MCL Report for:

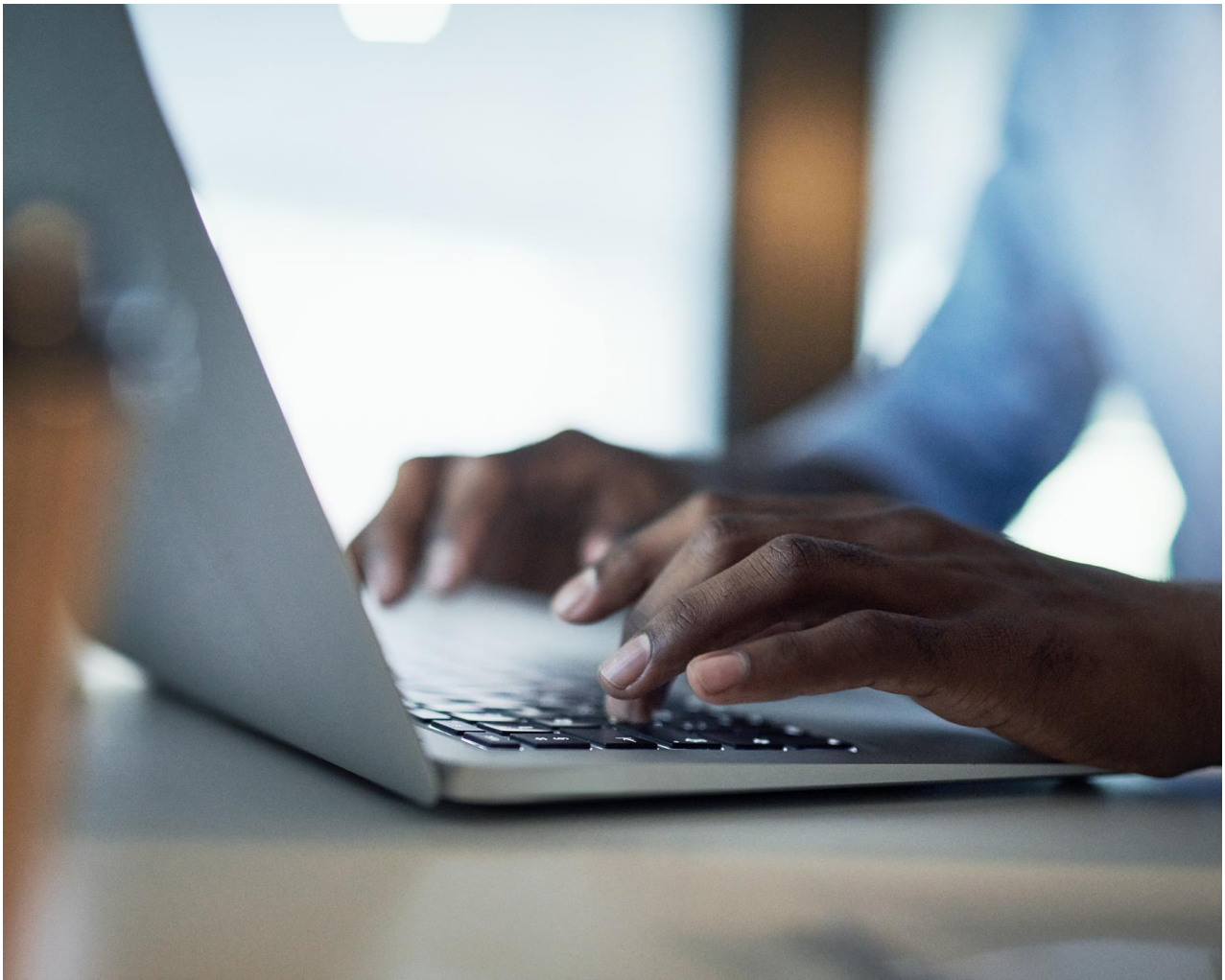
2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one, Bixlozone (ISO)

Proposal for mandatory classification and labelling (MCL) based on Annex VI, Part 2 of the retained CLP Regulation (EU) No. 1272/2008 as amended for Great Britain

EC Number: 701-330-4

CAS Number: 81777-95-9

Month: November 2022

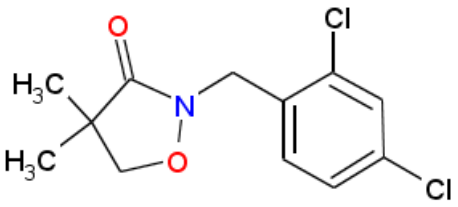


Contents

1. Identity of the substance

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one
Other names (usual name, trade name, abbreviation)	Not applicable
ISO common name (if available and appropriate)	Bixlozone (provisional)
EC number (if available and appropriate)	701-330-4
EC name (if available and appropriate)	Not applicable
CAS number (if available)	81777-95-9
Other identity code (if available)	Not applicable
Molecular formula	C12H13Cl2NO2
Structural formula	
SMILES notation (if available)	Not available
Molecular weight or molecular weight range	274.14 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum 960 g/kg

1.2 Composition of the substance

Bixlozone has no stereoisomers. There are a number of confidential impurities present, however, none are considered toxicologically relevant at the levels present and are not relevant to the classification and labelling of the substance. There are no additives present in technical grade bixlozone.

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current MCL on GB MCL list (if applicable)
2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one	≥ 96%	Not listed

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The impurity contributes to the classification and labelling?
No impurities of relevance to the classification and labelling.			

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current MCL on GB MCL list (if applicable)	The additive contributes to the classification and labelling?
No additives of relevance to the classification and labelling.				

2. Proposed mandatory classification and labelling

Table 5: Proposed mandatory classification and labelling according to the GB CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current GB MCL list entry	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Dossier submitters proposal	TBD	2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one, bixlozone	701-330-4	81777-95-9	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H400 H410	N/A	M-factor = 1 M-factor = 10	N/A
Resulting entry on GB MCL list	TBD	2-[(2,4-dichlorophenyl)methyl]-4,4-dimethyl-1,2-oxazolidin-3-one, bixlozone	701-330-4	81777-95-9	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H400 H410	N/A	M-factor = 1 M-factor = 10	N/A

Table 6: Reason for not proposing mandatory classification and status under public consultation

Hazard class	Classification / reason for no classification	Within the scope of public consultation
Explosives	*Data conclusive but not sufficient for classification*	Yes
Flammable gases (including chemically unstable gases)	*Hazard class not applicable*	No
Oxidising gases	*Hazard class not applicable*	No
Gases under pressure	*Hazard class not applicable*	No
Flammable liquids	*Hazard class not applicable*	No
Flammable solids	*Data conclusive but not sufficient for classification*	Yes
Self-reactive substances	*Data conclusive but not sufficient for classification*	Yes
Pyrophoric liquids	*Hazard class not applicable*	No
Pyrophoric solids	*Data conclusive but not sufficient for classification*	Yes
Self-heating substances	*Hazard class not applicable*	No
Substances which in contact with water emit flammable gases	*Hazard class not applicable*	No
Oxidising liquids	*Hazard class not applicable*	No
Oxidising solids	*Data conclusive but not sufficient for classification*	Yes
Organic peroxides	*Data conclusive but not sufficient for classification*	No
Corrosive to metals	*Not classified, data lacking*	Yes
Acute toxicity via oral route	*Data conclusive but not sufficient for classification*	Yes
Acute toxicity via dermal route	*Data conclusive but not sufficient for classification*	Yes
Acute toxicity via inhalation route	*Data conclusive but not sufficient for classification*	Yes
Skin corrosion/irritation	*Data conclusive but not sufficient for classification*	Yes
Serious eye damage/eye irritation	*Data conclusive but not sufficient for classification*	Yes
Respiratory sensitisation	*Not classified, data lacking*	Yes
Skin sensitisation	*Data conclusive but not sufficient for classification*	Yes
Germ cell mutagenicity	*Data conclusive but not sufficient for classification*	Yes
Carcinogenicity	*Data conclusive but not sufficient for classification*	Yes

Hazard class	Classification / reason for no classification	Within the scope of public consultation
Reproductive toxicity	*Data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-single exposure	*Data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-repeated exposure	*Data conclusive but not sufficient for classification*	Yes
Aspiration hazard	*Not classified, data lacking.*	Yes
Hazardous to the aquatic environment	***Aquatic Acute 1; H400*** ***Aquatic Chronic 1; H410***	Yes
Hazardous to the ozone layer	*Not classified, data lacking.*	Yes

3. History of the classification and labelling

Bixlozone (2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one, also known as F9600, CAS 81777-95-9) is a new pesticidal active substance in Great Britain (GB) under Regulation (EC) 1107/2009 as it has effect in Great Britain.

An 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) was concluded in 2015. The Netherlands have submitted a harmonised classification and labelling dossier (CLH) to the European Chemicals Agency (ECHA) which underwent public consultation in June 2022. The proposed classification was Aquatic Acute 1, H400, M factor 1 and Aquatic chronic 1, H410, M factor 10. At the time of preparing this MCL report, the Risk Assessment Committee had not yet considered the CLH proposal.

Bixlozone does not have an existing GB CLP mandatory classification/entry on the GB MCL list but is self-classified by 16 notifiers as aquatic acute 1, H400 and aquatic chronic 1, H410. The substance was not previously discussed by the Technical Committee for Classification and Labelling (TC C&L) under Directive 67/548/EEC.

.

4. Justification that action is needed

Bixlozone is a new pesticidal active substance in the meaning of Regulation EC 1107/2009 and, as such, shall normally be subject to mandatory classification and labelling process in accordance with Article 36 (2) and Article 37A of the retained CLP Regulation (EC) NO. 1272/2008 as amended for Great Britain (hereafter referred to as GB CLP). This report has been prepared by technical experts at HSE, acting in its capacity as the GB CLP Agency (hereinafter referred to as “the Agency”).

5. Identified uses

Bixlozone is a herbicidal active substance intended to be used 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.

6. Data sources

This MCL report relies exclusively on the data submitted in the context of the application for approval as an active substance under Regulation (EC) 1107/2009 as it applies in Great Britain.

Draft assessment report – DAR – Volume 3, Annex B.1; Identity of the Active Substance - 2022

Draft assessment report – DAR – Volume 3, Annex B.2; Physical & Chemical Properties – 2022

Draft assessment report – DAR – Volume 3, Annex B.4; Further Information - 2022

Draft assessment report – DAR – Volume 3, Annex B.5; Methods of Analysis - 2022

Draft assessment report – DAR – Volume 3, Annex B.6; Toxicology & Metabolism Data – 2022

Draft assessment report – DAR – Volume 3, Annex B.7; Residue Data – 2022

Draft assessment report – DAR – Volume 3, Annex B.8; Environmental Fate – 2022

Draft assessment report – DAR – Volume 3, Annex B.9; Ecotoxicology Data – 2022

Draft assessment report – DAR – Volume 4; Confidential information – 2022

At the time of preparation of this report, there was no REACH registration dossier for bixlozone (November 2022).

7. Physicochemical properties

Table 7: Summary of physicochemical properties

The physico-chemical properties of bixlozone pure (pure) and bixlozone technical (technical) are summarised below. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.2; Physical and Chemical properties. All studies were conducted to appropriate quality standards and were considered acceptable during the peer review.

Property	Value	Reference	Comment (e.g., measured or estimated)
Physical state at 20°C and 101,3 kPa	Bixlozone pure: A crystalline, white solid with no discernible odour. Bixlozone technical: A pale yellow/brown crystalline solid with no discernible odour.	Cowlyn, N. 2017a Cowlyn, N. 2017b DAR: B.2.3	Visual assessment EPA/OPPTS 830.302 EPA/OPPTS 830.6303 EPA/OPPTS 830.6304 GLP Purity: 99.8 % (pure) 96.0 % (technical)
Melting/freezing point	Bixlozone pure: 81.5 °	Cowlyn, N. 2017a DAR: B.2.1	EC Method A1 OECD 102 GLP Melting block confirmation by differential scanning calorimetry (DSC) Purity: 99.8 % (pure)
Boiling point	The boiling point of bixlozone was not determinable; the test item decomposed before boiling.	Cowlyn, N. 2017a DAR: B.2.1	EC Method A2 OECD 103 GLP Siwoloboff method Purity: 99.8 % (pure)
Relative density	The relative density (D_4^{20}) of bixlozone was found to be 1.37.	Cowlyn,N. 2017i B.2.14	EC Method A.3 OECD 109 GLP Purity: 99.8 % (pure)

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g., measured or estimated)</th></tr>
<tr><td>Vapour pressure</td><td>
<table>
<tr><th>Parameter</th><th>Value</th></tr>
<tr><td>Vapour pressure at 20°C</td><td>1.1 × 10^{-3} Pa</td></tr>
<tr><td>Vapour pressure at 25°C</td><td>2.3 × 10^{-3} Pa</td></tr>
</table>
Bixlozone is considered slightly volatile.
</td><td>Cowlyn N. 2016a
DAR: B.2.2</td><td>EC Method A.4
OECD 104
GLP
Vapour pressure balance
Purity: 99.9 % (pure)</td></tr>
<tr><td>Surface tension</td><td>90% saturated aqueous solution at 20°C: 66.5mN/m. Bixlozone is not considered surface active.</td><td>Cowlyn, N. 2017a
DAR: B.2.12</td><td>EC Method A.5
OECD 115
GLP
Purity: 99.8 % (pure)</td></tr>
<tr><td>Water solubility</td><td>The water solubility of bixlozone was not significantly affected by pH. Bixlozone is moderately soluble.
<table>
<tr><th>Media</th><th>Mean Solubility (mg/L)</th></tr>
<tr><td>Purified water</td><td>42.0 ± 0.3 mg/L</td></tr>
<tr><td>pH 4 buffer</td><td>42.3 ± 2.2 mg/L</td></tr>
<tr><td>pH 7 buffer</td><td>39.6 ± 1.6 mg/L</td></tr>
<tr><td>pH 9 buffer</td><td>41.9 ± 1.8 mg/L</td></tr>
</table>
</td><td>Cowlyn, N. 2016b
DAR: B.2.5</td><td>EC Method A.6
OECD Method 105
GLP
Shake flask method
Purity: 99.9 % (pure)</td></tr>
<tr><td>Partition coefficient n-octanol/water</td><td>Bixlozone has a Log Pow 3.3

The following results were obtained at 20°C:
<table>
<tr><th>Compound</th><th>Buffer solution</th><th>Pow</th><th>Log Pow</th></tr>
<tr><td rowspan="3">Bixlozone</td><td>pH 4</td><td>2100</td><td>3.3</td></tr>
<tr><td>pH 7</td><td>2160</td><td>3.3</td></tr>
<tr><td>pH 9</td><td>2060</td><td>3.3</td></tr>
</table>
</td><td>Cowlyn, N. 2016c
DAR: B.2.7</td><td>EC Method A.8
OECD Method 107
GLP
Shake flask method
Purity: 99.9 % (pure)</td></tr>
<tr><td>Flash point</td><td>Not applicable as bixlozone melting point is > 40°C.</td><td></td><td></td></tr>
</table>

Property	Value	Reference	Comment (e.g., measured or estimated)
Flammability	Bixlozone technical is not highly flammable. Bixlozone technical material melted and burned with a yellow sooty flame, which extinguished immediately after the removal of the burner flame. Combustion did not propagate along the train. As a negative result was obtained in the preliminary test, a definitive burning rate test was not required.	Cowlyn, N. 2017e DAR: B.2.9.1	EC Method A.10 GLP Purity: 96.0 % (technical)
Explosive properties	Bixlozone technical does not have explosive properties. - No shock sensitivity No reaction observed in six tests using BAM drop hammer (mass 10 kg, drop height 40 cm). - No friction sensitivity No reaction observed in six tests using BAM friction apparatus with a force of 360 N. - No thermal sensitivity No reaction observed in the range135-285 ^{o}C in a 6 mm or 2 mm orifice.	Cowlyn, N. 2017g DAR: B.2.11	EC Method A.14 GLP Purity: 96.0 % (technical)
Self-ignition temperature	The auto-ignition temperature of bixlozone technical was 382°C. Bixlozone is a solid hence Method A.16 should be used. However, bixlozone was observed to melt at around 81 °C which resulted in material flowing out of the wire mesh cage, preventing measurement. Test A.15 was used as it employed a glass vessel. The use of test A.15 is considered acceptable.	Cowlyn, N. 2017f DAR: B.2.9.2	EC Method A.15 GLP Purity: 96.0 % (technical)
Oxidising properties	Bixlozone technical has no oxidising properties. Mixtures of 2:1, 1:1 or 1:2 bixlozone /cellulose only burned slowly and did not burn to completion.	Cowlyn, N. 2017h DAR: B.2.13	EC Method A.17 GLP Purity: 96.0 % (technical)
Granulometry	No data		

Property	Value	Reference	Comment (e.g., measured or estimated)
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	Not applicable as bixlozone does not contain any groups that are ionisable within an environmentally relevant pH range. Water solubility and partition coefficient determinations at different pH's support this, as no pH dependence of these properties observed	Guo, J. 2018 DAR: B.2.8	Scientific justification
Viscosity	No data		

8. Evaluation of physical hazards

8.1 Explosives

Table 8: Summary of studies on explosive properties

Method	Results	Remarks	Reference
EC Method A14 GLP Test substance: Bixlozone technical Purity: 96.0 %	- No shock sensitivity No reaction observed in six tests using BAM drop hammer (mass 10 kg, drop height 40 cm). - No friction sensitivity No reaction observed in six tests using BAM friction apparatus with a force of 360 N. - No thermal sensitivity No reaction observed in the range 135-285 °C in a 6 mm or 2 mm orifice.	Bixlozone has no explosive properties.	Cowlyn, N. 2017g DAR: B.2.11

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Bixlozone was tested for explosive properties using EC Method A14 (Cowlyn, N. 2017g). The results showed no evidence of explosivity. Further, experience in handling and use indicates that it is not a pyrophoric solid and does not emit flammable gas on contact with water.

8.1.2 Comparison with the GB CLP criteria

Bixlozone has been adequately tested for explosivity but does not meet the criteria for classification under GB CLP.

8.1.3 Conclusion on classification and labelling for explosive properties

Not classified, data conclusive but not sufficient for classification.

8.2 Flammable solids

Table 9: Summary of studies on flammable solids

Method	Results	Remarks	Reference
EC Method A10 GLP Test substance: Bixlozone technical Purity: 96.0 %	Bixlozone technical melted and burned with a yellow sooty flame, which extinguished immediately after the removal of the burner flame. Combustion did not propagate along the train. As a negative result was obtained in the preliminary test, a definitive burning rate test was not required.	Bixlozone technical is not highly flammable.	Cowlyn, N. 2017e DAR: B.2.9.1

8.2.1 Short summary and overall relevance of the provided information on flammable solids

Bixlozone is a solid at room temperature but when tested for flammability using EC Method A10 it was shown to be not readily combustible and it did not contribute to fire through friction (Colwyn, N. 2017e). The results showed that bixlozone technical melted and burned with a yellow sooty flame which immediately extinguished after the removal of the burner flame. As a negative result was obtained in the preliminary test, a definitive burning rate test was not required.

8.2.2 Comparison with the GB CLP criteria

Bixlozone has undergone a screening test for flammability and does not meet the criteria for flammability under GB CLP.

8.2.3 Conclusion on classification and labelling for flammable solids

Not classified, data conclusive but not sufficient for classification.

8.3 Self-reactive substances

Table 10: Summary of studies on self-reactivity

Method	Results	Remarks	Reference
EC Method A.14 GLP Substance tested: Bixlozone technical Purity: 96.0 %	**No shock sensitivity** No reaction observed in six tests using BAM drop hammer (mass 10 kg, drop height 40 cm).	Bixlozone technical does not have explosive properties.	Cowlyn, N. 2017g DAR: B.2.11

Method	Results	Remarks	Reference
	No friction sensitivity No reaction observed in six tests using BAM friction apparatus with a force of 360 N. **No thermal sensitivity** No reaction observed between 135-285 °C in a 6 mm or 2 mm orifice.		
EC Method A.17 GLP Substance tested: Bixlozone technical Purity: 96.0 %	Mixtures of 2:1, 1:1 or 1:2 bixlozone /cellulose burned slowly and did not burn to completion.	Bixlozone technical has no oxidising properties.	Cowlyn, N. 2017h DAR: B.2.13

8.3.1 Short summary and overall relevance of the provided information on self-reactive substances

Bixlozone was tested for explosivity using EC Method A14 (Cowlyn N. 2017g). The results of this study showed there was no shock sensitivity, no friction sensitivity and no thermal sensitivity associated with bixlozone. Bixlozone has also been shown to have no oxidising properties in accordance with EC Method A17 (Cowlyn, N. 2017h). Bixlozone is not an organic peroxide and bears no chemical groups associated with explosive or self-reactive properties.

8.3.2 Comparison with the GB CLP criteria

Bixlozone is not a thermally unstable solid and is not liable to undergo exothermal decomposition in the presence or absence of oxygen. Bixlozone does not meet the criteria for a self-reactive substance.

8.3.3 Conclusion on classification and labelling for self-reactive substances

Not classified, data conclusive but not sufficient for classification.

8.4 Pyrophoric solids

8.4.1 Short summary and overall relevance of the provided information on pyrophoric solids

Experience in handling and use of bixlozone indicates that it is not pyrophoric. It does not ignite within five minutes of coming into contact with air.

8.4.2 Comparison with the GB CLP criteria

Bixlozone does not meet the criteria for a pyrophoric solid.

8.4.3 Conclusion on classification and labelling for pyrophoric solids

Not classified, data conclusive but not sufficient for classification.

8.5 Self-heating substances

Table 11: Summary of studies on self-heating substances

Method	Results	Remarks	Reference
EC Method A.15 GLP Test substance: bixlozone technical Purity: 96.0 %	The auto-ignition temperature of bixlozone technical was 382°C.	Bixlozone is a solid hence Method A.16 should be used. However, bixlozone was observed to melt at around 81 °C which resulted in material flowing out of the wire mesh cage, preventing measurement. Test A.15 was used as it employed a glass vessel. The use of test A.15 is considered acceptable.	Cowlyn, N. 2017f DAR: B.2.9.2

8.5.1 Short summary and overall relevance of the provided information on self-heating substances

Bixlozone was tested for auto-ignition using EC Method A15. The results of the study showed that the auto-ignition temperature of bixlozone technical was 382 °C. However, according to the Guidance on the Application of the CLP criteria (Version 5.0, July 2017) substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class as the melting process is endothermic and the substance-air surface is drastically reduced.

8.5.2 Comparison with the GB CLP criteria

Bixlozone should not be considered for this class.

8.5.3 Conclusion on classification and labelling for self-heating substances

Not classified – conclusive but not sufficient for classification

8.6 Substances which in contact with water emit flammable gases

8.6.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

The classification procedure for this class does not apply as chemical structure of bixlozone does not contain metals or metalloids.

8.6.2 Comparison with the GB CLP criteria

Bixlozone should not be considered for this class.

8.6.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified – conclusive but not sufficient for classification

8.7 Oxidising solids

Table 12: Summary of studies on oxidising solids

Method	Results	Remarks	Reference
EC Method A.17 GLP Substance tested: Bixlozone technical Purity: 96.0 %	Mixtures of 2:1, 1:1 or 1:2 bixlozone /cellulose burned slowly and did not burn to completion.	Bixlozone technical has no oxidising properties.	Cowlyn, N. 2017h DAR: B.2.13

8.7.1 Short summary and overall relevance of the provided information on oxidising solids

Bixlozone was tested under EC Method A17 in order to determine whether it has any oxidising properties (Cowlyn, N. 2017h). The results showed that when burned as a mixture with cellulose (2:1, 1:1 or 1:2) it burned slowly and did not burn to completion. Therefore, bixlozone did not enhance the combustion of cellulose and was not shown to have any oxidising properties.

8.7.2 Comparison with the GB CLP criteria

As bixlozone did not cause or contribute to the combustion of cellulose, it does not meet the definition as an oxidising solid.

8.7.3 Conclusion on classification and labelling for oxidising solids

Not classified – conclusive but not sufficient for classification.

8.8 Organic peroxides

8.8.1 Short summary and overall relevance of the provided information on organic peroxides

Bixlozone does not contain the bivalent -O-O- structure and can therefore, not be considered an organic peroxide.

8.8.2 Comparison with the GB CLP criteria

Bixlozone does not meet the criteria for classification as an organic peroxide.

8.8.3 Conclusion on classification and labelling for organic peroxides

Not classified – conclusive but not sufficient for classification.

8.9 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

There is no testing data on bixlozone with regards to its corrosivity to metals. However, bixlozone does not have an extreme pH. Experience in handling and use also indicates it is not corrosive to metals. 8.15.2 Comparison with the GB CLP criteria

There is no evidence to suggest that bixlozone is corrosive to metals, however a corrosivity test with steel or aluminium has not been carried out.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Not classified – data lacking.

9. Toxicokinetics (absorption, metabolism, distribution and elimination)

The ADME of bixlozone has been investigated in rats, via oral and iv dosing. Studies were conducted with both ^{14}C-phenyl bixlozone and ^{14}C-carbonyl bixlozone. Also available are two in vitro comparative metabolism studies using hepatocytes from humans, rats, dogs and mice exposed to both ^{14}C-phenyl and ^{14}C-carbonyl bixlozone. Together, these studies provide a thorough understanding of the ADME of bixlozone in experimental animals.

Reference should be made to the Draft Assessment Report – DAR- Volume 3, Annex B.6; Toxicology and Metabolism data – July 2022

Table 13: Summary of toxicokinetic studies

Method	Results	Remarks	Reference
Toxicokinetics **Rat** (Crl: CD9(SD) 4/sex/group in all groups except for single oral high dose with 8/sex/group GLP OECD 417 (2010) Deviations: None of significance Study no. FMC-P3773 Bixlozone technical, PL14-0163 Purity 99.8 % **[^{14}C-Phenyl]-bixlozone**, Batch CFQ42017; purity 99.6 %	<u>Single oral low dose (5 mg/kg bw):</u> C_{max} of bixlozone 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 % (bixlozone in plasma) for M /F <u>Single oral high dose (1000 mg/kg bw):</u> C_{max} of bixlozone 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 % (bixlozone in plasma) for M /F	**Acceptable**	**Anon, 2016h** **DAR : B.6.1.1.2**

Method	Results	Remarks	Reference
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>Multiple oral dose (5 mg/kg bw/day; 14 days)</u> C_{max} of bixlozone 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 % (BIXLOZONE in plasma) for M /F No indications of accumulation of total radioactivity or bixlozone. <u>Intravenous bolus dose (IV; 3 mg/kg bw):</u> C_{max} of bixlozone 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 % (bixlozone in plasma) for M /F <u>All groups</u>: Extensive metabolism of bixlozone and limited partitioning of bixlozone 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.		
Pharmacokinetics and metabolism (pilot study) **Rat** (Crl :CD9(SD), males & females, 3/sex/group) Not to GLP	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.	**Supplementary only**	**Anon, 2014j** **DAR : B.6.1.1.1**

Method	Results	Remarks	Reference
Not to OECD Guideline Deviations: N/A Report no. FMC-R2838 bixlozone technical, batch G3773-17 Purity: 99.25 % 25 mg/kg bw Single oral (gavage)	No bixlozone detected in urine and rat faeces Extensive metabolism to various isoxazolidinone ring-opened/modified analogues in both urine and faeces.		
Tissue distribution **Rat** (Crl :CD9(SD), males & females, 4/sex/group) GLP OECD 417 (2010) Deviations: None Study no. FMC-P4973 **[^{14}C-Phenyl]-bixlozone** Batch 77874-3-19; purity 100 % BIXLOZONE technical, PL14-0163 Purity 99 % Single oral low dose (5 mg/kg bw) Single oral high dose (500 mg/kg bw)	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.	**Acceptable**	**Anon, 2017d** **DAR : B.6.1.1.4**

Method	Results	Remarks	Reference
Multiple oral dose (5 mg/kg bw/day; 14 days)			
Radioactivity concentration in plasma and bone marrow Rat (Crl: CD9(SD) 4 males GLP OECD 417 (2010) Deviations: None Study no. FMC-P7354 **[^{14}C-Phenyl]-bixlozone** Batch CFQ43224; purity 99.8 % 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).	**Acceptable**	**Anon, 2017h** **DAR: B.6.1.1.4**
Excretion and Metabolism Rat (Crl :CD9(SD) 4 animals/sex/group GLP OECD 417 (2010) Deviations: None of significance Study no. FMC-P3887 **[^{14}C-Phenyl]-bixlozone**, batch	<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).	**Acceptable**	**Anon, 2018d** **DAR: B.6.1.1.5**

Method	Results	Remarks	Reference
77874-3-19; purity 100 % BIXLOZONE technical, batch PL14-0163 Purity 99.8 % 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)	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> Bixlozone was extensively metabolised; unchanged bixlozone 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). Proposed main metabolic pathway in rats: hydroxylation leading to the formation 5-OH-bixlozone and its derivatives. Other routes of metabolism included a combination of oxidation, decarboxylation and deamination followed by conjugation of oxidative derivatives.		
Metabolism (pilot study) **Rat** (Crl :CD9(SD) 2 males Not to GLP OECD 417 (2010) Deviations: N/A Study no. FMC-R3694 Bixlozone technical, batchPL14-0163 Purity 99.8 %	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.	**Supplementary only**	**Anon, 2017c** **DAR: B.6.1.1.1**

Method	Results	Remarks	Reference
[^{14}C-phenyl]-bixlozone, batch CFQ42017; purity 99.6 % 1000 mg/kg bw Single oral (gavage)			
Excretion and Metabolism Rat (Crl :CD9(SD) 4 animals/sex/group GLP OECD 417 (2010) Deviations: None of significance Study no. FMC-P4547 **[^{14}C-Carbonyl]-bixlozone**, batch CFQ42476; purity 99.9 % Bixlozone technical, batch PL G3773-17 Purity 99.5 % 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: > 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> Bixlozone was extensively metabolised; unchanged bixlozone 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). 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	**Acceptable**	**Anon, 2017e** **DAR: B.6.1.1.6**

Method	Results	Remarks	Reference
	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.		
Excretion and Metabolism (pilot study) Rat (Crl :CD9(SD) 2 animals/sex/group Not to GLP OECD 417 (2010) Deviations: N/A Study no. FMC-R3449 **[^{14}C-Carbonyl]-bixlozone**, batch CFQ42018; purity 99.9 % Bixlozone technical, batch G3773:62 (PL14-0163) Purity 99.5 % 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> Bixlozone was extensively metabolised; unchanged bixlozone 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).	**Supplementary only**	**Anon, 2018c** **DAR: B.6.1.1.6**
Mass balance – bile cannulated rats Rat (Crl :CD9(SD) 5 males Not to GLP – OECD 417 (2010)	<u>Excretion</u> M: > 90 % of the AD recovered in 5 days. 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	**Supplementary only**	**Anon, 2017f** **DAR: B.6.1.2**

Method	Results	Remarks	Reference
Deviations: None of significance Study no. FMC-P5709 **[^{14}C-Phenyl]-bixlozone**, batch 77874-3-19; purity 100 % Bixlozone technical, batch PL G3773-17 Purity 99.5 % IV low dose (3 mg/kg bw)	recovery in tissues and carcass was minimal (day 7). <u>Metabolism</u> Bixlozone was extensively metabolised; unchanged bixlozone 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.		
In vitro comparative interspecies metabolism (first study) hepatocytes pools (males, females) Mouse: 8 animals in male pool, 54 in female pool Rat: 12 animals in male pool, 21 in female pool Dog: 3 animals in male pool, 3 in female pool Human: 10 individuals in male pool, 10 in female pool Not to GLP however the scientific validity of such a qualitative study design is not	[^{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.	**Acceptable**	**Anon, 2017g** **DAR: B.6.1.3.1**

Method	Results	Remarks	Reference
compromised therefore this study is acceptable for regulatory purposes. Study no. FMC-R4547 **[^{14}C-Phenyl]-bixlozone**, batch 77874-3-19; purity 100 % **[^{14}C-Carbonyl]-bixlozone**, Batch CFQ42476; purity 99.9 % F9600 technical, batch PL G3773-17 Purity 99.5 % 20 µM			
In vitro comparative interspecies metabolism (second study) Mixed-sex mouse, rat, dog and human hepatocytes Mouse: 48 animals Rat: 36 animals Dog: 6 animals Human: 10 individuals GLP Study no. FMC-53482 **[^{14}C-Phenyl]-bixlozone,** batch	[^{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.	**Acceptable**	**Anon, 2020** **DAR: B.6.1.3.2**

Method	Results	Remarks	Reference
CFQ43508; purity 99.3 % **[^{14}C-Carbonyl]-bixlozone, b**atch CFQ43509; purity 99.6 %; specific activity 56 mCi/mmol **Bixlozone technical,** batch PL14-0163 Purity 99.8 %. 20 µM			

9.1 Short summary and overall relevance of the provided toxicokinetic information

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.

