg dw

[f] Refined value for 2,4-dichlorobenzoic acid , please refer to Volume 3CP B.8

Under current data requirements (Commission Regulation (EU) No 283/20130), studies with the technical a.s. are required for non-target soil meso- and macrofauna. However, for the current risk assessment, in the absence of toxicity data with the technical a.s., formulation data have been expressed in terms of a.s. content and used alongside the a.s. PEC_{soil} in the risk assessment. It is noted that F9600-4 SC is a single a.s. formulation, thererefore it is likely that the formulation assessment is protective of the risk from the a.s. In addition, the available toxicity data with earthworms (Table B.9.8-1) indicates that the toxicity of the formulation ($NOEC_{corrected}$: 40 mg F9600-4/kg soil dw) is not under representative of the a.s. alone ($NOEC_{corrected}$: 50 mg a.s./kg soil dw), therefore, the current risk assessment is considered to be protective of the risk from the a.s.

It is also noted that no data is available for the soil metabolite 2,4-dichlorobenzoic acid, therefore, 10x the toxicity of the parent (in this case 10x the toxicity of the formulation endpoint expressed in terms of the a.s. content) has been used in the risk assessment. This approach is considered to be protective as the available toxicity data for earthworms (Table B.9.8-1) indicates that the metabolite ($NOEC_{corrected}$: 29.15 mg/kg soil dw) is similar in toxicity to the technical a.s. ($NOEC_{corrected}$: 50 mg a.s./kg soil dw), therefore, the current risk assessment is also considered to be protective of the risk from the metabolite.

The majority of TER values are above the relevant regulatory trigger value of 5, therefore an acceptable chronic risk can be concluded for *Folsomia candida* for these groups, with the exception of those for 2,4-dichlorobenzoic acid in maize (refined TER: 4.0) and winter oilseed rape (refined TER: 3.2). It is also noted that in the available study with F9600-4 SC (Pavič, B., 2017a), it is not stated in the study report if the extraction efficiency was validated which presents some uncertainty regarding the reliability of the extraction method. This has been considered further by HSE.

HSE considers that the risk assessment for 2,4-dichlorobenzoic acid in this instance is very worst case. It is also noted that the margins of failure in the risk assessment are relatively narrow. In the absence of chronic formulation toxicity data with *Folsomia candida*, reference is made to the available toxicity data with earthworms (Table B.9.8-1) and aquatic invertebrates (Table B.9.8-5). It is noted that based on the earthworm data, the metabolite ($NOEC_{corrected}$: 29.15 mg/kg soil dw) is of similar toxicity to the formulation ($NOEC_{corrected}$: 40 mg F9600-4/kg soil dw) and the active substance ($NOEC_{corrected}$: 50 mg a.s./kg soil dw). A more relevant comparison has also been undertaken with the data available for aquatic invertebrates – with the acute toxicity data available for *Daphnia magna* and *Americamysis bahia* and chronic data available for *Chironomus riparius*. In each case the toxicity endpoint for bixlozone is lower than that obtained in the corresponding study conducted with the metabolite 2, 4-dichlorobenzoic acid. Therefore, it is expected that the metabolite would be of similar toxicity to *Folsomia candida* compared to the formulation, if toxicity data were available. With reference to the risk assessment (Table B.9.8-4), even if the metabolite was 6x more toxic than the formulation to *Folsomia candida* it would still pass. In light of the earthworm and aquatic invertebrate toxicity data, it seems very unlikely that this would be the case.

Overall, based on the available margins of safety in the risk assessment for bixlozone and F9600-4 SC, and the consideration of the earthworm and aquatic invertebrate data. HSE considers that the risk assessment is protective and a low risk to *Folsomia candida* can be concluded.

Table 2.9.9.6-3: First-tier assessment of the chronic risk for *Hypoaspis aculeifer* due to the use of F9600-4 SC

Use pattern	Test item	NOEC/EC_{10} Corr (mg/kg dw)	PEC_{soil} (mg/kg dw)[b]	TER_{LT} (criterion TER ≥ 5)
Maize 1 x 375 g a.s./ha	Bixlozone (active substance)	45[c]	0.780	57.7
	2,4-dichlorobenzoic acid	4.5[d]	0.544	8.3
	Bixlozone-4 SC	125[e]	1.402[e]	89.2
Winter oilseed rape 1 x 300 g a.s./ha	Bixlozone (active substance)	45[c]	0.624	72.1
	2,4-dichlorobenzoic acid	4.5[d]	0.435	10.3
	Bixlozone-4 SC	125[e]	1.121[e]	112
Winter cereals 1 x 200 g a.s./ha	Bixlozone (active substance)	45[c]	0.416	108
	2,4-dichlorobenzoic acid	4.5[d]	0.290	15.5
	Bixlozone-4 SC	125[e]	0.748[e]	167

[a] The $logK_{ow}$ values of bixlozone (3.3) and 2,4-dichlorobenzoic acid (2.82) are both greater than 2 (Section 3CA B2), therefore the NOEC/EC_{10} values have been corrected by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
[b] The maximum $PEC_{accumulation}$ value for bixlozone and maximum $PEC_{initial}$ values for 2,4-dichlorobenzoic acid and bixlozone-4 SC (Section 3CP B.8), have been used in the risk assessment.
[c] In the absence of toxicity data with the technical a.s., the formulation data have been expressed in terms of the a.s. content and used alongside the a.s. PEC_{soil} in the risk assessment.
[d] In the absence of toxicity data with the metabolite 2,4-dichlorobenzoic acid, it has been assumed in the risk assessment that the metabolite is 10x more toxic than the parent.
[e] mg formulation/kg dw

All TER values are above the relevant regulatory trigger value of 5, therefore an acceptable chronic risk can be concluded for *Hypoaspis aculeifer*.

Under current data requirements (Commission Regulation (EU) No 283/2013), studies with the technical a.s. are required for non-target soil meso- and macrofauna. However, for the current risk assessment, in the absence of toxicity data with the technical a.s., formulation data have been expressed in terms of a.s. content and used alongside the a.s. PECsoil in the risk assessment. It is noted that F9600-4 SC is a single a.s. formulation, therefore it is likely that the formulation assessment is protective of the risk from the active. In addition, the available toxicity data with earthworms (Table B.9.8-1) indicates that the toxicity of the formulation ($NOEC_{corrected}$: 40 mg F9600-4/kg soil dw) is not under representative of the a.s. alone ($NOEC_{corrected}$: 50 mg a.s./kg soil dw), therefore, the current risk assessment is considered to be protective of the risk from the a.s.

It is also noted that no data is available for the soil metabolite 2,4-dichlorobenzoic acid, therefore, 10x the toxicity of the parent (in this case 10x the toxicity of the formulation endpoint expressed in terms of the a.s. content) has been used in the risk assessment. This approach is considered to be protective as the available toxicity data for earthworms (Table B.9.8-1) indicates that the metabolite ($NOEC_{corrected}$: 29.15 mg/kg soil dw) is similar in toxicity to the technical a.s. ($NOEC_{corrected}$: 50 mg a.s./kg soil dw), therefore, the current risk assessment is also considered to be protective of the risk from the metabolite.

Consideration of potential volatilisation

Whilst the above risk assessments for earthworms and soil macro-organisms demonstrates acceptable risk, Environmental Fate have identified that bixlozone exceeds the relevant trigger values for volatilisation, therefore there is some uncertainty regarding the extent of exposure in these studies and hence the endpoints have the potential to underestimate the toxicity due to volatilisation.

The issue of volatilisation has been considered in Volume 3 CP Section B.9,8 and it is agreed that bixlozone is not volatile when incorporated into soil. Thus, it does not trigger the analysis of the test soils in any of the studies discussed above. Overall, based on the available information an acceptable risk to earthworms and other soil macro-organisms can be concluded for the proposed uses.

2.9.9.7. Risk assessment for soil nitrogen transformation

The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to the 'Guidance Document on Terrestrial Ecotoxicology' (SANCO/10329/2002).

The risk assessment for soil nitrogen transformation is summarised in Tables 2.9.9.7-1, for exposure to both the active substance, metabolites and representative formulation. Overall an acceptable risk was concluded for soil nitrogen transformation.

Risk assessment for soil micro-organisms

The potential risk to soil micro-organisms has been determined by comparing the maximum concentration at which effects on nitrogen activity were < 25% to the appropriate PEC_{soil}

Table 2.9.9.7-1: Assessment of the risk for effects on soil micro-organisms due to the use of F9600-4 SC

Use pattern	Test item	No effect >25% (mg/kg dw)	PEC_{soil} (mg/kg dw)[b]	Risk acceptable?
Maize 1 x 375 g a.s./ha	Bixlozone (active substance)	1000	0.780	Yes
	2,4-dichlorobenzoic acid	1.79	0.544	Yes
	F9600-4 SC	7.55[a]	1.402[a]	Yes
Winter oilseed rape 1 x 300 g a.s./ha	Bixlozone (active substance)	1000	0.624	Yes
	2,4-dichlorobenzoic acid	1.79	0.435	Yes
	F9600-4 SC	7.55[a]	1.121[a]	Yes
Winter cereals 1 x 200 g a.s./ha	Bixlozone (active substance)	1000	0.416	Yes
	2,4-dichlorobenzoic acid	1.79	0.290	Yes
	F9600-4 SC	7.55[a]	0.748[a]	Yes

[a] mg formulation/kg dw

The results show that bixlozone, 2,4-dichlorobenzoic acid and F9600-4SC had no effects ≥ 25% compared to the control on soil microbial activity up to a maximum tested concentrations of 1000 mg a.s./kg soil dw, 1.79 mg a.s./kg soil dw and 7.55 mg/kg soil dw, respectively after 28 days, which is higher than the maximum PEC_{soil}

values for each one. This supports the conclusion that under field conditions, use of F9600-4 SC poses an acceptable risk to non-target soil micro-organisms.

It is noted that in the study with the technical a.s. (Deslandes, L., 2014a), distilled water was used as an extraction solvent, where 0.1M KCl is typically used. Furthermore, the ratio of sampled test soil:extraction solvent deviated from the guideline-recommended 1:5 (w:v). As such there is uncertainty as to whether the extracted levels of nitrate measured represent accurately the levels formed in the control and test item soil replicates. The applicant proposed that as the nitrogen transformation study with the representative formulation (Hammesfahr, U., 2016) was conducted to cover the EU specific use rates and is based on the OECD guideline for agrochemicals, it could be relied upon in the risk assessment alongside this study. It is noted that F9600-4 SC is a single a.s. formulation therefore is likely the formulation assessment is protective of the risk from the a.s., in addition the risk assessment above indicates that the risk from the formulation is not under representative of the risk from the a.s. alone, this is also the case for earthworms for which the lowest TER for the formulation was 28.531 whilst the lowest TER for the technical a.s. was 64.103. It is also noted that the formulation endpoint expressed in terms of a.s (1.875 mg a.s./kg soil dw) is higher than the maximum accumulation PEC_{soil} for the a.s. (0.780 mg a.s./kg), which also demonstrates an acceptable risk, accounting for the fact that the formulation assessment doesn't cover accumulation.

It is also noted that in the study with the metabolite 2,4-dichlorobenzoic acid (Häuser, R., 2018), regarding the validity criteria the guideline specifies that the variation between replicate control samples in nitrate concentrations should be less than ± 15%. However, in the current study on Day 7 in both the solvent control and untreated control samples, the coefficient of variation was 22.3% and 16.3%, respectively. As the Day 7 values are not critical for the evaluation of the nitrogen formation rates of the last time interval and the margins of safety in the risk assessment are sufficient, the current risk assessment is considered to be protective of this level of uncertainty.

2.9.9.8. Risk assessment for terrestrial non-target higher plants

Spraydrift: The result of the risk assessment for the proposed uses of the representative formulation ('F9600 4SC') is summarised here. Risk assessments were conducted according to the 'Guidance Document on Terrestrial Ecotoxicology' (SANCO/10329/2002).

The risk assessment for the proposed uses is summarised in Table 2.9.9.8-1, where exposure occurs via spraydrift.

Table 2.9.9.8-1: Summary of the risk assessment for terrestrial higher plants due to exposure via spraydrift

Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**Toxicity value (g a.s./ha)**	**TER (criterion: TER ≥ 5) [a]**
Use on winter cereals at 200 g a.s./ha (1 x application)				
1	2.77	5.54	Vegetative vigour: 99.8 (1.5) [b]	18.01 (**0.27**) [b]
1	2.77	5.54	Seedling emergence: 19	**3.43**
Use on oilseed rape at 300 g a.s./ha (1 x application)				
1	2.77	8.31	Vegetative vigour: 99.8 (1.5) [b]	12.01 (**0.18**) [b]
1	2.77	8.31	Seedling emergence: 19	**2.29**
Use on maize at 375 g a.s./ha (1 x application)				
1	2.77	10.39	Vegetative vigour: 99.8 (1.5) [b]	9.61 (**0.14**) [b]
1	2.77	10.39	Seedling emergence: 19	**1.83**

[a] *TER values in **bold** are below the Annex VI trigger (< 5) and indicate an unacceptable risk*

[b] *Values in parentheses refer to phytotoxic effects. This endpoint has been used as a screen to establish if further consideration of phytotoxicity is required during product authorisation. TERs in **bold**, in parentheses, indicate the risk form phytotoxic effects has not been resolved and further consideration of this issue is required during product evaluation/authorisation.*

The risk is unresolved for exposure via spraydrift, including both vegetative vigour and seedling emergence assessments, unless mitigation is taken into account. To demonstrate an acceptable risk for exposure via spraydrift mitigation in the form of a label phrase is required, for all uses. The required label phrase is as follows:

"Extreme care must be taken to avoid spray drift onto non-crop plants outside of the target area."

