Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one

EC Number: -
CAS Number: 81777-95-9

CLH-O-0000007325-75-01/F

Adopted

8 June 2023

8 June 2023

CLH-O-0000007325-75-01/F

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

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

Chemical name: **bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one**

EC Number: **-**

CAS Number: **81777-95-9**

The proposal was submitted by **the Netherlands** and received by RAC on **3 June 2022.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 June 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 August 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anja Menard Srpčič**

Co-Rapporteur, appointed by RAC: **Annemarie Losert**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2023** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one	-	81777-95-9	**Add** Aquatic Acute 1 Aquatic Chronic 1	**Add** H400 H410	**Add** GHS09 Wng	**Add** H410	-	**Add** M = 1 M = 10	-
RAC opinion	TBD	bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one	-	81777-95-9	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	M = 1 M = 10	-
Resulting Annex VI entry if agreed by COM	TBD	bixlozone (ISO); 2-(2,4-dichlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one	-	81777-95-9	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410	-	M = 1 M = 10	-

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Based on the partitioning properties of bixlozone (= F9600) (log P_{OW} = 3.3) and its water solubility (~41 mg/L) the vehicles applied in different toxicological studies appear to be suitable i.e. 4:1 acetone / olive oil; DMSO; acetone; 0.5% (w/v) carboxymethylcellulose (CMC) in 5% tween 80.

The purity of bixlozone tested in the toxicological studies ranged from 95.9% - 99.9% and were considered adequate for testing.

Based on the information from several toxicokinetic studies in rats and mice, with and without radiolabelled bixlozone, using single low and high oral doses, repeated oral doses and single i.v. doses, the following can be concluded: Bixlozone is well absorbed by the oral route (oral absorption value 70% was derived for reference dose setting (see annexed DAR to the CLH report)), it is widely distributed and there is no evidence for accumulation. Rate and extent of exposure increased with dose, but not proportionally to the increase, indicating non-linear kinetics in rat and there were indications that metabolism of bixlozone is enhanced with time. Exposure of bone marrow was demonstrated. The substance was heavily metabolised and excreted mainly via the urine. Further, the results indicated that there was significant first-pass metabolism of bixlozone in rats.

Comparative *in vitro* metabolism assays in hepatocytes of rats, mice, dogs and humans indicated largely similar metabolite profiles across all tested species. However, one metabolite (4-hydroxymethyl-F9600) was formed in disproportionate amounts in human hepatocytes, compared with only trace amounts in rat and mouse hepatocytes. The CLH report stated based on QSAR-analysis that this metabolite does not present a genotoxicity concern.

There were no gender differences identified in the *in vivo* studies, or in the comparative *in vitro* studies, except that absorption in female rats was bit higher than in males, while the opposite was observed in mice.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The substance is a solid and the Dossier Submitter (DS) proposed no classification in any physical hazard class. Hazard classes for liquids and gases are not applicable.

Explosive properties

The DS proposed no classification for the substance based on a test according to EC Method A.14 as well as on an assessment of the chemical structure.

Flammable solid

The DS proposed no classification for the substance based on the results of a test according to EC Method A.10. For negative test results, this EC test is comparable to the CLP criteria that refer to the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria (UN RTDG, MTC).

Self-reactive substances and mixtures

The DS proposed no classification for the substance based on the assessment of the chemical structure, as well as melting and boiling point measurements.

Pyrophoric solids

The DS proposed no classification for the substance based on experience in use, as well as melting and boiling point measurements.

Self-heating substances and mixtures

The DS proposed no classification for the substance based on tests related to the melting and boiling point, as well as the relative self-ignition for solids.

Substances and mixtures which in contact with water emit flammable gases

The DS proposed no classification for the substance based on an assessment of the chemical structure and experience in handling, as well as tests on water solubility.

Oxidising solids

The DS proposed no classification for the substance based on an assessment of the chemical structure and a test according to EC Method A.17.

Organic peroxide

The DS proposed no classification for the substance based on an assessment of the chemical structure.

Corrosive to metals

The DS proposed no classification for the substance based on the physical state, the melting point as well as the chemical structure.

Comments received during consultation

During the commenting period, one supporting comment for non-classification in any physical hazard class was received from a company (manufacturer).

Assessment and comparison with the classification criteria

Explosive properties

RAC disagrees with the DS conclusion, as it is not possible to conclude on explosive properties based on the provided data. The assessment of the chemical structure shows that an N-O functional group is present in the substance. N-O is listed as an example for chemical groups that can exhibit explosive properties in appendix 6 to the UN RTDG, MTC. Furthermore, the oxygen balance is -166.3, which is higher than the cut-off value set at -200 (see Annex I, 2.1.4.3.(b)). No data on the exothermic decomposition energy was provided. Therefore, a test according to the CLP criteria (cf. to UN RTDG, MTC) is necessary to conclude on explosive properties. A test according to EC Method A.14 is provided, which refers to the UN RTDG and includes tests on thermal sensitivity, as well as sensitivity to shock and friction. Although both test methods investigate the afore-mentioned properties and the EC method is based on the UN RTDG, the conducted EC test A.14 is not considered acceptable for the purposes of classification according to Annex I, section 2.1.2. of the CLP regulation, as it is not identical to the UN RTDG

test series. Consequently, **no classification due to the lack of data is warranted for explosive properties**.

Flammable solid

RAC agrees with the DS conclusion that the substance **does not warrant a classification as flammable solid**. According to the CLP criteria the UN RTDG, MTC test N.1 should be conducted to conclude on the flammability of the substance. EC Method A.10 is provided, which gives a negative result, as the flame did not propagate along the sample train. This test is considered acceptable for the purposes of classification according to the CLP criteria (CLP, Annex I, 2.2.2.), as in both tests the principle of the method as well as the preparation of the test is comparable.

Self-reactive substances and mixtures

RAC disagrees with the DS conclusion, as it is not possible to conclude on self-reactive properties based on the data provided, as stipulated in Annex I, section 2.8.2. of the CLP regulation. The assessment of the chemical structure shows that an N-O functional group is present in the substance. N-O is listed as an example for chemical groups that can exhibit explosive properties in appendix 6 to the UN RTDG, MTC. No data on the exothermic decomposition energy was provided. Therefore, a test according to the CLP criteria (cf. to UN RTDG, MTC Test Series A to H) is necessary to conclude on self-reactive properties. As no test was provided, it is not possible to conclude on self-reactive properties of the substance. Consequently, **no classification due to the lack of data is warranted for self-reactive substances and mixtures.**

Pyrophoric solids

RAC agrees with the DS conclusion that the substance **should not be classified as pyrophoric solid**. The substance does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room temperature for prolonged periods of time (days)). This is based on experience and handling of the substance, which is in accordance with the CLP criteria, Annex I, section 2.9.2.

Self-heating substances and mixtures

RAC agrees with the DS conclusion that the substance is not classified as self-heating substance. According to the guidance on the application of the CLP criteria, Annex I, section 2.11.2., substances that are completely molten up to a temperature of ≤160°C shall not be considered for classification into this hazard class. This is due to the fact that the melting process is endothermic and the substance-air surface is drastically reduced. The melting range is reported as 81.5-83.5°C and therefore the substance does **not warrant classification as a self-heating substance in accordance** with the CLP criteria.

Substances and mixtures which in contact with water emit flammable gases

RAC agrees with the DS conclusion that the substance is not classified as substance, which in contact with water emits flammable gases. According to the CLP criteria (Annex I, section 2.12.2.) the classification procedure needs not be applied if the chemical structure of the substance or mixture does not contain metals or metalloids. As the chemical structure of the substance does neither contain any metals nor metalloids, the substance is **not classified as substance, which in contact with water emits flammable gases**.

Oxidising solids

RAC disagrees with the DS conclusion, as it is not possible to conclude on oxidising properties based on the provided data. The substance contains oxygen and chlorine, which are bound to carbon or hydrogen atoms (i.e. N-O functional group) and therefore testing is required. A test

according to EC Method A.17 is provided, which is, however, not comparable to the CLP criteria. Hence, a test according to the CLP criteria, Annex I, section 2.14.2. (cf. to UN RTDG, MTC test O.1 and O.3) is necessary to conclude on oxidising properties. Consequently, **no classification due to the lack of data is warranted as an oxidising solid.**

Organic peroxide

RAC agrees with the DS conclusion that the substance is not classified as organic peroxide. The substance does not contain bivalent –O-O- structures and is therefore **not an organic peroxide** according to the CLP criteria, Annex I, section 2.15.2.

Corrosive to metals

RAC agrees with the DS conclusion, as it is not possible to test a solid substance for corrosive to metals properties. According to the guidance on the application of the CLP criteria substances with melting points below 55°C must be considered for classification. The substance has a melting range from 81.5-83.5°C and therefore, the testing procedure cannot be applied. However, if the substance may become a liquid (e.g. by dissolving in water) testing should be considered. The substance as a solid is **not classified as corrosive to metals in accordance** with the CLP criteria (Annex I, section 2.16.2.) as there is no applicable testing procedure available.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for acute toxicity via the oral, dermal or inhalation routes.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

The data on acute toxicity presented in the CLH dossier covered the oral, the dermal and the inhalation routes of exposure. A concise overview of the studies can be found in table 2.6.2.1.-1. of the CLH report.

Acute toxicity – oral route

The oral studies consisted of an acute toxicity study according to OECD 425 (2008) and GLP in SD albino rats (2013TOX-ISX0999, 2014a), a dose range finding study for an acute neurotoxicity study (OECD 424) in Crl:CD(SD) rats (2013TOX-ISX1065, 2014b) which was not conducted according to guideline nor GLP (supplementary information) as well as an acute neurotoxicity study according to OECD 424 and GLP in Crl:CD(SD) rats (2013TOX-ISX1066, 2014c). The latter two studies had minor deviations from the guideline, which were not considered to have an impact on the study results.

No deaths occurred in any of the studies and only slight and transient signs of toxicity were observed. These observations consisted of:

- hypoactivity, irregular respiration and decreased defecation in 2 out of 3 animals at the top dose (2000 mg/kg bw), from which the animals fully recovered on day 2 (2013TOX-ISX0999, 2014a)
- sporadic incidences of slightly soiled fur in all treated groups and red deposits around the nose ≥ 1000 mg/kg bw/d (2013TOX-ISX1065, 2014b)
- yellow material around the urogenital area in top dose (2000 mg/kg bw) females (2012TOX-ISX1066, 2014c)
- transient lower mean total and ambulatory motor activity counts were observed in males and females in all treated groups (500, 1000, 2000 mg/kg bw) at the beginning of the test, although without dose-response (2012TOX-ISX1066, 2014c)

No additional effects on behavioural endpoints were reported and there were no test material related macroscopic or microscopic findings nor effects on brain weights or dimensions.

Based on these results it can be concluded that the acute oral LD_{50} value for bixlozone exceeds the upper cut-off level of 2000 mg/kg bw for Category 4 and no classification is warranted.

Acute toxicity – dermal route

The only acute dermal toxicity study available followed OECD 402 (1998) and GLP and was conducted in SD albino rats (2013TOX—ISX0998, 2014b). There was a deviation from the protocol, i.e. the dermal application site constituted less than 10% of the animals' surface area. It was reported that this was a consequence of the small quantity of test material applied.

No mortality, clinical signs or gross abnormalities were observed. The only finding was slight dermal irritation at the application site in 2 out of 5 males treated with a single dose of 2000 mg/kg bw on the day of application, which was fully reversed on the next day. No dermal irritation was observed in any females.

RAC notes that it can be assumed that greater amounts of the test material would have been absorbed if the required 10% of the animal's surface had been covered with the test material.

Nevertheless, as there is also a dermal 21-day study (2016TOX-ISX2425, 2016) available, which did not induce mortality up to 1000 mg/kg bw (see section on STOT RE), and given that also the acute toxicity studies using the oral and inhalation routes did not induce mortality, it can be concluded that the acute dermal LD_{50} value for bixlozone lies above the upper cut-off level of 2000 mg/ kg bw for Category 4 and no classification is warranted.

Acute toxicity – inhalation route

The only acute inhalation toxicity study available followed OECD 403 (2009) and GLP without deviations (2013TOX-ISX1000, 2014c). Five SD albino rats per sex were exposed to 2.11 mg/L (aerosol; MMAD: 2.84 µm GSD: 2.09) via nose only for 4 hours. No mortality was reported and the observed irregular respiration, which was seen in all animals following exposure, was fully reversed by day 3. Sporadic weight losses were noted, but they were not considered to be of toxicological significance, as at the end of the study the animals had gained the expected amount of weight.

It can be concluded that the LC_{50} exceeds the applied test concentration of 2.11 mg/L, however, as the upper cut-off value for classification in Category 4 is 5 mg/L it is obvious that the whole range of concentration relevant for classification was not covered in this study. There was no information whether any technical conditions prevented to generate and test a higher concentration. There was only a note that the target dose was selected based on US regulatory requirements. However, given the lack of mortality seen in the acute oral toxicity studies, supported by the toxicokinetic data presented in the CLH report and its annex, which

demonstrated sufficient absorption after oral exposure (mean oral absorption 70%) and the lack of severe findings at the tested dose of 2.11 mg/L, it appears justified to conclude that the LC_{50} is likely to exceed the cut-off value for Category 4 (5 mg/L) and no classification is warranted.

Overall RAC concurs with the DS that **no classification for acute toxicity via the oral, dermal or inhalation route is warranted**.

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT SE.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

The studies relevant for the assessment of STOT SE presented in the CLH report are the same as for the hazard class Acute Toxicity and consisted of one acute oral study, an oral neurotoxicity study and the related dose range study, an acute dermal toxicity study and an acute inhalation study. The details on these studies can be found in the section on acute toxicity above and a concise overview is included in table 2.6.2.1.-1. of the CLH report.

In order to be classified as a substance with a specific organ toxicity after single exposure, the significant non-lethal toxic effect should be observable on a specific organ at a certain dose level. Depending on the level of toxic effect, a substance can either be in Category 1 or Category 2.

The effects seen in the acute toxicity studies via oral, dermal and inhalation route are not considered specific and severe enough to fulfil these criteria.

For the oral and dermal route, the available studies cover the range of doses relevant for classification as STOT SE up to the upper guidance value for Category 2. However, for the available acute inhalation study the only concentration tested was 2.11 mg/L, which is below the upper guidance value for STOT SE 2 (i.e. 5 mg/L). As already described in the section on Acute Toxicity it is not known whether there were any conditions that prevented to generate and test higher concentrations by inhalation. As no relevant findings were observed at 2.11 mg/L it is not considered supportive for a classification as STOT SE.

Based on the results from the studies via oral, dermal and inhalation route it can be concluded that bixlozone does not have a significant toxic effect on any specific organ after single exposure and should therefore not be classified as STOT SE 1 or 2.

No effects relevant for classification as STOT SE 3, i.e. respiratory tract irritation or narcotic effects, were observed.

Overall, RAC concurs with the DS that **no classification for STOT SE is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin corrosion/irritation.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

The data presented in the CLH report consisted of an *in vitro* skin irritation test (SIT) using the Epiderm ™ skin model according to OECD TG 439 (2015) (2018TOX-IXS421, 2018) and *an in vivo* primary skin irritation study in New Zealand albino rabbits according to OECD TG 404 (2002) (2013TOX-ISX0995, 2014d). Both studies were conducted according to GLP and there were no deviations from the test methods. A concise overview of the studies can be found in table 2.6.2.4.-1. of the CLH report.

The *in vitro* study (2018TOX-IXS421, 2018) resulted in a prediction, under the conditions specified in the study, that bixlozone was not irritant to the skin. The cell viability of bixlozone treatment was 109.1% compared to the negative control. The positive control (5% SDS) gave the expected result (information from annex to the CLH report).

In the *in vivo* study (2013TOX-ISX0995, 2014d) no deaths or signs of toxicity were observed during the 72h observation period. All animals gained the expected amount of weight. The mean scores (24 – 72h) for erythema for each rabbit were 0, 0.67 and 0.33 and the mean scores for oedema were 0 for all three rabbits. The erythema reversed fully by 72h in all animals, indicating mild irritation.

RAC notes that also in the acute dermal toxicity study (2013TOX—ISX0998, 2014b) at the top dose (2000 mg/kg bw) irritation was observed in 2 out of 10 animals, which was fully reversible within 24h. However, no signs of dermal changes were reported in the dermal 21-day study up to a dose of 1000 mg/kg bw/d (2016TOX-ISX2425, 2016).

A substance is classified for skin corrosion, Category 1, if it induces irreversible skin damage, whereas skin srritation, Category 2, covers reversible skin damage.

The skin effects induced by bixlozone in animal skin were reversible, therefore a comparison with the criteria for Skin Irritation, Category 2 is indicated.

According to Annex I of CLP reversible damage is defined (dermal exposure for 4h) by the following severity criteria:

1) a mean score of ≥ 2.3 - ≤ 4 for erythema/eschar of for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72h after patch removal or, if reactions are delayed from grades on 3 consecutive days after the onset of skin reactions or
2) inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling or
3) in some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Effects described under (2) and (3) were not observed in the studies with bixlozone and in the rabbit study the scores achieved for erythema/eschar of oedema for each rabbit did not exceed 2.3, as would be required under (1). The reversible skin irritation described in the acute dermal toxicity study is not considered to fulfil the above criteria for classification and also the result of the *in vitro* tests predicted that bixlozone is not irritant.

Overall, RAC agrees with the DS that **no classification for skin corrosion/irritation is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for serious eye damage/irritation, based on the clearly negative results of an *in vivo* eye irritation study in New Zealand albino rabbits according to OECD TG 405 (2012) and an *in vitro* Epiocular ™ eye irritation test (EIT) according to OECD TG 492 (2017). According to the DS the *in vitro* Epiocular ™ eye irritation test (EIT) was not suitable to make any prediction as bixlozone reduced cell viability to 19.4%, which is below 60% cut-off level.

Comments received during consultation

One supporting comment was received from a manufacturer company.