Absorption

Oral absorption was rapid following low dose administration (C_{max} < ½ hours) but slower after high dose (C_{max} 15-24 hours). The extent of absorption was ≈ 60-70 % in male and ≈ 80-90 % in female rats, and the bioavailability was higher in females (86 %) compared to males (70 %) following low oral dosing. Possible saturation of absorption was observed following high dose administration. No accumulation was observed in plasma following repeated dosing in both sexes.

Distribution

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. Among all

tissues analysed, the GI tract accounted for about 60 % of the 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.

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

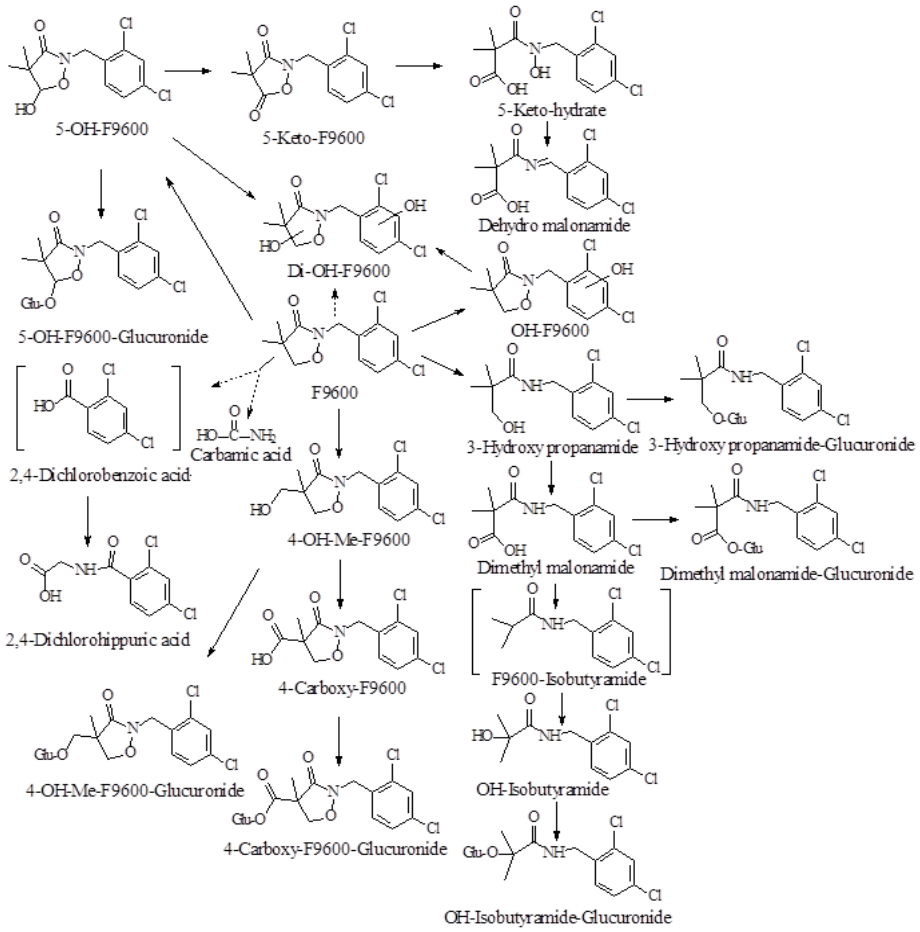
Comparative in vitro metabolism studies using rat, human, mouse and dog cryopreserved hepatocytes showed that bixlozone was extensively metabolised after 4 hours incubation in all hepatocytes species. No unique or label-specific metabolite was identified in human hepatocytes; however, a disproportionate 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.

Elimination

Excretion after a low oral dose was rapid (83-97 % of the administered dose (AD) excreted within 48 hours via the urine and faeces), with a higher elimination rate in females. 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 % AD with the phenyl label and 62-76 % AD with the carbonyl label), with faecal elimination accounting for 11-27 % and 16-34 % of AD for the phenyl and carbonyl label respectively. Elimination in expired air was very low with both labels.

The proposed metabolic pathways of bixlozone in rats are presented in Figure 1:

Figure 1: proposed metabolic pathways of bixlozone in rats



10. Evaluation of health hazards

10.1 Acute toxicity – oral route

There is one well-conducted study available to inform on the acute oral toxicity of bixlozone in rats. In addition, there is a dose range-finding acute neurotoxicity study (non-guideline and non-GLP) and a guideline neurotoxicity study conducted according to GLP available. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.6; Toxicology and metabolism data.

Table 14: Summary of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Effects**	**Reference**
Acute oral toxicity study, up-and-down procedure, gavage OECD 425 (2008) GLP Deviations: none	Rats SD albino Females (5 animals)	Bixlozone technical, batch PL13-0203 Purity: 98.5 % Vehicle: 0.5 % aqueous solution of CMC in 5 % Tween-80	430 (1 female), 1370 (1 female) & 2000 mg/kg bw (3 females) Observation period: 14 days	**> 2000 mg/kg bw**	430 and 1370 mg/kg bw No deaths and no clinical signs of toxicity. 2000 mg/kg bw No deaths. Day 1 only: hypoactivity, irregular respiration (3-5 hours) and ↓ defecation in 2/3.	**Anon, 2014d** **DAR: B.6.2.1**

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

The acute oral toxicity of bixlozone has been investigated in five fasted female Sprague-Dawley rats in accordance with the up-and-down procedure (OECD TG 425). An initial animal was given a single gavage dose of 430 mg/kg bw. This animal survived and did not display any signs of toxicity. Therefore, a second animal was dosed with 1370 mg/kg

bw of the test substance; the survival of this rat led to the dosing of a further three animals at the maximum recommended dose of 2000 mg/kg bw.

All three animals survived to the end of the 14-day observation period; the only signs of toxicity observed in 2 females were hypoactivity, reduced respiration (3-5 hours) and decreased defecation on the first day of treatment. These signs did not indicate a specific toxic effect and had fully reversed by day two; all 3 animals appeared active and healthy for the remainder of the 14-day observation period. All animals gained weight during the study and no gross abnormalities were noted upon necropsy.

The LD_{50} of bixlozone was determined to be > 2000 mg/kg bw.

In an acute neurotoxicity study performed according to guidelines and GLP, SD rats (5/sex/dose) received a single dose of either 0, 500, 1000 or 2000 mg/kg bw via oral gavage and were then observed for 14 days. There were no deaths during the course of this study. Similarly, in a range-finding study that preceded the main neurotoxicity study, no deaths were observed following a single dose of 0, 500, 1000 and 2000 mg/kg bw. The results of these studies support the findings of the guideline acute oral toxicity study in rats.

10.1.2 Comparison with the GB CLP criteria

In accordance with the CLP criteria, substances should be classified for acute oral toxicity when the LD_{50} (or ATE) has been reliably determined to be ≤ 2000 mg/kg bw. The LD_{50} of bixlozone was determined to be > 2000 mg/kg bw. Therefore, the substance does not meet the criteria for classification for acute oral toxicity (i.e., the ATE is not ≤ 2000 mg/kg bw).

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Not classified – conclusive but not sufficient for classification.

10.2 Acute toxicity – dermal route

There is one well conducted study available to inform on the acute dermal toxicity of bixlozone in rats. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.6; Toxicology and metabolism data – July 2022.

Table 15: Summary of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Effects	Reference
Acute dermal toxicity study Guideline: OECD 402 (1998) GLP Deviations: Application site constituted less than 10 % surface area	Rats, SD albino, 5/sex	Bixlozone technical, batch PL13-0203 Purity: 98.5 % Moistened with distilled water to a dry paste (70 % w/w)	2000 mg/kg Exposure period: 24 hours Observation period: 14 days	**> 2000 mg/kg bw**	Dermal irritation (erythema) at dose site in 2 out of 5 males only on day 1 (fully reversible by day 2).	**Anon, 2014e** **DAR: B.6.2.2**

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

The acute toxicity of bixlozone *via* the dermal route was investigated in a study in the rat conducted in accordance with OECD TG 402 and in compliance with GLP. The shorn skin of 5/sex Sprague-Dawley rats was exposed to a 2000 mg/kg bw dose of bixlozone technical (batch PL13-0203; purity 98.5%) for 24 hours, with a 14-day observation period. The dose was formulated as a 70 % w/w paste with distilled water and applied to the skin with a semi-occlusive dressing (taped onto an area of approximately 3.5 cm x 2.5cm, which corresponds to approximatively 8%). The applicant reported that this was the maximum area that could be covered owing to the small quantity of test material applied. Since signs of dermal irritation were minimal in the study and bixlozone was not found to be acutely toxic via the oral route (LD_{50} > 2000 mg/kg bw), the Agency considers that this deviation is not expected to have had a significant impact on the outcome of the present study.

There were no deaths or clinical signs of toxicity; all animals gained weight during the study and no gross abnormalities were noted upon necropsy. Dermal irritation (erythema) was noted at the dose site of 2 out of 5 males only on day one, which had fully reversed by day 2.

The LD_{50} was determined to be > 2000 mg/kg bw.

10.2.2 Comparison with the GB CLP criteria

In accordance with the CLP criteria, substances should be classified for acute dermal toxicity where the LD_{50} (or ATE) has been reliably determined to be ≤ 2000 mg/kg bw. The LD_{50} of bixlozone was determined to be > 2000 mg/kg bw. Therefore, the substance does not meet the criteria for classification for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified – conclusive but not sufficient for classification.

10.3 Acute toxicity – inhalation route

There is one well conducted study available to inform on the acute inhalation toxicity of bixlozone in rats. Reference should be made to the Draft Assessment Report – DAR – Volume 3, Annex B.6; Toxicology and metabolism data.

Table 16: Summary of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** **LC_{50}**	**Effects**	**Reference**
Acute inhalation toxicity study, nose-only Guideline: OECD 403 (2009) & U.S. EPA OPPTS 870.1300 (1998) GLP Deviations: Yes but not significant. An adequate method of analysis for the concentration tested is not available but	Rats, SD albino, males & females, 5/sex	Bixlozone technical aerosol, batch PL13-0385 Purity: 99.2 % MMAD: 2.84 µm	2.11 mg/L (maximum attainable concentration) Exposure : 4 hr	**> 2.11 mg/L**	There were no deaths. Clinical signs: irregular respiration in all animals following exposure (fully recovered by day 3).	**Anon, 2014f** **DAR: B.6.2.3**

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Effects	Reference
the method was concluded fit for purpose.						

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The acute inhalation toxicity of bixlozone was investigated in rats in a study conducted in accordance with OECD and GLP. Groups of Sprague-Dawley rats (56/sex) were exposed to 2.11 mg/L bixlozone technical as an aerosolised dust (4h, nose only exposure, MMAD 2.84µm). The concentration tested in this study (2.11 mg/L) is lower than the maximum concentration of 5 mg/L recommended in the OECD Guideline 403. Thus, the concentration tested did not cover all of the four hazard categories set in Annex I: 3.1.2.1. of the guidance on the Application of the CLP criteria for this endpoint (i.e., up to 5 mg/L). However, the testing conditions achieved in this study conformed with the recommendations of the OECD Guideline 403 regarding the testing of aerosols. Paragraph 30 of the OECD guideline specifies that it can be technically challenging to generate limit concentrations for aerosols. It also states that when testing aerosols, the primary goal should be to achieve a respirable particle size (MMAD of 1-4 µm), which is possible with most test articles at a concentration of 2 mg/L, which is the case for this study. Lastly, paragraph 30 also highlights that aerosol testing at greater than 2 mg/L should only be attempted if a respirable particle size can be achieved. These recommendations are also detailed in Annex I Section 3.1.2.3.2 of the CLP guidance, in that for dusts and mists a particle size range of 1-4 µm, corresponding to a maximum concentration of about 2 mg/l, would be tested in rats to achieve applicability of animal experiment conditions to human exposure. The Agency concluded that the study tested bixlozone technical at the maximum concentration for aerosols recommended by the OECD guideline and the CLP guidance. Therefore, the study is valid for the purpose of classification.

Following exposure, animals were observed for 14-days and examined for signs of toxicity and behavioural changes immediately following their removal from the chamber and at least once daily thereafter. There were no deaths reported; all rats exhibited irregular respiration following exposure which had fully recovered by day 3. Although there were minor individual body-weight losses observed at various weighting time-points, overall, by the end of the study, the animals had gained the expected amount of weight. The sporadic

weight losses observed were therefore not of toxicological significance. The LC_{50} was > 2.11 mg/L.

10.3.2 Comparison with the GB CLP criteria

In accordance with the criteria on CLP, classification for acute inhalation toxicity is appropriate where the 4-hour LC_{50} (ATE) is ≤ 5 mg/L (dust/mist). No deaths were reported in the study up to the maximum achievable concentration of 2.11 mg/L. Overall, bixlozone does not meet the classification criteria for acute inhalation toxicity noting that the study tested the aerosolised substance up to the maximum attainable concentration of 2.11 mg/L

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified – conclusive but not sufficient for classification.

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

Information relating to the potential for bixlozone to cause specific target organ toxicity was available from the acute toxicity data presented in Sections **Error! Reference source not found.**, **Error! Reference source not found.** & **Error! Reference source not found.**. In addition, an acute neurotoxicity study and it's dose-range finding study in the rat are available.

Table 17: Summary of animal studies on STOT SE

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, route of exposure, dose levels, duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Acute neurotoxicity study, gavage

Rats, Crl:CD(SD), males & females, 10/sex/group

Guideline: OECD 424

Deviations: none

GLP: yes

Study no. WIL-105114

Acceptable</td>
<td>Bixlozone technical, batch PL14-0049

Purity: 96 %

Vehicle: 0.5 % (w/v) carboxymethylcellulose in 5 % Tween® 80

Gavage administration

0, 500, 1000 & 2000 mg/kg bw</td>
<td>There were no deaths & no treatment-related clinical findings or body weight changes up to 2000 mg/kg bw.

There were no treatment-related changes in FOB or neuropathology parameters up to 2000 mg/kg bw.

Motor activity: statistically significant lower total and ambulatory motor activity counts in females on day 0 from 1000 mg/kg bw for total motor activity and from 500 mg/kg bw for ambulatory motor activity.

Cumulative total and ambulatory motor counts (0-60 minutes) in females during the pre-test and at day 0
<table>
<tr><th></th><th colspan="5">Dose (mg/kg bw)
N = 10</th><th>HCD</th></tr>
<tr><th></th><th></th><th>0</th><th>500</th><th>1000</th><th>2000</th><th></th></tr>
<tr><td>Mean total motor count</td><td rowspan="4">Pre-test</td><td>2033</td><td>2261</td><td>2257</td><td>2491</td><td></td></tr>
<tr><td>% difference from control</td><td>N/A</td><td>+11</td><td>+22</td><td>+22.5</td><td></td></tr>
<tr><td>Mean ambulatory motor count</td><td>469</td><td>552</td><td>582</td><td>681*</td><td></td></tr>
<tr><td>% difference from control</td><td>N/A</td><td>+18</td><td>+24</td><td>+45</td><td></td></tr>
<tr><td>Mean total motor count</td><td rowspan="3">Day 0</td><td>2699</td><td>2201</td><td>1884*</td><td>2076*</td><td>2026 [1070-2543]</td></tr>
<tr><td>% difference from control</td><td>N/A</td><td>-18.5</td><td>-30</td><td>-23</td><td></td></tr>
<tr><td>Mean ambulatory</td><td>736</td><td>508*</td><td>478*</td><td>503*</td><td>444 [206-</td></tr>
</table>
</td>
<td>Anon, 2014c

DAR: B.6.7.1</td>
</tr>
</table>

		motor count	599]
		% difference from control	N/A -31 -35 -32
		Significant at * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001. HCD: CRL:CD(SD) RAT - Date Range: Apr 2009 – Jun 2017. 406 animals, 39 studies	
Dose-range finding acute neurotoxicity study, Rats, Crl:CD(SD), males & females, 3/sex/group Not to guideline GLP: no Study no. WIL-105113 **Supplementary**	Bixlozone technical, batch PL13-0385 Purity: 99.2 % Vehicle: 0.5 % (w/v) carboxymethylcellulose in 5% Tween® 80 Gavage administration 0, 500, 1000, 1500 & 2000 mg/kg bw	There were no deaths. Clinical findings: one female of the 2000 mg/kg bw dose group presented yellow material around the urogenital, ventral and abdominal areas, red material on the forelimbs, nose and mouth and decreased defecation. Another female in this group also had the same yellow material around urogenital areas. There were no other treatment related clinical findings. Since there was no clear dose-related response with regard to clinical findings a time to peak effect could not be determined from these results.	**Anon, 2014b** **DAR: B.6.7.1**

10.4.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

The acute toxicity of bixlozone has been investigated in vivo in rats via the oral (OECD 425), dermal (OECD 402) and inhalation (OECD 403) routes. Moreover, the neurotoxic potential of bixlozone has been investigated in rats in a guideline oral acute neurotoxicity study (preceded by a range-finding study).

Bixlozone was tested up to the limit dose of 2000 mg/kg via the oral and dermal routes. In these studies there were few clinical signs and no macroscopic abnormalities and no mortality. In the oral study the only signs of toxicity observed in 2 females on the first day of treatment at 2000 mg/kg bw were hypoactivity, irregular respiration (3-5 hours post dose) and decreased defaecation volume. These signs did not indicate a specific toxic effect and had fully reversed by day 2.

In the inhalation toxicity study, bixlozone was tested at 2.11 mg/L only, the maximum concentration that could be obtained. At this dose, no mortality occurred and the few clinical signs noted did not indicate a specific acute toxic effect and had fully reversed by

day 3. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period

In a GLP acute neurotoxicity study (conducted according to test guidelines), a single oral gavage dose of bixlozone was administered to groups of Sprague Dawley rats 10/sex/dose after a pre-dosing observation period of 14 days (doses: 0, 500, 1000 or 2000mg/kg bw in 0.5 % (w/v) carboxymethylcellulose in 5 % Tween 80). Animals were observed for a period of 14 days following dose administration.

There were no treatment-related changes in FOB or neuropathology parameters. Regarding motor activity parameters, mean lower cumulative total and ambulatory motor activity counts were observed from 1000 mg/kg bw for total motor activity and from 500 mg/kg bw for ambulatory motor activity in females on day 0. The differences were statistically significant. However, there was no clear dose-response and the control values registered at day 0 (day of treatment) were much higher than those registered during the pre-test, with the values in the tested groups being similar to the values in the pre-treatment controls. In addition, there were no shifts in the pattern of habituation in any dose group at any time-point. Lastly, in isolation, without any effects on any other motor activity and FOB parameters, the finding is unlikely to be related to treatment. Therefore the Agency concluded that the reduced motor activity (both total and ambulatory) observed in females on day 0 was not treatment-related.

Findings in acute toxicity studies included hypoactivity, reduced respiration (3-5 hours post administration) and decreased defecation. These were reported in 2 out of 3 animals in the acute oral (gavage) toxicity study on the first day of treatment at 2000 mg/kg bw but 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 (Anon, 2014e). In the acute inhalation toxicity study (Anon, 2014f), 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, there was no evidence that bixlozone was neurotoxic after single administration or induced any effects that might be due to specific target organ toxicity.

10.4.2 Comparison with the GB CLP criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed and not specifically covered by the acute toxicity classifications should be included.

Classification in categories 1 and 2 is for substances causing non-lethal “significant and/or severe toxic effects”, with the dose level at which the effect occurs covering the basis for the categorisation. Classification with STOT-SE 3 is reserved for substances/mixtures causing “transient effects” following a single exposure, specifically respiratory tract irritation (RTI) and narcotic effects. In the acute and repeated dose studies available there was no evidence of specific target organ toxicity relevant for classification in category 3.

Bixlozone is a new active substance thus there is limited human data to rely on. According to data maintained by FMC Corporation on more than 1200 workers involved in the research and development of bixlozone, there is no evidence that bixlozone causes significant toxicity in humans following a single exposure. In studies in rats, bixlozone did not cause specific organ toxicity following a single exposure by the oral, inhalation or dermal routes. There is also no evidence that bixlozone is neurotoxic after single administration up to the limit dose of 2000 mg/kg bw.

Based on the clinical and behavioural observations in the described animal studies (rat), there were no effects that are indicative of specific target toxicity following a single administration of bixlozone. The limited human data did not report clinical cases and poisoning. Therefore, bixlozone does not meet the classification criteria for specific target-organ toxicity Category 1, 2 or 3 following a single administration.

10.4.3 Conclusion on classification and labelling for STOT SE

Not classified – conclusive but not sufficient for classification.

10.5 Skin corrosion/irritation

The skin irritation potential of bixlozone was investigated in an in vitro skin irritation test using reconstructed human epidermis (OECD 439; bottom-up approach) and an in vivo study in rabbits (OECD 404).

Table 18: Summary of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels, duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr><td>Primary skin irritation study

Guideline: OECD 404 (2002)

GLP

Deviations: none</td><td>Rabbits, New-Zealand albino, females, 3 animals</td><td>Bixlozone technical, batch PL13-0203

Purity: 98.5 %

Vehicle: Distilled water</td><td>0.5g</td><td>Not irritating

Mean scores over 24, 48 & 72 hours for each animal:

0, 0.67 & 0.33 (erythema)

0, 0 & 0 (oedema)

<u>Dermal irritation results</u>
<table>
<tr><th rowspan="2">Animal No.</th><th rowspan="2">Sex</th><th colspan="5">Individual skin irritation scores (erythema / oedema)</th></tr>
<tr><th colspan="4">Time After Patch Removal</th><th>Mean score</th></tr>
<tr><th></th><th></th><th>30-60 min</th><th>24 hrs</th><th>48 hrs</th><th>72 hrs</th><th></th></tr>
<tr><td>3501</td><td>F</td><td>1/0</td><td>0/0</td><td>0/0</td><td>0/0</td><td>0/0</td></tr>
<tr><td>3502</td><td>F</td><td>1/0</td><td>1/0</td><td>1/0</td><td>0/0</td><td>0.67/0</td></tr>
<tr><td>3503</td><td>F</td><td>1/0</td><td>1/0</td><td>0/0</td><td>0/0</td><td>0.33/0</td></tr>
</table></td><td>Anon, 2014g

DAR: B.6.2.4.2</td></tr>
</table>

Table 19: Summary of other studies relevant for skin corrosion/irritation

Type of study/ data	Test substance	Observations	Reference
In vitro skin irritation test (SIT) Epiderm™ skin model (RhE-based test	Bixlozone technical batch PL14-0049 Purity: 95.9 %	**Not irritating** Mean viability of bixlozone Technical = 109 %. Skin Irritation Prediction = Not a skin irritant.	**Anon, 2018a** **DAR: B.6.2.4.1**

<table>
<tr><th>Type of study/ data</th><th>Test substance</th><th>Observations</th><th>Reference</th></tr>
<tr><td>method)

Guideline: OECD 439 (2015)

Deviations: none

GLP</td><td>25 mg (powder)</td><td><u>Results of the in vitro skin irritation test</u>

<table>
<tr><th>Test item</th><th></th><th>Concentration</th><th>Mean Viability (%) ± SD</th><th>Skin Irritation Prediction</th></tr>
<tr><td>Bixlozone</td><td>Bixlozone Technical, powder</td><td>Neat

25 mg</td><td>109.1 ± 4.59</td><td>Non-Irritant</td></tr>
<tr><td>Positive Control</td><td>SDS</td><td>5 % w/v</td><td>2.59 ± 0.53</td><td>Irritant</td></tr>
<tr><td>Negative Control</td><td>CMF-DPBS</td><td>-</td><td>100.0 ± 3.45</td><td>Non-Irritant</td></tr>
</table>
</td><td></td></tr>
</table>

10.5.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

<u>In vivo skin irritation study</u>

In an in vivo skin irritation study, conducted according to test guidelines and GLP, bixlozone technical (batch PL13-0203, purity 98.5 %) was applied to the clipped dorsal skin of three female rabbits. A moist paste (70 % w/w volume) was achieved by mixing 0.5 g of the test substance with distilled water, which was then applied under a semi-occlusive dressing for a 4-hour exposure period.

There was no oedema observed at any treated site during this study. Within one hour of patch removal, all three treated skin sites exhibited slight erythema. The mean scores for erythema for each rabbit were 0, 0.67 and 0.33 and the mean scores for oedema were 0 for each rabbit. All observations had fully reversed by 72 hours.

Under the conditions of this study, bixlozone was non-irritating to the skin.

<u>In vitro skin irritation test (SIT)</u>

In an in vitro skin irritation test, using the Epiderm™ skin model, and conducted according to test guidelines and GLP, tissues samples moistened with 25 µL sterile Ca^{++}/Mg^{++} Free Dulbecco's Phosphate Buffered Saline (CMF-DPBS) were treated with 25 mg of bixlozone technical (powder). The positive control, sodium dodecyl sulphate (SDS, 5 %) and

negative control, CMF-DPBS were tested concurrently and applied to tissues in triplicate; all tissues were incubated for 60 minutes. The tissues were subsequently rinsed, blotted, and transferred to fresh medium. After a 24-hour post-exposure incubation, tissues were supplied with fresh medium and incubated for further 18 hours for a total 42-hour post exposure period.

According to the OECD Guideline 439 criteria, a test article is predicted to be an irritant/corrosive (Category 1 or 2) when the mean relative viability of the three treated tissues is ≤ 50 % of the mean viability of the negative control. As the mean viability of bixlozone was 109 % it was predicted to be non-irritating to the skin. The positive control, SDS (viability at 2.6 %; SD < 18 %) and the negative control, CMF-DPBS (mean OD_{570} at 1.922 equating to100 %; SD < 18 %) performed accordingly.

10.5.2 Comparison with the GB CLP criteria

Bixlozone was tested for its potential to cause skin corrosion/irritation both in vitro and in vivo.

In the in vitro study, bixlozone was not predicted to be a skin irritant. The RhE-based test method used for this study is able to distinguish between irritant and non-irritant chemicals when a bottom-up approach strategy is used, and can thus serve as stand-alone skin irritation method for non-corrosive substances (new guidance document on an Integrated Approach on Testing and Assessment (IATA) for skin corrosion and irritation (ENV/JM/MONO(2014)19)).

The results of the in vivo study also showed a lack of skin irritation when bixlozone was applied to the skin of New Zealand white rabbits. The mean scores for erythema and oedema were less than 2.3 in all animals, there was no inflammation or pronounced variation in the responses amongst the test animals and all findings were reversible within 72 h.

In addition, available data from the acute dermal toxicity study (Section **Error! Reference source not found.**) and the 21-day repeated dose toxicity study (Section **Error! Reference source not found.**) demonstrate that the substance is not expected to be corrosive.

Based on these data, bixlozone does not meet the criteria for classification as a skin irritant.

10.5.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified – conclusive but not sufficient for classification.

10.6 Serious eye damage/eye irritation

The eye irritation potential of bixlozone was investigated in a modern guideline in vivo study in rabbits and a modern guideline in vitro eye irritation test using the EpiOcular™ eye model. Both studies were conducted according to GLP.

Table 20: Summary of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Primary eye irritation study Guideline: OECD 405 (2012) Deviations: none GLP	Rabbits, New Zealand albino, females, 3 animals	Bixlozone technical, batch PL13-0203 Purity: 98.5 %	0.1mL (0.08g)	**Not irritating** Mean scores at 24, 48 and 72 hours for each animal Corneal opacity: 0,0,0 Iritis: 0,0,0 Conjunctival redness: 0,0,0 Conjunctival chemosis: 0,0,0	**Anon, 2014h** **DAR: B.6.2.5.2**

Table 21: Summary of other studies relevant for serious eye damage/eye irritation

<table>
<tr><th>Type of study/data</th><th>Test substance</th><th>Observations</th><th>Reference</th></tr>
<tr><td>In vitro eye irritation study

Epiocular™ eye model ((RhE-based test method)

Guideline: OECD 492 (2017)</td><td>Bixlozone technical batch PL14-0049

Purity: 95.9 %

25 mg (powder)</td><td>Prediction: irritating

Mean viability of bixlozone Technical = 19.4 %.

Eye Irritation Prediction = eye irritant/damaging.

<u>In vitro Eye Irritation results (6 hour exposure time)</u>
<table>
<tr><th colspan="2">Test items</th><th>Concentration</th><th>Mean Viability (%)</th><th>Ocular Irritation Prediction</th></tr>
<tr><td>Bixlozone</td><td>Bixlozone Technical</td><td>Neat</td><td>19.4</td><td>Irritant</td></tr>
</table></td><td>Anon, 2018e

DAR: B.6.2.5.1</td></tr>
</table>

Type of study/data	Test substance	Observations					Reference
Deviation: none GLP		**Positive Control**	Methyl acetate	Neat	13.4	Irritant	
		Negative Control	CMF-DPBS	-	100	Non-irritant	

10.6.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In vivo Study

Bixlozone technical (purity 98.5 %) was administered as a single instillation of 0.1 ml into the right eye of three New Zealand albino rabbits; the untreated left eye served as the negative control. Ocular irritation was evaluated using a high-intensity white light at 1, 24, 48 and 72 hours post instillation (with an additional fluorescein dye procedure at 24 hours to evaluate corneal damage).