Volatilisation: The active substance, bixlozone, is known to volatilise resulting in the potential for aerial transport and deposition (Vp = 10^{-5} Pa (plant) or 10^{-4} Pa (soil) at 20 ℃ as outlined in Reg. 283/2013). Volatilisation is not considered as part of the risk assessment as described in the Terrestrial Guidance Document (SANCO/10329/2002). However, volatilisation has been known to cause effects in non-target plants and therefore consideration of the potential risk from this route of exposure has been conducted.

The risk assessment for exposure via volatilisation has been based on the approach for exposure via spraydrift (SANCO/10329/2002). Exposure estimates are based on the data from the wind tunnel study Staffa (2016). The endpoint for risk assessment is the lower-tier phytotoxicity endpoint (1.5 g a.s./ha). The risk assessment is summarised in Table 2.9.9.8-2.

Risk mitigation is required to resolve the risk in all cases (i.e. a 5m buffer for use on winter cereals and a 10 m buffer for the uses on oilseed rape and maize). Use of buffer zones is proposed to address the risk for exposure via volatilisation, based on precedent from assessments of similar active substances (cinmethylin and clomazone). This risk assessment and the resulting mitigation requirements are not based on an agreed risk assessment scheme but are in-line with UK mitigation for other herbicide products when considering the risk to non-target plants from volatilisation.

Table 2.9.9.8-2: Assessment of the risk to non-target terrestrial plants due to exposure via volatilisation, following use of 'F9600-4 SC', including risk mitigation measures

Distance (m)	**Deposition (%) [a]**	**$PER_{volatilisation}$ (g a.s./ha)**	**Toxicity value (g a.s./ha)**	**TER (criterion: TER ≥ 5)**
Use on winter cereals at 200 g a.s./ha (1 x application)				
1	0.42	0.84	1.5	**1.79**
5	0.14	0.28		5.36
Use on oilseed rape at 300 g a.s./ha (1 x application)				
1	0.42	1.26	1.5	**1.19**
5	0.14	0.42		**3.57**
10	0.08	0.24		6.25
Use on maize at 375 g a.s./ha (1 x application)				
1	0.42	1.58	1.5	**0.95**
5	0.14	0.53		**2.83**
10	0.08	0.30		5

MAF: Multiple application factor; $PER_{volatilisation}$: Predicted environmental rate, due to volatilisation (= application rate x (deposition(%)/100)); TER: toxicity to exposure ratio. TER values shown in bold are below the relevant trigger and an acceptable risk has not been demonstrated.
[a] *Deposition as established in Staffa (2016)*

2.9.9.9. Risk assessment for biological methods for sewage treatment

The risk to biological methods for sewage treatment is considered acceptable for all proposed uses. Contamination of sewage treatment systems via the agricultural use of bixlozone in the representative formulation is considered to be low. The worst-case PECsw was 20.192 µg a.s./L for maize (CP. B.8.5.), which is over 202 times lower than the lowest endpoint for activated sludge.

It is noted that regarding the validity criteria, oxygen uptake was marginally less than 20 mg oxygen/g/h as specified in the guideline (observed: 19.7 mg/g/h). As the oxygen uptake rate was only slightly below the guideline limit, all of the other validity criteria were met and the margin of safety in the risk assessment is high, the risk assessment is considered to be protective of this uncertainty.

2.10. CLASSIFICATION AND LABELLING

Classification and labelling is currently under evaluation. A mandatory classification and labelling report is being prepared under GB CLP by HSE. Therefore, this section will be completed at a later stage following the aligned evaluation process and when the report is complete.

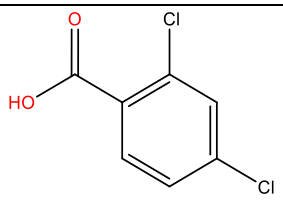
2.11. RELEVANCE OF METABOLITES IN GROUNDWATER

Under aerobic laboratory conditions, the major degradation route of bixlozone was *via* CO_2 (10.4-54.4 %AR after 120 days) and bound residues (peak values of 3.3-28.5 %AR after 120 days) in seven soils (KCA 7.1.1.1/01 Simmonds, R., 2015a, amended 2018). No metabolites were observed at levels above 5% of applied radioactivity in this study. However, concentrations of the metabolite **2,4-dichlorobenzoic acid** were observed at levels above 10% in the field dissipation studies; formation reached a maximum of 69.4% on a mass basis (99.53% on a molar basis). Therefore, 2,4-dichlorobenzoic acid was classed as a major soil metabolite. Furthermore, concentrations of the metabolite are predicted to occur in groundwater at concentrations above 0.1 µg/L. Assessment of the relevance of this metabolite according to the stepwise procedure of the EC guidance document SANCO/221/2000 –rev.10 is therefore required.

According to the PECgw assessments for the representative uses the following PECgw values are obtained for the metabolite 2,4-dichlorobenzoic acid:

Metabolite name	Maximum Predicted Concentration in Groundwater [µg/L]	Is an assessment for relevance required?
2,4-dichlorobenzoic acid	4.048 µg/L	Yes

Identification information for 2,4-dichlorobenzoic acid:

Compound	2,4-dichlorobenzoic acid (FMC-510224)
CAS N°	50-84-0
Chemical name (IUPAC)	2,4-dichlorobenzoic acid
Empirical formula	$C_7H_4Cl_2O_2$
SMILES	OC(=O)c1ccc(Cl)cc1Cl
Structural formula	

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

2,4-dichlorobenzoic acid does not meet the criteria for products of no concern as defined in Step 1 of the SANCO/221/2000-rev.10-final 2003 Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC and therefore, requires further assessment.

2.11.2. STEP 2: Quantification of potential groundwater contamination

PEC_{gw} calculations after leaching from soil for **2,4-dichlorobenzoic acid** were performed (see Vol 3CP, B8). The uses for which concentrations of 2,4-dichlorobenzoic acid were considered to exceed 0.1 µg/L. Details are given in the Vol 3CP, B8, section 8.3.

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

2.11.3.1. Step 3, Stage 1: Screening for biological activity

2,4-dichlorobenzoic acid was tested in a 96 well-plate assay at a rate equivalent to 1000 g/ha against four different weed species (Yellow Rocket, Bentgrass, Bermudagrass, and Tobacco). In this assay the metabolite showed no herbicidal control as indicated by Table 2.11.3.1-1.

Table 2.11.3.1-1: Summary of the biological activity of 2,4-dichlorobenzoic acid

	BARVU **Barbarea vulgaris** **Yellow Rocket**	**AGSPL** **Agrostis palustris** **Bentgrass**	**CYNDA** **Cynodon dactylon** **Bermudagrass**	**NIOTA** **Nicotiana tabacum** **Tobacco**
UTC	0	0	0	0
04194-000	0	0	0	0

UTC – untreated control
04194-000 – 2,4 dichlorobenzoic acid

It is therefore concluded that the metabolite 2,4 dichlorobenzoic acid is not herbicidally active. See report no. FMC-54343, submitted in MCA section 8 for more detail.

2.11.3.2. Step 3, Stage 2: Screening for genotoxicity

2,4-dichlorobenzoic acid is a common chemical with the CAS number 50-84-0. No harmonised classification under Regulation (EC) Nº1272/2008 (Annex VI to the CLP Regulation) or registration dossier is available for this metabolite; the notified classifications for Human Health effects[2] do not indicate any specific concerns with respect to genotoxicity; however HSE noted that genotoxicity data are lacking in the ECHA database.

The Applicant has provided a genotoxicity QSAR analysis; however, this is not sufficient, as according to the SANCO guidance, a standard battery of three in vitro tests is required.

Consequently, the applicant conducted the following tests: an Ames test, an in vitro mammalian cell micronucleus test in human peripheral lymphocytes and an in vitro HPRT mutation test using chinese hamster ovary cells. The tests have been evaluated by HSE and a summary of the findings are presented in Section CA B.6.8.1.1. of the DAR (Metabolite 2,4-dichlorobenzoic acid). All the tests were conducted to GLP and OECD guidelines, and were negative. Thus, the bixlozone metabolite 2,4-dichlorobenzoic acid is concluded not to be genotoxic.

2.11.3.3. Step 3, Stage 3: Screening for toxicity

The active substance bixlozone is not classified as acutely or chronically toxic or very toxic.

Bixlozone is not classified for carcinogenicity, reproductive toxicity or mutagenicity. On the basis of this information the metabolite 2,4-dichlorobenzoic acid is not defined as relevant according to this stage.
All metabolites passing stage 3 of step 3 are not considered as "relevant" and are subject to an exposure and/or risk assessment as outlined in the steps below to further consider potential relevance.

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

The metabolite 2,4-dichlorobenzoic acid is present in groundwater at 4.048 µg/L: as this exceeds the 0.75 µg/L threshold of toxicological concern, a refined risk assessment is required to further consider potential relevance (see Step 5).

2.11.5. STEP 5: Refined risk assessment

Dietary contribution for ,4-dichlorobenzoic acid
2,4-dichlorobenzoic acid was one of the metabolites of bixlozone selected for potential inclusion in the residue definitions based on its significant occurrence in the plant and livestock metabolism studies (DAR Volume 3 Section B.7) and a toxicological assessment was performed for this metabolite (DAR Volume 3 Section B.6.8.1.2). The overall conclusion on this assessment for ,4-dichlorobenzoic acid is presented below:

2,4-dichlorobenzoic acid is a putative major rat metabolite considered to be covered via its downstream glycine conjugate 2,4-dichlorohippuric acid, the latter being recovered in rat urine at levels > 10 % of the AD in both sexes following single low dose oral exposure (DAR Volume 3 Section B.6.1). On this basis, its toxicity profile could be considered 'covered' by the parent. However, specific data are available on this metabolite (acute oral toxicity studies in rat and mouse and modern in vitro genotoxicity studies). These data take precedence on the kinetic prediction and indicate that 2,4-dichlorobenzoic acid may be approximately 2-fold more toxic than bixlozone. On

[2] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/88971

this basis, it is concluded that 2,4-dichlorobenzoic acid is more toxic than the parent and a likely candidate for inclusion in the Residue Definition from a toxicological perspective.

For the purpose of dietary risk assessment, the dietary acute and chronic reference values of bixlozone should be used, adjusting the residue estimate of 2,4-dichlorobenzoic acid for a relative potency factor of 2. In addition, a modifying factor of 1.435 should also be applied to account for the molecular weight conversion between the metabolite and the parent. This will allow to express 2,4-dichlorobenzoic acid into parent bixlozone equivalents. For an example of the conversion/adjustment factors, please see the note $ with the table below.

Metabolites which have passed steps 1 to 3 and for which levels of estimated concentrations of metabolites in groundwater (as defined in Step 2) lie between 0.75 µg/L (from Step 4) and 10 µg/L will require a refined assessment of their potential toxicological significance for consumers. This applies to the metabolite 2,4-dichlorobenzoic (with a maximum PECgw value of 4.048 µg/L) and a refined risk assessment is required.

From the toxicological assessment performed for this metabolite for its potential inclusion in the residue definitions (DAR Volume 3 Section B.6.8.1.2), it was concluded that if a risk assessment were to be required, the dietary acute and chronic reference values of bixlozone should be used, adjusting the residue estimate of 2,4-dichlorobenzoic acid for a relative potency factor of 2. In addition, as indicated above, a modifying factor of 1.435 should also be applied to account for the molecular weight conversion between the metabolite and the parent. This will allow to express 2,4-dichlorobenzoic acid into parent bixlozone equivalents.

The estimates of dietary intake have been estimated for residues in food in section 2.7.9. The chronic dietary assessment intakes estimated for the various scenarios (arising from residues in food) were all low, <1% of the ADI for bixlozone of 0.3 mg/kg bw/day. This estimate took account of the proposed two fold toxicity of the metabolite 2,4-dichlorobenzoic acid, in that to compare to the ADI for bixlozone, the estimated residue contributions of 2,4-dichlorobenzoic acid to the total residue were doubled (also taking account of molecular weight adjustment to express residues of 2,4-dichlorobenzoic acid as bixlozone).

Dietary intake estimate of metabolite 2,4-dichlorobenzoic acid in drinking water.

The assessment of 2,4-dichlorobenzoic acid as a potential metabolite in drinking water is presented below in Table 2.11.5-1).