One MSCA commented on the DS's assessment of the Epiocular ™ eye irritation test (EIT) and in contrast to the DS was of the view that the test was clearly positive. The same MSCA further argued that no rationale was presented as to why the results of the negative *in vivo* Draize test should be superior to and overrule the results of the *in vitro* study. The MSCA also pointed out that according to OECD Guidance Document 263 the Draize test (OECD TG 404) is considered to have a high variability and the bottom-up procedure in OECD GD 263 could be applied, which would lead to a classification as Eye Irritant, Category 2.

Assessment and comparison with the classification criteria

The data presented in the CLH report consisted of an *in vitro* Epiocular ™ eye irritation test (EIT) according to OECD TG 492 (2017) (2018TOX-ISX4220, 2018) and an *in vivo* primary eye irritation study in New Zealand albino rabbits according to OECD TG 405 (2012) (2018TOX-ISX4220, 2014e). Both studies were conducted according to GLP and there were no deviations from the test methods. A concise overview of the studies can be found in the table below.

Table: Summary of studies on serious eye damage/irritation *(table 2.6.2.5.-1. from CLH report, supplemented with information from the annex to the CLH report)*

Method, Species, Guideline	**Test substance, doses**	**Results**	**Reference**
In vivo EpiOcular™ eye irritation test (EIT) OECD TG 492 (2017) Deviations: no relevant deviation GLP Cell viability was determined using MTT, interference of the test material with MTT detection was excluded. The test material is within the applicability domain of the test protocol.	Test substance: bixlozone Batch: PL 14-0049 Purity: 95.9%	Cell viability of 19.4% Positive control (Methyl acetate): 13.4%	2018TOX-ISX4220, 2018
In vivo eye irritation study Rabbits, New Zealand albino, 3 females OECD 405 (2012) Deviations: none GLP	Test substance: bixlozone Batch: PL 13-0203 Purity: 98.5% Dose: 0.1ml (0.08g) – applied as ground solid.	Mean scores over 24, 48, 72h for each animal Corneal opacity: 0, 0, 0 Iritis: 0, 0, 0 Conjunctival redness: 0, 0, 0 Conjunctival chemosis: 0, 0, 0 Conjunctival redness and chemosis were noted in all treated eyes to some extent, but only at one hour post-instillation, hence this did not affect the calculation of the mean scores (calculated for 24-72h).	2018TOX-ISX4220, 2014e

The result of the *in vitro* study (2018TOX-ISX4220, 2018) was a clear reduction of cell viability to only 19.4% compared to the negative control, which was close to the value achieved with the positive control (methyl acetate: 13.4%).

In the *in vivo* study (2018TOX-ISX4220, 2014e) no deaths or signs of toxicity were observed. 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-instillation, hence this did not affect the calculation of the mean scores (calculated for 24-72h). Mean scores for each animal for corneal opacity, iritis and conjunctival redness and chemosis over 24, 48 and 72 hours were 0 and bixlozone was not eye irritant under the conditions of this study.

While the *in vivo* OECD TG 405 study (2018TOX-ISX4220, 2014e) was clearly negative, the result of *in vitro* OECD TG 492 study (2018TOX-ISX4220, 2018) has to be interpreted as positive, with a cell viability of only 19.6% compared to the negative control. The OECD TG 492 specifies for the applied test system (EpiOcular™) that no classification is indicated for substances that reduce

cell viability above 60% level, whereas for substances that reduce cell viability below 60% level, no distinction can be made between Category 1 and 2 and no prediction can be made in isolation.

It is relevant to note that tests performed according to OECD TG 492 have a rather high rate of false positive results. For the EpiOcular™ test system it is as high as 37% (based on 55 chemicals), when compared to OECD TG 405 *in vivo* rabbit eye test data (see OECD TG 492, para 14). According to OECD TG 492 and OECD GD 263 a positive result of an OECD TG 492 test requires further testing with (an)other *in vitro* test method(s), or as a last option an *in vivo* test in rabbits (OECD TG 405). It is not known to RAC why in this case an *in vitro* test was carried out after a reliable *in vivo* study was available. The above described step-wise procedure would normally conclude with a result of a reliable *in vivo* study performed according to OECD TG 405.

RAC notes that the *in vivo* study (OECD TG 405) has strengths as compared to the *in vitro* studies (i.e. it formed the basis for the classification system, it reflects all possible modes of action, reversibility/persistence of effects can be directly observed), but also certain weaknesses: identification of Category 1 substances based on effects in a single eye has uncertainty, allocation of the scores might be subjective, uncertainties regarding the actual exposure duration influenced by species differences in relation to the type of test material or possibility that mechanical damage is induced if the test material is solid (see OECD GD 263). It is noted that the majority of these weaknesses are not relevant for the present *in vivo* study. The fact that the test material was applied as a solid is not considered to have an impact on the result, as it induced as the only effect conjunctival redness and chemosis observed only at 1h post-instillation, and no effects at all were seen after 24h or later. Therefore, identification of Category 1 is not relevant. It is further noted that "no effect" is less prone to subjectivity than grading of the degree of an effect.

In conclusion RAC, in line with the DS, is of the view that **no classification for serious eye damage/irritation is warranted**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification for skin sensitisation.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

The only study presented in the CLH report for the assessment of this hazard class was a mouse local lymph node assay (LLNA) conduced according to OECD TG 429 (2010) (2013TOX-ISX0997, 2014f) and GLP. No deviation from the test method was reported.

The appropriate dosing was determined in a preliminary study using 2 females per dose. A concentration of 25% was the highest dose which could technically be applied owing to the high viscosity of the test solution. No irritation was observed during the preliminary test. Based on these results the doses applied in the main study were 5%, 10% and 25% in acetone / olive oil (AOO; 4:1 v/v) on 3 consecutive days, using 5 females per dose. A single concentration of 25% w/w mixture of alpha-hexylcinnamaldehyde (HCA) in AOO (4:1 v/v) served as positive control.

There were no mortalities, but some treated animals failed to gain weight during the study. However, animals appeared active and healthy during the study. Very slight erythema (score 1) was evident at one positive control site on Day 2, at all sites on Day 3 and at one site on Day 6. Very slight oedema (score 1) was present at two dose sites on Day 3 and desquamation was present at all dose sites on Day 6 in the positive control.

Three days after the last dosing (Day 6) the mice were given an i.v. injection containing 20 µCi of ^{3}H -methyl thymidine and 5h later animals were euthanised, lymph nodes were harvested and prepared for analysis in the scintillation counter. The positive control gave the expected result, i.e. a stimulation index (SI) of 4.8 (as compared to the negative control). In contrast, the three doses tested resulted in SI < 3, i.e. 1.13, 1.32 and 1.57 for the 5%, 10% and 25% concentration, respectively. As none of the concentrations exceeded SI 3, bixlozone is considered negative under the conditions of this test.

The LLNA is the only study available for bixlozone for this hazard class, no other *in vitro* or *in vivo* studies nor human data are available.

According to the CLP criteria a substance is classified for skin sensitisation in Sub-category 1A if an EC3 value ≤ 2% is obtained in an LLNA. Sub-category 1B is reserved for substances that that result in an EC3 value > 2%.

SI ≥ 3 is the requirement to consider a substance as showing significant skin sensitising effects (i.e. Skin sensitiser 1), but none of the tested concentrations resulted in SI ≥ 3.

In conclusion, RAC in line with the DS is of the view that the available data **do not warrant classification of bixlozone for skin sensitisation**.

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

Summary of the Dossier Submitter's proposal

Based on the available data the DS concluded that liver and kidney were clear target organs of repeated bixlozone exposure, however, only at doses exceeding the relevant guidance values. Effects at lower doses were either not considered severe enough or were not repeated after longer exposure duration in the same species (dog).

The DS also summarised the findings in the thyroid, and did not propose a classification for this organ.

In addition, the DS concluded that bixlozone had an adverse impact on food consumption and body weight gain, and this was partly explained by the low palatability of bixlozone. The DS concluded that these findings were not severe enough at doses relevant for classification.

Overall, the DS concluded that the observations made in the different repeated dose toxicity studies were not supportive for a classification as STOT RE.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

The available data presented in the CLH report for the assessment of the hazard class STOT RE consisted of oral studies in rats, mice and dogs with duration of 28 days, 90 days and 1 year of exposure, as well as a dermal rat study with 21 days duration. In addition, the DS referred to three oral (dietary) 7-day studies in rats, mice and dogs and a 7-day capsule study in dogs, which were conducted to assess palatability of bixlozone and for dose range finding. The DS also referred to the two available carcinogenicity studies in rats and mice. RAC also assessed the available two-generation study as well as the two pre-natal developmental toxicity studies for this hazard class.

The table below summarises the relevant findings from the sub-acute and sub-chronic toxicity studies.

Table: Sub-acute and sub-chronic toxicity studies *(table 2.6.3.-1 from the CLH report, supplemented with information from the annex to the CLH report)*

Method, species, test substance	**Doses**	**Results** **- NOAEL/LOAEL** **- Critical effects at the LOAEL** **- Relevant guidance values STOT RE**	**Main effects**	**References**
28-day, dietary Rat, Crl:CD9(SD), males & females, 5/sex/toxicology group, 9/sex/toxico-kinetic group (3/sex/control group) GLP OECD 407 (2008) Deviations: None bixlozone, batch PL13-0385 Purity: 99.2%	0, 750, 2500, 5000, & 10000 ppm (for toxicology & toxicokinetic groups) Equivalent to: **Males:** 0, 57, 182, 359 & 740 mg/kg bw/d **Females:** 0, 61, 193, 379 & 733 mg/kg bw/d	**NOAEL** of 750 ppm (equivalent to 57 & 61 mg/kg bw/d in males & females respectively) Based on decreased body weight gain, decreased food consumption & liver weight increase, accompanied by hepatocellular hypertrophy in males & females at 2500 ppm. **LOAEL:** 2500 ppm (equivalent to 182 & 193 mg/kg bw/d in males & females respectively) **Guidance values:** STOT RE, Category 1: C ≤ 30 mg/kg bw/d STOT RE, Category 2: 30 < C ≤ 300 mg/kg bw/d	All animals survived to the scheduled necropsy. There were no clinical signs of toxicity at any dose. **10000 ppm** ↓ body weight (F: 18.2%), ↓ body weight gain (M: -13.6% and F: -58.6%), significant in F ↓ food consumption significant (F: -41% day 0-7, -17% day 7-14, -22% day 14-21 and -22% day 21-27; M: only Day 0-7 -20%) ↑ absolute and relative liver weights significant (M: +56.3% abs, +65.5% rel; and F: 32.2% abs, +60.9% rel) Hepatocellular hypertrophy: 5/5 mild (F) & 4/5 mild & 1/5 moderate (M) ↑ relative kidney weights significant (M: +14.5% & F: +14.4%)	2013 TOXISX 1073, 2015a

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
			↑ total protein (M: +13%), ↑ albumin (M: +11%), ↑ globulin (M: +12% and F: +15%), ↑ cholesterol (M: +79% and F: +91%), ↑ blood urea nitrogen (F: +45%), ↑ triglyceride (F: +86%) (significant) **5000 ppm** ↓ body weight gain (M: -24.1% day 0-7, -9.0% day 0-27 and F: significant -53.8% day 0-7, -25.7% day 0-27) ↓ food consumption (F: -24% day 0-7, -17% day 7-14, -17% day 14-21 & -11% (not sig.) day 21-27; M: only Day 0-7 -16%) ↑ absolute and relative liver weights (M: +10% abs (not sig.), +23.2% rel; and F: +18.9% abs, 29.0% rel) Hepatocellular hypertrophy: 3/5 minimal & 2/5 mild (M), 1/5 minimal & 4/5 mild (F) ↑ cholesterol (M: +32% not significant and F: +43% significant) **2500 ppm** ↓ body weight gain not significant (M: -20.3% day 0-7, -14.1% day 0-27 and F: -23.1% day 0-7, -22.9% day 0-27) ↓ food consumption (F: significant: -12% day 0-7, - 11% day 7-14) ↑ relative liver weight significant (M: +15.5% & F: +16.9%)	

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
			Hepatocellular hypertrophy: 3/3 minimal (M) & 4/4 minimal (F) ↑ cholesterol (M & F: exceeding 10%, but stat signif)) **750 ppm** No adverse effects	
28-day, dietary **Mouse**, Crl:CD-1, males & females, 5/sex/group GLP OECD 407 (2008) Deviations: none bixlozone, batch PL13-0385 Purity: 99.2%	0, 1000, 2000, 4000, and 5000 ppm Equivalent to: **Males:** 0, 187, 381, 788 & 985 mg/kg bw/d **Females:** 0, 289, 554, 984 & 1384 mg/kg bw/d	**NOAEL** of 2000 ppm (554 mg/kg bw/d females) Based on liver weight increases accompanied by hepatocellular hypertrophy in males & females at ≥ 4000 ppm **LOAEL**: 4000 ppm (788 & 984 mg/kg bw/d in males & females, respectively) **Guidance values:** STOT RE, Category 1: C ≤ 30 mg/kg bw/d STOT RE, Category 2: 30 < C ≤ 300 mg/kg bw/d	All animals survived to the scheduled necropsy. There were no clinical signs of toxicity at any dose. **5000 ppm** Clinical signs (M): yellow material around urogenital, ventral trunk and/or anogenital areas ↑ Relative liver weight (M: +13% and F: +23.7%) ↓ Relative heart weight (M: -14.5%) Hepatocellular hypertrophy: 4/5 M (2 minimal, 2 mild) & 3/5 F (2 minimal, 1 mild) ↑ ALT (M: +137%) **4000 ppm** Clinical signs (M): yellow material around urogenital, ventral trunk and/or ano-genital areas ↑ absolute and relative liver weight (F: +18.3% abs; +21.5% rel) ↓ relative heart weight (M: -18.1%) Hepatocellular hypertrophy: 1/5 M (minimal) & 2/5 F (minimal)	2013 TOXISX 1074, 2015b

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
			2000 ppm No adverse effects **1000 ppm** No adverse effects	
28-day, dietary **Dog, Beagle,** males & females, 2/sex/group bixlozone, batch PL14-0049 Purity: 96% Vehicle: acetone GLP Dose-range finding study (loosely follows OECD 409) No statistical analysis was performed as only two animals were used per dose group.	0, 1000, 3000, 10000 & 30000 ppm Equivalent to: **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 supple-mentation)	**No reliable NOAEL** could be set from this study	All animals survived to the scheduled necropsy. There were no clinical signs of toxicity at any dose. No test substance-related effects on haematology, coagulation and urinalysis parameters. At 30000 ppm dose group:	2013 TOXISX 1087, 2016a
Severely reduced food consumption leading to lower overall body weights and lower body weight gains; food supplementation resolved the palatability issues, but body weight gain at Day 27 still markedly lower than controls. Reduced body weight gain in all dose groups in F and in the 10000 and 30000 ppm dose groups in M. Increased liver weights: from 10000 ppm associated with hepatocellular hypertrophy indicative of liver enzyme induction in both males and females; considered adverse in nature. Kidney weights (relative and absolute) were increased at 30000 ppm in F and from 1000 ppm in M. Associated renal tubular hypertrophy and interstitial inflammation. → Considered treatment related and adverse at the two highest doses.				

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References

Changes in the weights of other organs were seen in both directions (up and down) and without relation to dose. Histological changes in the thymus (i.e. lymphoid depletion at 10000 ppm in 1 male (mild) and at 30000 ppm in 2 males (mild and moderate) might be indicative of a secondary effect as a consequence of stress (decrease in body weight / body weight gain at these doses).