There were no deaths or signs of toxicity and the animals gained the expected amount of weight by the end of the study period.

No corneal opacity or iritis was observed in any treated eye. Conjunctival redness and chemosis were noted in all treated eyes to some extent, but only at one hour post-installation (scores of 1 or 2). Mean scores for each animal for corneal opacity, iritis and conjunctival redness and chemosis over 24, 48 and 72 hours were 0, 0, 0 and 0.

In vitro study (Epiocular™ eye model (RhE-based test method))

Bixlozone is a solid and was tested as a powder in this study; the substance falls within the applicability domain of the test (paragraph 10 of the OECD Guideline 492). Tissue model inserts of stratified human keratinocytes were exposed to 50 mg of solid bixlozone technical for 6 hours in duplicate. The positive control methyl acetate and negative control CMF-DPBS were tested concurrently and applied to tissues in duplicate. Tissues were subsequently thoroughly rinsed, blotted, and transferred to fresh medium for 18 hours. Cell viability was assessed using the 3- [4,5 – dimethylthaizol-2-yl] – 2,5 – diphenyltetrazolium bromide (MTT) assay where the blue formazan salt formed in the test item-treated tissues was measured relative to the negative controls. Bixlozone was also tested to identify its potential in being a direct MTT reducer or for colour interference in contact with water or isopropanol. Bixlozone did not directly reduce MTT in absence of viable cells.

A test substance was predicted to have eye irritation/corrosion potential if the relative viability is ≤ 60 %. As bixlozone was shown to have mean viability at 19.4 %, it is predicted

to be an eye irritant. Both the positive control methyl acetate (viability at 13.4%) and the negative control CMF-DPBS met the laboratory criteria for a valid assay.

10.6.2 Comparison with the GB CLP criteria

In a well conducted in vivo eye irritation study, the mean scores for each animal for corneal opacity, iritis and conjunctival redness and chemosis over 24, 48 and 72 hours were all 0. As the observed scores were all 0 and no other effects were noted in the eyes, bixlozone was not considered irritating to the eye in this study.

In recently performed guideline in vitro eye irritation study using the EpiOcular™ test system, bixlozone was predicted to be an eye irritant with a cell viability of 19.4 %. A relative viability of ≤ 60 % leads to a prediction of eye irritation (no category). The criteria for classification is:

Mean tissue viability	UN GHS
> 60 %	No category
≤ 60 %	No prediction can be made

This type of study allows for the identification of substances that do not meet the classification criteria for eye irritation or serious eye damage. If the mean percent tissue viability after exposure and post-exposure incubation is less than or equal to the established tissue viability cut-off value (i.e., a 'positive' result, as in the case of bixlozone), then further information is required for classification purposes. This is because the test is known to produce a high rate of false positives (37%). In the case of a true positive, the test cannot resolve between Categories 1 and 2.

Taking into consideration all the information available on the eye irritating potential of bixlozone, it is considered that the result in the in vitro study most likely represents a false positive and that bixlozone does not meet the criteria to be classified for serious eye damage/irritation.

10.6.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Not classified – conclusive but not sufficient for classification.

10.7 Respiratory sensitisation

10.7.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There are no relevant data. However, it is noted that the substance is not classified for skin sensitisation.

10.7.2 Comparison with the GB CLP criteria

Not applicable, there are no data.

10.7.3 Conclusion on classification and labelling for respiratory sensitisation

Not classified – data lacking.

10.8 Skin sensitisation

Bixlozone has been tested for its potential to cause skin sensitisation in a single modern Local Lymph Node Assay (LLNA) in mice. The study was performed according to test guidelines and GLP.

Table 22: Summary of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels, duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Local lymph node assay (LLNA)

Guideline: OECD 429 (2010)

Deviations: none

GLP</td>
<td>Mice, CBA/J, females, 2/group (preliminary irritation), 5/group (main test), 5/group (vehicle and positive control)</td>
<td>Bixlozone technical, batch PL13-0203

Purity: 98.5 %

Vehicle: acetone/olive oil</td>
<td>5 %, 10 % & 25 %

Positive control: 25% HCA</td>
<td>Not sensitising

No dermal irritation observed for any of the vehicle control group sites or any of the test group sites.

<u>Results:</u>
<table>
<tr><th>Concentration (%)</th><th>Stimulation index (SI)</th></tr>
<tr><td>5</td><td>1.13</td></tr>
<tr><td>10</td><td>1.32</td></tr>
<tr><td>25</td><td>1.57</td></tr>
</table>
Positive & negative controls gave the expected results.</td>
<td>Anon, 2014i
DAR: B.6.2.6</td>
</tr>
</table>

10.8.1 Short summary and overall relevance of the provided information on skin sensitisation

The skin sensitising potential of bixlozone was investigated in a GLP and OECD compliant mouse local lymph node assay (LLNA).

A preliminary study was conducted testing bixlozone at concentrations of 5 %, 10 %, and 25 % in order to determine the highest achievable level that avoided overt systemic toxicity and excessive local irritation. Solutions in excess of 25 % were considered to be too viscous for application. No irritation was noted at any of these doses. Based on a combination of knowledge of the test substance (toxicity, solubility, irritancy and viscosity) and the findings of this preliminary toxicity study, the main study used concentrations of 0, 5, 10 and 25%. The positive control was 25% alpha-hexylcinnamaldehyde (HCA) (97.9 % purity).

There was no dermal irritation observed for any of the vehicle control group sites or any of the test group sites. Very slight erythema (score of 1) was evident at one positive control site on day 2, all sites on day 3 and one site on day 6; very slight oedema (score of 1) was present at two dose sites on day 3 and desquamation was present at all dose sites on day 6.

The stimulation index (SI) was recorded as 1.13, 1.32 and 1.57 at 5 %, 10 % and 25 % bixlozone respectively. The positive control produced an SI value of 4.83 thus confirming the validity of the study. The negative control gave the expected result.

10.8.2 Comparison with the GB CLP criteria

In a guideline and GLP-compliant LLNA, bixlozone showed no evidence of skin sensitisation in mice when tested up to a maximum attainable concentration of 25 % w/v. In order for a substance to meet the criteria for classification as a skin sensitiser, an SI of ≥ 3 is required. As the SI at all concentrations tested was ≤ 1.57, the criteria for classification are not met.

10.8.3 Conclusion on classification and labelling for skin sensitisation

Not classified – conclusive but not sufficient for classification.

10.9 Specific target organ toxicity – repeated exposure (STOT RE)

The repeated dose oral toxicity of bixlozone has been investigated in a number of studies in rats, mice and dogs. In rats, there is a 28-day and a 90-day study. Further information is also available from a two generation reproduction toxicity dose-range finding study, a two generation reproduction toxicity main study and a 2-year carcinogenicity study. In mice,

there is a 28-day and a 90-day study and an 18-month carcinogenicity study. In dogs there is a 28-day dose range finding study, a 90-day study and a 12-month study. A 21-day dermal study in rats is also available.

Table 23: Summary of animal studies on STOT RE

Note:
↑↓ denote an increase or decrease in a parameter with respect to the control value
*Statistical significance: * p ≤ 0.05, ** p ≤ 0.01*
abs. = absolute rel. = relative

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Oral rat studies			
7-day Rat, Crl:CD(SD), males & females 5/sex/group Non-guideline Non-GLP	Bixlozone technical, batch PL13-0385 Purity : 99.2% Dietary route 0, 4000, 7000 and 12000 ppm Equivalent to : Males (M) : 0, 441, 698 and 1067 mg/kg bw/day Females (F): 0, 434, 763 and 1250 mg/kg bw/day	There were no deaths. The only sign of clinical toxicity was the presence of yellow material around the urogenital area of males from 7000 ppm. **<u>12000 ppm (1067/1250 mg/kg bw/day M/F)</u>** ↓ mean food consumption (M): 28%** ↓ body weight gain (M): 45.5%** ↓ body weight gain (F): 45.5%** *Organ weights* ↑ absolute liver weights: 30%** (M) & 37%** (F) ↑ relative liver weights: 47%** (M) & 45%** (F) **<u>7000 ppm (698/763 mg/kg bw/day M/F)</u>** ↓ mean food consumption (M): 16%** ↓ body weight gain (M): 24%** *Organ weights* ↑ absolute liver weights: 30%** (M) & 31%** (F) ↑ relative liver weights: 37%** (M) & 35%** (F) **<u>4000 ppm (441/434) mg/kg bw/day (M/F)</u>** *Organ weights* ↑ absolute liver weights: 34.5%** (M) & 32%** (F) ↑ relative liver weights: 36%** (M) & 28.5%** (F)	**Anon, 2015e** **DAR: B.6.3.1.1**

28 day Rat, Crl :CD(SD), males & females 5/sex/toxicology group (inc. control) 9/sex/toxicokinetic group (3/sex/control group) GLP OECD 407 (2008) Deviations: None	Bixlozone technical, batch PL13-0385 Purity: 99.2% Dietary route 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/day Females (F): 0, 61, 193, 379 & 733 mg/kg bw/day	There were no deaths or clinical signs of toxicity **<u>10000 ppm (740 / 733 mg/kg bw/day M / F)</u>** ↓ 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) **<u>5000 ppm (359 / 379 mg/kg bw/day M / F)</u>** ↓ 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*	**Anon, 2015b** **DAR: B.6.3.2.1**

		Hepatocellular hypertrophy: 1/5 minimal & 4/5 mild (F); 3/5 minimal & 2/5 mild (M) *Clinical chemistry* ↑ cholesterol (43 %* F) **<u>2500 ppm (182 / 193 mg/kg bw/day M / F)</u>** ↓ 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) **<u>750 ppm (57 / 61 mg/kg bw/day M / F)</u>** No treatment-related findings.	
90-day (**Includes neurotoxicity and 28-day recovery phase**) Rat, Crl :CD9(SD), males & females 21/sex/group or 16/sex group (including neurotoxicity phase) GLP OECD 408 (1998) & OECD 424 (1997) Deviations: None	Bixlozone technical, batch PL14-0049 Purity: 96% Vehicle: acetone Dietary route 0, 500, 2000, and 8000 ppm (males) Equivalent to: 0, 29, 121 & 505 mg/kg bw/day (males) 0, 500, 2000, and 5000 ppm (females) Equivalent to: 0, 37, 150 & 351 mg/kg bw/day 90-days continuous dosing	One male (5000 ppm) was found dead on day 87 (undetermined cause); there were no clinical signs of toxicity at any dose. **<u>8000 / 5000 ppm M/F (505 / 351 mg/kg bw/day M / F)</u>** <u>1 death (M)</u> ↓ 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)	**Anon, 2016a** **DAR: B.6.3.3.1**

	Recovery period: 28-days (5/sex group)	↑ 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) Mild follicular cell hypertrophy: 3/10 (M) & 5/10 (F) *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) **<u>28-day recovery group (8000 / 5000 ppm)</u>** ↑ food consumption 11 %** (M) & 4.5 % (F) ↑ relative liver weight 10 % (M) ↑ relative kidney weight 22 %** (M) Mild macro vascular vacuolation 1/5 (M) ↑ cholesterol 31 %** (F) **<u>2000 ppm (121 / 150 mg/kg bw/day M / F)</u>** *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) **<u>500 ppm (29 / 37 mg/kg bw/day M / F)</u>** No treatment-related findings.	
24-month Rat (Crl:CD (SD) rats, males and females) GLP OECD 453 (2009) Deviations: none	Bixlozone technical Batch PL14-0049 Purity 96 % Dietary route 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	***<u>Chronic phase – 12 months</u>*** **<u>5000/3000 ppm (217 / 167 mg/kg bw/day)</u>** ↓ BW-gain in females (10%** days 1-344) *Organ weights* ↑ liver weight in F (17 %* absolute & 34 %** relative) ↑ liver weight in M (12 % absolute & 19 %** relative) **<u>1000 ppm & 250 ppm</u>** No treatment-related findings. ***<u>Carcinogenicity phase – 24 months</u>*** **<u>5000/3000 ppm (217 / 167 mg/kg bw/day)</u>** Dermal atonia and thin body condition in females ↓ body weight: 11 % (M) & 19 % (F) ↓ body weight gain: 14 % (M) & 19 % (F) *Organ weights* ↑ liver weight in M (20 %** absolute & 35 %** relative) *Histopathology - liver* Hepatocellular hypertrophy:	**Anon, 2017a** **DAR: B.6.5.1**

		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) *Clinical chemistry* ↑ Cholesterol 50.5 %** albumin +7 %* total protein +8 %* and calcium +4 %* (F) **1000 & 250 ppm** No treatment-related findings.	
2-generation reproductive toxicity **Dose range finding study** Rats, Crl:CD(SD), males & females, 10/sex/group	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: acetone 0, 300, 1000, 3000 ppm (0, 150, 500, 1500 ppm during lactation) Dietary route Equivalent to: Males: 0, 17, 56 and 176 mg/kg bw/day Females: 0, 20, 62 and 172 mg/kg bw/day (pre-mating), 0, 22, 74 and 217 mg/kg bw/day (gestation) & 0, 23, 86 and 251	***Parents*** **3000 ppm (176 / 217 mg/kg bw/day M / F)** ↓ body weight in F (-35 %, pre-mating) *Organ weights* ↑ liver weight in males (+ 15 % absolute & +19 % relative) ↑ relative kidney weight in males (+11 %) **1000 ppm & 300 ppm** No treatment-related findings. ***Offspring*** **3000 ppm (217 mg/kg bw/day M / F)** ↓ body weights in F (-10%) *Organ weights* ↑ relative liver weights (+29 % F & +23 % M)	**Anon, 2016b** **DAR: B.6.6.1.1**

	mg/kg bw/day (lactation)	↑ absolute liver weights in F (+15 %) **<u>1000 ppm (74 mg/kg bw/day M / F)</u>** *Organ weights* ↑ relative liver weights in F (+15 %) ↑ absolute liver weights in F (+15%) **300 ppm (22 <u>mg/kg bw/day M / F</u>)** No treatment-related findings.	
Two-generation reproductive toxicity Rats, Crl:CD(SD), males & females, 25/sex/group GLP Guideline: OECD 416 (2001) Deviations: none	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: acetone Dietary route 0, 150, 750 & 3000 ppm (reduced to 0, 75, 375 & 1500 ppm during lactation) Equivalent to (most conservative): Males: 0, 7, 34 & 140 mg/kg bw/day Females: 0, 10, 49 & 187 mg/kg bw/day	***<u>Parental (systemic) toxicity</u>*** ***<u>F_0 generation</u>*** There were no treatment related deaths or clinical signs of toxicity **<u>3000 ppm (140 / 187 mg/kg bw/day M / F)</u>** ↓ body-weight gains in F (-15 %** days 0-70) ↓ mean body weights in F (-9 %** at gestation) *Organ weights* ↑ absolute liver weights: +13 %** (M) & +12%** (F) ↑ relative liver weights: +19 %** (M) & +21 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %** (F) *Histopathology - liver* Hepatocellular hypertrophy in F *Histopathology - prostate* ↑ mononuclear cell infiltration (chronic inflammation) in the prostate **<u>750 (34 / 49 mg/kg bw/day M / F) & 150 ppm (7 / 10 mg/kg bw/day M / F)</u>** No treatment-related findings.	**Anon, 2016c** **DAR: B.6.6.1.2**

		F1 generation **3000 ppm (140 / 187 mg/kg bw/day M / F)** ↓ body-weight 11.3 % * M & 6.3 %* F ↓ body weights gains 11.6 % * M (NA for F) ↓ mean body weights in F (-7 %** at gestation) *Organ weights* ↑ absolute liver weights in F (+13 %**) ↑ relative liver weights: +14 %** (M) & +21 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %* (F) *Histopathology - liver* Hepatocellular hypertrophy in F *Histopathology - prostate* ↑ mononuclear cell infiltration (chronic inflammation) in the prostate **750 (34 / 49 mg/kg bw/day M / F) & 150 ppm (7 / 10 mg/kg bw/day M / F)** No treatment-related findings. ***Reproductive toxicity*** No specific treatment-related adverse effects. **Offspring toxicity** **3000 ppm (187 mg/kg bw/day)** *F1 pups* ↑ relative liver weights: +18 %* (M) *F2 pups*	

		↓ pup body weight-gain (PND 4-7 & 7-14) ↓ pup body weights (PND 14) **<u>750 ppm (49 mg/kg bw/day) & 150 ppm (10 mg/kg bw/day)</u>** No treatment-related findings.	
Oral mouse studies			
7-day Mouse, Crl:CD-1, males and females, 5/sex/group Non-guideline Non-GLP	Bixlozone Technical, batch PL13-0385 Purity: 99.2% Dietary route 0, 2000, 4000 and 6000 ppm Equivalent to: Males (M): 0, 404, 960 and 1348 mg/kg bw/day Females (F): 0, 476, 886 and 1460 mg/kg bw/day	There were no treatment-related deaths or clinical signs of toxicity. **<u>6000 ppm (1348/1460 mg/kg bw/day M/F)</u>** ↓ mean body weight (M): 11%** ↓ mean body weight gain (F): 42%** *Organ weights* ↑ relative liver weights: 16.5%** (M) & 24% (F) **<u>4000 ppm (960/886 mg/kg bw/day M/F) and 2000 ppm (404/476 mg/kg bw/day M/F)</u>** No statistically significant treatment-related findings.	**Anon, 2015d** **DAR: B.6.3.1.2**
28 day Mouse, Crl:CD-1, males & females, 5/sex/group GLP OECD 407 (2008) Deviations: none	Bixlozone Technical, batch PL13-0385 Purity: 99.2% Dietary route 0, 1000, 2000, 4000, and 5000 ppm Equivalent to: Males: 0, 187, 381, 788 & 985 mg/kg bw/day	There were no treatment-related deaths. **<u>5000 ppm (985 / 1384 mg/kg bw/day M / F)</u>** ↓ 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*	**Anon, 2015c** **DAR: B.6.3.2.2**

	Females: 0, 289, 554, 984 & 1384 mg/kg bw/day	↑ ALT: 137 %* (M) **<u>4000 ppm (788 / 984 mg/kg bw/day M / F)</u>** *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) **<u>2000 ppm (381 / 554 mg/kg bw/day M / F) & 1000 ppm (187 / 289 mg/kg bw/day M / F)</u>** No treatment-related findings.	
90 day Mouse, Crl:CD1(ICR), males & females, 10/sex/ toxicology group, 12/sex/toxicokinetic group GLP OECD 408 (1998) Deviations: none	Bixlozone technical, batch PL14-0049 Purity: 96% Vehicle: acetone Dietary route 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	There were no test-substance related deaths or clinical signs of toxicity. No clear treatment-related effects on body weight development or food consumption in all dose groups. **<u>5000 ppm (930 / 1185 mg/kg bw/day M / F)</u>** *Organ weights* ↑ absolute liver weights: 23 %** (M) & 20 %** (F) ↑ relative liver weights: 23 %** (M) & 21 %** (F) *Histopathology - liver* Hepatocellular hypertrophy in 10/10 M (1 minimal, 9 mild) Hepatocellular hypertrophy in 3/9 F (1 minimal, 2 mild) **<u>2250 ppm (414 / 583 mg/kg bw/day M / F)</u>** *Organ weights* ↑ absolute liver weights: 13 %* (F) ↑ relative liver weights: 17.5 %** (F)	**Anon, 2016f** **DAR: B.6.3.3.2**

		Histopathology - liver Hepatocellular hypertrophy in 4/10 M (3 minimal, 1 mild) Hepatocellular hypertrophy in 3/9 F (minimal) **<u>1000 ppm (180 / 257 mg/kg bw/day M / F)</u>** No treatment-related findings.	
18-month Mouse (Crl:CD1(ICR mice, males and females) GLP OECD 451 (2009) Deviations: none	Bixlozone technical Batch PL14-0049 Purity 96 % Dietary route 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	**<u>5000 ppm (647 / 834 mg/kg bw/day M / F)</u>** *Organ weights* ↑ relative liver weight > 15 %** (both sexes) *Histopathology - liver* Hepatocellular hypertrophy: 7/10 at 52 weeks & 79 % incidence at 104 weeks (M) *Histopathology - kidney* ↑ pelvis dilation of kidney (M) *Histopathology - stomach* ↑ inflammation of glandular stomach (M) *Histopathology - epididymes* ↑ incidence of reduced sperm in epididymes (M) **<u>1000 ppm (126 / 164 mg/kg bw/day M / F)</u>** *Histopathology - stomach* ↑ inflammation of glandular stomach (M) *Histopathology - epididymes* ↑ incidence of reduced sperm in epididymes (M) **<u>250 ppm (32 / 43 mg/kg bw/day M / F)</u>** No treatment-related findings	**Anon, 2017b** **DAR: B.6.5.2**

Oral dog studies			
7 day Dog, Beagle, males & females, 2/sex/group Non-guideline Non-GLP Animals were not sacrificed on completion of the study thus the clinical signs, body weight, food consumption, haematology and clinical (serum) chemistry could be measured.	Bixlozone technical, batch PL13-0385 Purity: 99.2% Dietary route: Initial dose groups: 0, 2500, 5000, 10000 ppm Additional dose group: 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	There were no treatment-related deaths. The only clinical sign of toxicity was the presence of a clear material around the mouths of males at 10000 ppm and females at 5000 ppm. **<u>30000 ppm (818/716 mg/kg bw/day M/F)</u>** ↓ food consumption: 69% (M) & 98% (F) on days 0-1 Improvement in food consumption led to an overall body weight gain that was comparable with controls; nevertheless, the mean body weight of these dogs was still lower than controls by the end of the study (7.4% (M) & 3.8% (F).	**Anon, 2015f** **DAR: B.6.3.1.3**
7 day Dog, Beagle, males & females, 2/sex/group Non-guideline Non-GLP Animals were not sacrificed on completion of the study thus the clinical signs, body weight, food consumption, haematology and clinical (serum) chemistry could be measured.	Bixlozone technical, batch PL14-0049 Purity: 96.0% Vehicle: none (capsule) 0, 150, 350 and 550 mg/kg bw/day	There were no treatment-related deaths and no clinical signs of toxicity. There were no effects on mean body weights, body weight gains and food consumption.	**Kappeler, K.V. (2016b)** **DAR: B.6.3.1.4**
28 day Dog, Beagle, males & females, 2/sex/group	Bixlozone technical, batch PL14-0049 Purity: 96%	There were no treatment related deaths No statistical analysis was performed	**Anon, 2016f** **DAR: B.6.3.2.3**

GLP Dose-range finding study (loosely follows OECD 409)	Vehicle: acetone Dietary route 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; ≈ 1015 / 1110 mg/kg bw/day M / F)	**<u>30000 ppm (≈ 1015 / 1110 mg/kg bw/day M / F)</u>** 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) **<u>10000 ppm (370 / 309 mg/kg bw/day M / F)</u>** ↓ body-weight gain: 17 % (M) and 54 % (F) ↓ food consumption in M & F *Organ weights* ↑ Relative liver weight: 28.5 % (F) and 20 % (M) ↑ 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) **<u>3000 ppm (134 / 108 mg/kg bw/day M / F)</u>** ↓ body weight gain: 45.5 % (F) *Organ weights* ↑ relative liver weight: 14 % (F) **<u>1000 ppm (38 / 39 mg/kg bw/day M / F)</u>** No treatment-related findings.	
90 day Dogs, Beagle, males & females, 4/sex/group GLP OECD 409 (1998)	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: none (capsule) 0, 30, 100, 300, and 750 mg/kg/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. **<u>750 mg/kg bw/day</u>** *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 *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 **<u>300mg/kg bw/day</u>** *Organ weights* ↑ relative liver weight: 21.5 %** (F)	**Anon, 2016g** **DAR: B.6.3.3.3**

		↓ abs (19%) and rel (26.5%) prostate weight and associated immaturity **<u>100 mg/kg bw/day</u>** *Organ weights* ↑ liver weights in F (27 %* absolute, 22 %** relative) **<u>30 mg/kg bw/day</u>** No treatment-related findings.	
12 months Dogs, Beagle, males & females, 4/sex/group GLP OECD 409 (1998) Deviations: none	Bixlozone technical, batch PL14-0049 Purity: 96% Vehicle: none (capsule) 0, 20, 100, and 500 mg/kg/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. **<u>500 mg/kg bw/day</u>** *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 **<u>100 & 20 mg/kg bw/day</u>** No treatment-related findings.	**Anon, 2017a** **DAR: B.6.3.4**
Dermal studies			
21 day Rat, Crl:CD9(SD), males & females, 10/sex/group GLP OECD 410 (1981) Deviations : None	Bixlozone technical, batch PL14-0049 Purity: 99.2% Dermal route 0, 100, 300, and 1000 mg/kg bw/day	There were no deaths or clinical signs of toxicity **<u>1000, 300 and 100 mg/kg bw/day</u>** No treatment-related findings.	**Anon, 2016i** **DAR: B.6.3.5**

10.9.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Target organ(s) identified in all species

The liver has been identified as a clear target organ in all species investigated. Increases in relative and absolute liver weights accompanied in some instances with minimal to moderate hepatocellular hypertrophy were repeatedly observed. The toxicological significance of the effects on the liver has been assessed by the Agency 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 and agreed at the Biocide Working Group-IV-2018 meeting (WGIV2018_TOX_6-2). The WoE approach for the evaluation of liver effects in repeated-dose toxicity studies has been described, 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 which should not be accompanied by adverse histopathology (necrosis, apoptosis, pigment deposition or hyperplasia), or by substantial changes in clinical chemistry indicative of liver toxicity (decreased albumin or increased activities of ALT, AST, ALP, GGT, bilirubin or cholesterol). In line with the TAB entry, relative liver weight increases up to 15 %, that are not accompanied by other signs of liver dysfunction, have been considered by the Agency to be an adaptive rather than an adverse response in the evaluation of the liver effects of bixlozone (see Table 24 below).

Following the above criteria, it can be seen that the effects exerted upon the liver by bixlozone become adverse in the rat at doses of ≥ 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, and that the female is the most sensitive sex across all species. These findings are 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 **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.

Table 24: 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.

Target organs identified in the rat oral studies

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 (Anon, 2015b) and from 150 mg/kg bw/day (females) in the 90-day study (Anon, 2016a). In addition, similar liver effects were seen from ≈ 180 / 220 mg/kg bw/d (mean dose males / females) in the 2-generation study (Anon, 2016c) and at the top dose of 217 / 176 mg/kg bw/day (males / females) in the 2-year carcinogenicity study (Anon, 2017a) These liver effects were associated with alterations of some clinical-chemistry parameters indicative of liver toxicity (e.g. increased cholesterol, 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 males, these weight changes were associated with chronic progressive nephropathy (CPN). 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, which were associated with CPN in males.

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. 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 from the top-dose of 984 mg/kg bw/day (females only) in the 28-day study (Anon, 2015c), 930 / 583 mg/kg bw/day (males / females) in the 90-day study (Anon, 2016f), and 647 / 834 mg/kg bw/day (males / females) in the 18-month carcinogenicity study (Anon, 2017b). 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 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 (Section **Error! Reference source not found.**).

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 (Anon, 2016f). In the following 90-day study (Anon, 2016g), the method of oral administration was changed from dietary to capsule owing to palatability issues noted in the 7-day (Anon, 2015f) 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 in 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 WBC, PT, 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.

10.9.2 Comparison with the GB CLP criteria

STOT RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/day (for a classification in category 2) in a 90-day rat study. 'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

The potential for bixlozone to cause specific organ toxicity following repeated exposure has been investigated in repeated-dose toxicity studies in rats, mice and dogs, where the liver and the kidney were identified as clear target organs. Supporting information has also been extracted from the chronic/carcinogenicity studies in rats and mice and a rat two-generation reproduction study. The effects occurring at doses relevant for classification are summarised in Table 25 below:

Table 25: Summary of the target organ adverse effects seen following repeated exposure to bixlozone at doses relevant for STOT RE classification.

Study **Guidance values for classification***	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
Rats (Crl: CD(SD), 7 days *Cat 1 ≤ 100 mg/kg bw/day* *100 < Cat 2 ≤ 1000 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	**7000 ppm (698/763 mg/kg bw/day M/F)** ↓ mean food consumption (M): 16%** ↓ body weight gain (M): 24% *Organ weights* ↑ absolute liver weights: 30%** (M) & 31%** (F) ↑ relative liver weights: 37%** (M) & 35%** (F) **4000 ppm (441/434 mg/kg bw/day M/F)** *Organ weights* ↑ absolute liver weights: 34.5%** (M) & 32%** (F) ↑ relative liver weights: 36%** (M) & 28.5%** (F)
Rats (Crl:CD9 (SD)), 28 days *Cat 1 ≤ 30 mg/kg bw/day* *30 < Cat 2 ≤ 300 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	**2500 ppm (182 / 193 mg/kg bw/day M / F)** *Liver weights* ↑ relative liver weight: 17 %** (F), 15.5 %** (M) *Histopathology – liver* Hepatocellular hypertrophy: 4/5 minimal (F) & 3/5 minimal (M)
Rats (Crl:CD9 (SD)), 90 days. *Cat 1 ≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None at 500 ppm (29 / 37 mg/kg bw/day M / F). At the next dose of **2000 ppm (121 / 150 mg/kg bw/day M / F)**, the findings were:

Study **Guidance values for classification***	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
		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)
Rats (Crl:CD9 (SD)), 2 years *Cat 1 ≤ 1.25 mg/kg bw/day* *1.25 < Cat 2 ≤ 12.5 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None at 250 ppm (equivalent to 10/13 mg/kg bw/day M/F). (Note: adverse liver effects seen at the top dose of 217 / 167 mg/kg bw/day only)
Rats (Crl:CD9 (SD)), 2 generation study – range-finding studies *Cat 1≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 mg/kg bw/day*	F_0 parents None; no doses relevant for Category 1 were tested. F_1 parents None; no doses relevant for Category 1 were tested. Offspring None; no doses relevant for Category 1 were tested.	F_0 parents None at 300/1000 ppm (17/20 and 56/62 mg/kg bw/day M/F). At the next dose of **3000 ppm 176/217 mg/kg bw/day (M / F)**, the following findings were: *Organ weights* ↑ liver weight in males (+ 15 % absolute & +19 % relative) ↑ relative kidney weight in males (+11 %) F_1 parents None. At the next dose of 3000 ppm 176/217 mg/kg bw/day (M / F), the following findings were: *Organ weights* ↑ relative liver weights (+29 % F & +23 % M)

Study **Guidance values for classification***	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
		↑ absolute liver weights in F (+15 %) <u>Offspring</u> **<u>1000 ppm (74 mg/kg bw/day M / F)</u>** *Organ weights* ↑ relative liver weights in F (+15 %) ↑ absolute liver weights in F (+15%)
Rats (Crl:CD9 (SD)), 2 generation study – main study *Cat 1≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 mg/kg bw/day*	<u>F_0 parents</u> None at 150 ppm (7/10 mg/kg bw/day M/F). <u>F_1 parents</u> None at 150 ppm (7/10 mg/kg bw/day M/F). <u>F1 pups</u> None at 150 ppm (7/10 mg/kg bw/day M/F). <u>F2 pups</u> None at 150 ppm (7/10 mg/kg bw/day M/F).	<u>F_0 parents</u> None at 34 / 49 mg/kg bw/day (M / F). At the next dose of 3000 ppm (**<u>140 / 187 mg/kg bw/day M / F</u>**)), the following findings were: *Organ weights* ↑ absolute liver weights: +13 %** (M) & +12%** (F) ↑ relative liver weights: +19 %** (M) & +21 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %** (F) *Histopathology – liver* Hepatocellular hypertrophy in F *Histopathology – prostate* ↑ mononuclear cell infiltration (chronic inflammation) in the prostate <u>F_1 parents</u> None at 34 / 49 mg/kg bw/day (M / F). At the next dose of 3000 ppm (**<u>140 / 187 mg/kg bw/day M / F</u>**)), the following findings were: *Organ weights*

Study **Guidance values for classification***	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
		↑ absolute liver weights in F (+13 %**) ↑ relative liver weights: +14 %** (M) & +21 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %* (F) *Histopathology - liver* Hepatocellular hypertrophy in F *Histopathology - prostate* ↑ mononuclear cell infiltration (chronic inflammation) in the prostate <u>F1 pups</u> None <u>F2 pups</u> None
Mouse (Crl: CD-1), 7 days *Cat 1 ≤ 100 mg/kg bw/day* *100 < Cat 2 ≤ 1000 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None at 4000 ppm (960/886 mg/kg bw/day M/F) or 2000 ppm (404/476 mg/kg bw/day M/F).
Mouse (Crl:CD-1), 28 days. *Cat 1 ≤ 30 mg/kg bw/day* *30 < Cat 2 ≤ 300 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None at 1000 ppm (equivalent to 180/257 mg/kg bw/day M/F). (Note: adverse liver effects seen from 788 / 984 mg/kg bw/day M / F)
Mouse (Crl:CD-1), 90 days. *Cat 1 ≤ 10 mg/kg bw/day* *10 < Cat 2 ≤ 100 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None (Note: adverse liver effects seen from 414 / 583 mg/kg bw/day M / F)
Mouse (Crl:CD-1), 18 month *Cat 1 ≤ 1.7 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None; no doses relevant for Category 2 were tested.