Table 2.11.5-1 Dietary intake estimate of 2,4-dichlorobanzoic acid in drinking water

Consumer group	**Water consumption (litres/kg bw/day)**	**Basis for the estimated intake**	**Estimated dietary intake of** 2,4-dichlorobenzoic acid arising from potential presence in drinking water at up to 11.62 µg/L[$] (mg/kg bw/day)
Adult (WHO)	0.033	2 litres water/day; 60 kg bw	0.00039 (0.1% of the ADI of 0.3 mg/kg bw/day)
Child (WHO)	0.100	1 litre water/day; 10 kg bw	0.00116 (0.4% of the ADI of 0.3 mg/kg bw/day)
Infant WHO)	0.150	0.75 litre water/day; 5 kg bw	0.00174 (0.6% of the ADI of 0.3 mg/kg bw/day)
Infant (EFSA, 2018 and used for UK assessments)	0.227	260 g/kg bw/day formula based on 33 g/kg bw powder and 227 ml water/kg bw/day	0.00264 (0.9% of the ADI of 0.3 mg/kg bw/day)

[$] Residues of 2,4-dichlorobenzoic acid doubled to account for this substance being twice as toxic as parent bixlozone. By doubling the residue levels for this metabolite, a risk assessment can be performed using the toxicological endpoints for parent bixlozone. (2,4-dichlorobenzoic acid residue 4.048 µg/L x 1.435 MW conversion x 2 to account for relative toxicological potency compared to parent bixlozone = 11.62 µg/L) It should be noted that although this value is >10 µg/L, this is due to the exposure being doubled to account for higher toxicity and enabling comparison to the parent toxicological end point. Additionally, this is due to the application of a MW conversion factor. The actual level of 2,4-dichlorobenzoic acid expected in ground water is 4.048 µg/L which is below the limit of 10 µg/L outlined in SANCO/221/2000 –rev.10.

Taking account of the possible presence of metabolite 2,4-dichlorobenzoic acid in food and drinking water, the co-exposures are expected to be low.

Overall, this is based on the low individual exposures as follows:

Estimation of long term (chronic) dietary exposures arising from foods (section 2.7.9) – total residues and associated intake across all consumer groups <1% of the ADI for parent of 0.3 mg/kg bw/day (this assessment accounts for the higher proposed toxicity of metabolite 2,4-dichlorobenzoic acid compared to parent).

Estimation of long term (chronic) dietary exposures arising from drinking water– metabolite 2,4-dichlorobenzoic acid <1% of the ADI for parent bixlozone of 0.3 mg/kg bw/day (for the critical consumer group infants). It should be noted that this estimation accounts for the higher toxicity of 2,4-dichlorobenzoic acid, considering twice the exposure.

Taken together these exposures are low.

2.11.6. Overall conclusion

The concentrations of the metabolite 2,4-dichlorobenzoic acid are predicted to occur in groundwater at concentrations above 0.1 µg/L. The assessment of the relevance of this metabolite was performed according to the stepwise procedure of the EC guidance document SANCO/221/2000 –rev.10 and concluded that 2,4-dichlorobenzoic acid is not of toxicological relevance at the maximum predicted concentration of 4.048 µg/L in groundwater. In terms of the risk assessment, this residue of 4.048 µg/L (2,4-dichlorobenzoic acid) has been assessed on the basis of 'parent bixlozone equivalents' as 11.62 µg/L. This takes account of the proposed two fold toxicological potency of residues of 2,4-dichlorobenzoic acid compared to parent bixlozone, and also includes an adjustment due to molecular weight (x 1.435).

The refined risk assessment above (at step 5 of the assessment) concludes that overall chronic dietary intakes from food and drinking water sources are low: metabolite 2,4-dichlorobenzoic acid from both drinking water (<1% of the ADI of 0.3 mg/kg bw/day for bixlozone); food sources ('total residues' dietary intakes assessed taking account of the higher toxicity of 2,4-dichlorobenzoic acid, all < 1% of the ADI of 0.3 mg/kg bw/day for bixlozone). Taken together these exposures are low.

As such, following the stepwise assessment, it is concluded that 2,4-dichlorobenzoic acid is not a relevant metabolite in groundwater.

2.12. CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.12.1. Identity and physical chemical properties

Not relevant; bixlozone does not show isomerism.

2.12.2. Methods of analysis

Not relevant; bixlozone does not show isomerism.

2.12.3. Mammalian toxicity

Not relevant; bixlozone does not show isomerism.

2.12.4. Operator, Worker, Bystander and Resident exposure

2.12.5. Residues and Consumer risk assessment

Not relevant; bixlozone does not show isomerism.

2.12.6. Environmental fate

Not relevant; bixlozone does not show isomerism.

2.12.7. Ecotoxicology

Not relevant; bixlozone does not show isomerism.

2.13. RESIDUE DEFINITIONS

2.13.1. Definition of residues for exposure/risk assessment

Food of plant origin:

Plant residue definition for risk assessment RD-RA:

For the intended (early application) use on oilseed rape, wheat, barley and maize:

Sum of residues of bixlozone and 2 x 2,4-dichlorobenzoic acid expressed as bixlozone

[the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. To express 2,4-dichlorobenzoic acid as bixlozone equivalence a molecular weight conversion of 1.435 also has to be applied. This then gives an overall factor of 2.87 to be applied to the level of 2,4-dichlorobenzoic acid].

For other crops and use patterns, no conclusion can currently be reached on a suitable residue definition (an updated TTC exposure assessment for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) will be required for future extensions of uses).

Food of animal origin:

A RD-RA (residue definition for dietary risk assessment) is not proposed at this time for products of animal origin

Soil: Bixlozone, 2,4-DBA

Groundwater: Bixlozone, 2,4-DBA

Surface water: Bixlozone, 2,4-DBA, 3-OH-propanamide, bixlozone-DMM, 4-COOH-bixlozone

Sediment: Bixlozone, 2,4-DBA, 3-OH-propanamide, bixlozone-DMM, 4-COOH-bixlozone

Air: Bixlozone

2.13.2. Definition of residues for monitoring

Food of plant origin: bixlozone

Food of animal origin: bixlozone

Soil: bixlozone

Groundwater: 2,4-dichlorobenzoic acid

Surface water: bixlozone

Sediment: bixlozone

Air: bixlozone

Level 3

Bixlozone

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 HSE considers that authorisation is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	Yes		It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with for bixlozone for use as a herbicide on oilseed rape, maize, barley and wheat (early application at up to post-emergence stage) (refer to Level 1, Table 1.5.1 for details of the representative uses considered).
3.1.1.2. Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	Yes		It is considered that a sufficiently complete dossier has been submitted which enables a regulatory decision on approval of bixlozone to be made and to establish that risks are acceptable and no critical areas of concern are identified. There are data gaps identified (see (ii) 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.	Yes		The data gaps identified at Level 3.1.4 are considered to be confirmatory in nature and are not required in support of approval of bixlozone. However, the following data should be provided to support product authorisation for cereal crops: • Storage stability data to cover cereal grain • The data/case for high temp hydrolysis of 2,4-DBA.
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.	Yes		*(a) the minimum degree of purity of the active substance;* Minimum purity 960 g/kg

				(b) the nature and maximum content of certain impurities; The following impurity identified in technical bixlozone is considered to be of toxicological or ecotoxicological relevance: **Maximum level of relevant impurity, (2,4-dichlorophenyl)methanol** (CAS 1777-82-8; 2,4-dichlorobenzyl alcohol), is **1.5 g/kg** *(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;* Not applicable *(d) type of preparation;* Not applicable *(e) manner and conditions of application;* • Due to the risk to non-target plants from volatilization a 5 m buffer zone (for winter wheat/barley at 200 g a.s./ha) is proposed; • Due to the risk to non-target plants from volatilization a 10 m buffer zone for winter oilseed rape (at 300 g a.s./ha) and maize at 375 g a.s./ha has been proposed. *(f) submission of further confirmatory information to the Competent Authority where new requirements are established during the evaluation process or as a result of new scientific and technical knowledge;* Not applicable *(g) designation of categories of users, such as professional and non-professional;* Not applicable *(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;*

				Not applicable *(i) the need to impose risk mitigation measures and monitoring after use;* • The following label replant restriction must be included to mitigate for any rotational crop residues related to the use of bixlozone. 'Leafy crops and above ground vegetables must not be planted until at least 10 months after application of bixlozone.' • As the risk from spraydrift to non-target plants was not resolved the following label mitigation is required: 'Extreme care must be taken to avoid spray drift onto non-crop plants outside of the target area.' *(j) any other particular conditions that result from the evaluation of information made available in the context of Regulation 1107/2009.* Not applicable
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).	Yes		The data submitted are sufficient to establish an Acceptable Daily Intake (ADI), an Acceptable Operator Exposure Level (AOEL) and an Acute Reference Dose (ARfD).
	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 (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing;	Yes		

	(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.	Yes		Yes (for all of the representative uses)
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.	Yes		The applicant has satisfactorily addressed all of the Efficacy related points outlined in SANCO/10054/2013. Effectiveness against a range of weed species was demonstrated for the representative uses. Crop safety of the proposed uses of bixlozone has been supported. Additionally, the resistance risk has been appropriately addressed. Further information will be examined at the product authorisation stage to ensure that the product itself fully complies with the data requirements for efficacy. (Refer to Volume 1, Section 2.3 for further details)
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.	Yes		Sufficient information provided for the representative uses.
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.	Yes		Acceptable data have been submitted to support the manufacturing sites of bixlozone and the proposed specification based on pilot scale manufacturing is considered supported by the available data. Following scale-up from pilot plant to full scale manufacture, data to confirm the commercial scale technical specification must be submitted. In addition, the toxicological significance of any changes in the impurity profile must be addressed.

				The following impurity identified in technical bixlozone is considered to be of toxicological or ecotoxicological relevance: **(2,4-dichlorophenyl)methanol** (CAS 1777-82-8; 2,4-dichlorobenzyl alcohol): Maximum **1.5 g/kg**.
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	N/A	N/A	There is currently no FAO Specification for bixlozone.
	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	N/A	N/A	There is currently no FAO Specification for bixlozone.
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.	Yes		Acceptable methods have been submitted for the determination of bixlozone and all significant and relevant impurities in the technical material as manufactured. (Refer also to Level 2, Section 2.1).
	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.	Yes		Acceptable methods have been submitted for the determination of bixlozone and selected metabolites in various matrices used in support of all areas of the risk assessment. Acceptable methods have been submitted for the determination of bixlozone and selected metabolites in various matrices for use in post-approval monitoring and control to support the representative uses. Fully validated methods for crops in the high water, high protein, and high starch (dry) crop groups are not available (for details see Level 3, Section 3.1.4.5), however these crop groups are not relevant for the representative uses. Validation data to support methods for crops in the high water, high protein, and high starch (dry) crop groups will be required for future product authorisations and/or MRL setting purposes.

				A method for the determination of the relevant impurity (2,4-dichlorophenyl)methanol in the plant protection product is required. (Relevant to representative product and therefore all representative uses.)
	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.	Yes		Refer to Level 2, Section 2.2 for further details.
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.	Yes		**ADI = 0.3-mg/kg bw/day** based on adverse effects on the liver observed at the LOAEL of 121/150 mg/kg bw/day (males/females) in the rat dietary oral 90-day repeated-dose toxicity study (NOAEL of 29 mg/kg bw/day). **AOEL = 0.2-mg/kg bw/day** based on adverse effects on the liver observed at the LOAEL of 121/150 mg/kg bw/day (males/females) in the rat dietary oral 90-day repeated-dose toxicity study (NOAEL of 29 mg/kg bw/day, bioavailability 70%). **ARfD = 0.75-mg/kg bw/day** based on adverse initial reduction in maternal body weight at 225 mg/kg bw/day in the the rat (oral gavage) developmental toxicity study (NOAEL of 75 mg/kg bw/day) adverse effects on the liver observed at the LOAEL of 121/150 mg/kg bw/day (males/females) in the rat dietary oral 90-day repeated-dose toxicity study.
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.**		No	Overall, it is concluded that bixlozone is not genotoxic in vivo and the data requirements of Regulation 283/2013 have been met. Therefore, classification of bixlozone for mutagenicity is not warranted.
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.**		No	Long term oral administration of bixlozone was not carcinogenic in the rat or mouse. Therefore, classification of bixlozone for carcinogenicity is not required.
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.			*N.A.*
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.**		No	No specific adverse effect on reproduction or development up to the highest dose tested. In accordance with Regulation (EC) Nº 1272/2008, classification of bixlozone for reproductive and developmental toxicity is not warranted
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.			*N.A.*
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**		No	Bixlozone should not be classified for carcinogenic category 2 and toxic for reproduction category 2.
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 HSE considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		No	Bixlozone does not meet the ED criteria of Regulation (EC) No 2018/605 of 19 April 2018, amending Annex II to Regulation (EC) No 1107/2009. HSE concludes that for the EATS-modalities bixlozone is not an ED and its ED potential has been sufficiently investigated and that no further information is required.
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.			*N.A.*
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.		No	A substance is deemed to meet the P criterion in a POP assessment if the DegT50 is > 2 months in water, > 6 months in sediment or > 6 months in soil. Best-fit non-normalised bixlozone $DegT_{50}$ values were calculated from 21 soil dissipation trials undertaken in four European countries and ranged from 6.90 days to 300 days (see Level 2 for further information). Of the 21 field DT_{50} values, 6 were above the 180 day trigger. The trials that recorded DT_{50} values >180 days were located in Italy, France and Germany. Therefore, bixlozone potentially fulfils the P criterion in soil. The total system bixlozone DegT50 values calculated for the two water/sediment test systems were 23.3 days and 24.8 days. Therefore,