Information from the annex to the CLH report (Table 6.3.1.7.-5):

	Males					Females				
Dosage (ppm)	**0**	**1000**	**3000**	**10000**	**30000**	**0**	**1000**	**3000**	**10000**	**3000**
(mg/kg bw/d)	**0**	**38**	**134**	**370**	**1015**	**0**	**39**	**108**	**309**	**111**
	Organ weights									
Terminal bodyweight (kg)	8.1 ± 0.28	7.9 ± 0.92	8.2 ± 0.78	8.1 ± 0.21	6.9 ± 0.99	7.5 ± 0.92	6.9 ± 0.49	7.2 ± 0.57	7.1 ± 0.71	6.8 ± 0.7
Kidney (g)	38.91 ± 0.15	44.04 ± 2.38	44.51 ± 3.67	47.53 ± 6.70	46.86 ± 8.78	38.11 ± 3.66	35.51 ± 1.37	36.04 ± 3.35	35.84 ± 3.72	48.63 0.2
Kidney (% difference from control)		13.2%	14.4%	22.1%	20.4%		-6.8%	-5.4%	-6.0%	27.6
R		17.1%	13.5%	22.9%	41.0%		1.2%	-2.5%	-1.6%	40.1
Liver (g)	233.33 ± 15.73	206.88 ± 1.41	246.35 ± 41.57	278.31 ± 11.62	303.93 ± 46.25	190.22 ± 48.92	188.49 ± 1.711	209.29 ± 18.095	230.05 ± 5.996	311.0 53.0
Liver (% difference from controls)		-11.3%	5.6%	19.27%	30.3%		-0.9%	10.0%	20.9%	63.5
R	-	-8.0%	4.3%	20.4%	53.0%		8.8%	14.6%	28.5%	79.6

R % change in relative weight (organ weight to body weight) from control
N=2 per dose

Information from the annex to the CLH report (Table 6.3.1.7.-6):

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References

	Males					Females				
Dosage (ppm)	**0**	**1000**	**3000**	**10000**	**30000**	**0**	**1000**	**3000**	**10000**	**3000**
(mg/kg bw/day)	**0**	**38**	**134**	**370**	**1015**	**0**	**39**	**108**	**309**	**1110**
	Microscopic findings									
Liver (N)	2	2	2	2	2	2	2	2	2	2
Hypertrophy, hepatocellular, diffuse	0	0	0	2	2	0	0	0	2	2
Minimal	-	-	-	2	1	-	-	-	2	0
Mild	-	-	-	0	1	-	-	-	0	2
Kidney (N)	2	2	2	2	2	2	2	2	2	2
Hypertrophy, renal tubular epithelium	0	0	0	1	1	0	0	0	0	0
Mild	-	-	-	0	1	-	-	-	-	-
Moderate	-	-	-	1	0	-	-	-	-	-
Inflammation, interstitial	0	0	0	0	1	0	0	0	0	2
Minimal	-	-	-	-	1	-	-	-	-	1
Mild	-	-	-	-	0	-	-	-	-	1
Thyroid (N)	2	2	2	2	2	2	2	2	2	2
Cysts	0	0	0	0	0	0	0	1	0	0
Decreased colloid	0	0	0	0	0	0	0	0	0	1
Mild	-	-	-	-	-	-	-	-	-	1
Hyperplasia, nodular, C-cell	0	0	0	0	0	0	0	0	0	1
Mild	-	-	-	-	-	-	-	-	-	1

90-day, dietary **including neurotoxicity phase** **Rat, Crl:CD9(SD), males & females,** 21/sex/group or 16/sex group bixlozone, batch PL14-0049 Purity: 96% Vehicle: acetone GLP OECD 408 (1998) & OECD 424 (1997)	**Males:** 0, 500, 2000, and 8000 ppm Equivalent to: 0, 29, 121 & 505 mg/kg bw/d **Females:** 0, 500, 2000, and 5000 ppm Equivalent to: 0, 37, 150 & 351 mg/kg bw/d 90 days continuous dosing Recovery period: 28 days (5/sex group)	**NOAEL** of 500 ppm (29/37 mg/kg bw/d in M/F) Based on increased kidney weights in males and increased liver weights accompanied by hepatocellular hypertrophy in females at 2000 ppm **LOAEL:** 2000 ppm (121/150 mg/kg bw/d in M/F) **Guidance values:** STOT RE, Category 1: C ≤ 10 mg/kg bw/d STOT RE, Category 2: 10 < C ≤ 100 mg/kg bw/d	One male (500 ppm) was found dead on day 87 (undetermined cause; not considered treatment related); There were no clinical signs of toxicity at any dose. There was no evidence of neurotoxicity associated with exposure to bixlozone. **8000 (M) / 5000 (F) ppm** ↓ body weight and body weight gain (M: -9.1% BW, -17.6% BWG; and F: -9.5% BW, -22.8% BWG)	2013 TOXISX 1085, 2016a

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
Deviations: none			↓ food consumption d0-7 (M: -12.6% and F: -26.3%) ↑ absolute and relative liver weights significant (M: +34.4% abs, +50.7% rel; and F: +21.0% abs, +34.0% rel) ↑ absolute and relative kidney weights significant in M: +21.5% abs, +36.6% rel & relative kidney weights significant in F: +16.6% Hepatocellular hypertrophy: 10/10 M (1 minimal, 6 mild, 3 moderate) & in 10/10 F (7 mild, 3 moderate) Macrovascular vacuolation (liver) 5/10 M (4 minimal, 1 moderate) Follicular cell hypertrophy thyroid (mild): 3/10 (M) & 5/10 (F) ↑ Cholesterol (M and F), ↑ globulin (F), ↑ calcium (F), ↓ glucose (F) **2000 ppm** ↑ kidney weights in males significant: +15% (absolute) & +15% (relative) ↑ liver weights in females significant: +15% absolute & +17% relative Hepatocellular hypertrophy 1/10 F (mild) ↑ Cholesterol (F), ↑ calcium (F)	

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
			500 ppm No adverse effects In animals with hepatocellular hypertrophy centrilobular hepatocytes were enlarged and had a prominent rough endoplasmic reticulum.	
90-day, dietary Mouse, Crl:CD1(ICR), males & females, 10/sex/ toxicology group, 12/sex/toxicokinetic Group bixlozone, batch PL14-0049 Purity: 96% Vehicle: acetone GLP OECD 408 (1998)	0, 1000, 2250, and 5000 ppm Equivalent to: **Males:** 0, 180, 414 & 930 mg/kg bw/d **Females:** 0, 257, 583 & 1185 mg/kg bw/d	**NOAEL** of 1000 ppm (180/257 mg/kg bw/d in M/F). Based on absolute and relative liver weight increases, accompanied by hepatocellular hypertrophy in both sexes at 2250 ppm. **LOAEL:** 2500 ppm (414/583 mg/kg bw/d in M/F) **Guidance values:** STOT RE, Category 1: C ≤ 10 mg/kg bw/d STOT RE, Category 2: 10 < C ≤ 100 mg/kg bw/d	There were no test-substance related deaths or clinical signs of toxicity. **5000 ppm** ↑ absolute and relative liver weights significant: (M: +23.1% abs, +22.8% rel; and F: +20.2% abs, +21.1% rel) ↓ adrenal weight significant (F: -15.9%) Hepatocellular hypertrophy in 10/10 M (1 minimal, 9 mild) and 3/9 F (1 minimal, 2 mild) ↓ neutrophil counts (F: -39.7%), ↑ serum glucose (F: +19.0%) **2250 ppm** ↑ absolute and relative liver weights, not significant for absolute weight males (M: +11.0% abs, +11.2% rel, and F: +12.8% abs, +17.5% rel) ↓ adrenal weight not significant (F: -12.1% abs, -10.8% rel) Hepatocellular hypertrophy in 4/10 M (3 minimal, 1 mild) and 3/9 F (minimal) ↓ neutrophil count (F: -42.3%) **1000 ppm** No adverse effects	2013 TOXISX 1086, 2016b

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
90-day, capsule Dogs, Beagle, males & females 4/sex/group bixlozone, batch PL14-0049 Purity: 96% Vehicle: none GLP OECD 409 (1998)	0, 30, 100, 300, and 750 mg/kg bw/d	**NOAEL** of 30 mg/kg bw/d Based on absolute and relative liver weight increases in females at 100 mg/kg bw/d **LOAEL:** 100 mg/kg bw/d **Guidance values:** STOT RE, Category 1: C ≤ 10 mg/kg bw/d STOT RE, Category 2: 10 < C ≤ 100 mg/kg bw/d	There were no treatment-related deaths or clinical signs of toxicity; there was no effect on body weight or food consumption at any dose. **750 mg/kg bw/d** ↑ absolute and relative liver weights (M: +21.3% abs (not sig), +20.3% rel; and F: +54.1% abs, +45.6% rel) Hepatocellular hypertrophy in 2/4 males (minimal) ↑ absolute and relative thyroid weight not significant (M: +42.6% abs, +42.9% rel) ↑ WBC (F: wk 6 +36.8% sig) and ↓ ALT (F: wk 6 -28.6%) **300 mg/kg bw/d** ↑ absolute and relative (significant) liver weights in F (+22.1% abs, +21.5% rel) ↑ absolute and relative thyroid weights not significant (M: +40.6% abs, +28.6% rel) **100 mg/kg bw/d** ↑ absolute and relative liver weights significant in F (+27.1% abs, +21.6%) **30 mg/kg bw/d** No adverse effects	2013 TOXISX 1088, 2016c

Method, species, test substance	Doses	Results - NOAEL/LOAEL - Critical effects at the LOAEL - Relevant guidance values STOT RE	Main effects	References
12 months, capsule **Dogs, Beagle, males & females** 4/sex/group bixlozone, batch PL14-0049 Purity: 96% Vehicle: none GLP OECD 452 (1998)	0, 20, 100 and 500 mg/kg bw/d	**NOAEL** of 100 mg/kg bw/d Based on decreased body weight gain at 500 mg/kg bw/d in F **LOAEL:** 500 mg/kg bw/d **Guidance values:** STOT RE, Category 1: C ≤ 2.5 mg/kg bw/d STOT RE, Category 2: 2.5 < C ≤ 25 mg/kg bw/d	There were no treatment-related deaths or clinical signs of toxicity; there was no effect on food consumption, serum chemistry parameters or microscopic parameters at any dose. **500 mg/kg bw/d** ↓ body weight gain not significant (F: -30%) **100 mg/kg bw/d** No adverse effects **20 mg/kg bw/d** No adverse effects	2013 TOXISX 1091, 2017
21-day, dermal; Rat, Crl:CD(SD), males & females, 10/sex/group bixlozone, batch PL14-0049 Purity: 96% Vehicle: deionized water GLP OECD 410 (1981)	0, 100, 300 and 1000 mg/kg bw/d	**NOAEL:** 1000 mg/kg bw/d (the highest dose level tested) was considered the NOAEL. **Guidance values:** STOT RE, Category 1: C ≤ 85 mg/kg bw/d STOT RE, Category 2: 85 < C ≤ 850 mg/kg bw/d	There were no test-substance related effects. An increased incidence of liver necrosis was observed across all groups including controls. This was considered secondary to wrapping of the torso as described by Perker & Gibbson (1995).	2016 TOXISX 2425, 2016

7-day studies with dietary exposure in rats, mice and dogs and capsule exposure in dogs, which were conducted to assess palatability of bixlozone and for dose range finding

From these studies it can be concluded that there were issues with palatability in rats and dogs, but not in mice. The observed lowered body weight gain, which was seen in several groups of all three species, was considered to be only partly related to low palatability. Liver toxicity was observed in all three investigated species. Increased liver weight was reported in rats and mice and was seen in all dose groups, but no remarkable macroscopic findings were described, and no blood biochemistry was determined in rats and mice. In dogs, liver weight and macroscopic changes were not determined as dogs were returned to the colony after the examination, but blood biochemistry was investigated and increases in cholesterol after the dietary exposure were detected in female dogs although without dose response (note: groups consisted of only 2

animals/sex/dose). No effects on blood cholesterol levels were observed in the 7-day dog study using capsule exposure. Further details on these 7-day studies can be found in the annex of the CLH report.

Carcinogenicity studies

There are two carcinogenicity studies available for bixlozone, a two-year combined chronic toxicity and carcinogenicity study in rats (OECD 453(2009), 2013TOX-ISX1089, 2017) and a 18-months carcinogenicity study in mice (OECD 451(2009), 2013TOX-ISX1090, 2017). For a detailed overview on these studies see the section on carcinogenicity, only the information relevant for STOT RE is summarised here.

In the rat study, there were slight effects on body weight and body weight gain as well as food consumption in males and females of the top dose. Also increased absolute and relative liver weights were seen in males and females, which was accompanied by hepatocellular hypertrophy and increased cholesterol levels as well as increased kidney weight at the top dose of 217/167 (M/F) mg/kg bw/d. This dose clearly exceeds the upper guidance value for classification as STOT RE 2 (i.e. 12.5 mg/kg bw/d).

In the mouse study there was slightly reduced body weight gain and food consumption in the top dose group. Like in the rat study there were increased absolute and relative liver weights in males and females, which was accompanied by hepatocellular hypertrophy in males (18/50) and necrosis in females (5/50) at the top dose of 647/834 (M/F) mg/kg bw/d. Hepatocellular hypertrophy was also seen in males (11/50) in the mid dose of 126/164 (M/F) mg/kg bw/d. Both the mid and top dose exceed the upper guidance value for classification as STOT RE 2 (i.e. 12.5 mg/kg bw/d).

Two-generation study – dose range finding study and main study

For a detailed overview on the rat two-generation study (2014TOX-ISX1294, 2016b) and the related dose range finding study (2014TOX-ISX1295, 2016c) see the section on reproductive toxicity, only the information relevant for STOT RE is summarised here.

<u>Dose range finding study</u>

Exposure duration for the F0 generation was 28 days for males and 57-69 days for females and for the F1 generation 7 days. The extrapolated guidance value for STOT RE 1 and 2 are 32.1 / 321, 16 / 160 and 128.5 / 1285 mg/kg bw/d, respectively. Therefore, the results from all dose groups up to the top dose could be relevant for classification as STOT RE 1 or 2.

In the top dose F0 generation there were slight effects on body weight, body weight gain and food consumption. Top dose kidney weights were increased in males (abs. 7%, rel. 10.8%) and liver weights were increased in males and females (M: abs. 14.9%, rel. 19.3%; F (not stat signif): abs. 14.4%, rel. 12.8%).

In the F1 generation top dose body weight was reduced by 10.2% in females and liver weights were increased in top dose males and females (M: abs. (not stat signif) 16.9%, rel. 23.4%; F: abs. 14.8%, rel. 29.3%).

<u>Main study</u>

Exposure duration for the F0 generation was 127 – 129 days for males and females and for the F1 generation 134 – 148 days for males and females. The extrapolated guidance value for STOT RE 1 and 2 are ~ 7 / 70 mg/kg bw/d, respectively.

No relevant effects were seen at the respective doses in the F0 generation or in the F1 generation.

Developmental toxicity studies

There are two prenatal developmental toxicity studies available, one in rats (2014TOX-ISX1291, 2016e) and one in rabbits (2014TOX-ISX1293, 2015), as well as their respective dose range finding studies (rat: 2014TOX-ISX1290, 2016d, rabbit: 2014TOX-ISX1292, 2014a). Further details on these studies can be found in the section on reproductive toxicity, only the information relevant for STOT RE is summarised here.

In the rat study, the exposure duration was from gestation day (GD) 6 to 19 and the extrapolated guidance value for STOT RE 1 and 2 are 69/690 mg/kg bw/d, respectively.

The top dose of the range finding study was 675 mg/kg bw/d and considered relevant for STOT RE 2. At this dose one animal had to be sacrificed on GD 11. Body weight (-7.5%) and gravid uterine weight (-8.9%) was slightly reduced and body weight gain (-45.3%) and food consumption (-29 – -59%) were considerably reduced at that dose. The liver weight was considerably increased (abs. 52.4%, rel. 64.1%).

In the main study, the top dose was 550 mg/kg bw/d, which is also relevant for STOT RE 2. Like in the preliminary study, body weight gain was considerably reduced (up to -90% on GD 6-9). Body weight and food consumption were less severely affected. Again, there was a considerable increase in liver weight (abs. 28.6%, rel. 38.2%) and there was hepatocellular hypertrophy (mild: 7/25; moderate: 18/25). Comparable, but less severe effects were also seen at the next lower dose of 225 mg/kg bw/d.

In the rabbit study exposure duration was from GD 7 to 28 and the extrapolated guidance value for STOT RE 1 and 2 is 43/430 mg/kg bw/d, respectively.

In the main and dose range finding rabbit study there were considerable effects on body weight gain and food consumption at doses relevant for STOT RE 2 (i.e. at 100 and 350 mg/kg bw/d in the range finding study and at 400 mg/kg bw/d in the main study), which however did not result in lowered body weight and there were no clinical signs reported (except a decrease in defecation during GD 13 – 20 in the main study).

Liver

In line with the DS, RAC is of the view that the liver was a main target of toxicity after repeated exposure to bixlozone.

The observed liver toxicity was characterised by dose dependent increase in absolute and relative liver weights, hepatocellular hypertrophy, occasionally accompanied by changes in related blood-biochemical parameters (i.e. increase in cholesterol, increase in triglycerides, increase in ALT). These findings are likely to be adaptive in nature, as also supported by the increase in rough endoplasmic reticulum in the 90-day rat study (2013TOXISX1085, 2016a) and the early onset (after 7 days already). However, liver microsomal enzyme induction was not investigated. Due to the considerable increases in liver weight at higher doses (increases up to 50 to 60% for absolute and relative liver weight in rats, around 20% for absolute and relative liver weight in mice and around 50% for absolute and relative liver weight in dogs), accompanied by hepatocellular hypertrophy and occasional changes in related blood-biochemical parameters, these findings are considered treatment related and adverse at higher doses. However, at doses relevant for classification as STOT RE 2 these findings were considered not severe enough for classification:

Rat

- 28-day study, dose relevant for STOT RE 2: 182/183 (M/F) mg/kg bw/d:
  Increase in relative liver weight: M: +15.5%; F: +16.9%
  Hepatocellular hypertrophy, minimal: M: 3/3; F: 4/4
- 90-day study, dose close to guidance value (GV) for STOT RE 2: 150 (F) mg/kg bw/d (above GV of 100 mg/kg bw/d, big gap to the next lower dose of 37 mg/kg bw/d):
  Increase in liver weight: F: absolute: +15%, relative: +17%
  Hepatocellular hypertrophy, mild: 1/10 F mild hepatocellular hypertrophy
  Blood biochemistry: F: increase in cholesterol in F
  (at the next higher dose the same effects were seen, but more severe and also in males)

Mouse

- 28-day study: no adverse effects at doses relevant for STOT RE 2 (300 mg/kg bw/d) and above (187/289 (M/F) mg/kg bw/d and 381/554 (M/F) mg/kg bw/d)
- 90-day study, no adverse effects at dose higher than GV of 100 mg/kg bw/d for STOT RE 2: 180/257 (M/F) mg/kg bw/d

Dog

- 28-day study: 370/309 (M/F) mg/kg bw/d, dose just above the GV for STOT RE 2 in F (300 mg/kg bw/d)
  Increase in liver weight: M: abs. 19.3%, rel. 20.4%; F: abs. 20.9%, rel. 28.5%
  Diffuse hepatocellular hypertrophy, minimal: M: 2/2; F: 2/2
  Increase in cholesterol (not dose dependent in M, increase in the two top doses in F): M: +42.5%, F: +29.9%
- 90-day study, dose relevant for STOT RE 2: 100 mg/kg bw/d (M & F):
  Increase in liver weight: F: abs. 27.1 %, rel. 21.6 %
  No histopathological correlations, no relevant changes in related blood-biochemical parameters.
- 12 months study, dose relevant for STOT RE 2: 20 mg/kg bw/d (M & F):
  No adverse effects were seen at this dose or the next higher dose (100 mg/kg bw/d).