Study **Guidance values for classification***	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
1.7 < Cat 2 ≤ 17 mg/kg bw/day		(Note: inflammation of glandular stomach and reduced sperm in epididymis seen from 126 mg/kg bw/day in M)
Dog (Beagle), 7 days (dietary) *Cat 1 ≤ 100 mg/kg bw/day* *100 < Cat 2 ≤ 1000 mg/kg bw/day*	None at 2500 ppm (equivalent to 67/79 mg/kg bw/day M/F).	None at 5000 or 1000 ppm (equivalent to 185/187 and 292/244 mg/kg bw/day in M/F, respectively). **30000 ppm (818/716 mg/kg bw/day M/F)** ↓ food consumption: 69% (M) & 98% (F) ↓ mean body weight at the end of the study: 7.4% (M) & 3.8% (F)
Dog (Beagle), 7 days (capsule) *Cat 1 ≤ 100 mg/kg bw/day* *100 < Cat 2 ≤ 1000 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None up to the top dose of 550 mg/kg bw/day.
Dog (Beagle), 28 days (dietary) *Cat 1 ≤ 30 mg/kg bw/day* *30 < Cat 2 ≤ 300 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	**3000 ppm (134 / 108 mg/kg bw/day M / F)** *Organ weights* ↑ relative liver weight: 15 % (F) **10000 ppm (370 / 309 mg/kg bw/day M / F)** *Organ weights* ↑ Relative liver weight: 28.5 % (F) and 20 % (M) ↑ 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)
Dog (Beagle), 90 days (capsule) *Cat 1 ≤ 10 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	**100 mg/kg bw/day** *Organ weights* ↑ liver weights in F (27 %* absolute, 22 %** relative)

Study **Guidance values for classification***	**Effects occurring below the guidance value for classification in category 1**	**Effects occurring below the guidance value for classification in category 2**
10 < Cat 2 ≤ 100 mg/kg bw/day		
Dog (Beagle), 1 year (capsule) *Cat 1 ≤ 2.5 mg/kg bw/day* *2.5 < Cat 2 ≤ 25 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None at 20 mg/kg bw/day. (Note: haematology changes seen at the top dose of 500 mg/kg bw/day in M)
Rats (Crl:CD9(SD)), 28 days (dermal) *Cat 1 ≤ 60 mg/kg bw/day* *60 < Cat 2 ≤ 600 mg/kg bw/day*	None; no doses relevant for Category 1 were tested.	None at 100 or 300 mg/kg bw/day.

**Guidance values for the rat have been used in absence of specific values for the dog, to aid comparison. Haber's rule was used to adjust the standard guidance values, which are for studies of 90-day duration, for studies of longer or shorter durations. The Guidance on the application of the CLP Criteria (version 5.0 July 2017) highlights that this rule should be used cautiously with due consideration of the nature of the substance in question and the resulting value produced.*

The classification for specific target organ toxicity-repeated exposure Category 1 applies for substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Adverse effects on the liver – Category 2 assessment

The liver has been identified as a clear target organ in all species investigated with increases in relative and absolute liver weights accompanied in some instances with minimal to moderate hepatocellular hypertrophy. Changes in clinical chemistry indicative of liver toxicity were observed at the highest dose levels.

In the rat, adverse liver effects occurring below the guidance values for classification in category 2 were reported in the 28-day study (182 / 193 mg/kg bw/day (M / F)) with increased relative liver weight and minimal associated histopathology. Similar findings were also noted at levels slightly above the guidance values in the 90-day study (at 121 / 150 mg/kg bw/day (M / F)) where the changes persisted following a 28-day recovery period, and in the 2-generation reproductive toxicity study (140 / 187 mg/kg bw/day M / F; F0 and F1 parents). Therefore, it cannot be entirely excluded that adverse liver effects could occur at 100 mg/kg bw/day in both studies. It is noted that there were no liver findings at the lowest dose range of 29 - 49 mg/kg bw/day.

In the dog increased relative liver weights was observed in the 28-day study (range-finding oral, dietary) in females at 134 / 108 mg/kg bw/day M / F and in both sexes at levels approaching the guidance values for classification with associated hepatocellular hypertrophy (370 / 309 mg/kg bw/day (M / F)). However the reliability of the finding is low as only 2 animals/sex/group were used in this study and animals experienced significant palatability issues leading to severe toxicity. The method of oral administration was consequently changed from dietary to capsule in the following 90-day study; yet, increased liver weights were also reported at 100 mg/kg bw/day in that study. Therefore, adverse liver effects occurred at levels relevant for classification in Category 2 in the dog. It is nevertheless noted that no liver-related adverse effects were noted in the 12-month (oral, capsule) dog study up to the top dose of 500 mg/kg bw/day; thus it appears the dog is relatively less sensitive to the toxic effect of bixlozone on the liver compared to the rat.

No adverse effects were seen in the mouse at levels relevant to the guidance values for classification in Category 2.

In conclusion, adverse liver findings are seen in the rat and the dog at doses below the guidance values for classification in category 2 in 28-day studies, and at levels equal or slightly above the guidance values for classification in category 2 in the respective 90-day studies. The Agency also noted that 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. This observation was supported by toxicokinetics evidence indicating that bixlozone and its metabolites did not accumulate in plasma or tissues following 14 days repeated dosing (Section **Error! Reference source not found.**). For this reason the standard guidance values adjusted according to Haber's rule for 28-day exposure studies should be used with caution (Guidance on the application of the CLP Criteria (version 5.0 July 2017)) especially for the dog study in which only 2 animals/ sex/group were used.

Thus, given the consistency of the adverse liver effects reported in the rat and dog following repeated-dosing of bixlozone at levels above the guidance values set for classification in Category 2 in the 90-day studies, the dog being less sensitive to liver effects compared to the rat and the apparent lack of increase in severity of the effects with

the duration of treatment in any species, the Agency concludes that bixlozone should not be classified for STOT RE Category 2 for liver effects.

Adverse effects on the kidney

The kidney was also identified as a target organ in rats, with males being the more sensitive sex. Increased relative kidney weights were observed, although this change was not accompanied with histopathology findings. These changes were first observed at levels where adverse liver effects also occurred. It is further noted that the relative kidney weights remained high in the rats treated for 90-days at levels close to the guidance values for classification (121 / 150 mg/kg bw/day M / F)) during the 28-day recovery period.

In the dog kidney weight changes were seen at 370 / 309 mg/kg bw/day (M / F) in the 28-day study (range-finding oral, dietary) but not in the 90-day and 12-month dog studies (oral, capsule). These changes were considered to be spurious findings as the likely consequence of the method of administration (dietary vs capsules) and associated severe toxicity due to palatability problems rather than the test substance itself.

In conclusion, the kidney changes seen in the rat and the dog are considered milder in severity than those seen in the liver and occurring at values above the guidance values set for classification in Category 2 for all species; thus no classification is warranted.

Other findings

There were no other specific organ findings (including neurotoxicity) or systemic toxicity noted at doses relevant for classification.

Overall conclusion

Overall, it is concluded that there is sufficient evidence in the relevant repeated-dose studies to conclude that bixlozone should not be classified for STOT-RE.

10.9.3 Conclusion on classification and labelling for STOT RE

Not classified – conclusive but not sufficient for classification.

10.10 Germ cell mutagenicity

The genotoxic potential of bixlozone was tested both in vitro and in vivo, in a range of modern (2018) genotoxicity assays, conducted in accordance with relevant OECD test guidelines and in compliance with GLP. The available in vitro studies are a bacterial reverse mutation assay (Ames test), a mammalian chromosomal aberration assay using Chinese hamster

ovary (CHO-K1) cells and a mammalian cell gene mutation assay (Mouse Lymphoma Assay). The available in vivo study is a mammalian erythrocyte micronucleus test in the rat.

Table 26: Summary of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
Ames test Plate incorporation methodology OECD Nº 471 (1997) Deviations: none GLP: yes	Bixlozone Technical Batch: JB-Bixlozone - 201603004 Purity: 96.82 %	*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and the WP2 *uvr*A strain of *E. coli* Concentrations: 0, 5, 15, 50, 150, 500, 1500 and 5000 µg / plate ± S9 (Aroclor 1254-induced rat liver S9) Vehicle control: DMSO Adequate positive controls used.	**Negative** Precipitation of the test item was observed in both experiments at 5000 µg per plate under all conditions; cytotoxicity was observed from 1500 or at 5000 µg per plate under most conditions. Reference positive controls mutagens in test produced a distinct increase in revertant colonies within HCD provided by the laboratory. Vehicle controls values were also within the HCD provided. No positive mutagenic responses observed with or without S9 up to the limit of cytotoxicity and / or solubility.	**Bruce, S. (2018)** **DAR: B.6.4.1.1**

Chromosomal aberration study CHO cells OECD Nº 473 (2016) Deviations: none GLP: yes	Bixlozone Technical Batch: JB-Bixlozone - 201603004 Purity: 96.82 %	Treatments: 4-h ± S9; 20-h - S9 Concentrations: 0, 20, 40, 80, 100, 120, 140, 160, 180 µg/mL used in triplicate in main experiment. Vehicle control: DMSO Positive controls: mitomycin C (MMC) for treatment without S9; cyclophosphamide (CP) for treatments with S9.	**Positive – evidence of clastogenicity** Cytotoxicity observed at concentrations ≥ 160 µg/mL in the 4 hour treatment without S9, ≥ 140 µg/mL in the 4 hour treatment + S9, ≥ 80 µg/mL in the 20 hour treatment condition without S9. All controls fulfilled the requirements for a valid test as described in the study report and in the OECD Guideline 473. Clastogenic following 4-h treatment with S9 at the top concentration of 140 µg/mL, at which extensive cytotoxicity occurred. **Summary results from the main chromosome aberration assay (n = 2):**	**Roy, S. (2018)** **DAR: B.6.4.1.2**

Treatment condition	Test Substance	Concentration (µg/mL)	Cytotoxicity (% from control)[a]	Aberrations per Cell[b,d] Mean ± SD	Aberrant Cells		Carrying exchanges	Total polyploid cells (Mean %)[e]
					Numerical (Mean %)[b]	Structural (Mean %)[c]		
4-h + 16 h Recovery Without S9	DMSO	NA	-	0.013 ± 0.115	1.3	1.3	0	1.3
	Bixlozone Technical	80	-6	0.010 ± 0.100	1.7	1.0	0	1.3
		120	26	0.023 ± 0.151	2.0	2.3	0	2.0
		160	50	0.017 ± 0.128	1.7	1.7	0.5	1.7
4-h + 16 h Recovery With S9	DMSO	NA	-	0.017 ± 0.0128	2.7	1.7	0	2.0
	Bixlozone Technical	40	7	0.010 ± 0.100	2.7	1.0	0.5	1.3
		80	33	0.033 ± 0.180	2.7	3.3	1	2.0
		140	57	0.133 ± 0.360	3.0	**12.7****	2.5	2.0

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference

20-h Without S9	CP	5	47	0.247 ± 0.491	2.3	**22.0****	6	2.3
	DMSO	NA	-	0.013 ± 0.115	1.7	1.3	0.5	1.7
	Bixlozone Technical	20	20	0.007 ± 0.082	3.0	0.7	0	2.7
		40	44	0.010 ± 0.100	2.7	1.0	0	2.7
		80	60	0.010 ± 0.100	1.3	1.0	0	1.3
	MMC	0.1	11	0.200 ± 0.505	0.7	**17.3****	8.5	0.7

DMSO: Dimethyl sulfoxide; MMC: Mitomycin C; CP: Cyclophosphamide; NA: Not Applicable; Fisher's Exact Test: ** $p \leq 0.01$.
a. Based on cell growth inhibition relative to solvent control.
b. Includes polyploid and endo-reduplicated cells.
c.. Does not include cells with only gaps
d. Severely damaged cells counted as 10 aberrations.
e. Does not include endo-reduplicated cell.
f. SD = Standard Deviation.

L5178Y/ TK+/- Mouse Lymphoma cells mutagenicity study OECD Nº 490 (2016) Deviations: none GLP: yes	Bixlozone Technical Batch: JB-Bixlozone - 201603004 Purity: 96.82 %	Concentrations: 0, 7.81, 15.6, 31.3, 62.5, 125, 175, 200 and 250 µg/mL 4-h treatment + S9 Concentrations: 0, 15.6, 31.3, 62.5, 125, 150 and 200 µg/mL 4-h treatment - S9 Concentrations: 0, 7.81, 15.6, 31.3, 62.5, 125, 175 and 200 µg/mL 24-h treatment – S9 Vehicle control: DMSO Positive controls: methyl methane sulfonate (MMS) for treatment without S9; 7,12-Dimethyl-benz(a)anthracene (DMBA) for treatments with S9.	**Negative** Precipitate observed at 250 µg/mL at the beginning of treatment in all tests. Bixlozone did not affect the pH of the cultures. The positive and negative controls were acceptable according to the OECD Guideline criteria. No induction of forward mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells, either with or without S9, up to cytotoxic concentrations.	**Dutta, A. (2018)** **DAR: B.6.4.1.3**

Table 27: Summary of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

<table>
<tr><th>Method, guideline, deviations if any</th><th>Test substance</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr>
<td>Rat micronucleus assay in vivo (oral gavage)

OECD Nº 474 (2016)

Deviations: none

GLP: yes</td>
<td>Bixlozone Technical

Batch: JB-Bixlozone - 201603004

Purity: 96.82 %</td>
<td>Concentrations: 0, 500, 1000 and 2000 mg/kg bw/day

Vehicle: 0.5 % (w/v) CMC (400 cPs) in 5 % (w/v) Tween® 80 in deionized water

Treatment on two consecutive days 24-h apart

The doses were chosen upon toxicological information provided by the applicant to the laboratory, and an additional 2 animals were dosed at 2000 mg/kg bw to cover for any possible mortality.

Positive control generated in a recent study from male rats treated once with cyclophosphamide monohydrate (CP) at 40 mg/kg.</td>
<td>The positive and negative controls values were within the HCD provided by the laboratory.

No increase in the incidence of MnPCEs in the bone marrow of male rats up to the limit dose of 2000 mg/kg bw.

<u>Summary of the mammalian erythrocyte micronucleus assay results in rats:</u>
<table>
<tr><th>Treatment</th><th>Dose (mg/kg/day)</th><th>No. of Animals/Group</th><th>% PCE (mean ± SD)</th><th>% change in % PCE compared to control</th><th>% MnPCE (mean ± SD)</th><th>MnPCE / PCE Scored</th></tr>
<tr><td>Vehicle control</td><td>0</td><td>5</td><td>52.7 ± 1.0</td><td>-</td><td>0.08 ± 0.02</td><td>16 / 20000</td></tr>
<tr><td rowspan="3">Bixlozone</td><td>500</td><td>5</td><td>52.1 ± 0.4</td><td>-1</td><td>0.07 ± 0.02</td><td>13 / 20000</td></tr>
<tr><td>1000</td><td>5</td><td>51.8 ± 0.7</td><td>-2</td><td>0.09 ± 0.02</td><td>17 / 20000</td></tr>
<tr><td>2000</td><td>5</td><td>50.1 ± 1.3**</td><td>-5</td><td>0.09 ± 0.02</td><td>18 / 20000</td></tr>
<tr><td>CP</td><td>40</td><td>5</td><td>43.2 ± 3.1**</td><td>-18</td><td>2.48 ± 0.21**</td><td>495 / 20000</td></tr>
</table>
*p < 0.05 or **p < 0.01, One-Way ANOVA with Post-Hoc Dunnett's Test or T-Test
24 Hrs MnPCE Male GLM P-value = 0.269, R-sqr = 21.21%</td>
<td>Anon, 2018b

DAR: B.6.4.2</td>
</tr>
</table>

10.10.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro studies

The genotoxic potential of bixlozone has been investigated in 3 in vitro studies, all conducted in accordance with appropriate OECD test guidelines and in compliance with GLP.

In a reverse mutation assay (Ames test) and in an in vitro L5178Y/TK+/- mouse lymphoma cells mutagenicity study there was no evidence of genotoxicity under any of the tested conditions up to the limit of cytotoxicity and / or solubility. Furthermore, there was no evidence of mutagenic potential with bixlozone in a mouse lymphoma cell mutation assay.

In the in vitro mammalian chromosomal aberration assay for the 4 and 20 hour treatments without S9, no statistically significant or dose-dependent increases in structural aberrations were observed at any concentrations. However, following the 4 hour treatment with S9 a dose-dependent increase in chromosomal and chromatid structural aberrations was observed. The increase in chromosomal aberrations was statistically significant at the top concentration of 140 µg/mL (12.7 % aberrant cells vs 1.7 % in negative control, $p \leq 0.01$ Fisher's Exact test and $p \leq 0.05$ Cochran-Armitage test) and above the historical 95 % control value (0.00 % to 3.88 %); cytotoxicity was extensive at this concentration (47 % from control). All controls fulfilled the requirements for a valid test as described in the study report and in the OECD test guidelines.

Overall, under the conditions of this study, there was evidence of a clastogenic potential of bixlozone in vitro, in the presence of metabolic activation at the top concentration of 140 µg/mL, at which cytotoxicity occurred. Thus, from the available studies, there is some evidence that bixlozone has the ability to damage chromosomes in vitro.

In vivo study

In a rat bone marrow micronucleus study, doses of 0, 500, 1000 or 2000 mg/kg bw of bixlozone technical were administered to 5 males per group by oral gavage on two consecutive days approximately 24 hours apart.

No mortality occurred at any dose level during the course of the definitive assay and there were indications of a dose-related systemic exposure to bixlozone and/or its metabolites. Clinical signs were seen in a dose-related manner; piloerection occurred in animals treated at 500 and 1000 mg/kg bw whilst piloerection, hunched position and diarrhoea were noted in animals treated at the maximum dose of 2000 mg/kg bw. There was also a dose-dependent decrease in body weight gain observed in the treated animals compared to the controls.

There was a statistically significant decrease (- 5 %) in the ratio of immature to mature erythrocytes observed at 2000 mg/kg/day dose group compared to the negative control group, indicating the test substance induced bone marrow toxicity. However, this reduction in this ratio did not exceed the OECD recommended value of – 20 % of the vehicle control proportion. Nevertheless, exposure of the bone marrow to the test item is further supported by kinetic studies in the rat showing that bixlozone and/or its metabolites were systemically available and reached the bone marrow (Section **Error! Reference source not found.** - study conducted by Anon, 2017h).

There were no statistically significant increase in the incidence of MnPCEs in the treated groups, when compared to the negative control group (ANOVA followed by Dunnett's post-hoc analysis, $p > 0.05$). The positive and negative controls values were within the HCD provided by the laboratory. Overall, there was no evidence of a clastogenic or aneugenic effect of bixlozone in this study up to the limit dose of 2000 mg/kg/day. Overall, it was concluded that bixlozone is not genotoxic in vivo.

10.10.2 Comparison with the GB CLP criteria

Bixlozone has been tested for its potential genotoxic properties in three *in vitro* assays and one in vivo test. From the available studies it appears that bixlozone has the ability to damage chromosomes in vitro. However, this finding was not reproduced in vivo in a micronucleus assay in rats (tested up to the limit dose of 2000 mg/kg bw/d). In accordance with the CLP regulation, positive results from in vitro studies alone are not sufficient to classify for germ cell mutagenicity. Therefore, bixlozone does not meet the requirements for classification for germ cell mutagenicity.

10.10.3 Conclusion on classification and labelling for germ cell mutagenicity

Not classified – conclusive but not sufficient for classification.

10.11 Carcinogenicity

The long term toxicity and carcinogenic potential of bixlozone was investigated in two carcinogenicity/chronic toxicity studies; one in the rat and one in the mouse. Both studies available were conducted according to guidelines and GLP.

Table 28: Summary of animal studies on carcinogenicity

Note:
↑↓ denote an increase or decrease in a parameter with respect to the control value
*Statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$*
abs. = absolute
rel. = relative

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Dietary 24-month **Rat** (Crl:CD (SD) rats, males and females) N = 50/sex/group + 10/sex/group for the chronic toxicity evaluation GLP yes OECD 453 (2009) Deviations: none The study was conducted following	Bixlozone technical Batch PL14-0049, Purity 96 % Doses: 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 The substance dose levels were selected from the previous 28- and 90-day oral dietary studies in	**Chronic phase – 12 months** **<u>5000/3000 ppm (217 / 167 mg/kg bw/d)</u>** ↓ BW-gain in females (10%** days 1-344) ↑ liver weight in F (18%*absolute & 11%* relative) **<u>1000 ppm & 250 ppm</u>** No adverse effects --- **Carcinogenicity phase – 24 months** ***Non-neoplastic findings*** **<u>5000/3000 ppm (217 / 167 mg/kg bw/d)</u>** 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)	**Anon, 2017a** **DAR: B.6.5.1**

<table>
<tr>
<td>the version of the OECD 453 i.e. adopted in 2009. The guideline has since been updated in June 2018. Changes from the 2009 version are considered minor</td>
<td>rats (Section Error! Reference source not found.). The high dose of 5000 ppm and 3000 ppm for males and females, respectively was estimated to approach the Maximum Tolerated Dose (MTD) based on the findings of the 90-day rat study</td>
<td>Hepatocellular vacuolation:

7/10 at 52 weeks & 32 % incidence at 104 weeks (M)

10/10 at 52 weeks & 74 % incidence at 104 weeks (F)

<u>1000 & 250 ppm</u>

No adverse effects

Neoplastic findings

None attributable to exposure to bixlozone up to the highest dose tested (5000/3000 ppm).

Selected neoplastic microscopic findings in the chronic/carcinogenicity rat study with bixlozone
<table>
<tr><th rowspan="2">Dose-levels (ppm)</th><th colspan="4">Males</th><th rowspan="2">HCD Mean % incidence (incidence range)</th><th colspan="4">Females</th><th rowspan="2">HCD Mean % incidence (incidence range)</th></tr>
<tr><th>0</th><th>250</th><th>1000</th><th>5000</th><th>0</th><th>250</th><th>1000</th><th>3000</th></tr>
<tr><td>Dose (mg/kg bw/day)</td><td>0</td><td>10</td><td>41</td><td>217</td><td></td><td>0</td><td>13</td><td>53</td><td>167</td><td></td></tr>
<tr><td>Skin
N examined</td><td>60</td><td>50</td><td>50</td><td>60</td><td></td><td>60</td><td>50</td><td>50</td><td>60</td><td></td></tr>
<tr><td>Fibroma, benign (% incidence)</td><td>0</td><td>0</td><td>0</td><td>1 (1.67)</td><td>2.3[c] (1.43-8.33)</td><td>0</td><td>0</td><td>0</td><td>0</td><td></td></tr>
<tr><td>Fibrosarcoma, malignant (% incidence)</td><td>0</td><td>0</td><td>0</td><td>3 (5.0)</td><td>1.26[c] (1.43 - 7.14)</td><td>1</td><td>0</td><td>0</td><td>0</td><td></td></tr>
<tr><td>1-sided pairwise comparison (p)[a]</td><td></td><td>1.000</td><td>1.000</td><td>0.1575</td><td></td><td colspan="4" rowspan="2">ND</td><td></td></tr>
<tr><td>1-sided trend test (p)[b]</td><td colspan="4">0.0206</td><td></td><td></td></tr>
<tr><td>Thyroid gland
N examined</td><td>58</td><td>50</td><td>47</td><td>60</td><td></td><td>60</td><td>50</td><td>49</td><td>60</td><td></td></tr>
</table>
</td>
<td></td>
</tr>
</table>

C cell adenoma, benign	7	10	4	5		5	5	6	5		
C cell carcinoma, malignant	1	0	0	0		2	1	0	1		
Combined C-cell carcinoma/ adenoma	8	10	4	5		7	6	6	6		
Follicular cell adenoma, benign (% incidence)	2 (3.44)	0 (0.00)	3 (6.4)	2 (3.33)		0 (0.00)	0 (0.00)	1 (2.04)	2 (3.33)	HCD 1999-2017 1.30[d] (0.0 – 6.12) HCD 2009-2017 (1.54 – 4.69)	
1-sided pairwise comparison (p)[a]	*ND*						*1.000*	*0.480*	*0.220*		
1-sided trend test (p)[b]						*0.0408*					
Follicular cell carcinoma, malignant	0	0	1	0		0	1	0	1 (1.7)	HCD 1999-2017 0.41[d] (0.0 – 3.33) HCD 2009-2017 (1.43 – 1.67)	
1-sided pairwise comparison (p)[a]	*ND*						*0.567*	*0.480*	*0.124*		
1-sided trend test (p)[b]						*0.0255*					

[a] 1-sided pairwise comparison of 0 ppm with active treatment group
[b] 1-sided trend test including 0 ppm and active treatment groups
Statistical Significance: Rare tumour - p<0.025 (trend), p<0.05 (pairwise); Common tumour - p<0.005 (trend), p<0.01 (pairwise)
* Statistically significant at the defined significance level
ND = Not determined
NA = not available
[c] HCD mean and range for Crl:CD(SD) male rats – sub-chronic (Charles River Ashland laboratory): 08 Feb 2001 - 08 Jan 2013; Number of Studies/Control Groups for males: 21 / 31;
[d] HCD mean and range for Crl:CD(SD) female rats – sub-chronic (Charles River Ashland laboratory): June 1999 - Jan 2017; Number of Studies/Control Groups for females: 32 / 48

<table>
<tr>
<td>Dietary 18-month

Mouse (Crl:CD1 (ICR mice, males and females)

GLP yes

OECD 451 (2009)

Deviations: none

The study was conducted following the version of the OECD 453 i.e. adopted in 2009. The guideline has since been updated in June 2018. Changes from the 2009 version are minor.</td>
<td>Bixlozone technical

Batch PL14-0049

Purity 96 %

Doses: 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</td>
<td>Non-neoplastic findings

<u>5000 ppm</u>

↓ cumulative body weight gain for F > 10 %

Organ weights

↑ relative liver weight > 15 %** (both sexes)

Histopathology findings

Hepatocellular hypertrophy: 7/10 at 52 weeks & 79 % incidence at 104 weeks (M)

↑ pelvis dilation of kidney (M)

↑ inflammation of glandular stomach (M)

↑ incidence of reduced sperm in epididymes (M)

Non-neoplastic histopathological findings in the 18-month oral carcinogenicity study in CD-1 mice (all types of death combined)
<table>
<tr><th rowspan="2">Diet concentration (ppm)</th><th colspan="4">Males (n = 50)</th><th colspan="4">Females (n = 50)</th></tr>
<tr><th>0</th><th>250</th><th>1000</th><th>5000</th><th>0</th><th>250</th><th>1000</th><th>5000</th></tr>
<tr><td>Bixlozone intake (mg/kg bw/day)</td><td>0</td><td>32</td><td>126</td><td>647</td><td>0</td><td>43</td><td>164</td><td>834</td></tr>
<tr><td colspan="9">non-neoplastic histopathology findings (all animals n = 50)</td></tr>
<tr><td colspan="9">Liver</td></tr>
<tr><td>Hypertrophy, hepatocellular</td><td>4</td><td>2</td><td>11</td><td>18</td><td>1</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Minimal</td><td>3</td><td>1</td><td>6[a]</td><td>7</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mild</td><td>0</td><td>0</td><td>1</td><td>6</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Moderate</td><td>1[a]</td><td>1</td><td>4[a]</td><td>5[a]</td><td>0</td><td>0</td><td>0</td><td>1[b]</td></tr>
<tr><td>% incidence</td><td>8</td><td>4</td><td>22</td><td>36</td><td>2</td><td>-</td><td>-</td><td>2</td></tr>
</table>
</td>
<td>Anon, 2017b

DAR: B.6.5.2</td>
</tr>
</table>

Epididymides									
Reduced sperm, luminal	**7**	**7**	**11**	**12**					
Minimal	1	1	none	none					
Mild	2	1	3	3					
Moderate	3	5	6	7					
Severe	1	none	2	2					
% incidence	14	14	**22**	**24**					
HCD (max)[c]	**13 (21.7 %)**								
Kidneys									
Pelvis dilation	4	4	4	**9**	0	0	0	0	
Minimal	4	1	4	3	0	0	0	0	
Mild	0	3	0	5	0	0	0	0	
Moderate	0	0	0	1	0	0	0	0	
% incidence	8	8	8	**18**	0	0	0	0	
HCD (max)[c]	**13 (21.7 %)**				-				
Stomach, glandular									
Chronic inflammation	5	7	**9**	**10**	0	1	2	1	
Minimal	5	6	9	10	0	1	2	1	
Mild	0	1	0	0	0	0	0	0	
% incidence	10	14	**18**	**20**	0	2	4	2	
HCD (max)[c]	**2 (3.33 %)**				**1 (2.04 %)**				

a – Associated with increased glycogen deposition
b – Found dead on study day 146
c - HCD (maximum numerical and % incidence) date range: Apr 2009 – May 2017; 8 studies/ 11 control groups; 630 animals

1000 ppm

Histopathology findings

↑ inflammation of glandular stomach (M)

↑ incidence of reduced sperm in epididymes (M)

Neoplastic findings

None attributable to exposure to bixlozone up to the highest dose tested (5000 ppm).