				bixlozone is not considered to meet the P criteria. However, no degradation was observed in a surface water aerobic mineralisation study and, therefore, bixlozone could be considered persistent in water. A substance fulfils the bioaccumulation crierion in a POP assessment where the bio-concentration factor or bioaccumulation factor in aquatic species is > 5000 or, in the absence of such data, that the log Pow is > 5. The lipid-normalised steady-state BCF is 77.5 L kg^{-1} (whole fish at 13.0 µg a.s./L) and 71.7 L kg^{-1} (whole fish at 130 µg a.s./L)). The log Pow is 3.3. Therefore, the bioaccumulation criterion is not met for bixlozone. Bixlozone has a calculated air DT_{50} of 0.498 days which is below the potential for long range transport threshold of 2 days.
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.		No	A substance is deemed to meet the P criterion in a PBT assessment if the DegT50 is > 40 days in fresh water, > 120 days in freshwater sediment or > 120 days in soil. Best-fit non-normalised bixlozone $DegT_{50}$ values were calculated from 21 soil dissipation trials undertaken in four European countries and ranged from 6.90 days to 300 days (see Level 2 for further information). Of the 21 trials, 10 recorded $DegT_{50}$ values > 120 days, with $DegT_{50}$ values > 120 days calculated in each test country. Therefore, as it cannot be concluded otherwise, bixlozone is considered to meet the P criterion in soil. The total system bixlozone DegT50 values calculated for the two water/sediment test systems were 23.3 days and 24.8 days. Therefore, bixlozone is not considered to meet the P criteria. However, no degradation was observed in a surface water aerobic mineralisation study and, therefore, bixlozone could be considered persistent in water. **Bioaccumulation** (B) – The active substance does not fulfil the bioaccumulation criterion as the bioconcentration factor for aquatic species is

				≤ 2000 (the lipid-normalised steady-state BCF is 77.5 L kg^{-1} (whole fish at 13.0 µg a.s./L) and 71.7 L kg^{-1} (whole fish at 130 µg a.s./L)). **Toxicity** (T) – The criteria for toxic classification is a long-term endpoint for a marine or freshwater organism of <0.01 mg/L. The long-term endpoints for aquatic plant *Myriophyllim spitacum* are a NOE_rC of 0.0096 mg a.s//L and an E_rC_{10} of 0.0071 mg a.s//L. **Therefore, it is classified as T.**
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.		No	A substance is deemed to meet the vP criterion in a vPvB assessment if the DegT50 is > 60 days in fresh water, >180 days in freshwater sediment or > 180 days in soil. As for the POP assessment above, of the 21 field DT_{50} values, 6 were above the 180 day 'very Persistent' trigger. The trials that recorded DT_{50} values >180 days were located in Italy, France and Germany. Therefore, the CA considers that bixlozone potentially fulfils the 'very Persistent' criteria. The total system bixlozone DegT50 values calculated for the two water/sediment test systems were 23.3 days and 24.8 days. Therefore, bixlozone is not considered to meet the vP criteria. However, no degradation was observed in a surface water aerobic mineralisation study and, therefore, bixlozone could be considered persistent in water. Bixlozone does not meet the criteria for very bioaccumulative. The lipid-normalised steady-state BCF is 77.5 L kg^{-1} (whole fish at 13.0 µg a.s./L) and 71.7 L kg^{-1} (whole fish at 130 µg a.s./L) ≤ 2000.
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	Yes		Acceptable risks have been demonstrated for all of the proposed representative uses (with the exception of non-target arthropods when considering use at the maximum rate proposed for maize), see below:

	a plant protection product containing the active substance, safener or synergist. HSE 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.			**Birds:** Based on the available data an acceptable risk to birds was demonstrated for all the proposed uses (see Section 2.9.9.1). **Mammals:** Based on the available data an acceptable risk to mammals was demonstrated for all the proposed uses (see Section 2.9.9.2). **Aquatic organisms:** Based on the available data an acceptable risk to aquatic organisms was demonstrated for all the proposed uses (see Section 2.9.9.3). **Bees:** Based on the available data an acceptable risk to bees was demonstrated for all the proposed uses (see Section 2.9.9.4). **Non-target arthropods (NTAs):** Based on the available data an acceptable risk to NTAs was demonstrated for the proposed uses on winter wheat/barley and winter oilseed rape (see Section 2.9.9.5). An acceptable risk has not been demonstrated for the proposed use on maize, at the maximum application rate of 375 g a.s./ha (see Section 2.9.9.5). However, it is noted that a range of application rates are proposed for use on maize (250-375 g a.s./ha). An acceptable in-field risk can be concluded for an application rate to maize of ≤ 367 g a.s./ha (as < 50% effects were reported at this concentration). **Soil meso- and macro-fauna:** Based on the available data an acceptable risk to earthworms and (other) soil macro-organisms was demonstrated for all the proposed uses (see Section 2.9.9.6). The risk via volatilisation was considered to be acceptable as it was concluded that the a.s. does not volatilise in soil. **Soil micro-organisms:** Based on the available data an acceptable risk to soil micro-organisms was demonstrated for all the proposed uses (see Section 2.9.9.7). **Non-target terrestrial plants (NTTPs):** Acceptable risks have been demonstrated for all proposed uses, assuming appropriate mitigation is applied. To address exposure via spray drift labelling mitigation is required for all uses (see Section 2.9.9.8). To address exposure via volatilisation a buffer zone of 5m is required for the use on winter wheat/barley (see Section 2.9.9.8). To address exposure via volatilisation a buffer zone of 10m is required for the use on winter oilseed rape and the use on maize (see Section 2.9.9.8). **Sewage treatment:** Based on the available data an acceptable risk to activated sludge micro-organisms was demonstrated for all the proposed uses (see Section 2.9.9.9).

	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.		No	<u>Overall HSE ecotoxicology conclusion for birds, reptiles and wild mammals</u> Overall, HSE concludes that based on current EFSA/ECHA 2018 guidance it is not possible to reach a conclusion for birds or reptiles when considering endocrine disruption (see Section 2.9.1.). For non-target wild mammals HSE concludes bixlozone does not meet the criteria of being an ED based on EAS or T modalities. Therefore, from an ecotoxicology perspective bixlozone is not an endocrine disruptor for wild mammals based on available data/information (see Section 2.9.9.9). <u>Overall HSE ecotoxicology conclusion for aquatic organisms</u> Overall, HSE concludes that based on current EFSA/ECHA 2018 guidance that bixlozone does not meet the criteria of being an endocrine disruptor (ED) for aquatic organisms when considering EAS and T modalities. Some uncertainties were identified by HSE in regard to study design, however, HSE still considers that bixlozone is not an endocrine disruptor for aquatic organisms when considering the EAS and T modalities (see Section 2.9.2.).
	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.		No	The proposed uses are not considered likely to result in negligible exposure. However, HSE concluded bixlozone is not an endocrine disruptor as described above.
	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.	Yes		Based on the available data an acceptable risk to bees was demonstrated for all the proposed uses (see Section 2.9.9.4).

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.	Yes		As detailed in section 2.13, residue definitions: **<u>For dietary risk assessment</u>** can only be proposed, taking account of the requested early application use on oilseed rape, wheat, barley and maize. **Food of plant origin:** RD-RA (residue definition for dietary risk assessment) (plants): Sum of residues of bixlozone and **2 x** 2,4-dichlorobenzoic acid expressed as bixlozone [the 2 x factor is to account for the relative toxicological potency compared to parent bixlozone. An overall factor of 2.87 to apply to a level of 2,4-dichlorobenzoic acid (determined on the metabolite equivalent basis) is derived from a combination of x 2 and x 1.435 (molecular weight conversion to express 2,4-dichlorobenzoic acid into parent bixlozone equivalents)]. <u>For other crops and use patterns, no conclusion can currently be reached on a suitable residue definition (an updated TTC exposure assessment for 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) will be required for future extensions of uses)</u>. **Food of animal origin:** A RD-RA (residue definition for dietary risk assessment) is not proposed at this time for products of animal origin. **Soil:** Bixlozone, 2,4-DBA **Groundwater:** Bixlozone, 2,4-DBA **Surface water:** Bixlozone, 2,4-DBA, 3-OH-propanamide, bixlozone-DMM, 4-COOH-bixlozone

				Sediment: Bixlozone, 2,4-DBA, 3-OH-propanamide, bixlozone-DMM, 4-COOH-bixlozone **Air:** Bixlozone **For monitoring:** **Food of plant origin:** bixlozone **Food of animal origin:** bixlozone **Soil:** bixlozone **Groundwater:** 2,4-dichlorobenzoic acid **Surface water:** bixlozone **Sediment:** bixlozone **Air:** bixlozone
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.	Yes		PEARL (4.4.4), PELMO (5.5.3) and MACRO (5.5.4) PECgw were calculated for bixlozone and 2,4-dichlorobenzoic acid (2,4-DBA) for all 4 GAPs. For all UK relevant scenarios, bixlozone PECgw was <0.001 µg/L. For 2,4-DBA, PECgw >0.1 µg/L were determined for all 4 GAPs for at least 1 UK relevant scenario. The max PECgw was 4.048 µg/L (wOSR, Hamburg) and so a groundwater relevance assessment according to the stepwise procedure of the EC guidance document SANCO/221/2000 – rev.10 has been carried out. The stepwise assessment concluded that 2,4-dichlorobenzoic acid is not a relevant metabolite in groundwater (for details see Section 2.11).

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	Yes		*[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,* -**No** *— it meets two of the criteria to be considered as a PBT substance* **Bixlozone fulfils 2 out of 3 of the criteria of a persistent, bioaccumulative and toxic substance (PBT) as laid out in Regulation 1107/2009 (for persistence and toxicity - see 3.1.1.4 above).** *— 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),* - **No** *— it contains a significant proportion of non-active isomers,* - **No** *— 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,* - **No** *— 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,* - **No** *— 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.]* - **No**

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.		No	Bixlozone cannot be considered a low risk substance because it is persistent in soil and because it is proposed to be classified for aquatic life as detailed below in accordance with Regulation (EC) No 1272/2008. — carcinogenic, - No classification proposed — mutagenic, - No classification proposed — toxic to reproduction, - No classification proposed — sensitising chemicals, - No classification proposed — very toxic or toxic, - Yes (Peer review proposal[3] for harmonised classification according to Reg (EC) No 1272/2008): Aquatic Acute 1; H400: Very toxic to aquatic life. Acute M-Factor of 1 Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects. Chronic M-Factor of 10 — explosive, - No classification proposed — corrosive. - No classification proposed In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), - Persistent in soil — has a bioconcentration factor higher than 100, No — is deemed to be an endocrine disrupter, or - No — has neurotoxic or immunotoxic effects. - No

[3] It should be noted that harmonised classification and labelling is formally proposed and decided in accordance with Regulation (EC) No 1272/2008. Proposals for classification made in the context of the evaluation procedure under Regulation (EC) No 1107/2009 are not formal proposals.

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				
Following scale-up from pilot plant to full scale manufacture new 5-batch analysis supporting the commercial scale technical specification must be submitted. In addition the toxicological significance of any changes in the impurity profile must be addressed.	Required for all representative uses.	X		
Full composition details of the co-formulants for which only 50 % and 2.5 % of the composition has been identified.	Relevant to representative product and therefore all representative uses. **These data should be provided to support a product authorisation.**	X		
3.1.4.2. Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
Data to address the UV/visible absorption spectra of the relevant impurity (2,4-dichlorophenyl)methanol are required.	Required for all representative uses.	X		
Data to address the content of the relevant impurity (2,4-dichlorophenyl)methanol in the product before and after storage are required.	Relevant to representative product and therefore all representative uses. **These data should be provided to support a product authorisation.**	X		

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**
3.1.4.3. Data on uses and efficacy				
None required.				
3.1.4.4. Data on handling, storage, transport, packaging and labelling				
None required.				
3.1.4.5. Methods of analysis				
A method for the determination of the relevant impurity (2,4-dichlorophenyl)methanol in the plant protection product is required.	Relevant to representative product and therefore all representative uses. **These data should be provided to support a product authorisation.**	X		
Independent laboratory validation data for the method for the monitoring of residues in plants for the high water and high starch (dry) crop groups are required.	Relevant to high water and high starch (dry) crop groups. **These data should be provided to support a product authorisation.**	X		
Validation and ILV of the method for the monitoring of residues in plants for the high protein crop group are required.	Relevant to high protein crop group. **These data should be provided to support a product authorisation.**	X		
3.1.4.6. Toxicology and metabolism				
None required.				