The increases in liver weight at doses relevant for STOT RE ranged between 10 and 20%, only in some instances accompanied by minimal hepatocellular hypertrophy and mostly not accompanied by related changes in blood biochemistry. One exception is the 28-day dog study, in which absolute and relative liver weights were clearly increased in all males and females (up to 28.5% for relative liver weight in females), accompanied by minimal hepatocellular hypertrophy in all animals and increases in cholesterol (although the latter finding was not strictly dose dependent). It is, however, noted that there were only 2 dogs per sex/group and no statistical analysis was performed, and that is why the DS considered this study as supplementary only. In addition, it is important to note that neither in the 90-day nor in the 12-month dog study liver findings were observed at a dose relevant for STOT RE 2 that would support classification.

In the two prenatal developmental toxicity studies in rat there were also considerable increases in liver weight which was accompanied by prominent increases in hepatocellular hypertrophy at doses relevant for STOT RE 2. The strong increase in liver weight and the high incidence of hepatocellular hypertrophy (mild to moderate) may be considered to be borderline to adversity. However, as these animals were pregnant, which may have influenced the liver reaction (liver in pregnant animals is already larger than in non-pregnant animals due to the higher demand during pregnancy) and in addition these animals had no clinical signs indicating that there was no functional impairment.

The liver effects seen in the two-generation study in rat were not severe enough at doses supporting classification and no effects in liver were seen in the rabbit studies.

Overall, RAC is of the view that the observed liver effects do not warrant classification for STOT RE.

Kidneys

Kidney was also identified as a target organ after repeated exposure to bixlozone. Increased absolute and relative kidney weights were observed in the 28-day and 90-day studies in rats and in the 28-day study in dogs, and although these increases were profound and considered adverse at higher doses, they were only mild or absent at doses relevant for STOT RE 2. In the 28-day rat study also increase in blood urea nitrogen was seen in females of the top dose group (+ 45%) and in the 28-day dog study hypertrophy of the renal tubular epithelium was seen in 1 out of 2 males each in the two highest dose groups (moderate and mild) and interstitial inflammation was seen in 1 out of 2 top dose males and all (2) top dose females. These findings were seen at doses exceeding or just exceeding the extrapolated guidance value for STOT RE 2. In dogs, the kidney findings were not reproducible in studies of longer duration (90-day and 12 months dog studies). No effects on kidneys were seen in the mouse studies (including the carcinogenicity study).

Overall, RAC concludes that the kidney effects do not warrant for classification as STOT RE.

Thyroid

Adverse effects on the thyroid were seen in rats and dogs, but not in mice.

Decreased colloid (mild) and nodular C-cell hyperplasia (mild) were seen in the 28-day dog study in one female at the top dose (1100 mg/kg/d, 2 females/dose), which is above the extrapolated guidance value of 300 mg/kg bw/d. In the 90-day study absolute and relative thyroid weights were increased only in males at doses ≥ 300 mg/kg bw/d (300 mg/kg bw/d: abs. 40.6%, rel. 28.6; 750 mg/kg bw/d: abs. 42.6 and rel. 42.9%), both doses are above the extrapolated guidance value of 100 mg/ kg bw/d. No effects on the thyroid were seen in the 1-year dog study up to a dose of 500 mg/kg bw/d.

In the 90-day rat study thyroid follicular cell hypertrophy (mild) was seen in top dose males and females (505/351 (M/F) mg/kg bw/d, 3/10 males and 5/10 females). This dose is above the extrapolated guidance value of 100 mg/kg bw/d. It is further noted that in females in the rat carcinogenicity study follicular cell adenoma were seen in the mid dose (1 (2%)) and in the top dose (2 (3.3%)), and one incidence of follicular carcinoma was seen in the top dose (1.7%). These findings were not accompanied by any other changes in the thyroid (like e.g. hypertrophy or hyperplasia) and they were within the available historical control data (HCD), also when adenomas and carcinomas were combined together. No tumours were seen in males.

Overall, the effects in thyroid were rather inconsistent, different effects were seen in different sexes and findings were not corroborated in studies of longer duration. In conclusion, no classification for STOT RE is warranted by the effects seen in the thyroid.

Heart

Decreases in mean relative heart weight were observed in the 28-day mouse study, but these effects were not dose dependent and were not repeated in the mouse studies with longer duration, therefore they are not considered supportive for classification.

Comparison with the classification criteria

A substance is classified for STOT RE if there is evidence of significant (Category 1) or severe (Category 2) toxicity. Relevant observations in humans are normally classified in Category 1. In the present case only animal studies are available. For observations made in animal studies the CLP regulation provides guidance values which are relevant for 90-day rat studies, i.e. STOT RE, Category 1: C ≤ 10 mg/kg bw/d, STOT RE, Category 2: 10 < C ≤ 100 mg/kg bw/d. For studies with different duration these values can be extrapolated using Haber's law, if applicable.

As analysed for the single organs (above) it can be concluded that the findings in the available repeated dose toxicity studies were either not severe enough or only seen at doses exceeding the guidance values for Category 2 or in other cases, were not confirmed in studies of longer duration.

No relevant toxicological findings were reported in the single study using the dermal route, i.e. a 21-day rat study (2016TOX-ISX2425, 2016).

In line with the DS RAC concludes that **no classification for STOT RE is warranted** for bixlozone.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

The DS presented three *in vitro* studies (one in bacteria, two in mammalian cells) and one *in vivo* study (erythrocyte micronucleus assay in rats) to assess the hazard class germ cell mutagenicity. All four studies were conducted according to guidelines without deviations and GLP. An overview of the studies is presented in the table below.

Table: Summary of the available genotoxicity/germ cell mutagenicity tests *(Tables 11 & 12 combined from the CLH report)*

Method, guideline, deviations if any	**Test substance**	**Organism / strain**	**Concentrations / dose tested, route**	**Observations / Results**	**Reference**
***In vitro* studies**					
Ames Bacterial Reverse Gene Mutation Assay. GLP	Bixlozone Batch: JB-F9600-201603004 Purity: 96.82%	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and	5.0, 15.0, 50.0, 150, 500, 1500 & 5000 µg per plate	Negative with and without metabolic activation. Precipitation at 5000 µg per plate Cytotoxicity ≥1500 µg per plate	2016 TOXISX 2999, 2018

OECD 471 (1997)	Solvent: DMSO	*Escherichia coli* WP2 uvrA. With and without S9 mix.			
Mouse lymphoma assay (MLA; L5178Y TK +/- forward mutation assay). GLP OECD 490 (2016)	Bixlozone Batch: JB-F9600-201603004 Purity: 96.82% Solvent: DMSO	L 5178Y - Mouse lymphoma cells thymidine kinase deficiency. With and without S9 mix.	4h -S9: 15.6, 31.3, 62.5, 125, 150 & 200 µg/mL 4h +S9: 7.81, 15.6, 31.3, 62.5, 125, 175, 200 & 250 µg/mL 24h –S9: 7.81, 15.6, 31.3, 62.5, 125, 175 & 200 µg/mL	Negative in the presence and absence of metabolic activation. Precipitation ≥ 500 µg/mL RSG (relative suspension growth): 4h, + S9: 16-77% 4h, -S9: 14-93% 24h, -S9: 28-109%	2017 TOXISX 3992, 2018
In Vitro Mammalian Chromosomal Aberration Assay GLP OECD 473 (2016)	Bixlozone Batch: JB-F9600-201603004 Purity: 96.82% Solvent: DMSO	Chinese hamster ovary cells (CHOK1). With and without S9 mix.	4h –S9, 20h –S9 & 4h +S9: 20, 40, 80, 100, 120, 140, 160, 180 µg/mL	Numerical aberrations: no indication Structural aberrations: 4 h, +S9 = positive (≥ 140 µg/mL) 4 & 20 h, -S9 = negative Precipitation ≥ 600 µg/mL Cytotoxicity (≥ 50% reduction): 4h, +S9: ≥ 140 µg/mL 4h, -S9: ≥ 160 µg/mL 20h, -S9: ≥ 80 µg/mL	2016 TOXISX 3672, 2018
***In vivo* studies**					
In vivo Mammalian Erythrocyte Micronucleus Assay in Rats to detect chromosome aberrations. GLP OECD 474 (2016)	Bixlozone Batch: JB-F9600-201603004 Purity: 96.82%	Rat, Sprague-Dawley (Hsd:SD), males	500, 1000 & 2000 mg/kg bw/d, 2 consecutive daily doses by gavage.	Negative. Bixlozone induced cytotoxicity at 2000 mg/kg bw/d. Evidence of bone marrow exposure provided from rats dosed at 500 mg/kg bw (2017METISX3950, 2017f – study according to OECD 417).	2017 TOXISX 3267, 2018

The results of the *in vitro* gene mutation assays in bacterial and mammalian cells were clearly negative. Also, the *in vitro* chromosome aberration test was negative for numerical aberrations and the assays without addition of metabolic activation, however, the result with S9 mix was positive at concentrations ≥ 140 µg/L. However, it is noted that also cytotoxicity was observed at concentrations which gave positive results, which might have influenced the result.

The *in vivo* micronucleus test in rat bone marrow was conducted as *in vivo* follow-up study for the partly positive *in vitro* chromosome aberration test. The result of the *in vivo* study was negative demonstrating that bixlozone did not induce chromosomal aberrations *in vivo*. On the basis of an ADME study according to OECD TG 417 (2017MET-ISX3950, 2017f) which was conducted in the same strain and with the same route as the micronucleus test, it can be concluded that under the test conditions of the micronucleus test, the bone marrow was exposed.

In conclusion, RAC concurs with the DS that **no classification for germ cell mutagenicity is warranted** for bixlozone.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS presented and analysed the two available carcinogenicity studies, one in rat (2013TOX-ISX10897, 2017) and one in mouse (2013TOX-ISX1090, 2017). Both studies were conducted according to OECD guideline and followed GLP and there were no relevant deviations from the guideline (see the table below).

The DS explained that during EFSA's evaluation procedure of the active substancea re-evaluation of specific tumours in both studies (rat and mouse) had been requested. The result of this re-evaluation was included in the DS's assessment.

Rat

An overview on the increased incidences of neoplastic findings in rat are given in the tables below.

Based on these incidences the DS concluded that there was an increase in the combined fibroma and fibrosarcoma of skin/subcutis in the top dose males and noted that based on the combined incidences of fibroma and fibrosarcoma together, there was a lack of dose response relationship. The DS also noted that the combined incidences did not exceed the provided HCD. The DS concluded that the slight dose-response effect on skin/subcutis fibrosarcoma is not relevant, although the finding exceeded HCD in the top dose, as no dose response was seen for fibroma or fibroma and fibrosarcoma combined.

In females there was an increased incidence of combined thyroid follicular cell adenoma/carcinoma, which the DS considered to be spontaneous in nature as it did not exceed the provided HCD, and there were no related non-neoplastic lesions in the thyroid (i.e. hypertrophy or hyperplasia) and as no tumours were seen in males.

Mouse

Table below summarises the increased neoplastic and non-neoplastic findings in mice.

The DS did not consider the observed histiocytic sarcomas in the top dose females as a relevant tumour finding, as the incidence was within HCD. The DS also pointed out that there was no big difference when compared to the concurrent control (12% vs 4% in the control).

Regarding the observed bronchioalveolar adenomas and carcinomas, the DS concluded that they were not treatment related at any dose group, as they were clearly below the HCD.

The DS did not consider the observed tumours in cervix/uterus as relevant. Although there was a slight increase in leiomyosarcoma and combined incidence of leiomyoma and leiomyosarcoma at the top dose and the combined incidence was slightly above the provided HCD, there was no clear dose response relationship, neither for leiomyosarcoma alone or in combination with the leiomyomas.

The overall conclusion of the DS was that there was no increased incidence of neoplasia nor alteration in the time of tumour onset and no induction of rare tumours in either of the two tested species. On this basis the DS proposed no classification for carcinogenicity.

Comments received during consultation

One supporting comment was received from a manufacturer company.

One MSCA commented and pointed out that the HCD used for the rat study might not be reliable because the information used would exceed the required 5 year period (i.e. 2009 to 2017 is 9 years) and because only the maximum values were presented. According to the MSCA this was not in line with the EFSA Administrative Guidance Requirements. The MSCA further commented that for the skin/subcutis-combined fibroma/fibrosarcomas seen in male rats, there was an unclear dose response, but clear increases compared to the concurrent control in both, the low dose and the high dose group. The MSCA questioned that this finding was incidental and asked for a weight of evidence assessment.

The same MSCA pointed out that that there was a monotonic increase with dose for combined thyroid follicular cell adenomas and carcinomas in female rats without such tumours in the concurrent control, and toxicokinetic information demonstrating that thyroid is exposed and thyroid hypertrophy observed in the 90-day dog and rat studies. The MSCA was of the view that more consideration is needed on these findings, especially in the light of the scarce information provided on the HCD.

The same MSCA pointed out that although ranges were provided for the HCD for mice, the information was still insufficient and stated that it was unclear why no HCD were provided for bronchiolo-alveolar adenoma and carcinoma combined, as this information was available for the tumour entities separately.

Finally, this MSCA noted that for both cervix/uterus leiomyosarcoma and leiomyoma / leiomyosarcoma combined the incidences were increased above HCD in the low as well as in the high dose groups.

The DS responded that that the applicant's dossier had been submitted prior to the EFSA Administrative Guidance was applicable. The DS also stated that the applicant had confirmed that the provided HCD were from the same conducting laboratory and that the same strains were used as in the studies on bixlozone.

Regarding the increased combined incidence of fibroma and fibrosarcoma of the skin/subcutis in male rats of the top dose group the DS pointed out that they were not considered treatment related as there was no increase with dose across the groups and the numbers were within the provided HCD (same strain, appropriate time period, same laboratory).

Regarding the thyroid tumours seen in females (Note: DS mistakenly mentions males) the DS considers them not treatment related as no follicular cell hypertrophy or hyperplasia was seen in

the same study, the incidences were within the provided HCD (same strain, appropriate time period, same laboratory) and were not corroborated by similar findings in the males. Therefore, the DS concluded that these findings were no supportive evidence for a carcinogenic effect.

With respect to the slight increase in the incidence of leiomyosarcoma and the combined incidence of leiomyoma and leiomyosarcoma the DS was of the view that they were not relevant despite exceeding the HCD, because no dose response was evident.

The DS did not specifically respond to the MSCA's comment on the lack of HCD for bronchiolo-alveolar adenomas and carcinomas combined.

Assessment and comparison with the classification criteria

The relevant information on the two available carcinogenicity studies in rats and mouse are presented in the table below.

Table: Summary table of long-term toxicity and carcinogenicity studies *(Table 13 of the CLH report, modified and supplemented with information from the annex to the CLH report)*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results:** **- NOAEL/LOAEL** **- target tissue/ organ** **- critical effect LOAEL**	**Reference**
Two-year oral (dietary) rat combined chronic toxicity and carcinogenicity study with toxicokinetics OECD 453 (2009) Deviations: None of Relevance GLP: yes Rat: Crl:CD (SD)	Bixlozone Batch: PL14-0049 Purity: 96.0% 52 wk (chronic toxicity): 10/sex/group 104 wk (carcinogenicity): 50/sex/group Target dose: 0, 250, 1000, and 5000/3000 (M/F) ppm (At the beginning on Day 49, dosage concentration of top dose females was reduced from 4000 ppm to 3000 ppm based on the lower mean body weights (approaching 10%), lower mean body weight gains (18-20%), and lower mean food consumption (9-12%) noted during	**Observations in top dose animals:** No mortality and no clinical signs were reported. <u>Reduced body weight:</u> M: -6.9% (sign) wk 52; -10.6% (not sign) wk 104 F: -14.5% (sign) wk 52; -14.2% (sign) wk 104 <u>Reduced body weight gain:</u> M: -14.2% not sign wk 0-104; F: -19.0% not sign wk 0-104), <u>Lower mean food consumption at the highest dose (M&F).</u> <u>Increased liver weights:</u> M: wk 52: +12.2% abs (not sign), +18.7% rel; wk 104: +19.9% abs, +35.6% rel F: wk 52: +16.9% abs, +34.1% rel; wk 104: -7.5% abs (not sign), +8.7% rel (not sign) <u>Hepatocellular hypertrophy:</u> M: 7/10; F 10/10 <u>Increased cholesterol:</u> M: no effect at wk 52 (-12.3% at wk 26) F: +50.5% sig wk 52 (-52.6% at wk 26) <u>gamma glutamyltransferase (GGT):</u> M: occasionally increased (also at mid dose); F: no increases	2013TOX-ISX 1089, 2017 and Pathology working group review (FMC-53830, 2020)

	the first 6 weeks of the study period. The high dose level for females is indicated as 3000 ppm in this summary.) Actual dose M: 0, 10, 41, and 217 mg/kg bw/d, Actual dose F: 0, 13, 53, and 167 mg/kg bw/d.	<u>Increased kidney weights:</u> M: wk 52: +1.7% abs (not sig), +7.5% rel (not sign); wk 104: +7.1% abs (not sign), +21.0% rel F: wk 52: +2.9% abs (not sign), +18.1% rel; wk 104: -1.3% abs (not sign), +17.5% rel (not sig) There was no treatment-related increase in incidence of neoplasms (for details see tables below). NOAEL for systemic toxicity of 1000 ppm for both males and females based on reduced body weight, reduced body weight gain, lower mean food consumption, increased liver weights (a/r), accompanied by hepatocellular hypertrophy and increased cholesterol and increased relative kidney weights at 5000/3000 ppm. NOAEL for carcinogenicity of 5000 ppm for the males and 3000 ppm for the females, equivalent to 217 and 167 mg/kg bw/d, respectively.	
18-month oral (dietary) mouse carcinogenicity study with toxicokinetics OECD 451 (2009) Deviations: None of relevance GLP: yes Mice: Crl:CD1(ICR)	Bixlozone Batch: PL14-0049 Purity: 96.0% Carcinogenicity: 50 mice/sex /group Toxicokinetics: 20 mice/sex/group Target dose: 0, 250, 1000, and 5000 ppm. Actual dose M: 0, 32, 126, and 647 mg/kg bw/d, Actual dose F: 0, 43, 164, and 834 mg/kg bw/d.	**Observations in top dose animals:** No mortality. Increased incidences of yellow material on the urogenital area and ventral trunk in males. Reduced body weight gain: F: -13.9% wk 0-78 Reduced food consumption: M: -13.6% at the highest dose Higher liver weights: M: +19.4% abs, +20.1% rel F: +21.0% abs, +27.1% rel Necrosis in F (5/50) Hepatocellular hypertrophy: M: 18/50 in the top dose (5000 ppm); also seen in the mid dose M: 11/50 (1000 ppm) There was no treatment-related increase in incidence of neoplasms (for details see table below). NOAEL for systemic toxicity of 250 ppm for males based on increased (severity of) hepatocellular hypertrophy and of 1000 ppm for females based on reduced body weight gain and higher liver weights accompanied by necrosis in some females at 5000 ppm. NOAEL for carcinogenicity of at 5000 ppm (highest dose tested).	2013TOX-ISX 1090, 2017 and Pathology working group review (FMC-53830, 2020)

Rats

In the rat study (2013TOX-ISX1089, 2017), there were no substance related effects on survival or incidence on palpable masses. Substance-related clinical observations were limited to yellow material on various body surfaces (uro- and anogenital area, ventral trunk and hindlimbs) in top dose males and dermal atonia and thin body condition in top dose females throughout the study. There were effects on body weight, body weight gain and food consumption as well as adverse liver effects (increased liver weight, hepatocellular hypertrophy, increased cholesterol) and increased kidney weights in top dose males and females (see the table above), indicating that exposure was sufficiently high.