Neoplastic histopathology findings in the 18-month oral carcinogenicity study in CD-1 mice

Diet concentration (ppm)	**Males (n = 50)**				**Females (n = 50)**				**HCD[#]**
	0	**250**	**1000**	**5000**	**0**	**250**	**1000**	**5000**	**Mean % incidence (range)**
Bixlozone intake (mg/kg bw/day	**0**	**32**	**126**	**647**	**0**	**43**	**164**	**834**	
Macroscopic findings (all animals n = 50)									
Cervical masses					1	0	0	5	
Lung nodules	0	5	2	0	0	1	1	5	
Incidence of neoplastic findings (all animals n = 50)									
Cervix									
Leiomyoma (benign)					1	0	0	1	**0.43 (0.0 – 2.9)**
% incidence					2	0	0	2	
Leiomyosarcoma (malignant)					**0**	**0**	**0**	**2**	**0.7 (0.0 – 5.8)**
% incidence					**0**	**0**	**0**	**4**	
Liver									
Hepatocellular carcinoma	2	1	3	2	1	0	0	0	
Hepatocellular adenoma	2	1	4	0	0	0	0	0	

Lung #											
Bronchiolo-alveolar hyperplasia	3	0	1	1	2	0	0	1			
Adenoma, bronchiolo-alveolar	**1**	**2**	**1**	**4**	2	1	0	0	**Males 9.85 (0.0 - 17)**		
% incidence	**2**	**4**	**2**	**8**	4	2	0	0			
Carcinoma, bronchiolo-alveolar (all)	3	4	3	2	1	1	2	5			
Minimal								2			
Mild				1		1					
Moderate	2	4	3	1			1				
Present					1		1	3			
Unscheduled deaths	2	0	0	1	0	1	1	2			
Scheduled necropsy	1	4	3	1	**1**	**0**	**1**	**3**	**Females 4.3 (0.0 - 14.3)**		
% incidence	6	8	6	4	**2**	**2**	**4**	**10**			
1-sided pairwise comparison (p)[a]	***N/D***				***-***	***0.8052***	***0.5207***	***0.0481***			
1-sided trend test (p)[b]					***0.0210***						
Systemic tumours											
Sarcoma, histiocytic (regardless of tissue present within)	**0**	**1**	**0**	**0**	**2**	**0**	**0**	**6**			
Unscheduled deaths		1			1			2			
Scheduled necropsy		0			1			4			
% incidence	**0**	**2**	**0**	**0**	**4**	**0**	**0**	**12**	**Females 6.5**		

									(0.0 - 18.33)
1-sided pairwise comparison (p)[a]	***N/D***				***-***	***1.000***	***1.000***	***0.0797***	
1-sided trend test (p)[b]					***0.0297***				
Uterus									
Leiomyoma (benign)					0	1	1	0	
Polyp, endometrial stromal (benign)					3	0	1	0	
Granular cell tumour (benign)					1	0	0	0	
Leiomyosarcoma (malignant)					1	2	0	1	
Sarcoma, endometrial stromal (malignant)					1	0	0	0	
Granular cell tumour (malignant)					0	1	0	0	
Total uterine neoplastic findings					6	3	2	1	

Laboratory HCD from carcinogenicity studies in CD-1 mice: Dates 1999-2017; for males, number of studies = 23; number of control groups = 34; for females, number of studies = 22; number of control groups = 33)
[a] 1-sided pairwise comparison of 0 ppm with active treatment group
[b] 1-sided trend test including 0 ppm and active treatment groups
Statistical Significance: Rare tumour - p<0.025 (trend), p<0.05 (pairwise); Common tumour - p<0.005 (trend), p<0.01 (pairwise)
* Statistically significant at the defined significance level
ND = Not determined
NA = not available

Comparison and combination of cervix and uterus incidence of leiomyoma and leiomyosarcoma in female mice

		Bixlozone diet concentration (ppm)	**0**	**250**	**1000**	**5000**
		Bixlozone intake (mg/kg bw/day)	**0**	**43**	**164**	**834**
		Cervix (No. Examined)	48	47	48	50
		Leiomyoma	1	0	0	1
		Leiomyosarcoma	0	0	0	2
		Uterus (No. Examined)	50	50	50	50
		Leiomyoma	0	1	1	0
		Leiomyosarcoma	1	2	0	1
		Combined Cervix and Uterus Leiomyomas	1	1	1	1
		% incidence	2	2	2	2
		Combined Cervix and Uterus Leiomyosarcomas	1	2	0	3
		% incidence	2	4	0	6

10.11.1 Short summary and overall relevance of the provided information on carcinogenicity

Rat chronic toxicity and carcinogenicity study

Bixlozone was administered *ad libitum* in the diet of Crl:CD (SD) rats for 52 and 104 consecutive weeks for the chronic toxicity (10 animals/sex/group) and carcinogenicity groups (50 animals/sex/group) respectively. Doses were 250, 1000, and 5000/3000 ppm (males/females), equating to consumed levels of 10, 41, and 217 mg/kg bw/day respectively for males and 13, 53, and 167 mg/kg bw/day respectively for females. The females were originally treated with 4000 ppm as the highest dose but at Day 49 (week 7), the dose administered was reduced to 3000 ppm based on severe adverse effects (body weight loss approaching 10 %, corresponding lower mean body weight gains by 18-20 % and lower mean food consumption by 9-12 %).

Results

There were no test substance-related deaths during the chronic toxicity or carcinogenicity phases of the study and no statistical difference in the survival between animal groups of the same sex. Test substance-related clinical observations noted throughout the study were limited to the top dose groups, with yellow material on various body surfaces (urogenital area and ventral trunk) in males and dermal atonia and thin body condition in females. A statistically significant adverse lower mean body weights and body weight

gains was observed in both sexes at the top-dose (5000 ppm in males and 3000 ppm in females). Thus, it was concluded that the MTD was reached at the top doses in both sexes.

Non-neoplastic findings

Treatment-related and adverse increases in liver weight were observed at week 52 in males and females at top-dose and at week 104 in males only. A dose-dependent increased incidence in hepatocellular hypertrophy was noted in males from 250 ppm and in females from 1000 ppm, and hepatocellular vacuolation was increased in males from 1000 ppm. Treatment-related increases in serum chemistry parameters indicative of adverse effects in the liver (serum cholesterol, albumin, calcium, total protein), were also observed in the top-dose females but not in males during the chronic toxicity phase; these effects correlated with the other effects seen on the liver in these animals (organ weight changes and histopathology). The findings are also consistent with the toxicokinetics data indicating that females are more systemically exposed to bixlozone than the males. The relevance of those non-neoplastic findings for the classification and labelling of bixlozone for STOT-RE is summarised in Section **Error! Reference source not found.**.

The survival rate at termination of the carcinogenicity phase of the study was low; mortality rates ranged from 54-76% across all groups, including controls. No dose-response relationship was identified for mortality rate. The Agency considers the study to have sufficient validity.

Neoplastic findings

There were no increases (relative to controls) in the tumour incidence in any of the main target organs identified in the repeated-dose studies (liver, kidneys) in both sexes; any incidences seen did not show a clear dose-response, were small in magnitude or were also seen in controls. Increased incidences of tumours were only seen for the skin in males and the thyroid in females and are discussed below.

Skin tumours in males

Regarding males, there was a non-statically significant increase in the incidence of skin fibrosarcoma (malignant tumours; 3/60 - 5% vs 0/60 in controls) and fibroma (1/60 - 1.7% vs 0/60 in controls) at the top-dose of 5000 ppm in comparison to the control, with 4 males bearing fibrosarcoma or fibroma in the skin. This is consistent with the increase in the number of top-dose males with palpable masses at the external examination. Fibrosarcoma was considered the cause of death for 2 of these animals (one euthanised *in extremis* on study day 234, and one that died on study day 708). The incidence of fibroma is even below the mean incidence of the laboratory HCD provided, whilst for

fibrosarcoma, the incidence is above the mean incidence value but well within the HCD range. There were no neoplastic findings noted at the lower doses.

The laboratory HCD provided with the study is extensive (derived from 31 male control groups in 21 studies conducted from February 2001 to July 2013). The CLP guidance specifies that the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study) since it is also known that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (Section 3.6.2.3.2.a of the CLP guidance). Therefore, HCD older than this should be used with caution and acknowledgement of its lower relevance and reliability. (RIVM, 2005; Fung et al, 1996; Greim et al, 2003). In this instance, the Agency considers that the HCD provided do not fully comply with the recommendations of the CLP Guidance. Nevertheless, these extended HCD, which can be taken into account in a WoE approach, suggest that skin fibromas are relatively common tumours since the HCD range (1.43 – 8.33 %) shows that at least 1 control animal in every study (included in the HCD) is found with such tumours. Regarding fibrosarcomas it is also noted that 1 concurrent control female showed a fibrosarcoma (with none in the treated female groups). Since the HCD range for this finding is 1.43 % - 7.14 % in males (with at least 1 control animal in every study found with such tumours) it suggests that the incidence in the concurrent control male group in this study (0/60) was unusually low.

Considering the biological plausibility of the finding, it is noted that the skin has not been identified as a target organ of toxicity of bixlozone in any other repeated-dose toxicity studies (Section **Error! Reference source not found.**) and bixlozone was found not to be acutely toxic via the dermal route or a skin irritant (Sections **Error! Reference source not found.** & **Error! Reference source not found.** respectively). Moreover, a tissue distribution study showed that bixlozone and/or its metabolites were only found at low levels in the skin after administration of an oral dose of 500 mg/kg bw (Section **Error! Reference source not found.**) which is much higher than the highest dose tested in males in this study (217 mg/kg bw/day).

In addition, the finding was sex specific, with an increased incidence observed in males only. However, in contrast, the available kinetics show a higher systemic exposure in females (Section **Error! Reference source not found.**), and yet females do not present any tumours up to the top dose (4000 / 3000 ppm). Lastly, no skin tumours were observed in mice.

Overall, considering the sex and species specificity of the response, the low biological plausibility of the finding, the inconsistency between the sex-specificity of the response and the higher systemic exposure in females, and the fact that the incidence was clearly within the range of the extended (2001-2013) HCD supplied, the Agency concludes that

these skin tumours in male rats at the top dose are chance findings, unrelated to treatment.

Thyroid tumours in females

Regarding females, there was a non-statistically significant but dose-related increase in the incidence of follicular cell adenomas (benign tumours; 2/60 - 3.3 % vs 0/60 in controls) and of the follicular cell carcinoma (1/60 - 1.7 % vs 0/60 in controls) in the thyroid gland at the top-dose of 3000 ppm in comparison to controls.

The applicant provided the maximum control numerical and % incidence values derived from studies conducted between 2009 and 2017 i.e. performed around the date of the current study (2014): the maximum numerical incidence was 3 (4.7 % incidence) for follicular cell adenoma and 1 (1.7 %) for follicular cell carcinoma. Thus the thyroid tumour incidences seen in the study in females at the top dose are consistent with the top of the range of the control groups monitored around the time of the study.

Significantly extended HCD (date range 1999 – 2017) were also provided for these tumour findings and show that at the top dose both tumour incidences are slightly higher than the mean % incidence but well within the HCD range. The data do not fully comply with the recommendations of the CLP Guidance (Section 3.6.2.3.2.a of the CLP guidance) however they are from the same laboratory and strain of rat and derived from a reasonable number of studies; thus, they can be considered by the Agency in a WoE approach.

The finding is sex specific, since no relevant or dose-related increase in incidence was observed in males. Moreover, it is noted that the incidence of follicular cell adenoma (benign) in the control male group is similar (3.4 %) to the incidence seen in the top dose female group (3.3 %) and that the incidence of follicular cell carcinoma was for both sexes very low (maximum of 1 case per 50 / 60 rats per group) and clearly not dose-related.

The available kinetics data appear to support the sex specificity of the response since in rats the females show a higher systemic exposure than males (Section **Error! Reference source not found.**). However, a tissue distribution study showed that less than 0.1 % of the administered dose (AD) was found in the thyroid in both sexes following oral administration of bixlozone with the thyroid of males being more exposed than females' following a single high oral dose (500 mg/kg bw) or a repeated low oral dose (5 mg/kg bw/day, 14 days).

Considering the biological plausibility of the finding, it is noted that there were no other associated findings noted in the thyroid (e.g. hyperplasia, hypertrophy) in the study to support the tumorigenic response. Yet, in the 90-day rat study (Section **Error! Reference source not found.**) the thyroid was identified as a target organ of toxicity with histopathology changes seen at doses higher than the top dose tested in this carcinogenicity study, with mild follicular cell hypertrophy observed in both sexes at the top

dose of 505 / 351 mg/kg bw/day only and without associated changes in the thyroid weights. There were no histopathology findings seen at the lower doses of 121 / 150 mg/kg bw/day. Therefore, the biological plausibility of these thyroid tumour findings appears to be low with no clear continuum of neoplastic transformation seen in the thyroids in the relevant rat studies.

Overall, considering the sex specificity of the response, the low incidence of the tumours and the low biological plausibility of the finding, the Agency concludes that the thyroid tumours observed in female rats at the top dose are chance findings unrelated to treatment.

To conclude, there were no treatment-related neoplastic findings identified in this study in the rat.

Mouse carcinogenicity study

In a GLP- and OECD-compliant carcinogenicity study, bixlozone was administered *ad libitum* in the diet of Crl:CD1(ICR) mice (50 animals/sex/group) for 18 months (78 weeks). Administered doses were 250,1000 and 5000 ppm for both sexes equating to consumed levels of 32,126 and 647 mg/kg bw/day respectively, for males and 43, 164 and 834 mg/kg bw/day respectively, for females. Concurrent toxicokinetic groups (20 animals/sex/group) were treated for 365 days (52 weeks). Administered doses were 250, 1000, and 5000 ppm for both sexes equating to consumed levels of 38, 150, 756 mg/kg bw/day, respectively, for males and 50, 202, and 1046 mg/kg bw/day, respectively, for females.

Results

Treatment-related increased incidences of yellow material on the urogenital area and ventral trunk were noted for males in the top-dose group (5000 ppm). These were not considered adverse by the Agency. All other observed signs were common for the age and strain of mice, did not show a clear dose-response and occurred in a small number of animals. The overall survival rates were acceptable in this study across all groups (> 50 % after 18 months) and were not affected by treatment.

Treatment-related and adverse body weight changes were observed in females at the top-dose of 5000 ppm (834 mg/kg bw/day), whilst body weights, body weight gains and food consumption were unaffected by bixlozone administration up to the top-dose of 5000 ppm (647 mg/kg bw/day) in males. Consistent with the toxicokinetic findings from the 90-day repeated-dose toxicity study in mice (Section **Error! Reference source not found.**) bixlozone mean concentrations were found to be 2- to 13-fold higher in males than in females on most sampling days at all dose levels.

Non-neoplastic findings

Treatment-related and adverse changes in liver weight were observed in males and females at the top dose of 5000 ppm (647 and 834 mg/kg bw/day in males and females respectively). It was pointed out by the study authors that the highest individual animal liver weights seen, regardless of sex or exposure group, were in mice afflicted with primary tumours (hepatocellular adenoma or carcinoma) or systemic tumours (malignant lymphoma or histiocytic sarcoma). The incidence of these organ weight outliers (since they contain tumours) was noted in the study report to be fairly equally distributed across all exposure groups; therefore, no individual animal organ weights were proposed to be excluded to calculate the mean weights. The approach was considered acceptable by the Agency and the results are thus appropriate for comparison purposes.

A treatment-related and dose-dependent increased incidence in hepatocellular hypertrophy (including glycogen accumulation and single cell necrosis) was noted in males only from 1000 ppm; however, these findings are considered adverse only at top-dose (5000 ppm; 647 mg/kg bw/day) since they correlate with clear and adverse liver weight increases. In addition, slightly higher incidences of reduced epididymal sperm and inflammation of the glandular stomach were seen in males from 1000 ppm, with pelvis dilation of the kidney occurring in males at the top dose of 5000 ppm. Overall, adverse non-neoplastic findings started to occur in males from the mid dose of 1000 ppm (126 mg/kg bw/day).

The relevance of those non-neoplastic findings for the classification and labelling of bixlozone for STOT-RE is summarised in Section **Error! Reference source not found.**.

Neoplastic findings

There was no increased incidence of benign or malignant liver tumours in both sexes up to the top-dose however increased incidences of histiocytic sarcoma, cervical and lung tumours were noted and are discussed below.

Histiocytic sarcoma in females

The incidence of systemic histiocytic sarcomas in animals, regardless of tissue present within, was 6/50 (12 %) in top dose females vs 2/50 (4 %) in controls, with no clear dose-response since there was no tumours in the mid and low doses. The increased incidence was not statistically significant and was well within the range of the laboratory HCD provided (0.0 – 18.3 %); the data suggest that the incidence of histiocytic sarcoma in CD-1 female mice is highly variable in controls, with a maximum level of 18.3 %. It is noted that the data do not fully comply with the recommendations of the CLP Guidance (Section 3.6.2.3.2.a of the CLP guidance) however they are from the same laboratory and strain of rat and derived from a reasonable number of studies; thus, they can be considered by the Agency in a WoE approach. This wide data range is also similar to the one reported by P.

Greaves 2012[1] (0 - 15 % for CD-1 mice). According to this review publication, histiocytic sarcoma tumours are uncommon until the age of 12 months, but they increase steeply after 18 months when they are generally more common in females than in males, which is the case in this study. Moreover, the tumour cells are metastatic, and deposits can be found in particular in the lungs and liver. This was also the case in this study, with 3 of the 6 sarcomas being found in the cervix, with metastasis/deposits localised in the lungs and liver.

It is also noted that the biological plausibility of this tumourigenic response is very low as the blood is not a target tissue of toxicity in mice. The finding was sex specific, with an increased incidence observed in females only, whilst in males a single incidence was noted at 250 ppm. This sex-specific response is in contrast to the higher systemic exposure to the test substance seen in males compared to females, further reducing the biological plausibility and possible relation to treatment of the finding. Lastly, no similar findings were seen in the combined chronic / carcinogenicity study in rats even though these tumours can be relatively common in both species. Finally, it is widely believed that this particular neoplasm lacks relevance in the identification of a human carcinogenic hazard. This neoplasm is very rare in humans and no chemical has been shown to increase the incidence of histiocytic sarcoma in the rat and only rarely in the mouse (Greaves P., 2012).

Overall, considering the sex and species specificity of the response, the low biological plausibility of the finding and the relatively high incidence of this tumour observed in control CD-1 mice in accordance with the laboratory extended HCD supplied and the HCD reported in the available literature, the Agency concludes that the incidence in histiocytic sarcomas observed in female mice at the top dose is overall unrelated to treatment with bixlozone.

Cervical tumours in females

Increased incidences of cervical tumours were seen in top dose females: 2 females (4%) showed cervical leiomyosarcoma (malignant tumours) vs none in controls. The laboratory HCD data derived from studies conducted between 2009 and 2017 (i.e performed ± 5 years around the date (2014) of the current study) with an incidence range of 1.54 - 2.04 % for cervical leiomyosarcoma confirm that the incidence seen at the top dose in this study is higher than the incidence range. In addition, significantly extended laboratory HCD (1999 – 2017) have been provided to help interpret the significance of this response, however they do not fully comply with the recommendations of the CLP Guidance (Section 3.6.2.3.2.a of the CLP guidance). Nevertheless, the data range (.0 – 5.8 %) show that the incidence observed in the study is within the incidence range, but significantly higher than

[1] Peter Greaves. Histopathology of Preclinical Toxicity Studies. Interpretation and Relevance in Drug Safety Studies. Book • 4th Edition • 2012

the mean incidence value of 0.7 %. Thus, both sets of HCD show that this tumour type is relatively rare in the mouse and confirm that the observed incidence of 4 % in this study is generally higher than the historical control incidences.

It is however noted that there was no increased incidence in leiomyosarcoma reported in the uterus which is adjacent to the cervix. The practical difficulties in isolating the cervix from the uterus in the mouse can often result in the uterine corpus and uterine cervix being collected and identified together. Moreover, there are no definitive gross or macroscopic features that clearly demarcate the uterine body (also known as the corpus) from the uterine cervix. Also, there are no histochemical stains, immunohistochemical stains, or ultrastructural features using transmission electron microscopic examination that can differentiate uterine body smooth muscle cells from uterine cervix smooth muscle cells. Further, the 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. As a result, it is not possible to determine if a uterine neoplasm arose in the uterine body or uterine cervix based on gross or microscopic features. On that basis, the use of the combined incidence of neoplastic lesions from both tissues (cervix and uterus) to assess the biological relevance of the finding is supported.

The combined uterus/cervix tumour incidences show that a dose-response is no longer apparent for leiomyomas and leiomyosarcomas up to the highest dose tested. Thus, the cervical/uterine tumours reported in females in this study are not considered to be attributable to exposure to bixlozone.

Lung tumours in both sexes

In the top dose females 5/50 animals had lung nodules compared to 0/50 in controls; the finding appears to correlate well microscopically with a higher incidence in bronchio-alveolar carcinoma seen in top dose females (5/50 – 10 %) compared to the controls (1/50 – 2 %). The increase is not statistically significant and is well within the range of the laboratory HCD provided (0 – 14.3 %). Although these extended HCD (1999 – 2017) are not compliant with the data requirements, the Agency notes that the range indicates that the incidence of bronchio-alveolar carcinomas is highly variable in controls, rising up to 14.3 %. It is further noted that there were no increases (rather decreases compared to controls) in bronchio-alveolar adenomas in the top dose females.

In top-dose males there were 4/50 bronchio-alveolar adenomas compared to 1/50 in controls, but only 2/50 bronchio-alveolar carcinomas compared to 3/50 in controls. None of the findings showed a clear dose-relationship or was statistically significant compared to the control group.

Moreover, it is noted that the lung has not been identified as a target organ in the mouse and that there is no clear pattern of pre-neoplastic lesions or progression of benign

tumours to malignant tumours at the top dose in both sexes. Thus, the biological plausibility of the finding is considered to be low.

Overall, the neoplastic findings observed in the lung in males and females are not considered to be attributable to exposure to bixlozone.

Overall conclusion

Overall, long term oral administration of bixlozone was not carcinogenic in the rat or mouse up to the highest dose tested where general toxicity occurred.

10.11.2 Comparison with the GB CLP criteria

As there is no evidence to suggest that bixlozone causes carcinogenicity in humans, classification with Category 1A is not considered appropriate.

Classification with category 1B is reserved for substances that are presumed to have carcinogenic potential in humans, largely based on animal evidence. There are no clearly significant tumour findings in the studies presented above to support classification with Category 1B.

Substances are placed in Category 2 on the basis of evidence of a carcinogenic effect in animal studies that is not sufficiently convincing to place the substance in Category 1A or 1B. Thus, in order for bixlozone to be classified in category 2, there must be evidence of a treatment-related increase in tumours in the available animal studies.

In animal studies were no neoplastic findings reported in the rat and the mouse that were considered to be attributable to exposure to bixlozone up to the highest dose tested where general toxicity occurred. Consequently, bixlozone should not be classified for carcinogenicity Category 2, based on no evidence of carcinogenicity in both species.

10.11.3 Conclusion on classification and labelling for carcinogenicity

Not classified – conclusive but not sufficient for classification.

10.12 Reproductive toxicity

10.12.1 Adverse effects on sexual function and fertility

A full assessment for the potential adverse effects on sexual function and fertility of bixlozone has been carried out with a GLP and OECD guideline compliant 2-generation reproductive dietary toxicity study in rats. A range-finding reproduction/developmental study is also available. Additional findings on reproductive organs from the short-term and long-term repeated dose toxicity studies are also taken into consideration.

Table 29: Summary of animal studies on adverse effects on sexual function and fertility

Note:
↑↓ denote an increase or decrease in a parameter with respect to the control value
*Statistical significance: * p ≤ 0.05, ** p ≤ 0.01*
abs. = absolute
rel. = relative
PND = post-natal day

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Dose range finding study **Rats**, Crl:CD(SD), males & females, 10/sex/group Not to guideline GLP: No	Bixlozone technical, batch PL14-0049 Purity: 96 % Oral dietary administration Vehicle: acetone 0, 300, 1000, 3000 ppm (0, 150, 500, 1500 ppm during lactation) Equivalent to: Males: 0, 17, 56 and 176 mg/kg bw/day Females: 0, 20, 62 and 172 mg/kg bw/day (pre-mating), 0, 22, 74 and 217 mg/kg bw/day (gestation) &	***Parents*** **3000 ppm (176 mg/kg bw/day M / 172-217-251 mg/kg bw/day F)** ↓ body weight (-35 %, pre-mating) body weight gain & food consumption in F *Organ weights* ↑ liver weight in M (+ 15 % absolute & +19 % relative) ↑ relative kidney weight in M (+11 %) **1000 ppm (56 mg/kg bw/day M / 62-74-86 mg/kg bw/day F) & 300 ppm** No treatment-related findings. ***Offspring*** **3000 ppm (217-251 mg/kg bw/day)** ↓ body weights in F (-10%) *Organ weights* ↑ relative liver weights (+29 % F & +23 % M) ↑ absolute liver weights in F (+15 %) **1000 ppm (74-86 mg/kg bw/day)** *Organ weights*	**Anon, 2016b** **DAR: B.6.6.1.1**

	0, 23, 86 and 251 mg/kg bw/day (lactation)	↑ relative liver weights in F (+15 %) ↑ absolute liver weights in F (+15%) **300 ppm (22-23 <u>mg/kg bw/day</u>)** No treatment-related findings.	
Two-generation reproductive toxicity Rats, Crl:CD(SD), males & females, 25/sex/group GLP Guideline: OECD 416 (2001) Deviations: none	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: acetone Oral dietary administration 0, 150, 750 & 3000 ppm (reduced to 0, 75, 375 & 1500 ppm during lactation) Equivalent to (most conservative): Males: 0, 7, 34 & 140 mg/kg bw/day Females: 0, 10, 49 & 187 mg/kg bw/day Actual mean test substance intakes are summarised in Table 30 below.	***<u>Parental (systemic) toxicity</u>*** ***<u>F_0 generation</u>*** There were no treatment related deaths or clinical signs of toxicity **<u>3000 ppm (140 / 187 mg/kg bw/day M / F)</u>** ↓ body-weight gains in F (-14 %** days 0-70) ↓ mean body weights in F (-9 %** at gestation) *Organ weights* ↑ absolute liver weights: +13 %** (M) & +12%** (F) ↑ relative liver weights: +19 %** (M) & +18 %** (F) ↑ relative kidney weights: +13 %** (M) & +10 %** (F) *Histopathology* Hepatocellular hypertrophy in F ↑ mononuclear cell infiltration (chronic inflammation) in the prostate **<u>750 (34 / 49 mg/kg bw/day M / F) & 150 ppm (7 / 10 mg/kg bw/day M / F)</u>** No treatment-related findings ***<u>F1 generation</u>*** **<u>3000 ppm (140 / 187 mg/kg bw/day M / F)</u>** ↓ body-weight gains in M** & F ↓ body weights in M** & F* *Organ weights* ↑ absolute liver weights in F (+13 %**)	**Anon, 2016c** **DAR: B.6.6.1.2**

		↑ 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* **750 (34 / 49 mg/kg bw/day M / F) & 150 ppm (7 / 10 mg/kg bw/day M / F)** No treatment-related findings. **Reproductive toxicity** No specific treatment-related adverse effects. **Offspring toxicity** **3000 ppm (187 mg/kg bw/day)** *F1 pups* ↑ relative liver weights: +18 % (M)* *F2 pups* ↓ mean body weights PND 14 (-8 %) **750 ppm (49 mg/kg bw/day) & 150 ppm (10 mg/kg bw/day)** No treatment-related findings.	

10.12.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Range-finding reproduction/developmental study

Groups of 10 Crl:CD(SD) rats /sex / dose were administered dietary concentrations of bixlozone at 0, 300, 1000 and 3000 ppm. In males this equated to test substance intakes of 0, 17, 56 and 176 mg/kg bw/day. In females this equated to test substance intakes of 0, 20, 62 and 172 mg/kg bw/day during the pre-mating period and 0, 22, 74 and 217 mg/kg bw/day during gestation; doses were halved during lactation to maintain consistent dose levels during anticipated increases in food consumption, and equated to 0, 23, 86 and 251 mg/kg bw/day. Four pups/sex/litter were randomly selected on the Post-Natal Day 4 (PND

4) to be exposed during lactation to milk from dams receiving the reduced test substance intakes (0, 150, 500 and 1500 ppm) and for a further 7 days following weaning to the original target concentrations (0, 300, 1000 and 3000 ppm).

Parental toxicity

There were no deaths or treatment-related signs of toxicity. In parental animals, reductions in food consumption, body weight and body weight gain were observed in females at the top dose of 3000 ppm. In males there were no statistically significant, treatment-related changes in the mean body weights or body weight gain at any dose tested; however increases in relative liver weights > 15 % compared to controls accompanied by histopathological findings (hepatocellular hypertrophy) and adverse kidney weight changes were noted at the top dose. No adverse effects were observed at the lower doses in both sexes.

Reproductive toxicity

There was no adverse effect on male or female fertility, mating, copulation, and conception indices, the numbers of days between pairing and coitus at any dose tested and compared to the controls; neither was there any effect on mean gestation duration or parturition performance (mean gestation lengths were 21.8, 21.6 and 21.5 days for the 300, 1000 & 3000 ppm groups respectively, compared with 21.8 days in the concurrent control group). The number of implantation sites was similar across the control and all treated groups.

Offspring toxicity

Bixlozone had no effect on the mean number of pups born, litter size, sex ratio or pup survival. All pups selected for rearing survived to the scheduled necropsy. The only clinical signs noted were a short stature in two animals (one male at 3000 ppm and one female at 1000 ppm) and a missing tail portion of one female at 3000 ppm; these isolated incidences are unlikely to be treatment-related.

Consistent with the toxicity observed in the parent animals, body weights and body-weight gain of pups in were affected by treatment with bixlozone at the top dose; there was no corresponding effect on food consumption. There were no treatment-related macroscopic findings in both sexes but there were adverse increases in liver weights in offspring from 1000 ppm. No adverse effects were observed at the lower doses.

Conclusion

In conclusion, in this reproductive range-finding study in which bixlozone was administered in the diet at 0, 300, 1000 and 3000 ppm, there were no adverse effects on reproductive parameters. Parental treatment-related and adverse effects were observed at 3000 ppm comprising lower mean body weights and increased liver and kidney weights. In offspring,

adverse increased liver weights were observed in females from 1000 ppm and in males at the top dose. In addition, lower pup body weights were noted at the top-dose of 3000 ppm.