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
3.1.4.7. Residue data				
To support cereal uses (and other crop uses), storage stability data on either cereal grain or representatives of the high water and high protein commodity groups (to cover all crops; data on representatives of all five commodity categories) should be generated in accordance with the OECD guideline 506 and using suitably validated analytical methods. These data should be provided to support a product authorisation for cereal crops. If stability in cereal grain (or a broad range of raw agricultural commodity types) is demonstrated, then the principle of extrapolation of these data to cereal processed fractions (or a range of broad commodity types) is considered reasonable.	Relevant to representative product to support cereal uses (and other crop uses). **These data should be provided to support a product authorisation for cereal crops.**	X		
To support cereal uses, further data on the nature of residues of 2,4-dichlorobenzoic acid under standard hydrolysis conditions are needed. These data should be provided to support a product authorisation for cereal crops. It may be possible for an improved case for non-submission of such radiolabelled hydrolysis data for 2,4-dichlorobenzoic acid to be provided, however such a case would need to be detailed and sufficient to address the potential fate of the	Relevant to representative product to support cereal uses. **These data should be provided to support a product authorisation for cereal crops.**	X		

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
molecule 2,4-dichlorobenzoic acid. New data should be generated in accordance with OECD test guideline 507.				
Depending on the residues in crops, further information to address the data requirement for the nature of residues in fish [metabolism], and if needed, magnitude of the residues in fish [feeding studies] will be required when guidance and agreed data on the diets of fish become available.	Relevant to representative product and therefore all representative uses.	-		
3.1.4.8. Environmental fate and behaviour				
None required.				
3.1.4.9. Ecotoxicology				
None required.				

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)
None	

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	

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.)
The material tested in the toxicological studies has been demonstrated to be representative of the technical specification.

Representative use		Winter Wheat Winter Barley BBCH 00-09 (200 g as/ha)	Winter Wheat BBCH 11-13 (200 g as/ha)	Winter Oilseed Rape BBCH 00-09 (200 – 300 g as/ha)	Maize BBCH 00-09 (250 – 375 g as/ha)
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				X[1]
	Assessment not finalised				
Risk to aquatic organisms	Risk identified				
	Assessment not finalised				
Groundwater exposure active substance	Legal parametric value breached				
	Assessment not finalised				
Groundwater exposure metabolites	Legal parametric value breached	X[2]	X[2]	X[2]	X[2]
	Parametric value of 10µg/L[(a)] breached				
	Assessment not finalised				
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

[1] An acceptable risk to non-target arthropods has not been established at the highest application rate proposed for maize (see Section 2.9.9.5)

2 PECgw values for metabolite 2,4-dichlorobenzoic acid exceed 0.1 µg/L in a number of scenarios for all uses of F9600-4 SC. Overall maximum PECgw for 2,4-dichlorobenzoic acid was 4.048 µg/L (PELMO, Hamburg, winter OSR) (see section 2.11 for the assessment of relevance of this metabolite in groundwater).

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
Human health - Carcinogenicity	In the (SD) rat study, a higher incidence of skin fibrosarcoma and fibroma in males and a non-statistically significant but dose-related increase in the incidence of thyroid follicular cell adenomas in females was seen at the top dose in comparison to the controls ([redacted] (2017), DAR Vol. 3 CA B6, section B.6.5.1, Table B 6.5.1.11, page 174). All neoplastic findings occurred in the presence of significant generalised toxicity (effects on body weight, body weight gain and adverse liver effects). In view of the sex-specificity of the findings, the low levels of radioactivity found in the skin and thyroid in the kinetic studies, the species specificity of the response (increased incidence of these tumours not seen in mice), the low biological plausibility of the finding (the skin is not a target organ and bixlozone is not toxic via the dermal route or a skin irritant; in the thyroid no other histopathological findings were noted in the study to support the tumorigenic response, even though mild follicular cell hypertrophy was observed in the 90-day rat study at a much higher dose in both sexes (section B.6.3.3.1)) and the fact that the incidences of these tumours were well within the extended historical control data (HCD) provided, HSE concluded that on balance the skin tumours in males and the thyroid tumours in females noted at the top dose in the rat were unrelated to treatment. In the (CD-1) mouse study, a higher incidence of systemic histiocytic sarcomas, cervical leiomyosarcomas and bronchio-alveolar carcinoma was seen in females at the top dose in comparison to the controls ([redacted] (2017), DAR Vol. 3 CA B6, section B.6.5.2, Tables B 6.5.2.8 & B 6.5.2.9, pages 186-188). All neoplastic findings occurred in the presence of significant systemic toxicity (inflammation of the glandular stomach, pelvis dilation of the kidney and increased liver weights). The lung has not been identified as a target organ in the mouse. The increase in bronchio-alveolar carcinoma was not statistically significant and was well within the range of the extended HCD provided (0 – 14.3 %) which indicates that the incidence is highly variable in control animals. There was also no clear pattern of pre-neoplastic lesions or progression of benign tumours to malignant tumours. Thus, the biological plausibility of the finding was considered to be low. The increased incidence of systemic histiocytic sarcomas was not statistically significant and was within the extended laboratory and literature HCD provided (range 0.0 – 18.3 %) which suggests that the incidence of this tumour is highly variable in control CD-1 mice. These tumours can be relatively common in rodent species yet no similar findings were seen in rats. The blood has not been identified as a target organ in the mouse and the sex-specific response was in contrast to the higher systemic exposure to

Area(s) where expert consultation is considered necessary	Justification
	bixlozone seen in males compared to females in the toxicokinetic studies (DAR Vol. 3 CA B6, Table B 6.5.2.4, page 180). Overall HSE concluded that the finding was unrelated to treatment. Regarding the cervical tumours (leiomyosarcomas), two sets of laboratory HCD showed that this tumour type is relatively rare in the mouse and confirmed that the incidence observed in this study at the top dose was generally higher than the HCD incidences. HSE requested further information from the applicant, who consulted an independent Pathology Working Group (PWG) panel to review the neoplasms identified in the study. The panel provided evidence that the cervix and uterus (body) are adjacent tissues that share gross and macroscopic features, making it challenging to differentiate the uterine body from the uterine cervix smooth muscle cells in mice using current histochemical, immunohistochemical or transmission electron microscopic methods. Moreover, neoplastic lesions arising from the uterine body and/or uterine cervix can frequently obliterate the normal architecture of these tissues and involve both of these regions, so it is not possible to determine if a uterine neoplasm arose in the uterine body or uterine cervix based on gross or microscopic features. Therefore, HSE agreed to combine the incidences from the cervix and uterus tissues together for analysis. The combined uterus/cervix tumour incidences showed that a dose-response was no longer apparent for leiomyosarcomas. HSE thus concluded that overall there were no tumours attributable to exposure to bixlozone in this mouse study up to the highest dose tested (5000 ppm). The ECP is invited to advise.
Human health - Thyroid toxicity	Thyroid weight changes, non-neoplastic and neoplastic histopathological findings were reviewed to determine if bixlozone exerts an adverse effect on the thyroid. In the dog, a dose-related and adverse increase in the thyroid/parathyroid weights was reported in both sexes in the 90-day oral (capsule) study ([redacted], 2016c; DAR Vol. 3 CA B6, section B.6.3.3.3); however thyroid weight was not affected in any other dog studies at similar or higher dose levels (28-day, 12-month studies), or in the rat and mouse in any of the studies investigating potential adverse effects on the thyroid. In the 90-day rat study ([redacted], 2016a, DAR Vol. 3 CA B6, section B.6.3.3.1), mild follicular cell hypertrophy (with no associated changes in organ weight) was noted at the top dose in both sexes; this occurred concomitantly with excessive systemic toxicity and was not observed at the end of the 28-day recovery period. No other occurrences of thyroid histopathology were reported in any other rat studies (28-day, 2-generation reproduction toxicity and 2-year studies) or in the mouse, dog and rabbit, including in studies where comparable/higher dose levels of bixlozone were tested. In the rat carcinogenicity study, there was a non-statistically significant but dose-related increase in the incidence of thyroid follicular cell adenomas in females at the top dose in comparison to the control group ([redacted]

Area(s) where expert consultation is considered necessary	Justification
	(2017), DAR Vol. 3 CA B6, section B.6.5.1). HSE concluded that on balance the thyroid tumours were unrelated to treatment. Overall, it was shown that repeated exposure to bixlozone in rats, mice and dogs was not associated with any clear effects on the thyroid gland, with only an isolated incidence of thyroid weight increase reported in the 90-day dog study and one of mild follicular cell hypertrophy described in the 90-day rat study. Therefore HSE concluded that there was no evidence of a clear pattern of adversity on the thyroid in any species. The ECP is invited to advise.
Human health - Endocrine disruption	HSE considers that the estrogen, androgen and steroidogenesis (EAS)-mediated adversity of bixlozone has been sufficiently investigated, based on a modern 2-generation reproduction toxicity study conducted by [redacted] (2016c, section B.6.6.1.2, page 196); the study was fully compliant with the OECD Guideline No. 416 (2001) and followed GLP standards. There were no specific adverse effects on reproduction (rat) and on development (rat, rabbit; DAR Vol. 3 CA B6, section B.6.6.2), or on reproductive organs and other endocrine organs related to EAS modalities following repeated exposure to bixlozone (all species; DAR Vol. 3 CA B6, section B.6.3). Overall, there was no clear pattern of adversity for the EAS modalities. Although no specific EAS activity studies are available for bixlozone, it was predicted not to bind to ER receptors using (Q)SAR profiling; thus no further data related to the EAS modalities were considered necessary. HSE consider that thyroid (T)-mediated adversity has been sufficiently investigated based on modern studies in which thyroid effects were investigated (repeated-dose toxicity in section B.6.3, long-term toxicity in section B.6.5 and reproduction toxicity studies in section B.6.6 of DAR Vol. 3 CA B6). Overall, the available data showed that bixlozone did not present a clear pattern of adversity for the T modality in relation to effects on the thyroid gland. No specific thyroid activity studies are available for bixlozone and none were considered necessary. Based on the ECHA/EFSA/JRC guidance (2018) for the identification of endocrine disruptors in the context of the retained Regulations (EU) 528/2012 and (EC) No 1107/2009, HSE concluded that bixlozone did not meet the ED criteria for the EATS-modalities and that these have been sufficiently investigated. The ECP is invited to advise.
Consumer risk assessment - Uncertainty in the residues metabolism package	All residue metabolism studies (wheat, canola/OSR, sugar beet, rice, rotational crops, poultry and ruminant) have involved storing samples in the freezer for much longer periods than is desirable, and have not been well supported by demonstration of stability of residues in the metabolism context. The ECP is invited to advise.

Area(s) where expert consultation is considered necessary	**Justification**
Consumer risk assessment - Applicant's proposal for natural origin of residues	The main residue found in cereals trials, was M118/1, 2,2-dimethyl-3-hydroxy propionic acid. The applicant is of the view that these residues, and also possible associated residues of M132/1, dimethyl malonic acid, are present as they are naturally derived. The presence of 2,2-dimethyl-3-hydroxy propionic acid was confirmed in a number of field trials control plots, commonly at a broadly similar level to that found in the corresponding treated samples. M118/1 (2,2-dimethyl-3-hydroxy propionic acid) was also found as a main radioactive residue in the treated metabolism samples. HSE considers that the residues were likely found in the cereal crops in field trials due to applications made in the field with bixlozone, possibly involving volatile transfer to untreated crop material in the vicinity. HSE has considered representations on this topic from the applicant to address this issue from the early admissibility stage, during evaluation and finally as the applicant presented a late position paper on this topic. The position paper has been assessed and HSE consideration of the points made explained in DAR Vol 1 Level 2 - end of section 2.7.4. HSE's opinion is unchanged. HSE proposes that an improved case for natural provenance, with data, would be needed in a future submission to justify that these residues are normally found in foods. It has now been confirmed that the metabolites 2,2-dimethyl-3-hydroxy propionic acid (M118/1) and dimethyl malonic acid (M132/1) can be initially screened using an exposure consideration versus a Threshold of Toxicological Concern (TTC) using the Cramer Class I classification (which presumes low toxicity). As such these residues do not need to be included in the residue definition for dietary risk assessment at the current time. The ECP is invited to advise.
Consumer risk assessment - Residue definition proposals	The ECP is invited to advise.
Fate and behaviour in the environment - Use of the EFSA degT50 calculator tool to conclude on the selection of modelling endpoints from field dissipation studies	Soil dissipation studies were undertaken at 7 sites (in France, Italy, Germany and the UK). The representative product is an SC formulation. For most sites, 4 separate trials were undertaken: SC formulation applied to bare soil, SC formulation applied to bare soil and then incorporated, CS (capsule suspension) formulation applied to bare soil and CS formulation applied to bare soil and then incorporated. Endpoints from 21 trials in total were determined. As part of the EFSA DegT50 guidance a calculator spreadsheet was developed to determine whether laboratory and field DT50 values come from the same population. HSE used the spreadsheet in a novel way to assess whether there was any statistical difference in the bixlozone endpoints determined by the different formulations and application methods. This analysis indicated for the SC formulation, the bare soil and the incorporated modelling DT50s were from the same population and could therefore be