Table: Incidence of selected neoplastic findings in male rats *(table from CLH report). Number of studies building the HCD was not indicated.*

Dose (ppm)	**0**	**250**	**1000**	**5000**	**Max HCD ± 5yrs (2009-2017) skin and subcutis combined**	**HCD studies from Jan 2012 – Jan/2015; incidence per study (range & mean)***
Skin and subcutis combined (no examined)	60	60	60	60	-	-
Skin/subcutis - fibroma	3	3	2	3	8 (12.31%)	1-8 (3.3)
Skin/subcutis – fibrosarcoma	0	1 (1.67%)	1 (1.67%)	3 (5%)	2 (4.08%)	1-2 (1.3)
Skin/subcutis – combined fibroma/fibrosarcoma	3 (5%)	4 (7.84%)	3 (5.88%)	6 (10%)	10 (16.39%)	1-10 (4)

* Communication from DS (April 2023): No information on number of underlying studies was provided for the newly submitted HCD for this tumour type.

In male rats there was an increased incidence in skin/sub-cutis fibroma and sarcoma (see the table above). RAC agrees with the DS that there was no dose response for skin/sub-cutis fibroma or fibroma/fibrosarcoma combined, and that the provided HCD was not exceeded. However, despite the low number of tumours, there seemed to be a dose response for fibrosarcoma alone and for top dose males the incidence for this lesion just exceeded the HCD and there was no incidence in the concurrent control.

When combining the incidences of both lesions (fibroma and fibrosarcoma) there is no dose response any longer and both lesions together are clearly below HCD. For the malign fibrosarcoma the HCD indicates that this finding is rather rare and the dose related increase exceeding the HCD in the top dose could therefore be meaningful. It is a drawback that the number of underlying studies is not known. Although the relevance of the increase in malign fibrosarcoma cannot be excluded, the absence of such tumours in females reduces the concern.

Table: Incidence of fibroma and fibrosarcoma in skin in male rats *(table provided in an up-date by the applicant to EFSA).*

Dose (ppm)	**0**	**250**	**1000**	**5000**	**Max HCD ± 5yrs (2009-2017) skin and subcutis combined**
Skin (no examined)	60	60	60	60	-
Skin - fibroma	0	0	0	1 (1.67%)	2.3% (1.43% - 8.33%)
Skin – fibrosarcoma	0	0	0	3 (5.0%)	1.26% (1.43% - 7.14%)
Skin – combined fibroma/fibrosarcoma	0	0	0	4 (6.67%)	-

The applicant further provided an analysis of the fibromas and fibrosarcomas in rat skin alone. The table above presents the incidences. An increase in top dose males was observed, which was statistically significant compared to the control. However, as the observed increase is clearly within the provided HCD this is considered less relevant, although again no details on the HCD is available. The DS noted however, that a pathology working group had concluded that it is not appropriate to separate skin and subcutis tumours, as it is not possible to distinguish the two appropriately (neither macroscopically nor microscopically).

Table: Incidence of selected neoplastic findings in female rats *(table from CLH report). Number of studies building the HCD was not indicated.*

Dose (ppm)	**0**	**250**	**1000**	**3000**	**Max HCD ± 5yrs (2009-2017)**	**HCD studies from Jan 2012 – Jan/2015; incidence per study (range & mean)***	
						incidence	%
Thyroid gland (no examined)	60	50	49	60	-	-	-
Follicular cell adenoma	0	0	1 (2%)	2 (3.3%)	3 (4.7%)	1-3 (1.6) [a]	1.4-4.7 (2.4) [a]
Follicular cell carcinoma	0	1 (1.7%)	0	1 (1.7%)	1 (1.7%)	1 (1.7) [b]	1.4-1.5 (1.5) [b]
Combined follicular cell adenoma/carcinoma	0	1 (1.7%)	1 (2%)	3 (5%)	4 (6.4%)	1-4 (6.4) [c]	1.4-6.3 (2.5) [c]

* Information from DS (April 2023): [a] 9 control groups; [b] 2 control groups; [c] 10 control groups

In female rats benign and malign thyroid tumours were slightly increased with one follicular cell adenoma in the mid dose and two in the top dose as well as one follicular cell carcinoma in the low and top dose each (see the table above). These observations were below the provided upper range of the HCD, however as for the previous lesion no range or median values and no information on the underlying studies (number) was provided for the HCD in the CLH report.

In April 2023 new HCD information was provided by the DS (which the DS received from the applicant). These studies were considered more appropriate as they were from a time period more closely related to the actual study dates (HCDs: 2012 – 2015; study date: Nov/2014 – Nov/2016). Despite the rather low incidence of these tumours, it is noted that for follicular cell carcinomas the incidence at the low and top dose exceeds the HCD percentage according to the new HCD information. Another issue is that only two studies built the basis for the HCDs for

follicular cell carcinoma and it was stated *"if incidence in the control group was 0, the study (and thus the tumour incidence of 0) is not displayed in the HCD document. This means that the number of studies differ between tumour type and that no 'real' overall mean % incidence can be calculated as studies with an incidence of 0% are missing*." The provided HCD are discussed under a specific heading further down for both the rat and the mouse study.

The DS did not consider these tumours as relevant as they did not exceed the HCD and no hypertrophy or hyperplasia was seen in the thyroids in this study. However, as also commented by a MSCA in the consultation, non-neoplastic lesions were seen in the thyroids of rats and dogs in other studies. While in a 28-day dog study decreased colloid (mild) and nodular hyperplasia in C-cells (mild) were seen in one female of the top dose (1100 mg/kg bw/d) (2 females / group) and in the 90-day dog study absolute and relative thyroid weight was increased in males at the two highest dose groups (300 and 750 mg/kg bw/d), there were no effects on the thyroid in the 1-year dog study (up to 500 mg/kg bw/d). In the 90-day rat study thyroid follicular cell hypertrophy (mild) was seen in males (3/10) and females (5/10) at the top dose of 505/351 (M/F) mg/kg bw/d. Overall, these effects are rather inconsistent with regard to the sex affected or the type of effect and no such findings were seen in the studies of longest duration. It is also noted that these effects were seen at higher doses than administered in the rat carcinogenicity study where the thyroid tumours were observed. In conclusion, there were only a few tumours in total, which were clearly below the provided HCD, no related non-neoplastic lesions were seen in the carcinogenicity study and the tumours were restricted to one sex. Overall, this lowers the concern for a carcinogenic effect.

Mice

In the mouse study (2013TOX-ISX1090, 2017) survival was unaffected by test substance administration and only slight clinical signs were reported consisting of increased incidences of yellow material on the urogenital area and ventral trunk in males of the top dose group. There were no substance-related effects on body weights, but lower body weight gain (11.7 - 13.6%) throughout the study was observed in the top dose females. At the end of the study food consumption was lower in the top dose males (-13.6%), but not in females. Clear adverse findings were seen in the liver in both males and females of the top dose, consisting of increased liver weight (M: abs. 19.4%, rel. 20.1%; F: abs. 21%, rel. 27.1%), necrosis in 5/50 F and hepatocellular hypertrophy in 18/50 M, indicating that exposure was sufficiently high.

Table: Incidence of selected neoplastic findings in male and female mice *(table from CLH report)*

Diet concentration (ppm)	**Males**				**Females**				**HCD Incidence range and (%)**	
									males	females
	0	250	1000	5000	0	250	1000	5000		
mg/kg bw/d	0	32	126	647	0	43	164	834		
No of animals	50	50	50	50	50	50	50	50		
Lung										
Bronchio-alveolar hyperplasia	3	0	1	1	0	0	0	0	-	-
Adenoma, bronchiolo-alveolar	0	3 (6%)	2 (4%)	3 (6%)	3 (6%)	1 (2%)	0	4 (8%)	2-11[a] (4-16.9%)	3-8[a] (6-12.3%)

Carcinoma, bronchiolo-alveolar	4 (6%)	3 (6%)	2 (4%)	3 (6%)	2 (4%)	1 (2%)	2 (4%)	2 (4%)	2-5[a] (3.1-8.3%)	3-8[a] (1.7-8.3%)
Combined adenoma and carcinoma, bronchioloalveolar	4 (8%)	6 (12%)	4 (8%)	6 (12%)	5 (10%)	2 (4%)	2 (4%)	6 (12%)	n.r.	n.r.
Systemic tumours										
Sarcoma, histiocytic	0	1 (2%)	0	0	2 (4%)	0	0	6 (12%)	-	2-7[b] (2-11.7%)
Cervix/uterus										
Leiomyoma	-	-	-	-	1 (2%)	1 (2%)	1 (2%)	1 (2%)		1[c] (1.7%)
Leiomyosarcoma	-	-	-	-	1 (2%)	2 (4%)	0	3 (6%)	-	1-3[c] (1.7-6%)
Combined leiomyoma/ leiomyosarcoma	-	-	-	-	2 (4%)	3 (6%)	1 (2%)	4 (8%)	-	2-3[c] (3.3-5%)

[a] 7-11 control groups, [b] 11 control groups, [c] 2-5 control groups; all CD-1 mouse, same lab, studies 2009 – 2017

n.r., not reported (no adequate HCD reported for the combined incidence)

Diet concentration (ppm)	**HCD Incidence range and % range***		**HCDIncidence range and mean/ % range and mean****	
	males	females	males	females
Lung				
Bronchio-alveolar hyperplasia	-	-	-	-
Adenoma, bronchiolo-alveolar	2-11[a] (4-16.9%)	3-8[a] (6-12.3%)	3-13[aa] (6)	3-9[aa] (5.1)
			6-20%[aa] (10.6%)	6-13.8%[aa] (9%)
Carcinoma, bronchiolo-alveolar	2-5[a] (3.1-8.3%)	3-8[a] (1.7-8.3%)	3–6[aa] (3.9)	1–5[bb] (2.8)
			3.1–10%[aa] (6.8%)	1.7-8.3[bb] (4.9%)
Combined adenoma and carcinoma, bronchioloalveolar	n.r.	n.r.	6–18[aa] (9.9)	3–12[aa] (7.6)
			12–27.7[aa] (17.3%)	6-18.5%[aa] (13.3%)
Systemic tumours				
Sarcoma, histiocytic	-	2-7[b] (2-11.7%)	-	1-11[aa] (4.7)
			-	2-18.3%[aa] (8.3%)
Cervix/uterus				
Leiomyoma		1[c] (1.7%)	-	1[cc]
			-	(1.7)[cc]

Leiomyosarcoma	-	1-3[c] (1.7-6%)	-	2-3[dd] (2.3)
			-	3.3-6%[d] (4.1%)
Combined leiomyoma/ leiomyosarcoma	-	2-3[c] (3.3-5%)	-	2-3[dd] (2.7)
			-	3.3-6%[dd] (4.7%)

* HCD from CLH report, studies from 2009 – 2017, [a] 7-11 control groups, [b] 11 control groups, [c] 2-5 control groups;
** HCD submitted by DS in April 2023, studies from 2012 – 2015; [aa] 7 control groups, [bb] 6 control group, [cc] 1 control group, [dd] 3 control groups

Bronchio-alveolar adenomas and carcinomas were seen in both, males and females of all dose groups, but no clear dose response was evident, also when adenomas and carcinomas were combined and a rather high incidence of carcinoma was seen in the concurrent controls in both males and females (see the table above). Though tumours were seen in both sexes, bronchiolo-alveolar hyperplasia was only seen in males and without dose response (highest incidence in controls). The tumour incidences were clearly within the provided HCD range, which consisted of 7-11 control groups. Overall, the observed lung tumours are not considered supportive for a carcinogenic effect.

In the top dose females six histiocytic sarcomas were reported, with two such tumours in the control group, but none in the low and mid dose groups (see the table above). In males only a single tumour was seen in the low dose group. The increase in the top dose females was just above HCD when percentage was considered, but within HCD when comparing to the absolute numbers (HCD: 2 – 7 (2 – 11.7%)). The HCD consisted of 11 studies, which is considered adequate. For the HCD range the median value was not provided. In this case the median value would be helpful to interpret the meaning of the six histiocytic sarcomas in the top dose females on the upper border of the HCD range. Overall, it can be concluded that the total lack of such tumours in the low and mid dose females and the absence of tumours in the mid and top dose males reduce the relevance of the finding.

In cervix/uteri of female mice leiomyomas and leiomyosarcoma were reported. There was a lack of dose response for leiomyoma, as there was a single incidence in each group including the control. Also, for leiomyosarcoma no dose response was obvious, but in the top dose the incidence was at the upper range of the HCD and when leiomyoma and leiomyosarcoma are combined the incidence in the top dose as well as in the low dose slightly exceeds the HCDs. Based on the available HCD, although consisting of 2-5 studies only, it may be concluded that leiomyomas and leiomyosarcoma are rare tumours. The presence of a single leiomyoma in each group, including the control, could be an indication of slightly higher incidence of this tumour type in the animals on the present study compared to the HCDs. However, the lack of dose response reduces the concern.

Quality of the provided HCD

As confirmed by the DS in its response to comments from consultation, the available HCDs were in the same strain from the conducting laboratory, but it seems they were not from appropriate time period. Upon RAC's request the DS clarified the actual study dates of both carcinogenicity studies (Rat study: Nov/2014 – Nov/2016; Mouse study: Nov/2014 – May/2016) and it can be concluded that the HCD from between 2009-2017 do not fulfil the ± 5 year requirement. The DS provided in April 2023 new HCD which were from between Jan/2012 and Jan/2015. For some lesions the number of underlying studies was rather low (e.g. 2 control studies for follicular cell carcinoma in the rat or 1 control study for cervix /uterus leiomyoma and 3 for cervix/uterus

leiomyosarcoma in the mouse). The DS also stated that only studies in which respective tumours were seen were included, but studies with no respective tumour incidence in the control were excluded. The DS concluded that this results in different numbers of studies available for different tumour types and that no "real" overall mean % incidence can be calculated as studies with an incidence of 0% were excluded. RAC agrees with this conclusion and is of the view that the reliability of the provided HCD is rather low.

Weight of evidence assessment for the single tumour types observed

In the table below the relevant points to be considered for deciding on the need for a classification for carcinogenicity are listed and assessed for bixlozone.