Two-generation study (main study)

The potential for bixlozone to adversely affect fertility and reproduction was investigated in a 2-generation reproductive toxicity study in the rat. Groups of 25 male and female Crl:CD(SD) rats were administered 0, 150, 750 or 3000 ppm bixlozone in the diet for 2 successive generations. Actual mean test substance intakes for males and females during pre-mating, mating, gestation and lactation for the F_0 and F_1 generations are summarised in Table 30 below. Parental (F_0) animals were administered the test item for 70 days before mating. Both F_0 and F_1 males continued to receive the test substance throughout mating and females throughout mating, gestation and lactation before termination and necropsy following weaning of their respective litters. Litters received the same diets as their parents following weaning.

Table 30: Test substance intakes for the two-generation rat study

Bold values are the most conservative doses to consider when setting the NOAELs and LOAELs

** The test substance concentration for the F0 and F1 generation females was halved during lactation to account for expected increases in food consumption during this period*

Test substance consumption (mg/kg bw/day)			
Phase of study, generation	**150 ppm**	**750 ppm**	**3000ppm**
Males			
Pre-mating (F_0 males)	10	49	200
Pre-mating (F_1 males)	12	60	238
Post-mating (F_0 males)	7	34	141
Post-mating (F_1 males)	**7**	**34**	**140**
Mean (males)	*9*	*44*	*180*
Females*			

Pre-mating (F_0 females)	11	53	209
Pre-mating (F_1 females)	12	59	241
Gestation (F_0 females)	10	50	203
Gestation (F_1 females)	**10**	**49**	**187**
Lactation (F_0 females)	12	62	261
Lactation (F_1 females)	12	59	255
Mean (females)	*11*	*55*	*226*

Parental toxicity (F_0 & F_1)

There were no treatment-related deaths found for the F_0 generation; 2 deaths occurred in the control and low-dose female groups only, they were not attributable to treatment with bixlozone. In the F_1 generation, a female of the mid-dose group was also found dead, but this was attributed to a mechanical head injury.

Observed clinical signs at F_0 and F_1 comprising hair loss on the forelimbs and facial area, decreased defecation and red material around the nose, occurred sporadically in all groups (including the control group) and hence were not considered treatment-related.

In relation to general toxicity in parental animals, adequate toxicity was achieved and in line with the findings of the repeated-dose toxicity studies (Section **Error! Reference source not found.**), 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) were also noted at the top dose in both generations. No adverse effects were observed at the lower dose of 34 / 49 mg/kg bw/day.

Reproductive parameters

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, this finding is considered of minimal toxicological significance. There was also no effect on litter size, sex ratio or pup survival up to the highest dose tested in the study.

It was noted that the age of attainment of vaginal opening of F1 pups was statistically significantly greater at 3000 ppm compared to the corresponding control (33.6 days compared with 31.7 days). The mean body weights of the female pups reached at the age of attainment were unaffected by treatment with bixlozone in all groups, 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, the Agency considers this finding the secondary consequence of reduced post-weaning female pup body weight development and not a specific reproductive effect of bixlozone.

Offspring toxicity (F_1 & F_2)

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. No adverse effects were observed at the lower dose of 34 /49 mg/kg bw/day in both generations.

Conclusion

In conclusion, in this 2-generation reproductive toxicity study bixlozone was administered in the diet at 0, 150, 750 or 3000 ppm; there were no adverse effects on reproductive parameters. Parental treatment-related and adverse effects were observed at the top-dose of 3000 ppm comprising lower mean body weights and increased liver and kidney weights which correlated with histopathology findings for the liver. In addition, mononuclear cell infiltration (chronic inflammation) in the prostate was observed in both generations. The administration of bixlozone did not have a specific effect on fertility, reproduction, pregnancy outcome or pup survival up to the top-dose tested of 3000 ppm. Consistent with the parental toxicity, body weights and body weight gain of pups in the F_2 generation were

affected by treatment with bixlozone at the top-dose, whilst relative liver weights were adversely increased in male pups of the F_1 generation.

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 (Section **Error! Reference source not found.**). 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. An increase in the number of malignant cervical tumours was also seen in females at the top dose of 834 mg/kg bw/day at terminal sacrifice in the 18-month mouse carcinogenicity study (see carcinogenicity section). Again, these findings, occurring during the reproductive senescence of the female mouse are 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 (Section **Error! Reference source not found.**). 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.

10.12.3 Comparison with the GB CLP criteria

Bixlozone has been tested in a modern guideline and GLP compliant 2-generation study in Crl:CD(SD) rats. Further information has also been provided by a preliminary dose range-finding study performed with the same strain of rats.

Bixlozone is a new active substance thus there is limited human data to rely on; however there is no evidence that bixlozone is a reproductive toxicant in humans, therefore classification in 1A is not appropriate.

Classification in Category 2 is reserved for substances where there is some evidence from human or experimental animals of an adverse effect on sexual function and fertility. Such effects should be observed in the absence of other toxic effects or if occurring together with other toxic effects the adverse effect is considered not to be a secondary non-specific consequence of other toxic effects. On the basis that there is no evidence that bixlozone causes any specific adverse effects to sexual function or fertility up to the top dose tested where parental toxicity occurs, it should not be classified in this category. Therefore no classification for this endpoint is warranted for bixlozone.

10.12.4 Adverse effects on development

The developmental toxicity of bixlozone has been investigated in GLP and 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.

Table 31: Summary of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Pre-natal developmental toxicity study **Rats**, Crl:CD(SD), females, 25/group Guideline: OECD 414 (2001) Deviations: None of significance ***Acceptable***	Bixlozone technical, batch PL14-0049 Purity: 96% Vehicle: 0.5% (w/v) carboxymethylcellulose (CMC) in 5% tween® 80 Oral gavage administration 0, 75, 225 & 550 mg/kg bw/day Dose volume: 10 mL/kg	***Maternal toxicity*** **550 mg/kg bw/day** Clinical signs: red, yellow and/or clear material on various body surfaces Early ↓ body-weight gain: -90 % (GD 6-9)** ↓ body-weight gain: -16 % (GD 6-19)** ↓ body weight: -6 % (GD 20)** ↓ net body-weight gain: -29.5 %** ↓ net body weight: -7 %** ↓ food consumption: -11.5 % (GD 6-20)** *Organ weights* ↑ liver weight: +29 %** (absolute) & +38 %** (relative) *Histopathology* Hepatocellular hypertrophy: 7/25 (mild) & 18/25 (moderate) **225 mg/kg bw/day** Clinical signs: red, yellow and/or clear material on various body surfaces	**Anon, 2016e** **DAR: B.6.6.2.2**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Early ↓ body-weight gain: -40 % (GD 6-9)* ↓ food consumption: -8 % (GD 6-20)* **<u>75 mg/kg bw/day</u>** No adverse effects **<u>Developmental toxicity</u>** No treatment-related findings.	
Dose-range finding pre-natal developmental toxicity study **Rats**, Crl:CD(SD), females, 8/group Not to guideline GLP: No ***Supplementary only***	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: 0.5 % (w/v) carboxymethylcellulose (CMC) in 5% tween® 80 Oral gavage administration 0, 25, 75, 225 and 675 mg/kg bw/day Dose volume: 10 mL/kg	***<u>Maternal toxicity</u>*** **<u>675 mg/kg bw/day</u>** One death (sacrificed on GD11) ↓ body weight (- 6.9 to 8.5 %) ↓ body-weight gain (-36 %) ↓ net body weight (-7.5 %) ↓ net body weight gain (-45 %) ↓ food consumption (-18 %) *Organ weights* ↑ liver weight: 64 % (relative) & 52 % (absolute) **<u>225 mg/kg bw/day</u>** *Organ weights* ↑ liver weight: 17 % (absolute & relative) **<u>75 mg/kg bw/day & 25 mg/kg bw/day</u>** No treatment-related findings ***<u>Developmental toxicity</u>***	**Anon, 2016d** **DAR: B.6.6.2.1**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		675 mg/kg bw/day ↓ foetal body-weights (- 8 % M & F)	
Pre-natal developmental toxicity study **Rabbits**, New Zealand White (Hra:(NZW)SPF), females, 25/group Guideline: OECD 414 (2001) Deviations: None GLP ***Acceptable***	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: carboxymethylcellulose (CMC) in 5% tween® 80 Oral gavage administration 0, 25, 75, 200 & 400 mg/kg bw/day	***Maternal toxicity*** There were no treatment-related deaths. **400 mg/kg bw/day** ↓ defecation ↓ body-weight gain -32 % (GD 13-20) ↓ food consumption -18 % (GD 13-20) **25, 75, 200 mg/kg bw/day** No treatment-related findings. ***Developmental toxicity*** No treatment-related findings.	**Anon, 2015a** **DAR: B.6.6.2.4**
Dose-range finding pre-natal developmental toxicity study **Rabbits**, New Zealand White (Hra:(NZW)SPF), females, 6/group Not to guideline GLP: No ***Supplementary only***	Bixlozone technical, batch PL14-0049 Purity: 96 % Vehicle: carboxymethylcellulose (CMC) in 5% tween® 80 Oral gavage administration 0, 100, 350, 750 & 1000 mg/kg bw/day	***Maternal toxicity*** **1000 mg/kg bw/day** There were two deaths on GD 17 & 19; all remaining animals were sacrificed on GD 19 due to severe toxicity ↓ body weight -13 % ↓ food consumption -85 % **750 mg/kg bw/day** There were two deaths; all remaining animals were sacrificed on GD 23 due to severe toxicity ↓ body weight -16 %	**Anon, 2014a** **DAR: B.6.6.2.3**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ food consumption -86 % **<u>350 mg/kg bw/day</u>** ↓ body-weight gain -27 % (not statistically significant) ↓ net body-weight gain ↓ food consumption -13 % **<u>100 mg/kg bw/day</u>** ↓ body-weight gain -42 % (not statistically significant) ↓ net body-weight gain ↓ food consumption -19 % ***<u>Developmental toxicity</u>*** No treatment-related findings.	

10.12.5 Short summary and overall relevance of the provided information on adverse effects on development

Rat studies

In a developmental range-finding study (Anon, 2016d), administration of bixlozone to rats throughout gestation resulted in maternal toxicity at the highest dose tested (675 mg/kg bw/day), characterised by 1 death, body weight loss, reduced body weight gain and increases in absolute and relative liver weights. Initial body weight loss and increased liver weight were also observed at 225 mg/kg bw/day. No external or visceral malformations or variations that were attributable to treatment with bixlozone were apparent but foetal weight was reduced at the top dose.

Based on the findings of this study, doses of 75, 225 and 550 mg/kg bw/day were selected for the main pre-natal developmental toxicity study (Anon, 2016e). Female Crl:CD(SD) rats (25/group) were administered bixlozone technical as a 10 mL/kg gavage dose, once

daily throughout gestation days (GD) 6-19; the concurrent control group received the same volume of vehicle (0.5 % [w/v] CMC in 5 % Tween® 80) under identical experimental conditions.

Parental toxicity

In the rat developmental toxicity study, 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.

GD 20 laparohysterectomy data

Bixlozone had no effect on intrauterine growth and survival according to any of the parameters measured (post-implantation loss, live litter size, mean foetal body-weights and foetal sex ratios). Furthermore, the mean number of corpora lutea and implantation sites and the mean litter proportions of pre-implantation losses were similar across all treated and control groups.

Foetal toxicity

The number of foetuses (litter) available for morphological examination were 391 (25), 390 (25), 384 (25) and 380 (24) in the control, 75, 225 and 550 mg/kg bw/d dose groups respectively. At these same respective dose-groups malformations were observed at a rate of 2 (2), 3 (2), 2(2) and 2 (2) foetuses (litter).

Some skeletal variations were apparent, but these were either not dose-related or only marginally increased above controls and thus considered of no toxicological significance (Table 32). An increased incidence of 14th rudimentary rib and sternebrae malaligned (slight or moderate) was observed at the top-dose. However these are common variations and they did not show a dose-related response. Moreover the increases were not statistically significant compared to the controls and occurred in the presence of significant maternal toxicity. Overall they are not considered to be of toxicological significance by the Agency.

In conclusion no evidence of developmental toxicity was observed in the rat at any dose tested and up to doses causing clear maternal toxicity.

Table 32: Summary of variations from the rat developmental toxicity study

Dose (mg/kg bw/day)	**0**	**75**	**225**	**550**
Total foetuses examined	**391**	**390**	**384**	**380**
Total litters examined	**25**	**25**	**25**	**24**
Detailed foetus (litter) incidence - Visceral variations (absolute number)				
Renal papilla(e) not developed and/or distended ureter(s)	2 (1)	3 (3)	0	0
Liver- accessory lobule(s)	2 (1)	1 (1)	0	0
Kidney(s)- small	0	2 (1)	0	0
Haemorrhagic ring around the iris	1 (1)	1 (1)	0	0
Detailed foetus (litter) incidence - Skeletal variations (absolute number)				
Cervical centrum #1 ossified	49 (16)	66 (15)	50 (19)	51 (18)
Sternebra(e) #5 and/or #6 unossified	54 (17)	55 (21)	50 (18)	51 (15)
Hyoid unossified	6 (3)	8 (5)	3 (3)	3 (3)
14th rudimentary rib(s)	31 (8)	14 (11)	28 (13)	49 (17)
% litter incidence	7.7	3.6	7.2	13.0
Sternebra(e) malaligned (slight or moderate)	3 (2)	1 (1)	2 (2)	6 (5)
% litter incidence	0.8	0.2	0.5	1.5
7th cervical rib(s)	2 (2)	3 (3)	5 (2)	2 (2)
Sternebra(e) #1,#2,#3 and/or #4 unossified	3 (3)	5 (4)	1 (1)	3 (2)
Reduced ossification of the vertebral arches	0	3 (2)	1 (1)	3 (3)
Reduced ossification of the skull	0	6 (3)	0	0
Bent rib(s)	0	5 (3)	4 (2)	3 (2)
Pubis unossified	1 (1)	0	0	2 (2)
27 presacral vertebrae	0	0	0	1 (1)
Reduced ossification of the 13th rib(s)	2 (2)	1 (1)	1 (1)	0
Spherical enlargement of the rib(s)	0	0	1 (1)	0
Ischium unossified	0	0	0	1 (1)
Litter incidence (%) of visceral Variations	1.3	1.9	0	0
Litter incidence (%) of skeletal Variations	35.1	35.2	37.7	34.9
Total litter incidence for variations	36.1	36.4	37.7	34.9

Rabbit studies

In a range-finding study (Anon, 2014a), administration of bixlozone to New Zealand white rabbits caused severe maternal toxicity at the top-doses of 1000 and 750 mg/kg bw/day that resulted in the early termination of these animals. Maternal toxicity was also evident at the lower doses of 100 and 350 mg/kg bw/day characterised by lower body-weight gain, net body-weight losses, reduced food consumption and reduced defecation. In contrast to previous studies in rats, lower absolute and relative liver weights were noted at 100 and 350 mg/kg bw/day (possibly a consequence of reduced food consumption at these doses).

Owing to the early sacrifice of the rabbits at the higher doses, no liver weights or caesarean section data was evaluated at these doses. There was no indication of developmental toxicity at any dose.

Based on the results of this study, doses of 25, 75, 200 and 400 mg/kg bw/day were selected for a main developmental toxicity study in rabbits (Anon, 2015a). In this GLP and guideline-compliant study, groups of 25 female New Zealand White rabbits were administered bixlozone with daily doses at 0, 25, 75, 200 and 400 mg/kg bw/day from gestation day (GD) 7 to GD 28 by gavage. The vehicle used was 0.5% [w/v] carboxymethylcellulose (CMC) in 5% Tween® 80 whilst the dosage volume for all groups was 5 mL/kg.

Parental toxicity

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 was noted in dams at the highest dose of 400 mg/kg bw/day. No adverse maternal effects were noted at lower doses.

GD 29 laparohysterectomy data

Intrauterine growth and survival were unaffected by test substance administration at all dosage levels. Parameters evaluated included post implantation loss, live litter size, mean foetal body weights, and foetal sex ratios. Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar across all groups. Differences from the control group were slight and not statistically significant.

Foetal toxicity

The numbers of foetuses (litters) available for morphological evaluation were 212(23), 211(24), 221(25), 199(22), and 210(24) in the control, 25, 75, 200, and 400 mg/kg bw/day groups, respectively. Malformations were observed in 5(4), 3(2), 5(4), 3(2), and 2(2) foetuses (litters) in these same respective groups and were considered by the Agency to be spontaneous in origin.

There were no external malformations (either treatment-related or otherwise) at any dose. No treatment-related soft tissue malformations were observed; however several spurious soft tissue malformations were noted and are summarised in Table 33. These soft tissue malformations were also present at a similar frequency in the control group; furthermore none of these incidences occurred in the high-dose group; hence the Agency considers that the effects were not related to treatment with bixlozone. There were no treatment-related skeletal malformations. All of these malformations occurred at no greater incidence than the concurrent control group and did not show a clear dose-response.

No treatment-related soft tissue (visceral) variations were noted; variations that occurred in all groups included accessory spleen(s), major blood vessel variation (no brachiocephalic trunk), extra papillary muscle in the heart or only 2 papillary muscles, retrocaval ureter, absent, small, or bilobed gallbladder, small or pale spleen, pale liver, accessory liver lobule, and/or haemorrhagic ring around the iris. These visceral variations did not occur in a dose-related manner, were noted similarly in the concurrent control group, and were therefore considered to be unrelated to treatment by the Agency.

No treatment-related skeletal variations were noted; findings across all groups included 13th full and/or rudimentary ribs, sternebrae (unossified, malaligned or misshapen), presacral vertebrae, extra ossification of sternebra, 7th cervical ribs or sternebra, bent hyoid arches, accessory skull bones, vertebral centra not fully ossified, reduced ossification of the skull and a hole in the xyphoid cartilage. These findings did not occur in a dose-related manner and were noted similarly in the concurrent control group; therefore, none of these were considered to be treatment-related by the Agency. The incidence of sternebrae with thread-like attachment was increased (3 in 3 litters vs 0 in controls) at the top dose. However, considering the very low incidence and the isolated nature of the observation (with no clear pattern of skeletal variations), this is most likely a chance finding unrelated to treatment with bixlozone.

A distended, gas-filled stomach was observed in one foetus of the 400 mg/kg bw/day group and cystic oviducts were observed for one foetus in the control group and two foetuses in the 75 mg/kg bw/day group. These findings were not classified as either malformations or variations and hence were not included in the summary tables. In any case, they were not treatment-related (they occurred infrequently, at similar frequencies in the control group, and/or in a manner that was not dose-related).

Overall, there was no treatment-related developmental toxicity noted in the rabbit up to the highest dose tested.

Table 33: Summary of the malformations found in the rabbit developmental toxicity study:

Dose (mg/kg bw/day)	**0**	**25**	**75**	**200**	**400**
Total foetuses examined	212	211	221	199	210
Total litters examined	23	24	25	22	24
Visceral malformation					
Foetal incidence	**3**	**3**	**3**	**1**	**0**
Litter incidence	**3**	**2**	**2**	**1**	**0**
Detailed foetus (litter) incidence - Visceral malformation					
Persistent truncus arteriosus	0	1(1)	1(1)	1(1)	0
Interventricular septal defects	0	1(1)	1(1)	1 (1)	0
Lungs – lobular agenesis	2(2)	2(2)	2(1)	0	0
Vena cava – malpositioned	1(1)	0	0	0	0
Skeletal malformation					
Foetal incidence	**3**	**0**	**2**	**3**	**2**
Litter incidence	**3**	**0**	**2**	**2**	**2**
Detailed foetus (litter) incidence - Skeletal malformation					
Sternebrae fused	1 (1)	0	0	0	1
Vertebral anomaly with or without associated rib anomaly	1 (1)	0	0	2 (1)	1 (1)
Vertebral central anomaly	1(1)	0	0	0	0
Severe maligned sternebrae	0	0	2(2)	1(1)	0
Costal cartilage anomaly	0	0	1(1)	0	0
Total malformations					
Total Foetal incidence	**5**	**3**	**5**	**3**	**2**
Total Litter incidence	**4**	**2**	**4**	**2**	**2**

Table 34: Summary of variations from the rabbit developmental toxicity study

Dose (mg/kg bw/day)	**0**	**25**	**75**	**200**	**4**
Total foetuses examined	212	211	221	199	210
Total litters examined	23	24	25	22	24
Detailed foetus (litter) incidence - Visceral variations (absolute number)					
Accessory spleen(s)	31 (14)	28 (15)	42 (18)	19 (12)	29 (14)
Heart- extra papillary muscle	9 (7)	12 (7)	7 (5)	12 (9)	7 (6)
Major blood vessel variation	7 (6)	24 (10)	8 (5)	7 (5)	15 (8)
Retrocaval ureter	1 (1)	0	4 (3)	6 (5)	1 (1)
Spleen- pale	1 (1)	2 (1)	0	0	0
Gallbladder - absent or small	0	0	0	1 (1)	0

Dose (mg/kg bw/day)	**0**	**25**	**75**	**200**	**4**
Total foetuses examined	212	211	221	199	210
Total litters examined	23	24	25	22	24
Heart - only two papillary muscles present	1 (1)	1 (1)	0	1 (1)	3 (1)
% per litter	0.5 ± 2.32	0.4 ± 1.86	0.0 ± 0.00	0.4 ± 1.94	1.4 ± 6.80
Liver - accessory lobule(s)	1 (1)	1 (1)	0	0	2 (1)
Spleen - small	0	0	0	1 (1)	0
Liver- pale	0	0	8 (1)	1 (1)	0
Haemorrhagic ring around the iris	1 (1)	2 (2)	0	0	1 (1)
Gallbladder- bilobed	0	0	0	1 (1)	0
Detailed foetus (litter) incidence - skeletal variations (absolute number)					
13th rudimentary rib(s)	36 (17)	37 (19)	25 (16)	43 (18)	31 (19)
Sternebra(e) #5 and/or #6 unossified	24 (12)	28 (13)	16 (10)	18 (7)	25 (10)
13th full rib(s)	50 (19)	90 (22)	84 (18)	78 (16)	65 (17)
% per litter	25.7 ± 23.4	42.6 ± 24.7	37.5 ± 30.0	37.1 ± 27.5	33.5 ± 31.7
Accessory skull bone(s)	1 (1)	0	0	1 (1)	0
Extra site of ossification anterior to sternebra #1	6 (4)	3 (3)	3 (2)	5 (3)	2 (2)
Sternebrae with thread-like attachment	0	0	1 (1)	0	3 (3)
% per litter	0.0 ± 0.00	0.0 ± 0.00	0.4 ± 1.8	0.0 ± 0.00	1.4 ± 3.8
27 presacral vertebrae	4 (2)	10 (6)	14 (12)	17 (7)	8 (7)
Hyoid arch(es) bent	2 (2)	1 (1)	3 (3)	5 (4)	1 (1)
7th cervical rib(s)	1 91)	0	10 (7)	7 (4)	0

Dose (mg/kg bw/day)	**0**	**25**	**75**	**200**	**4**
Total foetuses examined	212	211	221	199	210
Total litters examined	23	24	25	22	24
7th sternebra	1 (1)	0	1 (1)	0	0
25 presacral vertebrae	1 (1)	0	2 (2)	0	0
Sternebra(e) malaligned(slight or moderate)	2 (2)	0	5 (5)	3 (3)	0
Vertebral centra not fully ossified	0	0	1 (1)	2 (2)	0
Reduced ossification of the skull	0	0	3 (2)	0	0
Xyphoid cartilage- hole	0	0	0	1 (1)	0
Sternebra(e)- misshapen	0	0	0	1 (1)	0
Total Variations					
Litter incidence (%) of visceral Variations	**23.2**	**30.5**	**30.6**	**21.2**	**27.7**
Litter incidence (%) of skeletal Variations	**54.4**	**67.2**	**60.5**	**70.3**	**58.3**

Other studies

In the rat 2-generation study (Anon, 2016c), Section **Error! Reference source not found.**) there were no effects of treatment on pup survival, sex ratio, developmental landmarks and preputial separation up to the top dose of 140 mg/kg bw/day at which parental and offspring toxicity occurred.

10.12.6 Comparison with the GB CLP criteria

Bixlozone has been tested in guideline prenatal developmental studies in rats and rabbits. A specific effect on the development of the unborn fetus was not observed in either species.

Classification in Category 1A for effects on development is required when a substance is a known human reproductive toxicant. Bixlozone is a new active substance thus there is limited human data to rely on. Nevertheless, as there is no evidence to suggest that

bixlozone causes reproductive toxicity in humans, classification with Category 1A is thus not necessary.

In order to be classified in Category 1B, there must be clear evidence from animal studies of an adverse effect on development occurring in the absence of other toxic effects. As there was no clear evidence to suggest bixlozone should be a presumed human reproductive toxicant, classification in this category is not appropriate.

Classification in Category 2 is required if a substance is considered a suspected human reproductive toxicant. In guideline and GLP-compliant studies conducted in the rat (Anon, 2016e) and the rabbit (Anon, 2015a) there was no developmental toxicity noted up to the highest doses tested.

Overall, it can be concluded that bixlozone is not a developmental toxicant. Classification for this endpoint is not warranted for bixlozone.

10.12.7 Adverse effects on or via lactation

10.12.8 Short summary and overall relevance of the provided information on effects on or via lactation

One- or two-generation reproductive toxicity studies in animals can provide clear evidence of adverse effects on or via lactation in the offspring owing to transfer in the milk or adverse effects on the quality of the milk. Moreover absorption, metabolism, distribution and excretion studies can indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

In the relevant studies available for bixlozone, such as the 2-generation reproductive toxicity study in the rat and the dose range-finding study, there were no adverse effects on or via lactation observed in all treated groups compared to the control groups. There is no absorption, metabolism, distribution and excretion data available which indicate that the substance could be present in potentially toxic levels in breast milk (Section **Error! Reference source not found.**).

10.12.9 Comparison with the GB CLP criteria

Classification for effects on or via lactation might be assigned where: there is human evidence that indicates a hazard to babies during the lactation period; in the case of bixlozone, there is no human data to inform on this end-point since this is a new active substance. In the available two-generation study in rats, there was no evidence to suggest that bixlozone had an adverse effect on lactation or via lactation; therefore classification with this endpoint is not appropriate.

10.12.10 Conclusion on classification and labelling for reproductive toxicity

There was no evidence to suggest that bixlozone had an adverse effect on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring (including on lactation or via lactation), therefore classification with this endpoint is not warranted.

Not classified – conclusive but not sufficient for classification.

10.13 Aspiration hazard

There are no data available for this endpoint.

11. Evaluation of environmental hazards

Available environmental fate and hazard studies have been considered under the GB CLP Regulation; these are summarised in the Draft Assessment Report (DAR), GB 2022.

11.1 Rapid degradability of organic substances

Table 35: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradation OECD Guideline 301B, GLP	Not readily biodegradable - Maximum of 13% (single replicate) biodegradation after 29 days at 22 °C	Valid	Shannon, M., 2017 (section CA.B.8.2.2.1 in the DAR)
Aquatic hydrolysis OECD Guideline 111, GLP	Stable (<5% degradation) at pH 4 and 7 (50°C) and 9 (25°C)	Valid	Roohi, A.& Cooper, T., 2015 (section CA.B.8.2.1.1 in the DAR)
Aerobic mineralisation OECD Guideline 309, GLP	Not significantly degraded - <10% Applied Radioactivity mineralised after 63 days at 20°C	Valid	Simmonds, R., 2018 (section CA.B.8.2.2.2 in the DAR)
Water/sediment simulation OECD Guideline 308, GLP	$DegT_{50}$ of 23.3 to 24.8 days (20°C) based on whole system. Mineralisation: maximum 52% Applied Radioactivity	Valid	Cooper, J.; Challis, P., 2018 (section CA.B.8.2.2.3 in the DAR)
Aquatic photolysis OECD Guideline 316, GLP	Bixlozone DT_{50} of 44.0 – 54.4 days	Valid	O'Connell, C., 2015 (section B.8.2.1.2 in the DAR)

11.1.1 Ready biodegradability

The applicant submitted a ready biodegradability study in accordance with OECD Guideline 301B (CO_2 Evolution (Modified Sturm Test)) and to GLP. The study was undertaken over 29 days at 22°C in the dark on non-radiolabelled bixlozone, with sodium benzoate used as a reference substance. A sample of activated sludge was collected from a sewage treatment works which has a predominantly domestic waste-water catchment. Bixlozone showed limited biodegradation with a maximum replicate biodegradation of 13% during the study. This is less than the 60% of theoretical maximum CO_2 generation over 28 days used as a criterion for this study type, therefore, bixlozone cannot be considered readily biodegradable.

11.1.2 BOD_5/COD

Not applicable

11.1.3 Hydrolysis

The applicant submitted an aquatic hydrolysis study in accordance with OECD Guideline 111 and to GLP. 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 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.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not applicable

11.1.4.2 Inherent and enhanced ready biodegradability tests

Not applicable

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

A study of aerobic mineralisation in surface water was carried out in accordance with OECD Guideline 309 and to GLP. 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^{o}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 mineralisation in two UK water/sediment systems was carried out in accordance with OECD Guideline 308 and to GLP. 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. 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 exposure assessment was 16 days (Single First Order (SFO) kinetics), derived from Swiss Lake system. The longest non-normalised sediment DissT50, to be used in the UK spray drift calculations, is 35.2 days (SFO), derived from the Calwich Abbey test system. The total-system DegT50 for Calwich Abbey and Swiss Lake were 23.3 d (SFO) and 24.8 d (Hockey Stick (HS) kinetics) respectively.

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). No kinetic assessment was undertaken on the metabolites formed, with the applicant electing to use default values of 1000 d in the exposure assessment instead.

11.1.4.4 Photochemical degradation

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.

11.1.4.5 Conclusion on ‘rapid degradability’

Overall, bixlozone does not meet the CLP criteria to be classed as “rapidly degradable” in the environment as there is no evidence to suggest that the active substance is at least 70% degraded in the aquatic environment within 28 days. The active substance is not readily biodegraded and is stable to hydrolysis. Slow degradation was observed in the aerobic mineralisation study and moderate degradation in the aquatic photolysis study. Shorter total-system DT50 values were calculated in the water/sediment simulation study (23.3 d – 24.8 d), however, 70% degradation was not observed in either test system within 28 days. No kinetic assessment was undertaken on the metabolites formed in the water/sediment simulation study, with the applicant electing to use default values of 1000 d in the exposure assessment instead. Therefore, none of the metabolites are classed as being rapidly degradable either.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.3 Environmental fate and other relevant information

11.3.1 Volatilisation

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). A wind tunnel study determined the highest deposition of 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.

11.3.2 Soil adsorption and desorption

A sorption study was submitted by the applicant using [phenyl-U-^{14}C]-bixlozone in accordance with OECD Guideline 106 and to GLP. The study used the batch equilibrium method and was undertaken on 8 soils (5 of European origin and 3 of US origin). 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). 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.