Area(s) where expert consultation is considered necessary	Justification
	combined. However, for the CS formulation, the bare soil plots resulted in shorter DT50s and should be treated as separate populations. For the incorporated trials, the calculator indicated the SC formulation resulted in statistically shorter DT50s than the CS formulation. However, for the bare soil trials, there was no statistical difference between the two formulations. Based on the above analysis, the results of the combined SC formulation trials only were considered further in selecting an appropriate modelling endpoint for the representative SC formulation product. The geometric mean of all plots treated with the SC formulation was 54.4 d (n=13; treating each separate result as an individual replicate in determining the geometric mean). Information from plots treated with the CS formulation are not proposed for inclusion in the modelling endpoint selection for the representative SC formulation. The analysis above indicates that there may be a difference between the results from the SC and CS formulation for the incorporated plots. If applications for products based on a CS formulation are made in the future, HSE proposes that further analysis is undertaken to determine the appropriate endpoints. Further information is presented in DAR Vol 3 CA B8, section CA.B.8.1.2.3.22 (pages 305-307). The ECP is invited to advise.
Ecotoxicology - Endocrine disruption ([redacted] (2021b), Volume 3, CA, B.9.2.3., Bixlozone Technical (F9600) - An Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*). (Study KCA 8.2.3/02))	In regard to the analytical verification of the test item it is noted that OECD 231 specifies that ''*test solutions from each replicate tank at each concentration should be sampled for analyses at test initiation (day 0), and weekly during the test for a minimum of four samples''*. For the current study, samples on days 0, 14, and 21 were removed from replicates A and B and on test day 7 samples were removed from replicates C and D. This is not ideal as the full exposure profile of the test item over time is not available. Following a request for additional information, the following consideration of the analytical verification was provided by the applicant '*'OECD 231 indeed states that each concentration be tested at least once weekly and during the pre-test phase for four samples. The flow-through diluter system used in this study creates one concentration in a mixing chamber prior to delivery to each of the four replicates in the system from the mixing chamber. Taking samples of a substance known to be stable in water weekly but alternating between replicates to which the water is delivered from a common mixing chamber is sufficient, and, arguably, exceeds the requirements of the OECD 231. Testing in this manner evaluates the concentration, while also evaluating that the exposure is adequately maintained in each of the replicate exposure aquaria''*. HSE considers that this explanation is not ideal, whilst it is agreed that the flow-through diluter system is more likely to maintain desired test item concentration compared to other study designs (e.g. static single dose), the fact remains that the full exposure profile of the test item over time is not available due to the missing replicate data. However, it is acknowledged that measurements were taken from each concentration at test initiation (day 0), and weekly during the test for a minimum of four samples as OECD 231

Area(s) where expert consultation is considered necessary	Justification
	specifies, in addition, mean measured concentrations from those replicates tested remained within ±20% of the nominal value (93 to 100%) and variability between replicates was low. Therefore, this does give some confidence that the test item remained stable throughout the study. As such, this deviation is not considered by HSE to be cause to invalidate the study. The ECP is invited to advise.
…continued	The test organism feeding rates were reduced from the recommended feeding regime of Sera Micron in the Test Guideline (OECD 231, 2009). The applicant states that based on extensive experience with performing this study type, these rates employed with *Xenopus Express* Tadpole Food have consistently shown to support proper growth and development of *Xenopus laevis* tadpoles. However, without further data or evidence to support this claim, it is not possible to rule out the contribution of the reduced feeding rate to the effects observed. Following a request for additional information the applicant provided the following consideration of the feeding rates used in the study '*Excessive feeding has been shown to increase the prevalence of spinal deformities in the test organism for this study. The Larval Amphibian Growth and Development Assay (LAGDA) Guideline (OECD 241) expressly states that excessive feeding leads to an increased prevalence of scoliosis in developing Xenopus laevis. Consequently, the performing laboratories for the Amphibian Metamorphosis Assay often reduce the feeding rate to reduce the prevalence and severity of scoliosis.* [redacted]*, the laboratory performing the AMA being reviewed here, conducted their own internal study to determine the best feeding rate where the frogs developed as specified in the guideline (OECD 231), but minimized as much as possible the prevalence of scoliosis. Smithers subsequently found that feeding at the OECD 231 rates resulted in a scoliosis prevalence of greater than 80 percent. Additionally,* [redacted] *found that the frogs developed too quickly at the suggested OECD 231 feeding rate, and frequently >30% of the frogs exceeded NF60 by day 21. The feeding rate used here is a widely used rate of feeding for this study type, does not impact any of the study validity criteria (controls developed to the desired stage and gained weight during the conduct of the study), and therefore this should not be considered as contributing the reduced weight effect observed in the highest treatment*'. HSE notes that the 'internal study' referred to by the applicant has not been submitted, however, the explanation provided appears to be plausible. HSE does not consider that this deviation invalidates the study. The ECP is invited to advise.
…continued	In the current study there was a high rate of spinal deformity in the control and treatment groups. This was highest in the control (exposure total: 29%), followed by the 0.43 mg/L treatment group (exposure total 28%) and the 0.078 mg/L and 2.0 mg/L treatment groups which both had an exposure total of 21%. The study authors have justified this by stating that *"the incidence of spinal deformities was unrelated to treatment with bixlozone technical.*

Area(s) where expert consultation is considered necessary	Justification
	Incidences of spinal deformities can range widely and is not typically associated with overt toxicity (Coady et al., 2014)''. Following a request for additional information, the applicant provided the following consideration of the spinal deformity data ''*Coady, et al., (2014) is provided. Crooked tail (spinal deformations, bent tail, scoliosis) is a common occurrence in amphibian studies. It is not associated with a thyroid effect and is not considered relevant to this study as it is not dose dependent. Interestingly, it has been shown to be feeding rate dependent (see response below). Additionally, spinal deformities are not used as a justification for dose setting or interpreted as a toxicological effect in this study''.* HSE has considered Coady, *et al.*, (2014) in more detail. It is stated in the paper that *''an issue with the use of X. laevis tadpoles is the occurrence of bent tails (scoliosis), which can occur at a rate of up to 10 to 30% across an entire spawn of tadpoles for unknown reasons. In our experience, the occurrence of bent tails was not related to chemical exposure since the phenomenon occurred in controls and various treatment levels at the same incidence level. The bent tail phenomenon should be kept in mind when observing tadpoles at necropsy and interpreting study results''.* In the current study the occurrence of spinal deformity is in line with the rates observed in Coady, *et al.*, (2014), it is also noted that deformity was similar or greater in the controls than exposed tanks, therefore these results do not suggest overt toxicity. In addition there is no clear dose response relationship present and as such the incidence of spinal deformities do not seem to be treatment related, these findings are also in-line with Coady, *et al.*, (2014). In light of the additional information provided, HSE considers that the spinal deformity seen does not significantly impact study reliability. The ECP is invited to advise.
…continued	The raw data for each tadpole within control/treatment groups was not detailed in the study report for any parameter. HSE requested the raw data and ranges from the applicant in-order to verify the results seen, however, following a request for additional information the raw data was not provided. The applicant stated that *'these raw data would not provide additional context for the study report as sufficient data on the stage, weight and length data are provided in the study report'*. As the data cannot be verified by HSE, we consider that there is some uncertainty regarding the reliability of the endpoints. However, whilst this and the above points impact the reliability of the study somewhat, overall HSE consider that the study can be used in assessing the ED properties of the active substance. The ECP is invited to advise.
Ecotoxicology -	As for [redacted] 2021b with regard to the analytical verification of the test item, OECD 229 specifies that "*During the test, the concentrations of the test substance are determined at regular intervals, as follows: the flow rates of*

Area(s) where expert consultation is considered necessary	**Justification**
Endocrine disruption ([redacted] (2021a) Volume 3, CA, B.9.2.3. Bixlozone technical (F9600) - short-term reproduction assay with fathead minnow (*Pimephales promelas*). (Study KCA 8.2.3/01))	*diluent and toxicant stock solution should be checked preferably daily but as a minimum twice per week, and should not vary by more than 10% throughout the test. It is recommended that the actual test chemical concentrations be measured in all vessels at the start of the test and at weekly intervals thereafter*". This study has taken samples from alternate replicates: on day 0 and 14 samples were taken from replicates C and D and on day 7 and 21 samples were taken from replicates A and B. Therefore, samples have not been taken from all vessels and the full exposure profile of the test item over time is not available, leading to uncertainty that test item concentrations were maintained throughout the study. Following a request for further information, the following consideration of the analytical verification was provided by the applicant "*The OECD 229 indeed states that each concentration be tested at least once weekly and during the pre-test phase for four samples. The flow-through diluter system used in this study creates one concentration in a mixing chamber prior to delivery to each of the four replicates in the system from the mixing chamber. Taking samples of a substance known to be stable in water weekly but alternating between replicates to which the water is delivered from a common mixing chamber is sufficient, and, arguably, exceeds the requirements of the OECD 229. Testing in this manner evaluates the concentration, while also evaluating that the exposure is adequately maintained in each of the replicate exposure aquaria within the concentration".* Whilst not ideal, the fact that measured concentrations ranged from 97-110% of nominal means there can be a degree of confidence that the test chemical is stable and that the test concentration was maintained across replicates throughout the study. Additionally, the study is an intermittent flow through design, with the test medium being replaced over set periods during exposure. Compared to a static test design where a single dose is applied, the test medium concentration is more likely to be maintained when fresh doses are applied. Overall, the analytical measurements are not in line with guideline recommendations, however there is some degree of confidence that the treatment concentrations have been maintained, so this is not cause to dismiss the study. The ECP is invited to advise.
…continued	The OECD 229 guideline sets out the recommended wet weight of the adult fish to be used in the study. For *Pimephales promelas*, the recommended weight for adult females is 1.5 g ± 20% and for males is 2.5 g ± 20%. In this study, the mean wet weight of the adult fish was 3.0 g for females and 5.4 g for males, thus exceeding the guideline recommendations and leading to uncertainty over the suitability of these fish for use in the short-term reproductive assay. Following a request for information, the following consideration of fish weight was provided by the applicant. "*The purpose of the Fish Short Term Reproduction Assay is to screen for test substance effects on the reproductive success of fish. This study was conducted to both OECD 229 and OCSPP 890.1350, however the OECD 229 is the only guideline that has a recommended weight for the fish in the study. Both guidelines agree, however*

Area(s) where expert consultation is considered necessary	Justification
	that the weight of the fish should not vary by more than 20%. Wheeler, et al. (2019) (attached) found in an analysis of the control data from 65 FSTRAs (OECD 229 and/or OCSPP 890.1350) that the range of weights for control males and females were 1.95 g to 5.06 g and 0.79 to 2.05, respectively, thus deviating significantly from the recommendation for the fish weights in OECD 229 (see figure below). The average control weight at termination in this study was 4.56 g for males, and 2.24 g for females, and, importantly, Wheeler, et al. 2019 notes that this metric is not useful in determining adverse effects as a result of the treatment. Regardless, the validity criteria of the OECD 229 do not include weight, and all were met prior to initiation of the exposure or over the course of the study as necessary. This indicates that the fish were spawning according to the guideline prior to exposure, and this study is appropriate to evaluate the potential EAS effects of the test substance in fish." HSE has considered Wheeler *et al.* (2019) in more detail. It states: "*organism source and culture conditions may influence test organism size without compromising the performance of the study*". It is noted that the consideration of mean weights presented in Wheeler *et al.* (2019) refers to weight at test termination, whereas the concern of HSE was that the weight of fish at test initiation may be inappropriate. Additionally, the mean weights in this study exceed the range of weights presented in Wheeler *et al.* (2019) for both males and females. However, HSE accepts that weights were consistent within ± 20 % and the validity criteria show fish to be in active spawning prior to exposure. Additionally, the age of fish was consistent and within guideline recommendations. In light of the additional information provided, HSE considers that weight of fish used in this study is unlikely to have affected study reliability. The ECP is invited to advise.
Ecotoxicology - Aquatics - Fish – Long-term and chronic toxicity	EFSA 2013 aquatic guidance (EFSA Journal 2013;11(7):3290) outlines a preference for use of EC_{10} values over the NOEC in the aquatic risk assessment. However, given the magnitude of difference between the NOEC (0.38 mg a.s./L) and EC_{10} (4.6 mg a.s./L) in the fish early life stage study, the NOEC has been retained for use in the risk assessment as a more precautionary endpoint. The NOEC of 0.38 mg a.s./L was statistically determined and is considered acceptable; however it is noted that at concentrations up to 3.3 mg a.s./L only a 5.0 – 5.8% effect on fish total length was observed, with no corresponding effect on wet weight. The biological relevance of such a reduction in fish length is unknown. Therefore, HSE deemed it more appropriate to utilise the NOEC. Nevertheless, the chronic risk to fish does not drive the overall risk assessment which is driven by the risk to aquatic invertebrates. The ECP is invited to advise.
Ecotoxicology -	Four algal studies were submitted testing the active substance, however only one study, the study conducted with *Raphidocelis subcapitata* met all of the relevant validity criteria in accordance with OECD 201 (2011). The other