Table: *Analysis of the tumours observed in the rat and mouse carcinogenicity studies*

Factor	Evidence	Conclusion
Tumour type Considering background incidence and HCD	**Skin/subcutis fibroma/fibrosarcoma in male rats** <u>Skin/subcutis fibroma and fibroma/ fibrosarcoma combined:</u> increase not statistically significant; no clear dose response; within HCD. <u>Skin/subcutis fibrosarcoma</u>: not statistically significant; incidence of this lesion in top dose males just exceeded the HCD and there was no incidence in the concurrent control. HCD indicate that malign fibrosarcoma is rare and the dose related increase exceeding the HCD in the top dose could therefore be meaningful. HCD not exceeded for fibroma and fibroma/ fibrosarcoma combined. Insufficient information on HCD: only upper range, no median values, no information on number of underlying studies. Unknown mode of action (MoA).	Weakly supportive for classification in Category 2
	Thyroid follicular cell adenoma and carcinoma female rats: Weak increase in adenomas and carcinomas, not statistically significant, within the upper range of the HCD for adenomas, but just exceeding the HCD for carcinomas. Insufficient information on HCD: consists of only 2 studies for follicular cell carcinomas – studies with no incidence were excluded, indicating that the tumour incidence is actually lower than indicated by the numbers (no real mean value can be calculated) MoA – involvement of liver microsomal enzyme induction possible but not sufficiently investigated.	Not supportive for classification

	Bronchiolo-alveolar adenoma and carcinoma in male and female mice: Adenoma: Not dose dependent, not statistically significant, high incidence in concurrent control in females, within HCD. Carcinoma: Not dose dependent, comparable or higher incidence in concurrent control, within HCD. Adenoma and carcinoma combined: not dose dependent, high incidence in control, within HCD. Bronchiolo-alveolar hyperplasia only in males and without dose response.	Not supportive for classification
	Histiocytic sarcoma: Incidence in top dose females just outside HCD, not statistically significant, unclear dose response (low incidence in control, no cases in low and mid dose). Negative in males - single incidence in the low dose. HCD consists of 11 studies, range is indicated, but median value is missing.	Weakly supportive for classification in Category 2
	Cervix/uterus leiomyoma / leiomyosarcoma: Leiomyoma: Lack of dose response, single tumours in each dose group including control, at the upper range of the HCD Leiomyosarcoma and leiomyoma/ leiomyosarcoma combined: Lack of dose response, but incidences exceed HCD in the top dose (and in the low dose when both tumours are combined). HCD indicate that both tumours are rare, but HCD consist of only 1-3 studies. Presence of a single leiomyoma in each group, including the control, could indicate slightly higher incidences of this tumour type in the animals on the present study compared to the HCDs.	Weakly supportive for classification in Category 2
Multi-site responses	Yes	Increased concern
Progression of lesions to malignancy	**Malign tumours were observed:** Skin/subcutis fibrosarcoma Thyroid follicular cell carcinoma Bronchiolo-alveolar carcinoma Histiocytic sarcoma Leiomyosarcoma	Increased concern
Metastisation	Not observed	Decreased concern

Tumour – cause of death	In 2 of 3 top dose male rats with fibrosarcoma, the tumours were considered to be the cause of death.	Increased concern
Reduced tumour latency	Not indicated	Decreased concern
Whether responses are in single sex or both	1) Both sexes were affected in rats and mice, 2) but except for the bronicho-alveolar tumours these tumours were either seen in males or in females.	1) Increased concern 2) Decreased concern
Whether responses are in a single species or several	1) Tumours occurred in rats and mice, 2) but not the same tumours were seen in different species.	1) Increased concern 2) Decreased concern
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	No	-
Routes of exposure	Oral	-
Comparison of ADME between test animals and humans	No species-specific differences identified in the available toxicokinetic studies.	-
The possibility of a confounding effect of excessive toxicity at test doses	No	-
Mode of action and its relevance for humans	a) **Genotoxic MoA** – the available genotoxicity / mutagenicity studies are all negative, no classification for germ cell mutagenicity is proposed. b) **Thyroid tumours** – there are indications that bixlozone induces liver microsomal enzymes which is a well-known MoA resulting in thyroid tumour formation in rodents (increased thyroid hormone catabolism leading to thyroid stimulation via feedback mechanism). Dose dependent increase in liver weight was seen in most of the available studies in rats, mice and dogs, often accompanied by hepatocellular hypertrophy. In the 90-day rat study an increase in rough endoplasmatic reticulum was noted and liver weight increase was seen as early as after 7 days exposure, a common feature of chemicals inducing microsomal enzymes. Thyroid weight increase as well as hypertrophy and hyperplasia were only seen sporadically, at doses higher than those inducing thyroid tumours in females of the rat carcinogenicity study. No non-neoplastic lesions were seen in the thyroid of female rats on the carcinogenicity study, in which the thyroid tumours were seen. No mechanistic investigation is available – no assessment of liver microsomal enzyme induction, thyroid hormones not investigated. No other potential MoAs were proposed by the DS or were identified by RAC.	a) conclusive b) not sufficiently investigated

Classification in Category 1A for carcinogenicity is not justified as there is no evidence of bixlozone causing cancer in humans.

Substances should be classified in Category 1B where there is sufficient evidence of carcinogenicity in experimental animals and in Category 2 where there is limited evidence of carcinogenicity in experimental animals.

Bixlozone has been tested in two guideline carcinogenicity studies in rats and mice.

Based on the assessment of the relevant tumour findings (summarised in the table above), it can be concluded that the thyroid tumours observed in female rats and the bronchiolo-alveolar tumours in male and female mice are not considered supportive for classification.

Weak support for a carcinogenic response comes from the observed skin/subcutis fibrosarcomas in male rats, where a slight dose dependent increase was seen, with the top dose exceeding the HCD and with no incidence in concurrent controls. The information on the HCD is scarce (no information on number of studies, only upper range is available) and no respective findings were seen in females. Additionally, for the benign skin/subcutis fibroma no increase with dose was seen, nor was the HCD exceeded.

For histiocytic sarcoma the incidence exceeded the available HCD in the top dose female mice, but there was no dose response and in males the single incidence seen in the low dose was not considered related to the treatment.

In cervix/uterus the incidence of leiomyosarcomas was increased in the low and top dose female mice, but not in the mid dose and in the top dose the HCD were exceeded. When the incidences of leiomyosarcoma were combined with the leiomyomas, also the incidence at the low dose exceeded the HCD. However, no clear dose response was evident. Based on the pattern observed for leiomyomas (a single case in each dose including control) it may be concluded that the background occurrence of this tumour type in the present study was higher than in the HCD. However, the HCDs only consisted of 2-5 studies for this lesion.

Overall, the tumour increases were minor, although they occasionally exceeded the available HCD, but were mostly without dose response. The tumours were not present in both sex or in other species. Bixlozone is not genotoxic, which further decreases the concern. No other mode of action could be identified that might explain the observed tumour findings. Several of the tumours were maligant, but no metastases were observed and there was no decrease in tumour latency with respect to the occurrence of the tumours. Overall, the findings are not considered to present sufficient evidence to support a classification in Category 2.

In conclusion, in line with the DS, RAC considers that **classification of bixlozone for carcinogenicity is not warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS considered the available data consisting of a two-generation study in rats (2014TOX-ISX1295, 2016c) and its dose range finding (DRF) study (2014TOX-ISX1294, 2016b), a rat prenatal developmental toxicity study (2014TOX-ISX1291, 2016e) and its DRF study (2014TOX-ISX1290, 2016d) as well as a rabbit prenatal toxicity study (2014TOX-ISX1293, 2015) and its DRF study (2014TOX-ISX1292, 2014a) as adequate and reliable to assess reproductive toxicity of bixlozone. All studies followed GLP, and the main studies were conducted according to OECD guidelines.

The following conclusions were made by the DS:

Two-generation study and its DRF study:

- there were no adverse effects on reproductive parameters
- the reduced body weights in offspring were a consequence of maternal weight effects
- the delay in vaginal opening was a consequence of delayed growth, but not sufficient for classification.

Rat developmental toxicity studies:

- dosing was considered adequate, and to result in considerable maternal toxicity in the mid and top dose groups
- the increased incidence of the two variations 14th rudimentary rib and mild or moderately malaligned sternebrae was interpreted as treatment related, as the increase was dose dependent; the DS asked for further details on the HCD (mean litter incidence per study)

Rabbit developmental toxicity studies:

- dosing was considered adequate and maternal toxicity was demonstrated
- no relevant findings were observed in the offspring

The DS proposed no classification for sexual function and fertility, developmental toxicity or lactation.

Comments received during consultation

One supporting comment was received from a manufacturer company.

Assessment and comparison with the classification criteria

Sexual function and fertility

The studies presented by the DS consist of a two-generation study according to OECD TG 416 and GLP as well as the related non-guideline and non-GLP dose range finding study. The relevant information on these two studies is summarised in the table below.

Table: Summary table of two-generation studies *(Table 14 of the CLH report, modified and supplemented with information from the annex of the CLH Report)*

Method, species, test substance	**Doses**	**NOAEL / LOAEL**	**Main effects**	**Reference**
Dose range finding study, dietary Rats, Crl:CD(SD) males & females, 10/sex/group bixlozone, batch PL14-0049 Purity: 96%	0, 300, 1000 & 3000 ppm Reduced during lactation: 0, 150, 500 & 1500 ppm Equivalent to: <u>Males:</u> 0, 17, 56 & 176 mg/kg bw/d <u>Females:</u> premating: 0,	A NOAEL was not set by the DS from this dose-range finding study. No effect on reproductive parameters.	*F0 generation* **3000 ppm** <u>↓ body weight gain:</u> M: >10% throughout the study, not sig F: 35.3% during pre-mating, related to lower food intake, did not result in strong effects on body weight <u>↓ body weight gain, during gestation:</u>	2014 TOXISX 1294,2016b

Method, species, test substance	Doses	NOAEL / LOAEL	Main effects	Reference
Vehicle: acetone No guideline, non-GLP	20, 62 & 172 mg/kg bw/d gestation: 0, 22, 74 & 217 mg/kg bw/d lactation: 0, 23, 86 & 251 mg/kg bw/d		F: 8% (not sign) <u>↓ body weight:</u> M & F: day 0-20: 4%-5% (not sign) <u>↓ food consumption</u>: F: days 0-7 pre-mating (-20% sign) <u>↑ kidney weight:</u> M: 7.0% abs & 10.8% rel <u>↑ liver weight:</u> M: 14.9% abs & 19.3% rel F: 14.4% abs & 12.8% rel (both not sign) **1000 ppm** <u>↓ body weight gain:</u> M: -14.7% (not sign) <u>↑ liver weight:</u> M: 11.8% rel **300 ppm** No adverse effects *F1 generation* **3000 ppm** <u>↓ body weight:</u> F: -10.2% (sign) <u>↑ liver weights:</u> M: +16.9% abs (not sign) & 23.4% rel F: +14.8% abs & 29.3% rel **1000 ppm** <u>↑ liver weights:</u> F: +15.3% abs & +15.4% rel **300 ppm** No adverse effects	
Two-generation reproductive toxicity study, dietary Rats, Crl:CD(SD) males & females, 25/sex/group	0, 150, 750 & 3000 ppm Reduced during lactation: 0, 75, 375 & 1500 ppm Equivalent to: <u>Males, F0:</u>	**Parental toxicity** NOAEL: 750 ppm LOAEL: 3000 ppm **Fertility** NOAEL: 3000 ppm	**<u>Parental toxicity</u>** ***<u>F0 generation</u>*** There were no treatment related deaths or clinical signs of toxicity (1 F sacrificed & 1 F found dead in low dose group; clinical signs of hair loss on forelimbs and	2014 TOXISX 1295, 2016c

Method, species, test substance	Doses	NOAEL / LOAEL	Main effects	Reference
bixlozone, batch PL14-0049 Purity: 96% Vehicle: acetone GLP Guideline: OECD 416 (2001) Deviations: none	Premating: F0: 0, 10, 49 & 200 mg/kg bw/d After mating: 0, 7, 34 & 141 mg/kg bw/d <u>Females, F0:</u> Premating: 0, 11, 53 & 209 mg/kg bw/d Gestation: 0, 10, 50 & 203 mg/kg bw/d Lactation: 0, 12, 62 & 261 mg/kg bw/d <u>Males, F1:</u> Premating: 0, 12, 60 & 238 mg/kg bw/d After mating: 0, 7, 34 & 140 mg/kg bw/d <u>Females, F1:</u> Premating: 0, 12, 59 & 241 mg/kg bw/d Gestation: 0, 10, 49 & 187 Lactation: 0, 12, 59 & 255 mg/kg bw/d	**Offspring toxicity** NOAEL: 750 ppm LOAEL: 3000 ppm	decreased defecation, sporadically and all dose groups) **3000 ppm** <u>↓ body-weight gains:</u> F: days 0-7: -40.7%, days 0-70: -14.8% <u>↓ body weights:</u> F: during gestation (8-9%) <u>↓ food consumption:</u> F: pre- and post-mating; ~12%; during gestation max -9.1% <u>↑ liver weights:</u> M: +22.1% abs, +19.2% rel F: +11.8% abs, +18.4% rel <u>Hepatocellular hypertrophy:</u> F: 18/25 <u>↑ relative kidney weights:</u> M: +13% F: +9.7% <u>↑ mononuclear cell infiltration (chronic inflammation) in prostate:</u> 10/25, not sign **150 and 750 ppm** No adverse effects ***F1 generation*** **3000 ppm** <u>↓ body weight:</u> M: 7-11% F: 6-9% <u>Body weight during gestation:</u> F: (6.5%-10%) <u>↓ body weight gain:</u> M: ~11% F: 5-6%) <u>↓ food consumption:</u> M: occasionally, decrease mostly ≤ 10% F: only during gestation (-9.1%)	

Method, species, test substance	Doses	NOAEL / LOAEL	Main effects	Reference
			↑ relative liver weights: M: +13.8% F: 20.8% Hepatocellular hypertrophy: F: 20/25 ↑ relative kidney weights: M: 12.7% F: 10.2% ↑ mononuclear cell infiltration (chronic inflammation) in prostate: 12/25 (sign) **150 and 750 ppm** No adverse effects **Fertility** No treatment-related adverse effects **Offspring toxicity** 3000 ppm *F1 pups* ↑ age of attainment of vaginal opening: 33.6 days vs 31.7 days *F2 pups* ↓ pup body weight-gain (PND 4-7): M: -14.3% F: -10.4% **150 and 750 ppm** No adverse effects	

Dose range finding (DFR) study (2014TOXISX1294, 2016b)

All animals of the F0 and F1 generation survived until scheduled necropsy and there were no noteworthy clinical findings, except that one female of the control group had total litter loss on lactation day 6 and animals of the top dose group had dried red material around the nose.

For the F0 generation there were slight reductions in body weight, body weight gain and food consumption in the top dose animals of both sexes, but those rarely exceeded 10%. For the F1 generation the only significant effect was reduction of female body weights (~10%).

Like in other repeated dose toxicity studies with bixlozone the top dose animals of both generations were affected by increased liver weights (liver weight increases in males and females ranged between ~13 to 20% for the F0 generation and between ~15 to 30% for the F1

generation). Kidney weight was only increased in the top dose males of the F0 generation (abs. 7%, rel. 10.8%). The only effects seen at the next lower dose was increased liver weight in F0 males (11.8% rel.) and F1 females (~15% rel. and abs.). Only macroscopic examinations were performed on the animals.

Based on these results it seems that slightly higher doses might have been tested without inducing excessive toxicity in the animals. In rat studies with comparable duration, doses up to ~ 500 mg/kg bw/d were well tolerated (e.g. in the 90-day rat study), although liver and kidney toxicity was clearly more pronounced at high doses. In addition, it has to be considered that the females of the two-generation study were pregnant (in contrast to the 90-day study) and might therefore have a different susceptibility.

As some liver toxicity was seen at the top dose in this dose range finding study, it can be concluded that dosing was acceptable, also for the main study where a comparable dosing regime was applied.

No effects on reproductive parameters were observed in this dose range finding study.

Main two-generation study according to OECD 416 (2014TOX-ISX1295, 2016c)

Like in the DRF study, no treatment related deaths or clinical signs were observed. Again, there were slight effects on body weight, body weight gain and food consumption in the top dose animals of both generations which were statistically significant in females of the F0 generation and in males and females of the F1 generation (see the table above). Increased liver weight was seen in top dose males and females of both generations, which was accompanied by hepatocellular hypertrophy in females (see the table above) and relative kidney weight was slightly increased in top dose males and females of both generations (~10%).

No adverse effects on reproductive parameters were reported, but there were some effects in offspring (F1 and F2 pups). In F1 pups there was an increase in the age of attainment of vaginal opening (VO), i.e. 33.6 days in top dose vs 31.7 days in control females (stat. signif., see the table below). The DS concluded that as the body weight was not affected in females on the day of VO, the delay in VO was caused by bixlozone induced growth delay, although no information on the size of the pups was available. However, RAC notes that slight decreases in body weight /body weight gain were indicated for F1 animals in the post-weaning period (see the table below). RAC is of the view that slight body weight effects on the top dose animals could be the underlying cause of the slight delay of VO:

- PND 28: decrease on female body weight in the top dose: -7.4% statistically significant
- PND 21 – 28: decrease on female body weight gain in the top dose: -5.4%, statistically significant
- The body weight/body weight gain changes on PND 28 / PND 21-28 are considered relevant. (The numbers for PND 31 and 33 were not provided in the CLH report or its annex, see also the table below).

At the same time period there was also reduced body weight / body weight gain in male F1 pups, and a delay in balano-preputial separation of 1.5 days (45.6 days at top dose vs 44.1 days in control), however, not statistically significant. Overall, it is concluded that the finding in females was not severe (delay of VO 1.9 days) and therefore not considered supportive for classification.

Table: Developmental landmarks of F1 pups *(table B.6.6.1.2.-18 from the annex to the CLH report)*

Dose level (ppm)	**0**	**150**	**750**	**3000**	**0**	**150**	**750**	**3000**
Developmental landmark	**Males (preputial separation)**				**Females (vaginal patency)** *Historical control range (31.2 to 37.0 days; Mean: 33.6 days)†* *Mean body weight at Attainment (100.5 – 126.8 g Mean: 111.0g)*			
Age at attainment (days)	44.1	44.5	44.0	45.6	31.7	32.4	32.0	**33.6****
Mean body weight at age of attainment (g)	252.1	246.6	246.7	240.5	115.6	120.1	118.1	117.3

** Statistically significant at p<0.01, † HCD - Jan 2009 – Mar 2018; 18 studies/ 34 control groups, Crl:CD(SD) rat, same laboratory

Table: Body weight development of F1 animals post weaning *(table 6.6.1.2.-4 from the annex of the CLH report)*

Dose Level	**Males**				**Females**			
(ppm)	**0**	**150**	**750**	**3000**	**0**	**150**	**750**	**3000**
Mean body weight g (%)								
PND 21	59	57 (-3.4%)	58 (-1.7%)	55 (-6.8%)*	57	56 (-1.8%)	56 (-1.8%)	52 (-8.8%)**
PND 28	104	100 (-3.8%)	101 (-2.9%)	95 (-8.7 %)**	94	94 (0.0%)	93 (-1.1%)	87 (-7.4%)**
PND 91	530	515 (-2.8%)	517 (-2.5%)	476 (-10.2%)**	277	280 (1.1%)	284 (2.5%)	259 (-6.5%)*
PND 153	683	664 (-2.8%)	664 (-2.8%)	606 (-11.3%)**	318	319 (0.3%)	321 (0.9%)	298 (-6.3%)*
Mean body weight gain g (%)								
PND 21-28	45	42 (-6.7%)	43 (-4.4%)	40 (-11.1%)**	37	38 (2.7%)	37 (0.0%)	35 (-5.4%)*
PND 21-91	471	458 (-2.8%)	459 (-2.5%)	421 (-10.6%)**	220	224 (1.8%)	228 (3.6%)	207 (-5.9%)
PND 21-153	623	606 (-2.7%)	606 (-2.7%)	551 (-11.6%)**	NA	NA	NA	NA

NA Not applicable.
* Statistically significant at 0.05 compared to the control group using Dunnett's test.
** Statistically significant at 0.01 compared to the control group using Dunnett's test.