11.4 Bioaccumulation

One study has been performed measuring the bioaccumulation of bixlozone in fish. This study is summarised in Table 36 below and further discussion is included in Section 11.4.2.

Table 36: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioaccumulation in fish OECD 305 (2012) OCSPP Draft Guideline 850.1730 GLP 17-day exposure phase, 7-day	77.5 L kg^{-1} (whole fish at 13.0 µg a.s./L) 71.7 L kg^{-1} (whole fish at 130 µg a.s./L)	Study considered suitable for use in hazard classification.	**Anon, 2016k** **DAR: B.9.2.2.3**

Method	Results	Remarks	Reference
depuration phase, flow-through exposure	Values are lipid normalised (5.35 %) Geometric mean of 74.5 L kg^{-1}		
	Log K_{ow}: 3.3 at pH 4, 7, 9 (20 ^{o}C)		Colwyn (2016c)

11.4.1 Estimated bioaccumulation

Not applicable, measured partition coefficient and bioaccumulation test data available.

11.4.2 Measured partition coefficient and bioaccumulation test data

The log K_{ow} was determined using the shake flask method in accordance with OECD 107. The measured partition coefficient is a log K_{ow} of 3.3 at 20 ^{o}C (pH 4, 7 and 9).

The bioconcentration and metabolism study with bluegill sunfish (*Lepomis macrochirus*) was conducted to GLP and was evaluated according to OECD 305 (2012) and OCSPP Draft Guideline 850.1730. The study met the relevant validity criteria. However, it should be noted that there are a number of deviations from the OECD 305 guideline recommendations which is not ideal. The major issues are outlined below:

The fish in this study were only acclimated to the study conditions for 7 days, rather than 14 days which is the recommended minimum time in OECD 305. However, there was very little mortality in the acclimation period, which meant that the fish were clearly acclimated and settled into the environmental conditions before the test was initiated.

It is noted that the age of the fish was not reported. OECD 305 states that age of a fish may have a significant effect on BCF values, so all fish should be from the same year-class. Although the study protocol states that the fish should be a similar age, this was not confirmed in the study report. It is noted that the fish were obtained as a single batch from the supplier and a sub-sample of 30 fish had a weight range of 2.81 – 4.26 g, and a length range of 45-69 mm, which supports the possibility that these fish were of a similar age. Following a request for additional information, the applicant confirmed that the specific ages of the fish were not determined but that juvenile fish from the same age class were used. This is considered acceptable.

Lipid content was determined in fish that were not the same as those used to determine the concentration of the test substance. OECD state that in this case, the fish should at least be of a similar weight and the same sex. However, the study report does not state the sex of the fish so it is unclear whether the lipid analysis results are representative of the fish population in the tanks. Following a request for additional information, the applicant confirmed that the sex of the fish was not determined as this would require

sacrifice and direct observation of gonads. This adds to uncertainty in the lipid analysis result of 5.35 % as being representative of those fish sampled to measure active substance concentrations and ascertain the bioconcentration factor, however it does not invalidate the test.

The study protocol says that the flow rate will be checked daily, which is in accordance with the OECD 305 guideline (2012). However, this is not mentioned in the study report. OECD 305 also states that the flow through rate through each test chamber should not vary by more than 20 % either within or between chambers. This cannot be confirmed by data included in the study report. However, the delivery system was reported as calibrated to deliver a flow of 420 mL/min of dilution water. Following a request for additional information, the applicant confirmed that the flow rate was checked twice daily and toxicant/solvent used recorded in the raw data.

Fish loading exceeds 1g of fish (wet weight) per litre of water per day on several occasions during the test. However, these occasions are few and only exceed the loading rate by a small amount. The oxygen saturation was also kept above 60 % throughout the exposure and depuration phase of the test. Therefore, this deviation was not considered to invalidate the test.

Whilst the deviations outlined above are not ideal, they are not considered to invalidate the study and warrant further vertebrate testing, noting that the BCF value is well below the trigger of 500. **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 measured log K_{ow} for bixlozone is 3.3 (see Section 7, Table 7 and Section 11.4, Table 36), according to the guidance on the application of the CLP criteria (ECHA, 2017) this is below the log K_{ow} criterion of 4 and indicates a low bioaccumulation potential for aquatic hazard classification purposes. Also according to this guidance, a measured whole fish BCF should be used in preference when available to conclude on the bioaccumulation potential of a substance (BCF ≥ 500 indicates bioaccumulation potential). As the measured BCF values are < 500, it can therefore be concluded that bixlozone does not meet the CLP criteria as a bioaccumulative substance

11.5 Acute aquatic hazard

A summary of the suitable aquatic toxicity studies for bixlozone (both short- and long-term), under Reg. (EC) 1107/2009 are provided in Table 37. All the listed studies have been conducted according to GLP. The studies have been evaluated, considered valid according to the criteria of the respective test guidelines and deemed suitable for hazard classification purposes. Some of the available studies were considered unreliable and hence have not been included here. Only studies submitted testing the technical substance, bixlozone, have been summarised below (formulation studies submitted in the

context of the active substance renewal under Reg. (EC) 1107/2009 have not been considered further here). The following metabolites of bixlozone: 2,4-dichlorobenzoic acid, 4-carboxyl-F9600, F9600-dimethyl-malonamide and F9600-3-OH-propanamide were also tested in short-term aquatic toxicity studies that were reviewed under Reg. (EC) 1107/2009. None of these metabolites exhibit equivalent toxicity to bixlozone so are not considered relevant for hazard classification (see Annex I for a list of metabolite toxicity endpoints).

Table 37: Summary of relevant information on acute aquatic toxicity of technical bixlozone

Method[1]	Species	Endpoint	Results	Remarks	Reference
Fish					
Acute toxicity to fish OECD 203 (1992) GLP 96-hours, static exposure	*Oncorhynchus mykiss*	Mortality	**LC_{50} 9.8 mg a.s./L (mm)**	Study considered suitable for use in hazard classification.	**Anon, 2015g** **DAR: B.9.2.1**
Acute toxicity to fish OECD 203 (1992) GLP 96-hours, static exposure	*Lepomis macrochirus*	Mortality	LC_{50} 13 mg a.s./L (mm)	Study considered suitable for use in hazard classification.	**Anon, 2015h** **DAR: B.9.2.1**
Acute toxicity to fish OECD 203 (1992) GLP 96-hours, static exposure	*Cyprinodon variegatus*	Mortality	LC_{50} 14 mg a.s./L (mm)	Study considered suitable for use in hazard classification.	**Anon, 2015i** **DAR: B.9.2.1**
Fish early-life stage toxicity OECD 210 GLP	*Pimephales promelas*	Hatchability, average days to hatch, rate of developmental abnormality, survival rate, body weight, **total length**	EC_{10}, total length 4.6 mg a.s./L (mm) EC_{20}, total length 7.6 mg a.s./L (mm)	Study considered suitable for use in hazard classification.	**Anon, 2016j** **DAR: B.9.2.2.1**

32 days Flow-through			**NOEC, total length 0.38 mg a.s./L (mm)**		
Aquatic invertebrates					
Acute toxicity OECD 202 (2004) GLP 48-hours, static exposure	*Daphnia magna*	Immobility	EC_{50} >2.6 mg a.s./$L_{(mm)}$	Study considered suitable for use in hazard classification.	**Shaw (2015)** **DAR: B.9.2.4.1**
Acute toxicity OCSPP Draft Guideline 850.1035 GLP 96-hours, static exposure	*Americamysis bahia*	Mortality	LC_{50} 0.14 mg a.s./$L_{(mm)}$	Study considered suitable for use in hazard classification.	**Shaw (2016a)** **DAR: B.9.4.2**
Acute toxicity OECD 202 (2004)/OECD 235 (2011) GLP 48-hours, static exposure	*Caecidotea communis*	Immobility	EC_{50} >1.6 mg a.s./$L_{(mm)}$	Study considered suitable for use in hazard classification.	**Mason (2017a)** **DAR: B.9.2.4.2**
Acute toxicity OECD 235 (2011) GLP 48-hours, static exposure	*Chironomus riparius*	Immobility	EC_{50} 1.9 mg a.s./$L_{(mm)}$	Study considered suitable for use in hazard classification.	**Mason (2017d)** **DAR: B.9.2.4.2**
Acute toxicity OECD 202 (2004)/OECD 235 (2011)	*Pycnopsyche gentilis*	Immobility	EC_{50} 0.33 mg a.s./$L_{(mm)}$	Study considered suitable for use in hazard classification.	**Mason (2018a)** **DAR: B.9.2.4.1**

GLP 48-hours, static exposure					
Acute toxicity OECD 202 (2004)/OECD 235 (2011) GLP 48-hours, static exposure	*Hexagenia limbata*	Immobility	EC_{50} 1.5 mg a.s./$L_{(mm)}$	Study considered suitable for use in hazard classification.	**Mason (2018b)** **DAR: B.9.2.4.2**
Acute toxicity OECD 202 (2004)/OECD 235 (2011) GLP 48-hours, static exposure	*Thamnocephalus platyurus*	Immobility	**EC_{50} 0.11 mg a.s./$L_{(mm)}$**	Study considered suitable for use in hazard classification.	**Mason (2018c)** **DAR: B.9.2.4.2**
Reproduction study GLP 28-days, flow-through	*Americamysis bahia*	Survival, post-pairing survival, 28-day survival, **offspring per female**, body length of parent, dry weight of parent, F1 survival 96 h post-release	**NOEC 0.12 mg a.s./L (mm)**	Study considered suitable for use in hazard classification.	**Marini (2017)** **DAR: B.9.2.5.2**
Mortality and growth GLP OCSPP 850.1735 (2016) 10-days, static-renewal	*Hyalella azteca*	Mortality and growth (dry weight)	10-day LC/EC_{50} (based on growth and survival): >84 mg a.s./kg sediment dry weight > 8.2 mg a.s./L (mm)	Study considered suitable for use in hazard classification.	**Staggs (2016)** **DAR: B.9.2.5.4**

			NOEC 84 mg a.s./kg sediment dry weight (mm) 8.2 mg a.s./L (mm)		
Reproduction study OECD 218 (2004) GLP 28-days Dosed via sediment	*Chironomus riparius*	**Emergence**, development rate	**EC_{10} 69 mg a.s./Kg dw sediment (mm)** **EC_{10} 3 mg a.s./L (mm)** NOEC 49 mg a.s./Kg dw sediment (mm) NOEC 1.3 mg a.s./L (mm)	Study considered suitable for use in hazard classification	**Snow (2019)** **DAR: B.9.2.5.4**
Algae					
Freshwater algal growth inhibition OECD 201 (2011) GLP 96-hours, static exposure	*Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*)	**Growth rate**, yield, biomass	**72 hour E_rC_{50} 14 mg a.s./L (mm)** **72 hour E_rC_{10} 4.5 mg a.s./L (mm)** **72 hour NOE_rC 0.92 mg a.s./L (mm)**	Study considered suitable for use in hazard classification.	**Softcheck (2015a)** **DAR: B.9.2.6.1**
Aquatic plants					
Growth inhibition test OECD 221 (2006) GLP 7-days, static, water only	*Lemna gibba*	Growth **frond number**, growth dry weight, yield frond number, Yield dry weight	$E_rC_{50, \text{frond density}}$ 21 mg a.s./L (mm) $E_rC_{10, \text{frond density}}$ 2.4 mg a.s./L (mm) NOEC frond density 1.6 mg a.s./L (mm)	Study considered suitable for use in hazard classification.	**Kirkwood (2015a)** **DAR: B.9.2.7**

Water-Sediment Toxicity Test OECD 239 (2014) GLP 14-days, static renewal, water-sediment system dosed via water	*Myriophyllum spicatum*	**Growth rate total shoot length**, yield total shoot length, growth rate wet weight, yield wet weight, growth rate dry weight, yield dry weight	**E_rC_{50}, shoot length** 3.2 mg a.s./L (im) **E_rC_{20}, shoot length 0.033 mg a.s./L (im)** **E_rC_{10}, shoot length 0.0071 mg a.s./L (im)** **NOE_rC, shoot length 0.0096 mg a.s./L (im)**	Study considered suitable for use in hazard classification.	**Kirkwood (2015b)** **DAR: B.9.2.7**

[1] All studies were conducted with batch PL14-0049, Purity: 96.0%

Bixlozone was referred to as F9600 in the study reports

Bold entries are the endpoints considered most suitable to set the hazard classification for the active substance for each group of organisms.
mm = mean measured concentration nom = nominal concentration im = initial measured concentration

11.5.1 Acute (short-term) toxicity to fish

Three acute toxicity studies were conducted with fish using the following species: *Oncorhynchus mykiss, Lepomis macrochirus* and *Cyprinodon variegatus.* All three studies were performed to the OECD 203 (1992) guideline, according to GLP and were deemed valid for use in hazard classification.. The lowest endpoint is from the study with *O. mykiss;* an **LC_{50} value of 9.8 mg a.s./L** (mean measured). It is noted that fish are not the most acutely sensitive group of organisms and therefore not critical for hazard classification purposes.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

A total of 9 acute aquatic invertebrate toxicity studies using the active substance were conducted with the following species: *Daphnia magna, Americamysis bahia, Caecidotea communis, Chironomus riparius, Pycnopsyche gentilis, Hexagenia limbate, Thamnocephalus platyurus, Brachionus calyciflorus* and *Gammarus fasciatus*. All 9 studies were conducted to GLP. The studies testing *D. magna*, *A. bahia*, and *C. riparius* were conducted to standard guidelines (OECD 202 (2004), OCSPP draft 850.1035, OECD 235 (2011), respectively). There were no deviations or observations of note and the studies are considered suitable for use in hazard classification. Studies testing *C. communis*, *P. gentilis*, *H. limbate*, and *T. platyurus*, *B. calyciflorus* and *G. fasciatus* were conducted with wild caught test organisms, no specific guidelines are available for testing these species

but the OECD 202/235 guideline was used as a reference. These studies were also considered valid for use in hazard classification with the exception of the studies testing *B. calyciflorus* and *G. fasciatus.* For *B. calyciflorus* this was due to a lack of reference item being tested; according to ASTM 1440-91 (2004), copper is a suitable toxic reference item and the lack of reference item indicates that the sensitivity of the test organisms cannot be confirmed. In addition, the endpoint relates to only 24 hours of exposure. Regarding *G. fasciatus,* this study was conducted in accordance with OECD 202/235, however there is an available EPA guideline for this species (850.1020, 2016). It was noted that there were several deviations from this guideline, most notably the test duration, which is stated should be a minimum of 96 hours, whereas this study was conducted for 48 hours. This raises concerns as to whether the endpoint derived from this study is worst-case, noting that mortality doubled between 24 and 48 hours (mortality was observed in treatment groups at 1.3, 2.4 and 5.4 mg a.s./L; respectively, mortality increased from 5, 20 and 55 % at 24 hours to 10, 40 and 100 % at 48 hours in these treatment groups). Whilst these two studies are not considered reliable, it should be noted that the results do not indicate an increased sensitivity in comparison to the other species tested as the lowest concentrations where any mortality was observed was 2.2 mg a.s./L and 1.3 mg a.s./L for *B. calyciflorus* and *G. fasciatus* respectively, which is more than an order of magnitude higher than the lowest derived EC_{50} (0.11 mg a.s./L).

Table 38: Summary of reliable acute toxicity data for aquatic invertebrates

Group	Test substance	Time-scale (Test type)	End point	Toxicity[1]
Aquatic invertebrates				
Daphnia magna	Bixlozone	48 h (static)	Mortality, EC_{50}	>2.6 mg a.s./$L_{(mm)}$
Americamysis bahia	Bixlozone	96 h (static)	LC_{50}	0.14 mg a.s./$L_{(mm)}$
Caecidotea communis	Bixlozone	48 h (static)	Mortality, EC_{50}	>1.6 mg a.s./$L_{(mm)}$
Chironomus riparius	Bixlozone	48 h (static)	Mortality, EC_{50}	1.9 mg a.s./$L_{(mm)}$
Pycnopsyche gentilis	Bixlozone	48 h (static)	Mortality, EC_{50}	0.33 mg a.s./$L_{(mm)}$
Hexagenia limbata	Bixlozone	48 h (static)	Mortality, EC_{50}	1.5 mg a.s./$L_{(mm)}$
Thamnocephalus platyurus	Bixlozone	48 h (static)	Mortality, EC_{50}	0.11 mg a.s./$L_{(mm)}$

[1] mm = mean measured concentration

The lowest reliable endpoint was for *T. platyurus,* with an **EC_{50} of 0.11 mg a.s./L** (mean measured). Aquatic invertebrates are the most acutely sensitive group to exposure to

bixlozone based on the information available and will be used for acute hazard classification purposes.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Algae

Four algal studies were submitted testing the active substance and all were conducted to GLP. 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 studies conducted with different species of algae did not meet all of the validity criteria specified in the study guidelines (OECD 201, 2011) at any time point during the test. The mean coefficient of variation (CV) for section-by-section specific growth rates in the water control replicates was 67.3 % (96 hours) and 68.8 % (72 hours) for *Skeletonema costatum*. This value exceeds the guideline requirement (35 %) and the average CV based on historical data determined for *S. costatum* at Smithers Viscient (43 % based on N=5). It also failed one of the EPA OSCPP 850.4500 (2012) validity criteria at 72 hours, the co-efficient of variation of mean yield was 18.1 % compared with criteria of < 15 %. For *Navicula pelliculosa,* the CV between 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 water control replicates exceeded 35 % (actual: 110 % at 96 hours) 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 %, thus exceeding the 35 % specified in the guidance. This value also exceeds 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.

Regarding the valid study conducted with *R. subcapitata,* it was noted that 96-hour endpoints were not based on the raw data from the study and are incorrect. The applicant was given the opportunity to provide corrected values but opted not to. Therefore, only endpoints at 72-hours are considered valid. The **72-hour E_rC_{50} was 14 mg a.s./L and the 72-hour E_rC_{10} was 4.5 mg a.s./L** based on mean measured concentrations. **The 72 hour NOE_rC was 0.92 mg a.s./L** based on mean measured concentrations. It is noted that ultimately algae are not the most acutely sensitive group and therefore not critical for hazard classification purposes**.**

According to Commission Regulation (EU) 283/2013, where an active substance is known to exhibit herbicidal activity, "*a second species from a different taxonomic group shall be performed such as a diatom, for example Navicula pelliculosa"*. Therefore, as bixlozone is

a herbicide, a valid study with a second algal species should be provided. Nevertheless, the Agency has 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 19.29 mg a.s./L) and *S. costatum* (17 mg test item/L equivalent to 6.18 mg a.s./L).

Table 39: Summary of reliable data for algae

Test substance	Test organism	Test system	Endpoint (mg/L)[1]		Reference
Toxicity to algae					
Bixlozone	*Raphidocelis subcapitata*	Static, 96-hours	72 h E_rC_{50} 72 h E_rC_{10} 72 h NOE_rC	14 mm 4.5 mm 0.92 mm	Softcheck (2015a)
'F9600 4SC'	*Raphidocelis subcapitata*	Static, 96-hours	72 h E_rC_{50} 72 h E_rC_{10} 72 h NOE_rC	53 / 19.29 nom 19 / 6.9 nom 13 / 4.73 nom (f.p. / a.s.)	Softcheck (2017a)
'F9600 4SC'	*Skeletonema costatum*	Static, 96-hours	72 h E_rC_{50} 72 h E_rC_{10} 72 h NOE_rC	17 /6.18 mm 7.5 / 2.7 mm 6.1 / 2.22 mm (f.p. / a.s.)	Softcheck (2016a)

[1] mm = mean measured concentration nom = nominal concentration f.p. = formulated product

This comparison indicates that the formulation is of similar toxicity to *R. subcapitata* as the active substance when considering the 72 hour E_rC_{50} values. 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 acute toxicity endpoint to that based on growth rate for *Myriophyllum spicatum*. However, given the magnitude of difference in the endpoints derived for algae and aquatic plants when compared with aquatic invertebrates, the Agency considers the provision of an additional algal species study would be highly unlikely to alter the acute hazard classification driven by the toxicity to aquatic invertebrates.

<u>Aquatic plants</u>

Two studies were conducted with aquatic macrophytes; one with *Lemna gibba* and one with *Myriophyllum spicatum*. Both studies were conducted to GLP and considered valid for regulatory purposes.. The study with *L. gibba* was conducted in accordance with OECD 221 (2006) and the study with *M. spicatum* was conducted in accordance with OECD 239

(2014). For both studies the validity criteria were met, but there were deviations from the guideline recommendations. The following deviations relate to the study conducted with *M. spicatum* which was the more sensitive of the two species tested.

No toxic reference standard was included in the test; it is stated in OECD 239 (2014) that a reference substance such as 3,5-dichlorophenol should be periodically tested to check the performance of the test system over time. Therefore, this adds some uncertainty to the sensitivity of the test system, but is not an absolute requirement.

According to the OECD 239 (2014), the artificial sediment should be made up in line with OECD 219. The study report refers to OECD 218, which has the same composition as that in OECD 219 so is considered acceptable; however both guidance documents state that fine sand should predominate with > 50 % of particles between 500 and 200 µm. It is not clear if this is the case as the proportions of fine and coarse sand are not stated in the study report and no reference is made to the particle size. However, the controls met the validity criteria (with the exception of the replicate excluded from analysis) indicating that this did not have a significant impact on plant health.

One replicate (D) in the water control group was observed to have substantially lower yields and growth rates for all endpoints, in comparison to the remaining three control replicates. It was also noted that the CV for yield based on shoot wet weight in the pooled controls was 42 %, slightly exceeding the guideline criterion of < 35 %. For this reason, the study author performed Grubb's Test (U.S. EPA, 2002) for statistical outliers for all endpoints using the 14-day termination data, and control replicate D was determined to be an outlier for the growth rate based on shoot wet weight and shoot dry weight endpoints. Although no definitive reason could be identified for this replicate being an outlier, the plants were observed to have extensive filamentous algal growth present at test termination, which can be indicative of poor plant health (filamentous algae was not observed in control replicate D on the plants during the day 7 biological observations).

The pH of the control media increased from 7.2 to 10.0 from the start to the end of the test. This is more than the 1.5 units specified in the test guidelines, however as the validity criteria were met, it is not considered to invalidate the test.

The nominal test concentrations in the study were 0.0098, 0.039, 0.16, 0.63, 2.5 and 10 mg a.s./L. Test concentrations were measured in the new solutions on days 0 and 7 and in the aged test solutions on days 7 and 14. The initial measured concentrations (Day 0) were 0.0096, 0.034, 0.17, 0.58, 2.4 and 8.8 mg a.s./L. The corresponding mean measured concentrations were 0.0094, 0.033, 0.15, 0.56, 2.3 and 9.2 mg a.s./L and hence mean concentrations measured in the water phase were in the range 85-97 % of nominal. The lowest recovery was 82 % of nominal (in one of the day 7 aged samples). No analytical measurements were made in the sediment phase. However, the recovery in the water phase indicated that concentrations were maintained within 80-100 % of nominal in

the water phase. Therefore, the basing of results on initial measured concentrations is deemed acceptable.

Phytotoxic effects were observed at every treatment concentration tested. It is noted that when considering the overall effects due to the test item based on the number of plants affected and the percentage coverage of the plants affected that ≥ 50 % effects were not observed for chlorosis. However, % effects were not reported for root growth and apical bud damage. In the 0.0096 mg a.s./L treatment level eight plants were observed to be healthy while four plants were observed to be chlorotic at exposure termination (see Table 40 below).

Table 40: Morphological observations for controls and 0.0096 mg a.s./L treatment

Initial Measured Concentration (mg/L)	Replicate	Plant	Morphological Observations					Root Biological Observations[a]
			Day 0	Day 7	%Effect	Day 14	%Effect	
Control	A	1	H	H	0	H	0	4
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	B	1	H	H	0	H	0	4
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	C	1	H	H	0	H	0	4
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	D	1	H	H	0	FA/H	0	2
		2	H	H	0	FA/H	0	2
		3	H	H	0	FA/H	0	2
Solvent	A	1	H	H	0	H	0	4
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	B	1	H	H	0	H	0	4
		2	H	H	0	H	0	3
		3	H	H	0	H	0	3
	C	1	H	H	0	H	0	4
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	D	1	H	H	0	H	0	3
		2	H	H	0	H	0	2
		3	H	H	0	H	0	4
	E	1	H	H	0	H	0	3
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	F	1	H	H	0	H	0	4
		2	H	H	0	H	0	3
		3	H	H	0	H	0	3
	G	1	H	H	0	H	0	4
		2	H	H	0	H	0	4
		3	H	H	0	H	0	4
	H	1	H	H	0	H	0	4
		2	H	H	0	H	0	3
		3	H	H	0	H	0	4

Initial Measured Concentration (mg/L)	Replicate	Plant	Morphological Observations					Root Biological Observations[a]
			Day 0	Day 7	%Effect	Day 14	%Effect	
0.0096	A	1	H	H	0	H	0	3
		2	H	H	0	H	0	3
		3	H	H	0	C	5	3
	B	1	H	H	0	H	0	3
		2	H	H	0	H	0	3
		3	H	H	0	H	0	3
	C	1	H	H	0	C	5	3
		2	H	H	0	C	5	2
		3	H	H	0	C	10	3
	D	1	H	H	0	H	0	2
		2	H	H	0	H	0	2
		3	H	H	0	H	0	3

[a] Root biological observations are denoted as follows: 2 = few roots, 3 = moderate root development, 4 = very good root development.

H = Healthy
FA = Extensive filamentous algae observed on plant.

Table 41: Summary of results for shoot length, wet weight and dry weight

Initial Measured Concentration (mg test item/L)	Shoot Length (cm)		Shoot Wet Weight (g)[c]		Shoot Dry Weight [d] (g)	
	Mean (SD)	% Inhibition[b]	Mean (SD)	% Inhibition[b]	Mean (SD)	% Inhibition
Control [a]	20.2 (1.9)	n.a.	0.5444 (0.0417)	n.a.	0.0479 (0.0011)	n.a.
Solvent Control	18.7 (4.6)	n.a.	0.4865 (0.1625)	n.a.	0.0461 (0.0106)	n.a.
0.0096	16.8 (3.7)	12	0.4935 (0.0569)	2	0.0486 (0.0040)	-4
0.034	13.5 (2.4)	29	0.3872 (0.0879)	23	0.0414 (0.0106)	11
0.17	14.8 (2.0)	23	0.4407 (0.0549)	12	0.0488 (0.0071)	-5
0.58	11.0 (1.5)	42	0.2728 (0.0469)	46	0.0369 (0.0050)	21
2.4	9.4 (1.7)	51	0.2603 (0.0588)	48	0.0347 (0.0025)	25

Initial Measured Concentration (mg test item/L)	Shoot Length (cm)		Shoot Wet Weight (g)[c]		Shoot Dry Weight [d] (g)	
	Mean (SD)	% Inhibition[b]	Mean (SD)	% Inhibition[b]	Mean (SD)	% Inhibition
8.8	7.3 (1.7)	62	0.2572 (0.0891)	49	0.0412 (0.0081)	12

[a] Replicate D was removed as it was determined to be a statistical outlier

[b] Percent inhibition is calculated relative to the pooled control

[c] Wet weight of representative sample (N = 15) at exposure initiation = 0.0885 g

[d] Dry weight of representative sample (N= 15) at exposure initiation = 0.0092 g.

n.a = not applicable

Table 42: Mean values for yield and growth rate based on shoot length after 14 days exposure

Initial measured concentration (mg test item/L)	Yield (SD)	% Yield inhibition [b]	Average Growth rates (SD)	% Growth Rate inhibition [b]
Control [a]	13.6 (1.6)	n.a.	0.0796 (0.0057)	n.a.
Solvent Control	12.7 (3.9)	n.a.	0.0802 (0.0148)	n.a.
0.0096	10.5 (3.1)	19	0.0692 (0.0106)	14
0.034	8.0 [c] (1.6)	38	0.0638 [d] (0.0079)	20
0.17	8.6 [c] (2.0)	33	0.0623 [d] (0.0110)	22
0.58	5.3 [c] (1.5)	59	0.0465 [d] (0.0109)	42
2.4	4.3 [c] (1.5)	66	0.0445 [d] (0.0145)	44
8.8	1.9 [c] (0.9)	85	0.0224 [d] (0.0121)	72

[a] Replicate D was removed as it was determined to be a statistical outlier.

[b] Percent inhibition is calculated relative to the pooled control.

[c] Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test

[d] *Significantly reduced compared to the pooled control, based on William's Multiple Comparison Test*

n.a = not applicable

Table 43: Mean values for yield and growth rate based on shoot wet weight after 14 days exposure

Mean Measured concentration (mg test item/L)	Yield (SD)	% Yield inhibition[b]	Average Growth rates (SD)	% Growth Rate inhibition [b]
Control [a]	0.4559 (0.0417)	n.a,	0.1296 (0.0056)	n.a,
Solvent Control	0.3980 (0.1625)	n.a,	0.1181 (0.0248)	n.a,
0.0096	0.4050 (0.0569)	2	0.1224 (0.0082)	-1
0.034	0.2987 (0.0879)	28	0.1041 (0.0159)	14
0.17	0.3522 (0.0549)	15	0.1142 (0.0090)	6
0.58	0.1843 [c] (0.0469)	55	0.0796 [c] (0.0125)	34
2.4	0.1718 [c] (0.0588)	58	0.0757 [c] (0.0162)	38
8.8	0.1687 [c] (0.0891)	59	0.0724 [c] (0.0279)	40

[a] *Replicate D was removed as it was determined to be a statistical outlier.*

[b] *Percent inhibition is calculated relative to the pooled control.*

[d] *Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test*

n.a = not applicable

Table 44: Mean values for yield and growth rate based on shoot dry weight after 14 days exposure

Mean Measured concentration (mg test item/L)	Yield (SD)	% Yield inhibition [b]	Average Growth rates (SD)	% Growth Rate inhibition [b]
Control [a]	0.0387 (0.0111)	n.a.	0.1177 (0.0017)	n.a.
Solvent Control	0.0369 (0.0106)	n.a.	0.1132 (0.0170)	n.a.
0.0096	0.0394 (0.0040)	-5	0.1185 (0.0058)	-4

0.034	0.0322 (0.0106)	14	0.1057 (0.0169)	8
0.17	0.0395 (0.0071)	-6	0.1184 (0.0102)	-3
0.58	0.0277 (0.0050)	26	0.0986 (0.0094)	14
2.4	0.0255 [c] (0.0025)	32	0.0946 [c] (0.0052)	17
8.8	0.0320 (0.0081)	14	0.1058 (0.0147)	8

[a] *Replicate D was removed as it was determined to be a statistical outlier.*

[b] *Percent inhibition is calculated relative to the pooled control.*

[c] *Significantly reduced compared to the pooled control, based on Dunnett's Multiple Comparison Test*

n.a = not applicable

Shoot length and phytotoxic effects are the most sensitive parameters measured in the *M. spicatum* study.