Area(s) where expert consultation is considered necessary	Justification
Aquatics - Algae	studies conducted with different species of algae did not meet all of the validity criteria specified in the study guidelines (OECD 201) at any time point during the test. The mean coefficient of variation (CV) for section-by-section specific growth rates in the control replicates failed by a large margin. It was 67.3% and 73.1% for *Skeletonema costatum* in the water and solvent controls respectively. These values exceed the guideline requirement (35%) and the average CV based on historical data determined for *S. costatum* at Smithers Viscient (43% based on N=5). For *Navicula pelliculosa,* the CV between negative water control replicates for the section-by-section growth rate exceeds the limit of 35% set out in OECD 201 (2011) (observed: 72-hours, 41%; 96-hours, 35.1%). Additionally, the results from the solvent control fail to meet the validity criterion at either 72- or 96-hours (observed: 72-hours, 64.3%; 96-hours, 52.9%). For *Anabaena flos-aquae,* the mean CV for section-by-section specific growth rates in the control replicates exceeded 35% (actual: 110% and 107.3% in the water and solvent controls respectively), therefore this criterion was not met. Following a request for additional information, the applicant stated that the other two OECD 201 validity criteria were met at 72 hours, which the endpoints were based on. However, according to OECD 201, the mean CV for section-by-section specific growth rates in the control cultures was 116% and 123.8% in the water and solvent controls respectively, thus exceeding the 35% specified in the guidance. These values also exceed the average CV based on historical data determined for *A. flos-aquae* at Smithers Viscient (97% based on N=5). Therefore, the study and its endpoints are not considered valid for use in risk assessment. According to Commission Regulation (EU) 283/2013, where an active substance is known to exhibit herbicidal activity, a second algal species should be tested. Therefore, as bixlozone is a herbicide, a valid study with a second algal species should be provided. Nevertheless, we have considered this issue further by comparing the toxicity endpoint derived for *R. subcapitata* in the active substance study (72 hour E_rC_{50} of 14000 µg a.s./L (mm) with the corresponding endpoints derived from the formulation studies conducted with *R. subcapitata* (72-hour, E_rC_{50} = 53 mg test item/L equivalent to 19290 µg a.s./L) and *S. costatum* (17 mg/test item/L equivalent to 6180 µg a.s./L*)*. This comparison indicates that the formulation is of similar toxicity to *R. subcapitata* as the active substance. The toxicity endpoint for *S. costatum* is approximately 3.1 fold lower than that obtained for *R. subcapitata* when tested with the formulation. Therefore, it is anticipated that if a valid study conducted with the active substance was available for *S. costatum* it would likely result in a lower endpoint than that obtained with *R. subcapitata* (this is also indicated by the results from the invalid study with *S. costatum*) – if it were 3-4 fold lower then this would result in a similar toxicity endpoint to that based on growth rate for *Myriophyllum*. However, given the magnitude of difference in the endpoints derived for algae and aquatic plants when compared with aquatic invertebrates, we consider the provision of an additional algal study would be highly unlikely to alter the overall risk assessment which is driven by the risk to aquatic invertebrates. The ECP is invited to advise.
Ecotoxicology -	In the risk assessment for (other) soil macro-organisms (see DAR Vol 3 CP B9, section B.9.8.), the long-term TER values for *Folsomia candida* and the

Area(s) where expert consultation is considered necessary	Justification
Soil - Soil macro-organisms	metabolite 2,4-dichlorobenzoic acid were marginally below the regulatory trigger value of 5 for maize (refined TER: 4.03) and winter oilseed rape (refined TER: 3.2). This has been considered further by HSE. HSE considers that the risk assessment for 2,4-dichlorobenzoic acid in this instance is very worst case (Table B.9.8-4). In the absence of toxicity data with the metabolite and the a.s., the formulation endpoint expressed in terms of the a.s. has been used as a surrogate, this has then been divided by 10, as is standard procedure. This approach is considered to be protective as the available toxicity data for earthworms (Table B.9.8-1) indicates that the metabolite ($NOEC_{corrected}$: 29.15 mg/kg soil dw) is similar in toxicity to the technical a.s. ($NOEC_{corrected}$: 50 mg a.s./kg soil dw), therefore, the current risk assessment is also considered to be protective of the risk from the metabolite. With reference to the risk assessment (Table B.9.8-4), even if the metabolite was 6x more toxic than the formulation to *Folsomia candida* it would still pass. In light of the earthworm toxicity data, it seems very unlikely that it would be 6x more toxic. Overall, based on the available margins of safety in the soil risk assessment and the consideration of the chronic earthworm data, HSE considers that the risk assessment is protective. The ECP is invited to advise.
Ecotoxicology - Soil - Soil micro-organisms	In the nitrogen transformation study with the metabolite 2,4-dichlorobenzoic acid (Häuser, R., 2018), for the validity criteria to be met the guideline specifies that the variation between replicate control samples in nitrate concentrations should be less than ± 15%. However, in the current study on day 7 in both the solvent control and untreated control samples, the coefficient of variation was 22.3% and 16.3%, respectively. HSE considers that as the day 7 values are not critical for the evaluation of the nitrogen formation rates (given we are more concerned with the rate of transformation at the end of the study), and the margins of safety in the risk assessment are sufficient (Table B.9.10-3), the current risk assessment is considered to be protective in spite of this uncertainty. The ECP is invited to advise.
…continued	In the available study with activated sludge (Hammesfahr, U., 2016), one of the validity criteria marginally failed. Oxygen uptake was less than 20 mg oxygen/g/h as specified in the guideline (observed: 19.7 mg/g/h). This has been considered further by HSE below. In the risk assessment (see DAR Vol 3 CP B9, section B.9.13.), the worst-case PEC_{sw} was 20.192 µg a.s./L for maize (Volume CP. B.8.5.), which is

Area(s) where expert consultation is considered necessary	Justification
	over 202 times lower than the lowest endpoint for activated sludge ($NOEC_{Total\ respiration}$: 100 mg/L). HSE considers that as the oxygen uptake rate was only slightly below the guideline limit, all of the other validity criteria were met and the margin of safety in the risk assessment is high, the risk assessment is considered to be protective of this uncertainty. The ECP is invited to advise.

3.2. PROPOSED DECISION

It is proposed that:

Bixlozone (F9600) can be approved as a candidate for substitution under Regulation (EC) No 1107/2009

It is considered that the following is specified in Part A of the approval of the active substance:

The following impurity identified in technical bixlozone is considered to be of toxicological or ecotoxicological relevance:

(2,4-dichlorophenyl)methanol (CAS 1777-82-8; 2,4-dichlorobenzyl alcohol): Maximum 1.5 g/kg.

It is considered that the following specific provision should be included in Part B of the approval as areas requiring particular attention when evaluating applications for product authorisation(s):

The risk to aquatic organisms and non-target plants.

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

It is considered that the following areas require particular attention when evaluating applications for product authorisation(s):

- Identity, physical and chemical properties and methods for the product (see Level 3.1.4.1, 3.1.4.2 and 3.1.4.5)
- Methods for monitoring of residues in plants (see Level 3.1.4.5)
- Residues storage stability and nature of residues (see Level 3.1.4.7)

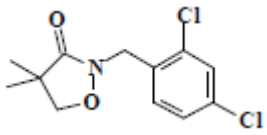
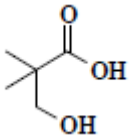
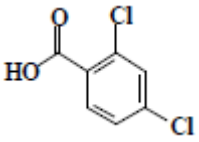
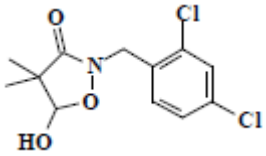
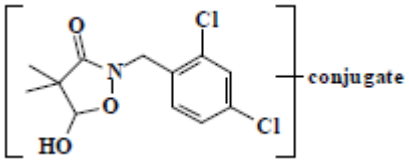
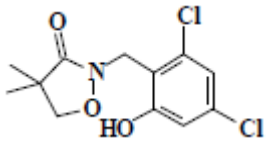
3.3. RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

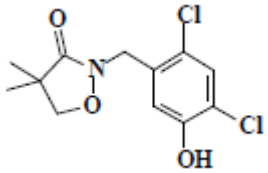
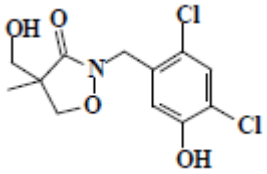
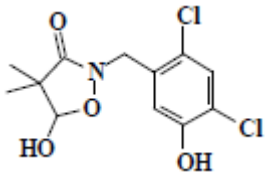
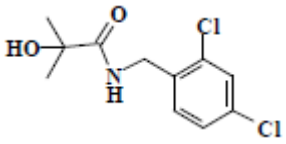
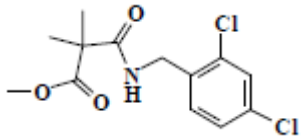
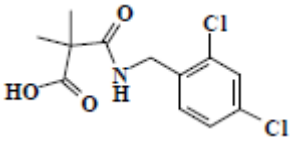
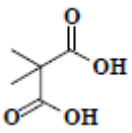
3.3.1. Particular conditions proposed to be taken into account to manage the risks identified

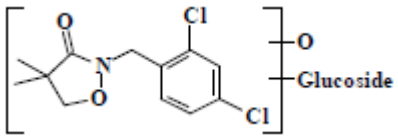
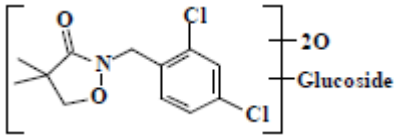
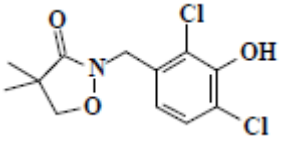
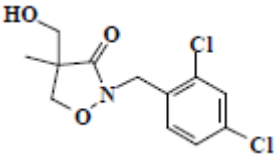
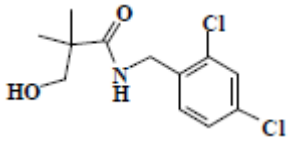
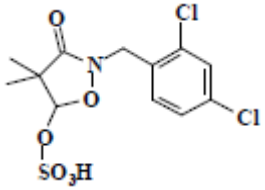
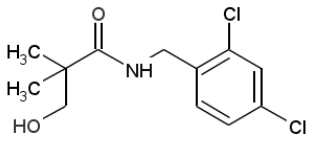
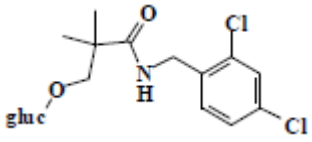
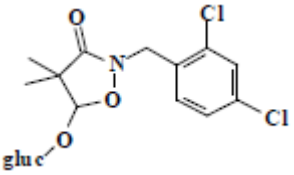
Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
The following label replant restriction must be included in the approval of bixlozone to mitigate for any rotational crop residues related to use of bixlozone. **'Leafy crops and above ground vegetables must not be planted until at least 10 months after application of bixlozone.'** [the applicant can generate further data to suggest removal or refinement of this restriction/to propose suitable MRLs covering the potential for rotational crop residues].	All proposed uses
To address the risk to aquatic plants from the active substance via spraydrift exposure the following risk mitigation is proposed: **5 m buffer zone**	For use on maize at 375 g a.s./ha
As the risk from spraydrift to non-target plants was not resolved the following label mitigation is proposed: **'Extreme care must be taken to avoid spray drift onto non-crop plants outside of the target area.'**	All proposed uses.
Due to the risk to non-target plants from volatilization a **5 m buffer zone** has been proposed.	For use on winter wheat/barley (at 200 g a.s./ha)
Due to the risk to non-target plants from volatilization a **10 m buffer zone** has been proposed.	For use on winter oilseed rape (at 300 g a.s./ha) and maize (at 375 g a.s./ha)

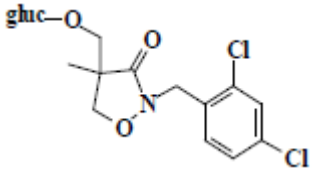
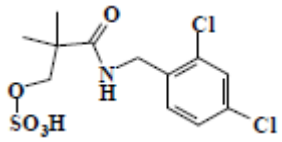
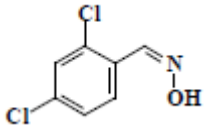
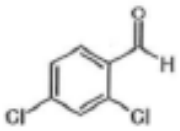
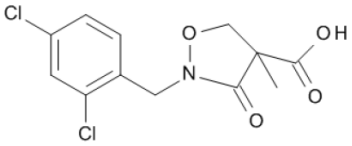
3.4. APPENDICES