Overall, the observations made in the two-generation study and the related range finding study are not considered supportive for a classification for sexual function and fertility.

Adverse effects in male or female reproductive organs can also be supporting evidence for a classification for sexual function and fertility. Weight changes have been observed in male and female reproductive organs in several repeated dose toxicity studies with bixlozone:

- <u>28-day dog study:</u> dose dependent decrease in absolute and relative prostate weight, decreased ovary, uterus and epididymis weight – without dose dependence.

- 90-day rat study: increase in ovary, uterus and testis weights, although not dose dependent for ovaries and uterus.
- 90-day mouse study: changes in ovary, uterus and epididymis weight, but without dose response; decrease in relative testis weight in the top dose considered to be related to lower body weight at this dose.
- 90-day dog study: dose dependent decrease in absolute and relative prostate weight – but in the two highest dose groups each there were 1 out of 2 animals with immature prostate, which correlates with lower weight (all animals were at the edge to attain maturity). Decreases in the weights of epididymides, ovaries and uteri were not dose dependent.
- 2-year rat carcinogenicity study: increase of relative epididymis weight was seen in the top dose males, but was considered to be related to the observed decrease in body weight at that dose (-14.7%); slight decreases in uterus weights was not dose dependent and was related to normal cyclic variation (within HCD).
- 18-month mouse study: slight decreases in uterus weights was not dose dependent and was related to normal cyclic variation (within HCD).
- Two-generation study, rat: chronic inflammation of the prostate was observed in top dose males of both generations (F0: 10/25; F1: 12/25), which was considered adverse.

Overall, it is noted that only few of the organ weight changes were dose dependent, and sometimes the changes went in opposite directions, whereas sometimes not even observed in studies of longer duration. Additionally, for none of the organ weight changes was there a histopathological correlate, except for prostate. Although the effects on prostate were considered relevant (i.e. dose dependent for weight decrease, adverse for inflammation in top dose F0 and F1 males in the rat two-generation study) it is noted that for the dog study sexually mature and immature animals were on the study and likely had an influence on the prostate weight. Regarding the chronic inflammation in the prostate seen in the two-generation study, it is noted that despite this observation, there was no adverse impact on fertility and no effect on any of the sperm parameters.

Overall, the findings in reproductive organs are not considered supportive for a classification for sexual function and fertility.

Based on the total lack of adverse effects on reproductive parameters in the two-generation study as well as its DRF study and the absence of relevant findings in reproductive organs in repeated dose toxicity studies in rats, mice and dogs, RAC is of the view that **no classification for sexual function and fertility is warranted.**

Developmental toxicity

Table: Summary table of developmental toxicity studies *(Table 15 of the CLH report, modified and supplemented with information from the annex of the CLH Report)*

Method, species, test substance	Doses	NOAEL/LOAEL	Main effects	Ref-erence
Dose-range finding (DRF) prenatal developmental toxicity study, gavage Rats, Crl:CD(SD) females, 8/group bixlozone, batch PL14-0049	0, 25, 75, 225 & 675 mg/kg bw/d Dose volume: 10 mL/kg bw	A NOAEL was not set from this DRF study	**Maternal toxicity** **675 mg/kg bw/d** One death (sacrificed in extremis on GD11, marked body weight loss and reduced food consumption on GD 8-10)	2014T OXISX 1290, 2016d

Method, species, test substance	Doses	NOAEL/LOAEL	Main effects	Ref-erence
Purity: 96% Vehicle: 0.5% (w/v) carboxymethylcellulose (CMC) in 5% tween 80) GLP: yes			↓ body weight: GD 20: 7.5% ↓ body-weight gain: GD 9-20: 45.3% ↓ gravid uterine weight: 8.9%, not significant ↓ food consumption (29-59%) ↑ liver weight: 52.4% abs & 64.1% rel **225 mg/kg bw/d** ↑ liver weight (16.6% abs and rel) **25 & 75 mg/kg bw/d** No adverse effects **Developmental toxicity** **675 mg/kg bw/d** ↓ foetal body-weights: M: 7.9% F: 8.3% M+F: 8.1%; significant for females	
Pre-natal developmental toxicity study, gavage Rats, Crl:CD(SD) females, 25/group bixlozone, batch PL14-0049 Purity: 96% Vehicle: 0.5% (w/v) carboxymethylcellulose (CMC) in 5% tween® 80 Guideline: OECD 414 (2001) GLP: yes Deviations: none	0, 75, 225 & 550 mg/kg bw/d Dose volume: 10 mL/kg bw GD 6-19	**Maternal toxicity:** **NOAEL** of 75 mg/kg bw/d; based on reduced net body weight gain, reduced food consumption and liver weight increase a/r accompanied by hepatocellular hypertrophy at 225 mg/kg bw/d **Developmental toxicity:** **NOAEL** of 225 mg/kg bw/d based on increased incidence of skeletal variations at 550 mg/kg bw/d	**Maternal toxicity** **550 mg/kg bw/d:** Clinical signs: red, yellow and/or clear material on various body surfaces; hair loss ↓ body-weight gain: GD 6-9: -90%, GD 6-20: -16.0%) ↓ net body-weight gain (without gravid uterus): -29.3% ↓ body weight: GD 20: -5.9% ↓ net body weight (without gravid uterus): -6.9% ↓ food consumption: GD 6-20: -11.5% ↑ liver weight: 28.6% abs, 38.2% rel	2014 TOXISX 1291, 2016e

Method, species, test substance	Doses	NOAEL/LOAEL	Main effects	Ref-erence
			Hepatocellular hypertrophy: 7/25 (mild) & 18/25 (moderate) **225 mg/kg bw/d:** Clinical signs: red, yellow and/or clear material on various body surfaces ↓ body-weight gain: GD 6-9: -40% ↓ net body-weight gain: -9.7% ↓ food consumption: GD 6-9: -12.5% ↑ liver weight: 12.3% (absolute) & 14% (relative) Hepatocellular hypertrophy: 9/25 (minimal) & 12/25 (mild) **75 mg/kg bw/d:** No adverse effects **Developmental toxicity** 550 mg/kg bw/d Increased skeletal variations: 14th rudimentary ribs and malaligned sternebrae	
Dose-range finding (DRF) prenatal developmental toxicity study, gavage Rabbits, New Zealand White (Hra:(NZW)SPF) females, 6/group bixlozone, batch PL14-0049 Purity: 96% Vehicle: carboxymethylcellulose (CMC) in 5% tween 80 GLP: yes	0, 100, 350, 750 & 1000 mg/kg bw/d Dose volume: 5 mL/kg bw	A NOAEL was not set from this DRF study	**Maternal toxicity** **1000 mg/kg bw/d:** There were two deaths on GD 17 & 19; all remaining animals were sacrificed on GD 19 due to severe maternal toxicity ↓ body weight: -12.9%) Liver weight not determined ↓ food consumption: -60-85% **750 mg/kg bw/d:** There were two deaths; all remaining animals were sacrificed on GD 23 due to severe maternal toxicity	2014 TOXISX 1292, 2014a

Method, species, test substance	Doses	NOAEL/LOAEL	Main effects	Ref-erence
			↓ body weight: -15.7% ↓ food consumption: - 60-86% Liver weight not determined **350 mg/kg bw/d:** ↓ body-weight gain: GD 10-13: -66.3% GD 13-20: -64.2% ↓ net bodyweight gain: -26.9% ↓ food consumption: -13.4% Liver weight: abs – 7.5%, rel – 4.1% Decreased defecation in 3 females: GD 16-21 (3 instances each) **100 mg/kg bw/d:** ↓ body-weight gain: GD 10-13: -49.4% GD 13-20: -108.2% ↓ net bodyweight gain: -42% ↓ food consumption: -18.7% Liver weight: abs – 11%, rel – 5.4% 1 female aborted three dead and two live foetus on GD 28. **Developmental toxicity** No treatment-related effects (only 100 and 350 mg/kg bw/d evaluated)	
Pre-natal developmental toxicity study, gavage Rabbits, New Zealand White Hra:(NZW)SPF), females, 25/group	0, 25, 75, 200 & 400 mg/kg bw/d Dose volume: 5 mL/kg bw GD 7-28	**Maternal toxicity:** 200 mg/kg bw/d **Developmental toxicity:** ≥ 400 mg/kg bw/d	**Maternal toxicity** There were no treatment-related deaths 400 mg/kg bw/d: ↓ body weight gain at the beginning of treatment: GD 7-10: -21.8%	2014 TOXISX 1293, 2015

Method, species, test substance	Doses	NOAEL/LOAEL	Main effects	Ref-erence
bixlozone, batch PL14-0049 Purity: 96% Vehicle: Carboxymethylcellulose (CMC) in 5% tween 80 Guideline: OECD 414 (2001) Deviations: None GLP: yes			↓ body weight gain: GD 13-20: -34.2% ↓ food consumption: GD 13-20: -18.9% ↓ defecation: during GD 13-20; transient **Developmental toxicity** No treatment-related effects up to the highest dose tested.	

Rat, dose range finding prenatal developmental toxicity study, non-guideline but GLP (2014TOX-ISX1290, 2016d)

In the top dose of 675 mg/kg bw/d one animal was sacrificed in extremis. There was a higher incidence of animals with red and / or yellow material on the body surface compared to control and an increased occurrence of clear or red material around the mouth (~1h after dosing), which was also seen at the next lower dose (225 mg/kg bw/d) and there was an increased incidence of hair loss in the ventral trunk and urogenital areas in the top dose. Body weight and body weight gain were reduced over the whole dosing period in top dose females (-7.5% and -45.3%, respectively), while these two parameters were affected in females of the next lower dose only the day after dosing started (after that no difference to control). Food consumption was also affected in the top dose group over the whole dosing period and in the next lower dose only on the two days after dosing started. Absolute and relative liver weights were also increased at 675 mg/kg bw/d (52.4% abs., 64.1% rel.) and at 225 mg/kg bw/d (abs. and rel. 16.6%). In the top dose females gravid uterine weight was reduced by 8.9% (not statistically significant) which might be related to the only findings in offspring, i.e. lower foetal body weight (in males -7.9% and females -8.3%, significant only in females). In total there were 2 foetuses with malformations (at 675 mg/kg bw/d: 1 foetus with soft tissue malformation (aortic arch) and at 75 mg/kg bw/d: one foetus with gastroschisis). These findings were considered incidental.

Rat, main prenatal developmental toxicity study according to OECD 414 and GLP (2014TOX-ISX1291, 2016e)

In this main study no deaths were reported in any of the groups including control, but clear signs of toxicity were noted in the mid and top dose, i.e. red and / or yellow material on various body surface areas and hair loss was seen in the top dose group. These findings started as early as GD 7. Net final body weight in the top dose females was lowered by 6.9% compared to controls, despite considerable effects on body weight gain during the dosing period (up to -90% on GD 6-9, directly after the start of dosing). Body weight gain was also affected in the mid dose (net body weight gain: -9.7%) but did not result in lower net body weight. Food consumption was reduced in the top dose group over the whole treatment period (GD 6-20: -11.5%) and only after start of the treatment in the mid dose group (GD 6-9: -12.5%). Liver weight increase and related hepatocellular hypertrophy was also seen in the mid and top dose groups (i.e. the mid dose: liver weight: abs. 12.3%, rel. 14%; hepatocellular hypertrophy: 9/25 minimal, 12/25 mild; the top dose: liver weight: abs. 28.6%%, rel. 38.2%; hepatocellular hypertrophy: 8/25 mild, 18/25 moderate). No adverse effects were seen in the low dose group. It can be concluded that there

was a dose dependent increase in general and evident liver toxicity in the mid and top dose females.

There were no effects on mean litter size, sex ratio, post implantation loss or early resorptions and in contrast to the DRF study no treatment related effect was seen on foetal weight. Also, no effects on gravid uterine weight were reported at any dose tested.

Malformations were observed in 2(2), 3(2), 2(2) and 2(2) foetuses (litters) in control, low, mid and top dose, respectively, and were considered spontaneous findings. The detected developmental variations were also considered incidental, except for a dose dependent increase in 14th rudimentary rib and malaligned sternebrae (mild and moderate). The table below lists the incidences across groups.

Table: Variations observed in the main rat pre-natal developmental toxicity study*(extracted from table B.6.6.2.2.-4 of the annex to the CLH report)*

Variations				
mg/kg bw/d	0	75	225	550
Total foetuses examined	390	390	384	380
Total litters examined	25	25	25	24
Fœtal incidence of 14th rudimentary rib(s)	31	14	28	49
Litter incidence of 14th rudimentary rib(s)	8 (32%)	11 (44%)	13 (52%)	17 (71%)
Fœtal incidence of malaligned sternebrae (slight or moderate)	3	1	2	6
Litter incidence of malaligned sternebrae (slight or moderate)	2 (8%)	1 (4%)	2 (8%)	5 (20%)

In the annex of the CLH report HCD were presented for these two variations, which were from the same strain and the same laboratory. HCD had a sufficient number of studies, however, as only the range of the HCDs was provided, the Rapporteur Member State (=DS) asked the applicant to provide also the mean litter incidence per study in order to help better interpret these observations.

Table: The available Historical Control Data (HCD) Ranges from Charles River Ashland Covering +/- 5 Years for the Rat Developmental Toxicity Study *(extracted from table B.6.6.2.2.-5 of the annex to the CLH report)*

Malformation or variation	**Incidence per litter ***
14th rudimentary rib(s)	Overall mean incidence: 8.17% Range: 18%-87% litters affected (4-20 litters affected per study) Mean litter incidence per study: not provided.
malaligned sternebrae (slight or moderate)	Overall mean incidence: 0.78% Range: 0-20% litters affected (0-5 litters affected per study) Mean litter incidence per study: not provided

* Based on 128 studies between 2009 and 2017, Crl:CD(SD) rats, same laboratory, 45025 fetuses and 3075 litters

Rabbit, dose range finding prenatal developmental toxicity study, non-guideline but GLP (2014TOX-ISX1292, 2014a)

In the two highest dose groups (750 and 1000 mg/kg bw/d) severe maternal toxicity was observed, including two deaths at each dose group, resulting in early termination of the study groups on GD 19 at 1000 mg/kg bw/d and on GD 23 at 750 mg/kg bw/d. Maternal toxicity was also evident at the lower dose characterised by lower body-weight gain, net body-weight losses, reduced food consumption and reduced defecation , but actual mean body weight for these dose groups was similar to controls for the duration of treatment. In contrast to the previous studies in rats, slightly lower absolute and relative liver weights were noted in the rabbits at 100 and 350 mg/kg bw/d and the DS concluded that this possibly was 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 and gravid uterine weights at 100 and 350 mg/kg bw/d were similar to those of the control group. Based on the results of this study, doses 25, 75, 200 and 400 mg/kg bw/d were selected for a definitive developmental toxicity study in rabbits.

Rabbit, main prenatal developmental toxicity study, according to OECD 414 and GLP (2014TOX-ISX1292, 2014a)

There were no treatment related deaths, but two animals at 200 mg/kg bw/d and one animal in the control group died due to intubation errors. Clinical signs were occasionally observed but were not dose dependent and were also seen in the controls.

Reduction in food consumption during the second week of dosing (GD 13-20) with a corresponding reduction in body-weight gain and decrease in defecation at the highest dose tested of 400 mg/kg bw/d. However, these effects did not persist past the second week of dosing and were not considered adverse.

There were no external malformations (either treatment-related or otherwise) noted at any dose. No treatment-related soft tissue or skeletal malformations were observed; however, several spurious malformations were noted. Some of the malformations that arose in the treated groups were also present at a similar frequency in the control group and/or were within historical control data. The HCD are from the conducting laboratory, the same strain (Hra:(NZW)SPF New Zealand White Rabbits (GD 29, Time-Mated, 100% Vis/Ske, Full) and consisted of 145 studies between 2009 – 2018, however, as for the rat studies, only the range and no mean value was presented. Further, no dose response was observed and most of the observed malformations were not present in the highest dose group. The malformations present in the highest dose group, were also observed in the control group.

RAC notes that the effects seen at the top dose of 400 mg/kg bw/d, i.e. transient decrease in body weight gain (-34.2%) and reduction in food consumption (-18.9%) during GD 13-20 are not severe. It appears that rabbits are less sensitive than rats and that they would have tolerated higher doses in this study. Unless limited by the physical/chemical nature or biological properties of the test chemical, the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity like clinical signs or a decrease in body weight, but not death or severe suffering. Therefore, it can be questioned whether the requirements for top doses according to OECD TG 414 were fulfilled in the rabbit study. In the DRF study no severe findings were seen at 100 and 350 mg/kg bw/d i.e. some decrease in body weight gain (at 350 mg/kg bw/d net body weight gain: -26.9%) and food consumption (at 350 mg/kg bw/d: -13.4%). In this respect it should be noted that body weight gain is not considered a good parameter to assess maternal toxicity, especially in rabbits, which is explicitly stated in the CLP regulation, Annex I, Section 3.7.2.4.4: "*In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy*." and further

supported by van Berlo *et al.* (2022). The single incidence of abortion seen in the DRF study at the low dose (100 mg/kg bw/d) is considered incidental. It is, however, acknowledged that it is difficult to find an appropriate dosing when the dose response relation is rather steep (hardly any toxicity at 350 mg/kg bw/d and severe toxicity and mortality at 750 mg/kg bw/d). In this sense, it is considered acceptable that the top dose in the main study was slightly higher than the highest dose of the DRF, which did not show much toxicity.

Discussion:

As dosing was questionable in the available rabbit study, the assessment concentrates on the available rat studies, including a rat two-generation study and its DRF study as well as the prenatal developmental toxicity study and DRF study.

In the DRF for the two-generation study body weight of F1 females of the top dose were reduced by ~10% on PND 28 and considerable increases in absolute and relative liver weights were seen at that dose. At the same dose also F0 body weights were decreased (< 10%) and animals were affected by liver toxicity.