The study conducted with *M. spicatum* in a water-sediment system resulted in the lowest standard endpoint based on a reduction in shoot length; a 14 day E_rC_{50} of 3.2 mg a.s./L based on initial measured concentrations. **However, given the effects on yield, phytotoxicity and morphology, a more precautionary endpoint – an E_rC_{20} of 0.033 mg a.s./L has been selected.**

It is noted that aquatic plants are not the most acutely sensitive group, but are the most sensitive chronic group for hazard classification purposes**.**

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data are available for other taxa.

11.6 Long-term aquatic hazard

11.6.1 Chronic toxicity to fish

A long-term toxicity early life stage (ELS) study was conducted with fathead minnow (*Pimephales promelas*) in accordance with OECD 210 (2012). The study was considered valid, however it is noted that the statistically-derived NOEC (0.38 mg a.s/L) based on total length was significantly lower than the corresponding EC_{10} value (4.6 mg a.s/L). The study summary can be found in Annex 1. Whilst only a 5.0 – 5.8 % effect on fish total length was observed, with no corresponding effect on wet weight, it was statistically significant and the biological relevance of this impact on fish length is unknown. Given the magnitude of difference between the NOEC and EC_{10}, the NOEC of 3.8 mg a.s./L has been retained as a more precautionary endpoint. Nevertheless, it is noted that fish are not the most chronically sensitive group for hazard classification purposes**.**

11.6.2 Chronic toxicity to aquatic invertebrates

Two long-term studies with aquatic invertebrates were conducted; one with *Daphnia magna* and one with *Americamysis bahia.* However, only the study conducted with *A. bahia* was considered valid. In *the D. magna* study there were long intervals where no chemical analysis took place (e.g. no analysis between day 5 and day 16, a duration in which a number of renewals have taken place) hence a full picture of the behaviour of the test item is not available. As a consequence, there is uncertainty in the exposure to test media over the duration of the study and hence in the endpoint derived. The study conducted with *A. bahia* was a flow-through design and although the study was conducted to EPA OPPTS 850.1350, it was evaluated using validity criteria from ASTM E 1191-03a (2008) as there was limited guidance on the acceptability of the test in 850.1350. The study was considered valid according to both sets of guidelines. The only deviation from the guideline was the photoperiod (16:8 light:dark in this test, the recommended photoperiod in 850.1350 is 14:10 light:dark). However, this is not thought to have affected the results of the study as all validity criteria were met.

No chronic study was conducted with *T. platyurus* the most sensitive of the invertebrate species tested for acute toxicity. However, it is noted that the acute toxicity endpoints for *T. platyurus* and *A. bahia* were very similar (0.11 and 0.14 mg a.s./L respectively). As *A. bahia* is more sensitive than *D. magna* (> 2.6 mg a.s./L) and appears to be one of the most sensitive species tested (based on acute toxicity) and comparable to *T. platyurus*, a chronic test with *A. bahia* is deemed acceptable. Only a NOEC could be derived for *A. bahia* as the only statistically significant effect was at the highest test concentration and at the preceding concentration, an effect of <10 % was observed. The derived **28 day NOEC is 0.12 mg a.s./L** (mean measured), noting that aquatic invertebrates are not the most chronically sensitive group for hazard classification purposes**.**

11.6.3 Chronic toxicity to algae or other aquatic plants

The available studies related to algae and aquatic plants have been summarised in Section 11.5.3 .

Aquatic plants are the most sensitive chronic group based on the available data and are driving the chronic classification. The study conducted with *M. spicatum* in a water-sediment system resulted in the lowest endpoint based on a reduction in shoot length and phytotoxicity; t**he 14 day NOEC based on shoot length and phytotoxicity was 0.0096 mg a.s./L and the E_rC_{10} based on shoot length was 0.0071 mg a.s./L based on initial measured concentrations** (E_rC_{10} value was extrapolated below the lowest treatment based on available data as a consequence the 95 % confidence limit is wide 0.0019-0.17 mg a.s./L). Whilst a 14 % reduction in growth rate and 19 % reduction in yield (based on shoot length after 14 days) were obtained in the 0.0096 mg a.s./L treatment (initial measured) neither of these reductions were statistically significant. Statistically significant

reductions in yield and growth rate were obtained in the next highest treatment of 0.034 mg a.s./L – see Table 42.

Based on the 'Guidance on the Application of the CLP Criteria' (ECHA, 2017) the E_rC_{10} value has been used preferentially over the NOE_rC to determine the chronic hazard classification, and noting that the E_rC_{10} is more conservative.

11.6.4 Chronic toxicity to other aquatic organisms

Three studies conducted on *Chironomus* species were submitted. However, only one study conducted with *Chironomus riparius* (Snow 2019) was considered valid. OECD 218 validity criteria were not met in the other two studies and there were a number of deviations from guideline recommendations. The Snow 2019 study was conducted according to OECD 218 (2004) and met all the validity criteria. The mass balance of the test system at day 0 ranged between 99-106 % and at day 28 ranged between 73-90 %, as such results are based on geometric mean measured concentrations. The **28 day EC_{10} based on midge development is 69 mg a.s./Kg dw sediment and 3.0 mg a.s./L overlying water**. The corresponding NOEC is 49 mg a.s./Kg dw sediment and 1.3 mg a.s./L overlying water.

One study conducted on *Hyalella azteca* was also submitted. This study was conducted according to OCSPP 850.1735 (2016) and following GLP. This study is considered valid and the endpoints derived are **10-day LC/EC_{50} (based on growth and survival): >84 mg a.s./kg dw sediment** based on mean measured concentrations in sediment. The 10-day NOEC was 84 mg a.s./Kg dw sediment. The corresponding 10-day **LC/EC_{50} based on mean measured pore water concentrations: > 8.2 mg a.s./L.** The 10-day NOEC was 8.2 mg a.s./L.

Sediment dwelling aquatic invertebrates are not the most chronically sensitive group for hazard classification purposes.

11.7 Comparison with the GB CLP criteria

11.7.1 Acute aquatic hazard

Reliable acute aquatic toxicity data on technical bixlozone are available for fish, invertebrates, algae and other aquatic plants (i.e. there are appropriate data for all three trophic levels that need to be assessed for CLP classification). The lowest LC_{50}/EC_{50} value is the mean measured 48-hour EC_{50} of 0.11 mg a.s./L for the aquatic invertebrate *Thamnocephalus platyurus* (Mason 2018c). This EC_{50} is > 0.1 mg/L but ≤ 1 mg/L, therefore bixlozone meets the criteria for classification as Aquatic Acute Category 1 with an acute M-factor of 1.

Information presented in Annex I indicates the metabolites of bixlozone are less toxic than the parent and are therefore not considered relevant for hazard classification.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Rapid degradability

Bixlozone is considered to be 'not rapidly degradable' according to the CLP criteria, this decision has been explained in full in the environmental fate section (Section 11.1.1).

Bioaccumulation

Bixlozone has a measured log K_{ow} of 3.3, which is lower than the CLP trigger of ≥ 4, therefore, for hazard classification purposes, it does not indicate a potential for bioaccumulation.

An experimental fish bioaccumulation study is available and the lipid-normalised BCF values for bixlozone (from Anon, 2016k) are 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). According to the guidance on the application of the CLP criteria (ECHA, 2017) a measured whole fish BCF should be used in preference when available to conclude on the bioaccumulation potential of a substance (BCF ≥ 500 indicates bioaccumulation potential). As the measured BCF values are < 500, it can therefore be concluded that bixlozone does not meet the CLP criteria for potential bioaccumulation.

Chronic toxicity

Reliable long-term aquatic toxicity data on technical bixlozone are available for fish, invertebrates, algae and other aquatic plants (i.e. there are appropriate data for all three trophic levels that need to be assessed for CLP classification). Based on the Guidance on the Application of the CLP Criteria (ECHA, 2017) the EC_{10} values are generally used preferentially over NOEC values to determine the chronic hazard classification. The lowest overall EC_{10} value considered valid (see Section 11.6.3) is the mean measured 14-day E_rC_{10} of 0.0071 mg a.s./L based on shoot length for the aquatic plant *Myriophyllum spicatum* (derived from Kirkwood 2015b). This is > 0.001 mg/L but ≤ 0.01 mg/L, therefore since bixlozone is considered to be 'not rapidly degradable' it meets the criteria for classification as Aquatic Chronic Category 1 with a chronic M-factor of 10. The chronic classification and M factor would be the same if the NOEC was used instead of the E_rC_{10}.

11.8 Conclusion on classification and labelling for environmental hazards

Based on the information evaluated above; bixlozone is considered to be ‘not rapidly degradable’ and does not meet the CLP criteria for potential bioaccumulation. It is sufficiently toxic to warrant the highest CLP classifications for both acute (H400) and chronic (H410) hazards to the aquatic environment, with acute and chronic M-factors of 1 and 10 respectively.

Classification:

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

12. Evaluation of additional hazards

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

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, bixlozone is considered to have a low risk of long-range transport and, therefore, no hazard to the ozone layer.

12.1.2 Comparison with the GB CLP criteria

A substance shall be classified as hazardous to the ozone layer (Category 1) if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer. Any substances having an Ozone Depleting Potential (ODP) of ≥ 0.005 of the substances currently listed in Annex I of CLP should be classified as hazardous to the ozone layer.

There are no data available to suggest that bixlozone presents a danger to the ozone layer and the substance is not currently listed as having an Ozone Depleting Potential (ODP) in Annexes to the Montreal Protocol. However, there is no ODP available for bixlozone.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified (data lacking)

13. Additional labelling

No additional labelling is proposed.

It should be noted that the GB evaluation of bixlozone as a pesticide is not finalised.

14. References (non-confidential)

Nb. Confidential references are in a separate Annex (Annex II).

General:

Reference	Author and date
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 Published Available at https://echa.europa.eu/	ECHA (2017)

Physico-chemistry:

DAR	Reference	Author and date
B.2.2	Title: F9600: Vapour Pressure GLP Unpublished	Cowlyn, N (2016a)
B.2.5	Title: F9600: Water Solubility GLP Unpublished	Cowlyn, N (2016b)
B.2.7	Title: F9600: Partition Coefficient(n-octanol/water) GLP Unpublished	Cowlyn, N (2016c)

B.2.1 **B.2.3** **B.2.12**	F9600: Physicochemical Properties GLP Unpublished	Cowlyn, N (2017a)
B.2.3	F9600 Technical: Appearance GLP Unpublished	Cowlyn, N (2017b)
B.2.9.1	Title: F9600: Flammability (solids) GLP Unpublished	Cowlyn, N (2017e)
B.2.9.2	Title: F9600: Auto-Ignition Temperature GLP Unpublished	Cowlyn, N (2017f)
B.2.11	Title: F9600: Explosive Properties GLP Unpublished	Cowlyn, N (2017g)
B.2.13	Title: F9600: Oxidising Properties GLP Unpublished	Cowlyn, N (2017h)
B.2.14	Title: F9600: Relative Density GLP Unpublished	Cowlyn, N (2017i)

B.6.3.4 **B.6.5.2**	Histopathology of Preclinical Toxicity Studies. Interpretation and Relevance in Drug Safety Studies (4th Edition).	Greaves, P (2012)
B.2.8	F9600: Waiver Request for Study on Dissociation Constants in Water Non-GLP, Unpublished	Guo, J (2018)

Human Health:

DAR	Reference	Author and date
B.6.6.2.3	An Oral (Gavage) Dose Range-Finding Prenatal Developmental Toxicity Study of F9600 Technical in Rabbits Non-GLP	Anon, (2014a)
B.6.7.1	An Oral (Gavage) Dose Range Finding Acute Neurotoxicity Study of F9600 Technical in Rats Non-GLP	Anon, (2014b)
B.6.7.1	An Oral (Gavage) Acute Neurotoxicity Study of F9600 Technical in Rats GLP	Anon, (2014c)
B.6.2.1	F9600 Technical: Acute Oral Toxicity – Up-and-Down Procedure in Rats GLP	Anon, (2014d)
B.6.2.2	F9600 Technical: Acute Dermal Toxicity Study in Rats GLP	Anon, (2014e)
B.6.2.3	F9600 Technical: Acute Inhalation Toxicity in Rats GLP	Anon, (2014f)
B.6.2.4.2	F9600 Technical: Primary Skin Irritation Study in Rabbits GLP	Anon, (2014g)

B.6.2.5.2	F9600 Technical: Primary Eye Irritation in Rabbits. GLP	Anon, (2014h)
B.6.2.6	F9600 Technical: Local Lymph Node Assay (LLNA) in Mice GLP	Anon, (2014i)
B.6.1.1.1	Pharmacokinetics and metabolism of F9600 in male and female Sprague-Dawley rats Non-GLP	Anon, (2014j)
B.6.6.2.4	An Oral (Gavage) Prenatal Developmental Toxicity Study of F9600 Technical in Rabbits GLP	Anon, (2015a)
B.6.3.2.1	A 28-Day Oral (Dietary) Toxicity and Toxicokinetic Study of F9600 Technical in Sprague Dawley Rats. GLP	Anon, (2015b)
B.6.3.2.2	A 28-Day Oral (Dietary) Toxicity Study of F9600 Technical in CD-1 Mice GLP	Anon, (2015c)
B.6.3.1.2	A 7-Day Oral (Dietary) Palatability Study of F9600 in CD-1 Mice	Anon, (2015d)
B.6.3.1.1	A 7-Day Oral (Dietary) Palatability Study of F9600 Technical in Sprague-Dawley Rats	Anon, (2015e)
B.6.3.1.3	A 7-Day Oral (Dietary) Palatability Study of F9600 Technical in Beagle Dogs	Anon, (2015f)
B.6.3.3.1	A 90-Day Dietary Combined Toxicity and Neurotoxicity Study of F9600 in Rats GLP	Anon, (2016a)
B.6.6.1.1	A Dose Range Finding Oral (Dietary) Reproduction/Developmental Study of F9600 Technical in Sprague-Dawley Rats Non-GLP	Anon, (2016b)
B.6.6.1.2	A Dietary Two-Generation Reproductive Toxicity Study of F9600 Technical in Rats	Anon, (2016c)

	GLP	
B.6.6.2.1	An Oral (Gavage) Dose Range-Finding Prenatal Developmental Toxicity Study of F9600 in Rats Non-GLP	Anon, (2016d)
B.6.6.2.2	An Oral (Gavage) Prenatal Developmental Toxicity Study of F9600 in Rats GLP	Anon, (2016e)
B.6.3.3.2 **B.6.3.1.4** **B.6.3.2.3**	A 90-Day Oral (Dietary) Toxicity and Plasma Concentration Measurement Study of F9600 Technical in CD-1 Mice GLP A 7-Day Oral (Capsule) Toxicity Study of F9600 Technical in Beagle Dogs. Non-GLP A 28-Day Oral (dietary) toxicity Study of F9600 Technical in Beagle Dogs GLP	Anon, (2016f)
B. 6.3.3.3	A 90-Day Oral (Capsule) Dose Toxicity Study of F9600 Technical in Beagle Dogs GLP	Anon, (2016g)
B.6.1.1.2	Pharmacokinetics of [14C-Phenyl]F9600 in Male and Female Sprague-Dawley Rats Following Single, Multiple Oral and Intravenous Bolus Doses GLP	Anon, (2016h)
B.6.3.5	A 21-Day study of F9600 by Dermal Application in Sprague-Dawley Rats GLP	Anon, (2016i)

B.6.3.4 **B.6.5.1**	A 12-Month Oral (Capsule) Dose Toxicity Study of F9600 Technical in Beagle Dogs GLP A 2-Year Oral (Dietary) Combined Chronic Toxicity and Carcinogenicity Study with Toxicokinetic Measurements of F9600 Technical in Sprague Dawley Rats GLP	Anon, (2017a)
B.6.5.2	An 18-month Oral (Dietary) Carcinogenicity Study with Toxicokinetic Measurements of F9600 Technical in CD-1 Mice GLP	Anon, (2017b)
B.6.1.1.1	Metabolism of [14C-phenyl]F9600 in male Sprague-Dawley rats - Pilot study Non-GLP	Anon, (2017c)
B.6.1.1.4	Tissue distribution of [14C-Phenyl]F9600 at peak concentration (Tmax) in male and female Sprague-Dawley rats GLP	Anon, (2017d)
B.6.1.1.6	Excretion routes and metabolism of [14C-carbonyl]F9600 in male and female Sprague-Dawley rats following a single oral dose GLP	Anon, (2017e)
B.6.1.2	Mass Balance of [14C-phenyl]-F9600 in male bile duct cannulated Sprague-Dawley rats following a single intravenous dose Non-GLP	Anon, (2017f)
B.6.1.3.1	Comparative in vitro Metabolism of [14C] F9600 (Phenyl and Carbonyl-labelled) in mouse, rat, dog and human hepatocytes Non-GLP	Anon, (2017g)
B.6.1.1.4	Radioactivity concentration in plasma and bone marrow at Tmax after oral administration of [14C-Phenyl]F9600 to Sprague-Dawley rats GLP	Anon, (2017h)

B.6.2.4.1	F9600 Technical: In vitro skin irritation test (SIT) using the EpidermTM skin model GLP	Anon, (2018a)
B.6.4.2	In Vivo Mammalian Erythrocyte Micronucleus Assay in Rats with F9600 Technical GLP	Anon, (2018b)
B.6.1.1.6	Metabolism of [14C-carbonyl]F9600 in male and female Sprague-Dawley rats – pilot study Non-GLP	Anon, (2018c)
B.6.1.1.5	Excretion routes and metabolism of [14C-phenyl]F9600 in male and female Sprague-Dawley rats following single or multiple oral doses GLP	Anon, (2018d)
B.6.2.5.1	F9600 Technical: Epiocular™ eye irritation test (EIT) for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage GLP	Anon, (2018e)
B.6.1.3.2	In Vitro Comparative Metabolism of [14C-Phenyl]-and [14C-Carbonyl]-F9600 in Mixed Gender Mouse, Rat, Dog and Human Cryopreserved Hepatocytes GLP	Anon, (2020)
B.6.4.1.1	Bacterial Reverse Mutation Assay with F9600 Technical	Bruce, S (2018)
B.6.4.1.3	In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay) with F9600 Technical	Dutta, A (2018)
B.6.4.1.2	In Vitro Mammalian Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) Cells with F9600 Technical	Roy, S (2018)

Ecotoxicology:

DAR	Reference:	Author and date

B.9.2.1	F9600: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)	Anon, 2015g
B.9.2.1	F9600: Acute toxicity to Bluegill Sunfish (*Lepomis macrochirus*)	Anon, 2015h
B.9.2.1	F9600: Acute toxicity to Sheepshead Minnow (*Cyprinodon variegatus*)	Anon, 2015i
B.9.2.2.1	F9600 - Early Life-Stage Toxicity Test with Fathead Minnow (*Pimephales promelas*)	Anon, 2016j
B.9.2.2.3	F9600 – Flow-Through Bioconcentration and Metabolism study with Bluegill Sunfish (*Lepomis macrochirus)*	Anon, 2016k
B.9.2.5.4	2,4-Dichlorobenzoic acid: Assessment of Side Effects on the Larvae of the Midge, *Chironomus riparius*, with the Laboratory Test Method – Spiked Sediment Test	Dabrunz, A. (2018a)
B.9.2.5.4	4-Carboxyl-F9600: Assessment of Side effects on the Larvae of the Midge, *Chironomus riparius*, with the Laboratory Test Method – Spiked Sediment Test	Dabrunz, A. (2018b)
B.9.2.5.4	F9600-Dimethyl-Malonamide: Assessment of Side Effects on the Larvae of the Midge, *Chironomus riparius*, with the Laboratory Test Method-Spiked Sediment Test	Dabrunz, A. (2018c)
B.9.2.7	4-Carboxyl-F9600: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System	Dill, M. (2018a)
B.9.2.7	F9600-Dimethyl-malonamide: Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System	Dill, M. (2018b)
B.9.2.7	F9600: 7-Day Toxicity Test with Duckweed (*Lemna gibba*)	Kirkwood, A. (2015a)
B.9.2.7	F9600: Growth Inhibition of the Aquatic Macrophyte *Myriophyllum spicatum* in a water-sediment system	Kirkwood, A. (2015b)
B.9.2.7	2,4-Dichlorobenzoic Acid – Growth Inhibition of the Aquatic Macrophyte *Myriophyllum spicatum* in a Water-Sediment System	Kirkwood, A. (2018)
B.9.2.4.1	2,4-Dichlorobenzoic acid: Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test-Static)	Kümmich, F (2018)

B.9.2.5.2	F9600: Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*)	Marini, J.P. (2017)
B.9.2.4.2 **B.9.2.4.2**	F9600 Technical - Acute Toxicity to Freshwater Isopods (*Caecidotea communis*) Under Static Conditions F9600-3-OH Propanamide – Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions	Mason, J (2017a)
B.9.2.4.2	F9600 Technical – Acute Toxicity to Midge (*Chironomus riparius*) Under Static Conditions	Mason, J (2017d)
B.9.2.4.2	F9600 Technical – Acute Toxicity to Caddisflies (*Pycnopsyche gentilis*) Under Static Conditions	Mason, J (2018a)
B.9.2.4.1 **B.9.2.4.2**	F9600-Dimethyl-Malonamide – Acute Toxicity Test with Water Fleas (*Daphnia magna*) Under Static Conditions F9600 Technical - Acute Toxicity to Mayflies (*Hexagenia limbata*) Under Static Conditions	Mason, J (2018b)
B.9.2.4.2	F9600 Technical: Acute Toxicity to Freshwater Beavertail Fairy Shrimp (*Thamnocephalus platyurus*) Under Static Conditions 2,4-Dichlorobenzoic acid – Acute Toxicity to Mysids (*Americamysis bahia*) Under Static-Renewal Conditions	Mason, J. (2018c)
B.9.2.4.2	4-Carboxyl-F9600 – Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions Study no. 72809215. GLP, unpublished.	Mason, J. (2018d)
B.9.2.4.2	F9600-Dimethyl-Malonamide – Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions	Mason, J. (2018e)
B.9.2.6.2	2,4-Dichlorobenzoic acid: Toxicity to the Marine Diatom *Skeletonema costatum* under Laboratory Conditions	Obert-Rauser, P (2018b)
B.9.2.6.1	2,4-Dichlorobenzoic acid: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindäk under Laboratory Conditions	Obert-Rauser, P. (2018a)

B.9.2.4.1	F9600: Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions	Shaw, A.C (2015)
B.9.4.2	F9600: Acute Toxicity to Mysids (*Americamysis bahia*)	Shaw, A.C. (2016a)
B.9.2.5.4	F9600 Technical - Toxicity Test Exposing Sediment-Dwelling Midges (*Chironomus riparius*) to a Test Substance Applied to Sediment Under Static Conditions	Snow, B. (2019)
B.9.2.6.1	F9600: 96-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*	Softcheck, K.A. (2015a)
B.9.2.6.1	F9600-3-OH-Propanamide: 96-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata*	Softcheck, K.A. (2017)
B.9.2.6.2	F9600-3-OH-Propanamide: 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*	Softcheck, K.A. (2017b)
B.9.2.6.1	4-Carboxyl-F9600 - 96-Hour Toxicity Test with the Freshwater Green Alga, *Raphidocelis subcapitata*	Softcheck, K.A. (2018a)
B.9.2.6.1	F9600-Dimethyl-Malonamide: 96-Hour Toxicity Test with the Freshwater Green Alga, *Raphidocelis subcapitata*	Softcheck, K.A. (2018b)
B.9.2.6.2	4-Carboxyl-F9600 - 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*	Softcheck, K.A. (2018c)
B.9.2.6.2	F9600 Dimethyl Malonamide - 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*	Softcheck, K.A. (2018d)
B.9.2.5.4	F9600 Technical: 10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to a Test Substance Applied to Sediment under Static-Renewal Conditions	Stags, M.L. (2016)

15. Annexes

Annex I – Summary of the aquatic toxicity of degradants of bixlozone

The available acute toxicity data available for the degradants of bixlozone (2,4-dichlorobenzoic acid, 4-Carboxyl-F9600, F9600-dimethyl-malonamide and F9600-3-OH-propanamide) considered relevant under Regulation 1107/2009 are summarised below in Table 1. This information is included for information only, none of the degradants exhibit equivalent toxicity to bixlozone. Therefore they are not considered to impact the hazard classification of bixlozone.

Table 1: Summary of toxicity data available for the degradants of bixlozone

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
Acute toxicity to invertebrates					
2,4-dichlorobenzoic acid	*Daphnia magna*	Static, 48-hours	EC_{50}	> 100 nom.	Kümmich (2018)
4-Carboxyl-F9600	*Daphnia magna*	Static, 48-hours	EC_{50}	> 100 nom.	Mason (2018a)
F9600-dimethyl-malonamide	*Daphnia magna*	Static, 48-hours	EC_{50}	> 100 nom.	Mason (2018b)
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)

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
F9600-3-OH-propanamide	*Americamysis bahia*	Static, 96-hours	LC_{50}	22 m.m.	Mason (2017a)
Toxicity to sediment dwelling invertebrates					
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)	((2018a)
			NOEC	104.88 mg /kg sed. dw m.m (93.26 mg/L)	
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)
			NOEC	494.54 mg /kg sed. dw m.m. (42.75 mg/L)	
F9600-dimethyl-malonamide	*Chironomus riparius*	Static, water-sediment system (dosed via sediment), 28-days	EC_{10} / EC_{20}	≥ 502 mg /kg sed. dw ini. (89.5 mg/L)	Dabrunz (2018c)
			NOEC	502 mg /kg sed. dw ini. (89.5 mg/L)	
Toxicity to algae					
2,4-dichlorobenzoic acid	*Raphidocelis subcapitata*	Static, 96-hours	E_rC_{50} NOE_rC E_yC_{50} NOE_yC	90.1 / 100 nom 31.3 / 31.3 nom 60.6 / 59.9 nom 31.3 / 31.3 nom	Obert -Rauser (2018a)

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
				(72 hours / 96 hours)	
4-Carboxyl-F9600	*Raphidocelis subcapitata*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	77 / 71 m.m. 63 / 56 m.m. 56 / 51 m.m. 24 / 49 m.m. 62 / 65 m.m. 49 / 52 m.m. 42 / 44 m.m. 24 / 49 m.m. (72 hours / 96 hours)	Softcheck (2018a)
F9600-dimethyl-malonamide	*Raphidocelis subcapitata*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	71 / 71 m.m. 57 / 56 m.m. 53 / 52 m.m. 49 / 49 m.m. 69 / 67 m.m. 56 / 53 m.m. n.r. / n.r. 49 / 49 m.m. (72 hours / 96 hours)	Softcheck (2018b)
F9600-3-OH-propanamide	*Raphidocelis subcapitata*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC	> 84 / > 84 m.m. 61 / ≥84 m.m. 45 / - [a] m.m. 33 / 33 m.m.	Softcheck (2017a)

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
			E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	63 / 66 m.m. 43 / - [a] m.m. 38 / - [a] m.m. 33 / 33 m.m. (72 hours / 96 hours)	
2,4-dichlorobenzoic acid	*Skeletonema costatum*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	> 100 / > 100 nom. > 100 / > 100 nom. > 100 / > 100 nom. 31.3 / 31.3 nom. > 100 / > 100 nom. n.r. / n.r. n.r. / n.r. 31.3 / 31.3 nom. (72 hours / 96 hours)	Obert -Rauser (2018b)
4-Carboxyl-F9600	*Skeletonema costatum*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	86 / > 110 m.m. 59 / 67 m.m. n.r. / 55 m.m. 48 / 48 m.m. 75 / 83 m.m. n.r. / 60 m.m. n.r. / n.r. 48 / 48 m.m. (72 hours / 96 hours)	Softcheck (2018c)

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
F9600-dimethyl-malonamide	*Skeletonema costatum*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	> 100 / > 100 m.m. > 100 / > 100 m.m. > 100 / > 100 m.m. 48 / 48 m.m. > 100 / > 100 m.m. n.r. / > 100 m.m. 51 m.m. / n.r. 48 / 48 m.m. (72 hours / 96 hours)	Softcheck (2018d)
F9600-3-OH-propanamide	*Skeletonema costatum*	Static, 96-hours	E_rC_{50} E_rC_{20} E_rC_{10} NOE_rC E_yC_{50} E_yC_{20} E_yC_{10} NOE_yC	> 85 [b] m.m. 47 [b] m.m. 32 [b] m.m. 13 [b] m.m. 70 [b] m.m. 30 [b] m.m. 16 [b] m.m. 13 [b] m.m. (72 hours)	Softcheck (2017b)
Toxicity to aquatic macrophytes					
2,4-dichlorobenzoic acid	*Myriophyllum spicatum*	Static renewal, water-sediment system (dosed via water), 14-days	$E_rC_{50, \text{ shoot length}}$ $E_rC_{20, \text{ shoot length}}$ $E_rC_{10, \text{ shoot length}}$ $NOE_rC_{, \text{ shoot length}}$ $E_yC_{50, \text{ shoot length}}$	24 m.m. 4.3 m.m. 1.1 m.m. 0.92 m.m. 11 m.m.	Kirkwood (2018)

Test substance	Test organism	Test system	Endpoint (mg/L)		Reference
			$E_yC_{20,\ shoot\ length}$	3 m.m.	
			$E_yC_{10,\ shoot\ length}$	1.1 m.m.	
			$NOE_yC_{,\ shoot\ length}$	3.3 m.m.	
4-Carboxyl-F9600	*Myriophyllum spicatum*	Static renewal, water-sediment system (dosed via water), 14-days	$E_rC_{50,\ shoot\ length}$	>1.3 m.m.*	Dill (2018a)
			$NOE_rC_{,\ shoot\ length}$	1.3 m.m.	
			$NOE_yC_{,\ shoot\ length}$	1.3 m.m.	
F9600-dimethyl-malonamide	*Myriophyllum spicatum*	Static renewal, water-sediment system (dosed via water), 14-days	$E_rC_{50,\ shoot\ wet\ weight}$	> 100 nom.	Dill (2018b)
			$E_rC_{20,\ shoot\ wet\ weight}$	17.9 nom.	
			$E_rC_{10,\ shoot\ wet\ weight}$	6.09 nom.	
			$NOE_rC_{,\ shoot\ length}$	3.05 nom.	
			$E_yC_{50,\ plant\ dry\ weight}$	38.7 nom.	
			$E_yC_{20,\ plant\ dry\ weight}$	5.69 nom.	
			$E_yC_{10,\ plant\ dry\ weight}$	n.r.	
			$NOE_yC_{,\ shoot\ length}$	3.05 nom.	

n.r. = not reported; nom. = nominal concentration; m.m. = mean measured concentration; ini. = initial measured concentration

[a] *Suitable values for risk assessment not available for effects at 96-hours*

[b] *The endpoints relate to the data at 72-hours, as the results at 96-hours are not considered suitable*

**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).*

Further information

For information about health and safety, or to report inconsistencies or inaccuracies in this guidance, visit the Agency website.

You can order the Agency priced publications at the Agency books website.

the Agency priced publications are also available from bookshops.

This publication is available on the Agency website.

Published by the Health and Safety Executive