3.4.1. Metabolites and their codes

Chemical Name	Alt Name	Code	Structure	Found in?
2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one	Bixlozone	F9600		Poultry, rotational crops, soil, water, sediment
2,2-dimethyl-3-hydroxypropionic acid	-	M118/1		Wheat, Canola, Sugar beet, Rice, Goat, poultry
2,2-dimethyl-3-hydroxypropionic acid conjugate	-	M118/1 conjugate	* *Unknown conjugation*	Rice
2,4-dichlorobenzoic acid	2,4-DBA	M190/1		Wheat, Canola, Sugar beet, Rice, Goat, rotational crops, poultry, soil, water, sediment
2-(2,4-dichlorobenzyl)-5-hydroxy-4,4-dimethylisoxazolidin-3-one	5-hydroxy-F9600	M289/1		Sugar beet, Goat, poultry
2-(2,4-dichlorobenzyl)-5-hydroxy-4,4-dimethylisoxazolidin-3-one conjugate	5-hydroxy-F9600 Conjugate	M289/1 conjugate		Sugar beet,
2-(2,4-dichloro-6-hydroxy benzyl)-4,4-dimethylisoxazolidin-3-one	6'-hydroxy-F9600	M289/5		Wheat

Chemical Name	**Alt Name**	**Code**	**Structure**	**Found in?**
2-(2,4-dichloro-5-hydroxy benzyl)-4,4-dimethylisoxazolidin-3-one	5'-OH-F9600 5'-hydroxy-F9600	M289/3		Wheat, rotational crops
5'-hydroxy-F9600 Conjugate	5'-hydroxy-F9600 Conjugate	M281/3 conjugate	* *Unknown conjugation*	Wheat,
4-hydroxymethyl-5'-hydroxyl-F9600	4-OH-Me, 5'-OH-F9600	M305/1		Wheat, Sugar beet **No structure in SB report*
5-hydroxy-5'-hydroxy-F9600	5-OH-5'-OH-F9600	M305/2		Wheat,
N-(2,4-dichlorobenzyl)-2-hydroxy-2-methylpropanamide	F9600-hydroxy-Isobutyramide, also termed bixlozone (F9600)-dimethyl-isobutyramide	M261/1		Canola, Rotational crops
F9600-dimethyl-malonamide methyl ester	-	M303/1		Canola,
F9600-Dimethyl-malonamide	-	M289/2		Canola, Sugar beet, Rice, Goat, Rotational crops, poultry, water, sediment
Dimethyl malonic acid	-	M132/1		Sugar beet, Rice, rotational crops, poultry
Dimethyl malonic acid conjugate	-	M132/1 conjugate	* *Unknown conjugation*	Rice

Chemical Name	Alt Name	Code	Structure	Found in?
F9600-[O, glucoside]	-	M451/1 and M451/2 (both glucoside conjugates)		Sugar beet, Rotational crops
Dihydroxy -F9600 conjugate	Di-OH-F9600 conjugate	M467/1		Sugar beet, rotational crops
3'-hydroxy-F9600	3'-hydroxy-F9600	M289/6		Sugar beet, rotational crops
2-(2,4-dichlorobenzyl)-4-(hydroxymethyl)-4-methylisoxazolidin-3-one	4-hydroxy-methyl-F9600	M289/4		Goat, sugar beet, canola, rotational crops, poultry
N-[(2,4-dichlorophenyl)methyl]-3-hydroxy-2,2-dimethylpropanamide	F9600-3-OH-Propanamide	M275/1		Goat, Rice, poultry
5-hydroxy-F9600-sulfate	5-hydroxy-F9600-sulfate	M369/1		Goat, poultry
F9600-3-OH-propanamide	Bixlozone-3-OH-propanamide	-		Water and sediment
F9600-3-OH-propanamide-Gluc	F9600-3-OH-propanamide-Gluc	M451/3		Goat, poultry
5-hydroxyl-F9600-Gluc	5-hydroxyl-F9600-Gluc	M465/1		Goat, poultry

Chemical Name	Alt Name	Code	Structure	Found in?
4-hydroxyl-F9600-Gluc	4-hydroxyl-F9600-Gluc	M465/2		Goat, poultry
F9600-3-OH-propanamide-sulfate	F9600-3-OH-propanamide-sulfate	M355/1		Goat
2,4-dichlorobenzaloxime	-	M189/1		Poultry, goat
2,4-dichlorobenzamine	-	M175/1		Poultry
4-carboxy-F9600	4-carboxy-bixlozone	-		Water and sediment

3.4.2. GUIDANCE DOCUMENTS USED IN THIS ASSESSMENT

Identity, Physical chemical properties, method of analysis

- Manual on development and use of FAO and WHO specifications for pesticides, 1st edition, 3rd revision; World Health Organisation and Food and Agriculture Organisation of the United Nations, Rome 2016

- Guidance document on significant and non-significant changes of the chemical composition of authorised plant protection products under Regulation (EC) No 1107/2009 of the EU Parliament and Council on placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. SANCO/12638/2011, rev. 2, 20 November 2012

- Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (Part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. SANCO/3030/99 rev. 4, 11 July 2000

- Guidance document for the generation and evaluation of data on the physical, chemical and technical properties of plant protection products under Regulation (EC) No. 1107/2009 of the EU Parliament and Council on placing plant protection products on the market, Final Draft. HSE, 13 July 2018.

- OECD, 2007, Guidance document on the pesticide residue analytical methods, (ENV/JM/MONO(2007)17), Series on testing and assessment No. 72 and Series on pesticides No. 39

- Residues: Guidance document for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (Part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. SANCO/3029/99 rev.4, 11/07/200.

- EU Guidance document on pesticide residue analytical methods. SANCO/825/00 rev. 8.1, 16/11/2010.

- Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods. SANTE/2017/10632 rev. 3, 22 November 2017

Toxicology

- CLP:
  ECHA 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 5.0, July 2017)

- Skin irritation and corrosion:
  OECD new guidance document on an Integrated Approach on Testing and Assessment (IATA) for skin corrosion and irritation - Series on Testing & Assessment N°203 (ENV/JM/MONO(2014)19)

- Eye irritation and damage:
  OECD Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation - Series on Testing & Assessment N°263 (ENV/JM/MONO(2017)15)

- Repeated-dose and carcinogenicity studies:
  OECD Guidance Document No. 116 on the Design and Conduct of Chronic Toxicity and Carcinogenicity Studies (ENV/JM/MONO(2011)47)

- Residue definition for dietary risk assessment:
  EFSA Guidance on the establishment of the residue definition for dietary risk assessment adopted 22 July 2016 - EFSA Journal 2016;14(12):4549)

- Endocrine disruption:
  1. ECHA/EFSA/JRC guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018;16(6):5311)

2. OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disruptors (OECD Revised Guidance Document 150, 2018b)

- Open Literature:
  EFSA guidance on the 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)

- Impurities:
  Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003 – rev. 10.1, 13 July 2012.

- ECHA document agreed upon at the Biocidal Products Committee (BPC)-31 on interpreting the definition of relevant impurities (June 2019)

- Dermal absorption:
  EFSA Guidance on dermal absorption: EFSA Journal 2012;10(4):2665

Residues

- EC (European Commission), 2010. Classes to be used for the setting of EU pesticide Maximum Residue Levels (MRLs). SANCO 10634/2010 Rev. 0, finalized in the Standing Committee on the Food Chain and Animal Health at its meeting of 23-24 March 2010.

- EC (European Commission), 2016. Appendix D. Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. 7525/VI/95-rev.10.3.

- FAO (Food and Agriculture Organization of the United Nations), 2009. Submission and evaluation of pesticide residues data for the estimation of Maximum Residue Levels in food and feed. Pesticide Residues. 2nd Ed. FAO Plant Production and Protection Paper 197, 264 pp.

- OECD, 2007, OECD Guidelines for the testing of chemicals – Metabolism in crops. No. 501, OECD, Paris 2007.

- OECD, 2007, OECD Guidelines for the testing of chemicals – Metabolism in rotational crops. No 502, Paris 2007.

- OECD, 2007, OECD Guidelines for the testing of chemicals – Metabolism in livestock, No. 503, OECD, Paris 2007.

- OECD, 2007, OECD Guidelines for the testing of chemicals – Residues in rotational crops (limited field studies). No 504, Paris 2007.

- OECD, 2007. OECD Guidelines for the testing of chemicals – Stability of pesticide residues in stored commodities. No 506, OECD, Paris 2007.

- OECD, 2007. OECD Guidelines for the testing of chemicals – Nature of the pesticide residues in processed commodities, high temperature hydrolysis. No 507, Paris 2007.

- OECD, 2008. OECD Guidelines for the testing of chemicals – Magnitude of pesticide residues in processed commodities. No 508, Paris 2008.

- OECD, 2009. OECD Guidelines for the testing of chemicals – Crop field trial. No 509, 2009 and 2021 update.

- OECD, 2009, Guidance document on the definition of residue, (ENV/JM/MONO(2009)30), Series on testing and assessment No. 63 and Series on pesticides No. 31

- OECD, 2008, Guidance document on magnitude of pesticide residues in processed commodities, (ENV/JM/MONO(2008)23), Series on testing and assessment No. 96

- OECD, 2018, Guidance document on residues in rotational crops, (ENV/JM/MONO(2018)9), Series on testing and assessment No. 279 and Series on pesticides No. 97

- Residues trials and MRL calculations, Proposals for a harmonised approach for the selection of the trials and data used for the estimation of MRL, STMR and HR, EFSA, September 2015

- Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, EFSA, September 2015

- Guidance Document on the assessment of the relevance of metabolites in groundwater of substances regulated under the Council Directive 91/414/EEC. SANCO 221/200 rev. 10 - final, 25 Feb 2003.

- EFSA guidance on the 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)

Environmental Fate and Behaviour

- OECD, 2002. OECD Guidelines for the testing of chemicals, Aerobic and Anaerobic Transformation in Soil. No. 307, April 2002.

- FOCUS (kinetics), 2014. Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. v1.1, 18 December 2014.

- OECD, (draft) 2002. OECD Guidelines for the testing of chemicals, Phototransformation of Chemicals on Soil Surfaces. Draft, January 2002.

- EPA, 2008. Fate, Transport and Transformation Test Guidelines. OPPTS 835.6100 Terrestrial Field Dissipation. October 2008.

- EFSA, 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662.

- OECD, 2016. Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies. ENV/JM/MONO(2016)6. 4 March 2016.

- OECD, 2007. OECD Guidelines for the testing of chemicals – Stability of pesticide residues in stored commodities. No 506, October 2007.

- SANCO, 2012. Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides. 25 September 2012 – rev.3.

- OECD, 2000. OECD Guidelines for the testing of chemicals, Adsorption - Desorption Using a Batch Equilibrium Method. No. 106, January 2000.

- OECD, 2004. OECD Guidelines for the testing of chemicals, Hydrolysis as a Function of pH. No. 111, April, 2004.

- OECD, 2008. OECD Guidelines for the testing of chemicals, Phototransformation of Chemicals in Water – Direct Photolysis. No. 316, October 2008.

- OECD, 1992. OECD Guidelines for the testing of chemicals, Ready Biodegradability (CO_2 Evolution (Modified Sturm Test)). No. 301B, July 1992.

- OECD, 2004. OECD Guidelines for the testing of chemicals, Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test. No. 309, April 2004.

- OECD, 2002. OECD Guidelines for the testing of chemicals, Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. No. 308, April 2002.

- BBA, 2002. Assessment Scheme for the Short Range Transport of Plant Protection Products – Environmental Exposure by Airborne Routes (Spray Drift, Volatilisation and Deposition), Federal Biological Research Centre for Agriculture and Forestry, Report 110 (2002), Braunschweig, Germany.

- FOCUS (air), 2008. Pesticides in air: considerations for exposure asssessment. Report prepared by the FOCUS Working Group On Pesticides in Air (FOCUS Air Group). SANCO/10553/2006 Rev 2 June 2008.

- FOCUS (groundwater), 2014. Generic Guidance for Tier 1 FOCUS Ground Water Assessments. v2.2, May 2014.

- FOCUS (surface water), 2015. Generic guidance for FOCUS surface water Scenarios. v1.4, May 2015.

Ecotoxicology

- Birds & Mammals:
  EFSA (2009). Guidance document on risk assessment for birds and mammals. EFSA Journal 2009;7(12):1438

- Aquatic Organisms:
  EFSA (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290

- Bees/Soil organisms/Non-target plants:
  SANCO/10329/2002 (rev 2 final). Guidance document on terrestrial ecotoxicology under council directive 91/414/EEC.

- Non-target arthropods:
  ESCORT 2 (Candolfi *et al.*, 2001). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods.

- Endocrine disruption:
  ECHA/EFSA/JRC guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018;16(6):5311)

3.5. REFERENCE LIST

Physical-chemical properties

None.

Efficacy

None.

Analytical Methods

None.

Toxicology

EFSA (2012) Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07):2799

Residues

None.

Environmental Fate and Behaviour

None.

Ecotoxicology

None.