Similar effects on body weight were also seen in the main two-generation study for adult F1 animals. Body weight of F1 animals in the top dose group were statistically significantly lower from PND 21 (-6.8% M/-8.8% F) to PND 153 (-11.3% M/-6.3% F). For F1 and F2 pups conflicting results were obtained for body weight, as slight decreases were seen for F2 pups but not for F1 pups (the actual numbers for F1 pups were not listed in the CLH report). In F2 pups, slight effects were seen in the mid and top dose animals not exceeding -7.8% decrease for body weight and -14.3% decrease for body weight gain. The findings were statistically significant only in the top dose males for body weight decrease on PND 14 and for reduction of body weight gain in the top dose males on PND 4-7 and 7-14 and in the top dose females on PND 4-7. Overall, these findings are likely related to maternal body weight effects and are not considered supportive for a classification for developmental toxicity.

The slight delay in vaginal opening (VO) in top dose females is not considered supportive for developmental toxicity classification (see discussion under the two-generation study).

In the rat prenatal developmental toxicity study and its DRF study the only relevant findings observed were the increase in the 14th rudimentary rib and malaligned sternebrae (mild and moderate), which was dose dependent and therefore considered treatment related.

For both lesions the classification as variation is in line with the ECETOC monograph no. 031 (2002). In contrast to severely malaligned sternebrae, mild and moderate forms as observed in this study, are considered variations only.

For the 14th rudimentary rib a dose dependent increase was seen for the litter incidence and in the top dose group the incidence was close to the upper range from the provided HCD. Factors reducing the relevance are: the concurrent controls had a rather high incidence, finding is a variation not a malformation and HCD consisted of a considerable number of studies (despite the lack of complete information on HCD).

For mild and moderately malaligned sternebrae there was a clear increase in the top dose compared to the other doses, which was at the upper range of the HCD. Both effects were seen in the presence of mild maternal toxicity.

As these were the only relevant findings seen in a complete data set of reliable developmental toxicity studies and as they were variations only, RAC concludes that this information is not providing sufficient justification for a classification.

In line with the DS, RAC is of the view that **no classification for developmental toxicity is warranted**.

Lactation

No information on the potential presence of bixlozone in human or animal milk is available.

Decreased body weight was observed in F2 pups of the mid and top dose groups of the two-generation study. The decrease did not exceed 7.8% for body weight and 14.3% for body weight gain and the finding was only statistically significant in the top dose males for body weight on PND 14 and for body weight gain in the top dose males on PND 4-7 and 7-14 and in the top dose females on PND 4-7. Overall, these findings are likely related to the effect on maternal body weight, but might also be caused by effects induced by bixlozone on or via the milk. It is, however, noted that lower body weights were also seen after weaning in F1 animals and the extent of the effect remained at similar level until PND 153 and was comparable to the effect seen in F0 and F1 adult animals. The CLH report states that no decrease in body weight was seen in F1 pups. However, body weights of F1 pups were consistently lower from PND21 to PND 153 (overall ranging between -6.3% to -11.3%). The numbers from PND 0 – 20 were not presented in the CLH report.

Overall, these observations do not indicate an adverse effect on or via lactation and RAC is of the view that **no classification for lactation is warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Bixlozone is an active substance in plant protection products used as herbicide, with representative uses in winter wheat, winter barley, winter oilseed rape and maize. The substance is currently not included in Annex VI of Regulation (EC) No 1272/2008.

The Dossier Submitter (DS) proposed to classify the substance as:

- **Aquatic Acute 1 (H400) with M-factor of 1** based on a 48 h EC_{50} value of 0.11 mg/L for invertebrate *Thamnocephalus platyurus* and
- **Aquatic Chronic 1 (H410) with M-factor of 10** based on 14 d E_rC_{50} value of 0.0071 mg/L for aquatic plant *Myriophyllum spicatum*a and the substance being not rapidly degradable.

Degradation

A hydrolysis GLP study according to OECD TG 111 was run at pH 4, 7 and 9 in the dark in sterile aqueous buffered solutions. In a preliminary test [phenyl-U-14C]-bixlozone and [carbonyl-14C]-bixlozone were added to sterile buffer solutions at pH 4, 7 and 9. Duplicate samples were incubated at 50°C for five days. 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 over 30 days at the environmentally relevant temperature of 25°C with expected $DegT_{50}$ values > 1 year. The rate and extent of degradation, however, increased with increasing temperature.

The GLP study on aqueous photolysis of bixlozone in sterile aqueous buffered solutions at pH 7 at 25°C was conducted according to OECD TG 316 and U.S. EPA OPPTS 835.2240 up to 13 days. The degradation rate of [carbonyl-14C]-bixlozone and [phenyl-U-14C]-bixlozone under irradiated conditions was slow. The concentration of [carbonyl 14C]- and [phenyl-U-14C]-bixlozone at the final time-point was 56.7% AR and 64.5% AR respectively. Appropriate controls confirmed that there was no degradation in darkness. Bixlozone was slowly degraded to many minor photoproducts after 13 days continuous irradiation. All of these degradation products were < 5% AR at each sampling point. The first-order $DegT_{50}$ values were 44.0 and 54.4 days for [carbonyl-14C]- and [phenyl-U-14C]-bixlozone, respectively, under natural summer sunlight at latitude 30-50°N. It was not possible to determine the quantum yield for F9600 due to the very low UV absorption at wavelengths > 290 nm.

One ready biodegradability test was available according to OECD TG 301B and in compliance with GLP. The degree of biodegradation (carbon dioxide evolution) over the 28 days test duration was < 13%. The substance is therefore not readily biodegradable under test conditions.

In an aerobic mineralisation in surface water study performed according to OECD TG 309, the degradation of [phenyl-U-14C]-bixlozone was studied in a pelagic test system (natural fresh water collected from Carsington Water, UK) under aerobic conditions for 62 days at 20±2°C in the dark. Mineralisation of the bixlozone was found to be low for both test concentrations, reaching maximum mean values of 2.0 % and 1.0 % AR (sum of CO_2 and organic volatiles) for the 10 and 100 µg/L test concentrations respectively. Bixlozone represented 94.1 % AR and 91.7 % AR at the end of the study in the 10 µg/L and 100 µg/L test systems respectively. Unknown degradation products were observed through the course of the study, but none exceeded 2.9 % at any time point. $DegT_{50}$ and $DegT_{90}$ values for the degradation of bixlozone were determined according to FOCUS recommendations using CAKE v 3.3. SFO was selected as the best fit for both test concentrations. $DegT_{50}$ values at 20 °C of 1040 and 818 days, and $DegT_{90}$ values of 3450 and 2720 days were determined for the 10 µg/L and 100 µg/L test systems respectively. $DegT_{50}$ values normalised to 12 °C of 2220 and 1746 days were calculated for the 10 µg/L and 100 µg/L test systems respectively.

Two water/sediment simulation studies carried out according to OECD TG 308 and in compliance with GLP, using two different radioactivity label positions, were run for 100 and 98 days at 20°C in the dark using two aerobic water-sediment systems (Calwich Abbey and Swiss Lake). In both studies, the material balances were good for both water-sediment systems with mean values ranging from 94.9 to 96.8% AR in the first study and 95.5% AR to 97.0% AR in the second study. In the first study the vast majority of the volatile radioactivity at the end of the study was confirmed to be $^{14}CO_2$, reaching 6.67-51.55% AR, while in the second study total volatiles reached 0.4- 4.2% AR. Low levels of bound residues were observed in both sediments (≤14.2% AR and ≤10.3% AR) in both studies. In first study bixlozone degraded steadily *via* four major metabolites; F9600-3-OH-propanamide (max. 8.4% AR), which was predominantly associated with the sediment phase, and 2,4-dichlorobenzoic acid (max. 40.9% AR), F9600-dimethyl malonamide (max. 15.6% AR), and 4-carboxy-F9600 (max. 21.3% AR), which were found in both the water and sediment phases. Other minor metabolites were detected but none were >5% AR at two consecutive time-points or >5% and increasing at the end of the study. In the second study, bixlozone degraded rapidly *via* four major metabolites; 2,4-dichlorobenzoic acid (max. 7.4% AR), F9600-3-OH-propanamide (max. 68.6% AR) and F9600-isobutyramide (max. 7.3% AR) which were found in both the water and sediment phases, and F9600-dimethyl malonamide (max. 10.3% AR) which was largely associated with the sediment phase. Other minor metabolites were detected, but none exceed 5 % AR at any time point (maximum individual unknown 4.5%).

In both studies the dissipation rates of bixlozone from the water phase and degradation rates in the total system were calculated following the recommendations of the FOCUS work group using

CAKE version 3.3. Combined data for phenyl and carbonyl labelled systems were used. In the first study, bixlozone dissipated from the water phase of the two water-sediment systems with $DissT_{50}$ values of 13.6 days (Calwich Abbey, SFO model) and 13.9 days (Swiss Lake, HS model). Degradation in the total water-sediment systems resulted in best-fit $DegT_{50}$ values of 23.3 days (Calwich Abbey, SFO model) and 24.8 days (Swiss Lake, HS model). Normalized to 12 °C, the corresponding $DegT_{50}$ values are 49.6 days (Calwich Abbey, SFO model) and 52.8 days (Swiss Lake, HS model). These $DegT_{50}$ values were determined with nonextractable residues attributed to degradation.

In the second study, bixlozone dissipated from the water phase of the two water-sediment systems with $DissT_{50}$ values of 8.97 days (Calwich Abbey, SFO model) and 9.22 days (Swiss Lake, SFO model). Degradation in the total water-sediment systems resulted in best-fit $DegT_{50}$ values of 9.57 days (Calwich Abbey, SFO model) and 10.1 days (Swiss Lake, SFO model). Normalized to 12 °C, the corresponding $DegT_{50}$ values are 20.5 days (Calwich Abbey, SFO model) and 21.5 days (Swiss Lake, SFO model). These $DegT_{50}$ values were determined with nonextractable residues attributed to degradation.

The DS concluded that bixlozone is considered to be not rapidly degradable in aquatic environment.

Bioaccumulation

In the study performed according to OECD TG 305 the bluegill sunfish (*Lepomis macrochirus*) were tested in a flow-through system at nominal concentrations of 13 and 130 µg/L of [phenyl-U-14C]-bixlozone for 28 days followed by 14 days depuration period. The determined kinetic bioconcentration factor (BCF_K) for whole fish lipid normalized was 77 L/kg.

For bixlozone, measured octanol-water partition coefficient (log K_{OW}) determined according to OECD TG 107 and OPPTS 830.7550 was 3.3 at 20°C and pH 4, 7 and 9.

Based on the data presented DS concluded that bixlozone has a low potential for bioaccumulation.

Aquatic Toxicity

Reliable aquatic toxicity data for each trophic level (fish, invertebrates, algae and aquatic plants) on bixlozone are available.

Acute toxicity

The summary of the relevant information on acute toxicity of bixlozone is provided in Table 19 of the CLH report.

For fish, three acute toxicity studies with three different fish species were available. Rainbow trout *Oncorhynchus mykiss* was the most sensitive fish species tested in the acute studies performed according to OECD TG 203, with mean measured 96 h LC_{50} value of 9.8 mg/L.

Nine acute toxicity studies with nine different invertebrate species were provided for aquatic invertebrates. The lowest study value, according to OECD 202 and OECD 235 resulted in a mean measured 48 h EC_{50} of 0.11 mg/L for freshwater beavertail fairy shrimp, *Thamnocephalus platyurus*. In addition, two other aquatic invertebrate species were shown to be equally sensitive. The first study was a study performed according to OCSPP Draft Guideline 850.1350 with saltwater mysid *Americamysis bahia* with mean measured 96 h LC_{50} of 0.14 mg/L and the second study was a study performed in line with OECD TG 202 and OECD TG 235 with caddisfly larvae *Pycnopsyche gentilis* with mean measured 48 h EC_{50} of 0.33 mg/L.

Three acute toxicity studies with three different algae species were available. The freshwater diatom *Navicula pelliculosa* was the most sensitive species tested in algae acute studies performed according to OECD 201 and OCSPP 850.4500, with mean measured 72 h E_rC_{50} of 13.0 mg/L.

There were two toxicity studies available for aquatic plants. The aquatic macrophyte *Myriophyllum spicatumin* was the most sensitive species tested in aquatic plants studies conducted according to OECD TG 239, with mean measured 14 d E_rC_{50} value of 3.2 mg/L.

From the available aquatic toxicity data for bixlozone, the DS concluded that invertebrates are the most acutely sensitive taxonomic group, therefore the acute aquatic classification proposed by the DS was based on the freshwater beavertail fairy shrimp *Thamnocephalus platyurus* (48 h EC_{50} = 0.11 mg/L). The most conservative study value is supported with two other results, 96 h LC_{50} value of 0.14 mg/L for *Americamysis bahia* and 48 h EC_{50} value of 0.33 mg/L for *Pycnopsyche gentilis*. DS proposed **Aquatic Acute 1** (H400) with **M-factor of 1** ($0.1 < L(E)C_{50} \leq 1$ mg/L).

Chronic toxicity

The summary of the relevant information on chronic toxicity of bixlozone is provided in Table 20 of CLP report.

There was only one study available for fish, conducted according to OECD TG 210, OCSPP Draft Guideline 850.1400 and ASTME E 1241-05, with a mean measured 32 d NOEC value of 0.38 mg/L for fathead minnow (*Pimephales promelas*).

Three chronic toxicity studies with three different species were provided for aquatic invertebrates but only in two studies the values are reported as mg/L. The saltwater mysid *Americamysis bahia* was the most sensitive species tested in chronic studies performed according to OCSPP Draft Guideline 850.1350, with a mean measured 28 d NOEC of 0.12 mg/L.

Three chronic toxicity studies with three different algae species were available. The diatom *Navicula pelliculosa* was the most sensitive species tested in algae chronic studies performed according to OECD TG 201 and OCSPP 850.450, with a mean measured 72 h E_rC_{10} of 3.3 mg/L. In addition, a mean measured 72 h E_rC_{10} value of > 0.63 mg/L for marine diatom *Skeletonema costatum*, according to OECD TG 201 and OCSPP Draft Guideline 850.4500, was reported.

There were two toxicity studies available for aquatic plants. The aquatic macrophyte *Myriophyllum spicatumin* was the most sensitive species tested in aquatic plants studies conducted according to OECD TG 239, with a mean measured 14 d E_rC_{10} value of 0.0071 mg/L.

Based on the results from the long-term aquatic toxicity studies using bixlozone, the DS concluded that aquatic plants are the most sensitive taxonomic group. Therefore, the chronic aquatic classification proposed by DS was based on the *Myriophyllum spicatumin* toxicity study (14 d E_rC_{10} = 0.0071 mg/L). The DS proposed **Aquatic Chronic 1** (H410), with **M-factor of 10** ($0.001 < NOEC \leq 0.01$ mg/L) along with the understanding that the substance is not rapidly degradable.

Comments received during consultation

Two Member States (MS) and one company-manufacturer provided comments. All agreed with the proposed by the DS classification for environmental hazards.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider bixlozone as not rapidly degradable:

- The substance was stable to hydrolysis at environmental relevant temperatures and pH.
- The substance was not readily biodegradable. Biodegradation in the OECD TG 301 B test was less than 13 % after 28 days.
- In the surface water simulation test (OECD TG 309), the mineralization was low (< 2%) and DT_{50} values of 818 to 1040 days were determined. No significant degradation products were observed.
- The DT_{50} in the whole system in water/sediment system studies (OECD TG 308) for two labels were from 9.57 to 23.3 days (Calwich Abbey) and from 10.1 to 24.8 days (Swiss Lake). Mineralization was 0.4 to 51.55%. Five main metabolites were formed.

Bioaccumulation

RAC agrees with the DS that bixlozone has a low potential for bioaccumulation in aquatic organisms based on the available bioconcentration study in bluegill sunfish showing a kinetic BCF (whole fish, lipid normalized) value of 77 being below the decisive CLP Regulation criterion of 500. Additionally, measured log K_{OW} value of 3.3 is below the CLP Regulation criterion of 4.

Aquatic toxicity

RAC is of the opinion that reliable aquatic acute and long-term toxicity data for bixlozone are available for fish, invertebrates, algae and aquatic plants.

Acute toxicity

According to the toxicity data presented in Table 19 of the CLP report, invertebrates are the most sensitive trophic level, and the lowest result is a mean measured 48 h EC_{50} value of 0.11 mg/L for freshwater shrimp *Thamnocephalus platyurus*. This result is supported by two other studies, which provide toxicities within the same range. The first study is a study performed with saltwater mysid *Americamysis bahia* with 96 h LC_{50} of 0.96 mg/L and the second study is a study performed with caddisfly larvae *Pycnopsyche gentilis* with 48 h EC_{50} of 0.33 mg/L. Based on these test results, RAC concludes that bixlozone meets the classification criteria as **Aquatic Acute 1** (H400) with an **M-factor of 1** ($0.1 < L(E)C_{50} \leq 1$ mg/L).

Chronic toxicity

According to the toxicity data presented in Table 20 of the CLP report, aquatic plants are the most sensitive trophic level and the most conservative result is a mean measured 14 d E_rC_{10} value of 0.0071 mg/L for the aquatic macrophyte *Myriophyllum spicatumin*. Bixlozone was not rapidly degradable and had a low potential for bioaccumulation. Consequently, RAC concludes that bixlozone meets the classification criteria as **Aquatic Chronic 1** (H410) with **M-factor of 10** ($0.001 < NOEC \leq 0.01$ mg/L).

Additional references

Parker, G.A.; Gibson W. B. Liver lesions in rats associated in wrapping of the torso. *Toxicologic Pathology* **1995,** *23*, pp 507-512

van Berlo et al. 10% body weight (gain) change as criterion for the maximum tolerated dose: a critical analysis. *Regul Toxicol Parmacol* (2022 Oct)

ANNEXES:

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

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