Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

benzobicyclon (ISO); 3-[2-chloro-4-(methylsulfonyl)ben zoyl]-4- (phenylthio)bicyclo [3.2.1]oct-3-en-2- one

EC Number: -
CAS Number: 156963-66-5

CLH-O-0000007414-76-01/F

Adopted

14 March 2024

14 March 2024

CLH-O-0000007414-76-01/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 on **14 March 2024** by **consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **benzobicyclon (ISO); 3-[2-chloro-4- (methylsulfonyl)ben zoyl]-4- (phenylthio)bicyclo [3.2.1]oct-3-en-2- one**

EC Number: **-**

CAS Number: **156963-66-5**

Rapporteur, appointed by RAC: **Bogusław Barański**

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

Administrative information on the opinion

Malta has submitted on **28 April 2023** 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 **8 May 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 July 2023**.

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 following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

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	benzobicyclon (ISO); 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one	-	156963-66-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M = 100 M = 100	
RAC opinion	TBD	benzobicyclon (ISO); 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one	-	156963-66-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M = 100 M = 100	
Resulting Annex VI entry if agreed by COM	TBD	benzobicyclon (ISO); 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one	-	156963-66-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M = 100 M = 100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Benzobicyclon is a new active substance, a systemic herbicide (absorbed principally by the roots), used to control monocotyledonous (grass and non-grass) weed species in rice. Benzobicyclon is currently not included in Annex VI to the CLP Regulation.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

The chemical structure of benzobicyclon does not contain hazardous groups (such as nitro-groups). As there are no functional groups associated with explosive properties in the molecule, the DS concluded that no classification was warranted.

Flammable solids

No independent burning or glowing over the length of the pile was observed in the preliminary test performed according to method A.10. The test substance was found to be not flammable. If available data from an A.10 test method indicate that the substance is not flammable, a classification as a flammable solid does not apply (result: not highly flammable). According to the DS, no classification as a flammable solid was warranted.

Self-reactive substances

The self-reactivity of benzobicyclon has been evaluated with method A.16. The purpose of this test is to provide preliminary information on the auto-flammability of solid substances at elevated temperatures. No self-ignition was observed at temperatures up to the melting point. According to the DS, no classification as a self-reactive substance was warranted.

Pyrophoric solid

Benzobicyclon does not self-ignite at temperatures prior to the melting point in test A.16. Furthermore, the experience in use with benzobicyclon over a long period of time demonstrates that it is stable. According to the DS, no classification as a pyrophoric solid was warranted.

Self-heating substances

The self-heating properties of benzobicyclon have been evaluated using data derived from method A.16 described in Regulation (EC) No 440. The substance had melted during the temperature raise. The barometric pressure was 766.2 mm Hg at the beginning and ending of the test. Results of the EC method A.16 performed on benzobicyclon exclude self-heating of the substance up to the melting point. According to the DS, no classification as a self-heating substance was warranted.

Substances which in contact with water emit flammable gases

Benzobicyclon does not fulfil the criteria for classification in this hazard class as the chemical structure does not contain metals or metalloids. According to the DS, no classification as a substance which in contact with water emits flammable gases was warranted.

Oxidising solids

No test has been performed with the substance. Benzobicyclon contains oxygen bonded to atoms other than carbon and hydrogen, therefore the screening criteria according to the Annex I part 2 point 2.14.4.1 of Regulation (EC) No. 1272/2008 are not met. In the absence of an appropriate test or any empirical data the DS concluded there is a lack of data to conclude on classification of this substance as an oxidising solid**.**

Organic peroxides

Benzobicyclon does not contain the bivalent organic peroxide –O–O– structure so it is not considered for classification in this hazard class. According to the DS, this information indicates that no classification is warranted.

Corrosive to metals

No studies have been submitted. Benzobicyclon has a melting point of 187 °C (99.0 %), thus it shows a melting point higher than 55 °C and is not to be considered for classification in this hazard class. According to the DS, no classification as corrosive to metals is warranted.

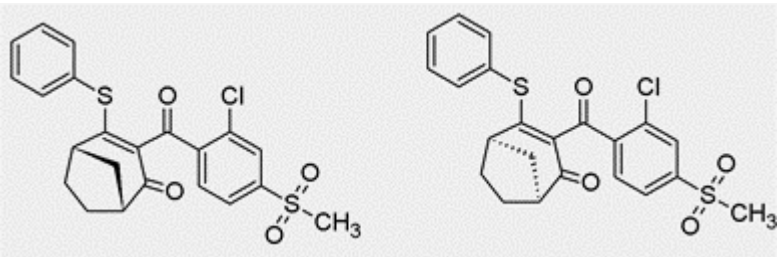
Comments received during consultation

No comments on physical hazards were received during public consultation.

Assessment and comparison with the classification criteria

Explosives

The substance has the following formula:



According to CLP Annex I, 2.1.4.3a and Guidance on the Application of the CLP Criteria Version 6.0 – January 2024 (hereafter the CLP guidance v6.0), a substance or mixture is not classified as explosive when there are no chemical groups associated with explosive properties present in the molecule. Since benzobicyclon does not have any such chemical groups, RAC agrees with the DS that benzobicyclon does not warrant classification as an explosive.

Flammable solid

As data from the available A.10 test shows no flammability of the test material, RAC agrees with the DS that benzobicyclon does not warrant classification as a flammable solid.

Self-reactive substances

According to CLP Annex I section 2.8.2.1(d) substances shall be considered for classification unless:

(c) their heat of decomposition energy is less than 300 J/g.

The guidance on the Application of the CLP criteria (v6.0) also indicates some chemical groups that might support self-reactive properties. The guidance also refers to additional guidance in the UN-MTC (Seventh revised version, 2019), which indicates that the classification procedures for self-reactive substances need not to be applied if there are no chemical groups present in the molecule associated with explosive or self-reactive properties. Examples of such groups are given in UN-MTC tables A6.1 and A6.3.

The molecule of benzobicyclon does not contain chemical groups associated with explosive or self-reactive properties such as C-C unsaturation, C-metal, N-metal, contiguous nitrogen atoms, contiguous oxygen atoms, N-O, N-halogen or O-halogen listed in the UN-MTC.

UN-MTC table A6.3 does list the S=O group together with examples of molecules where sulphonyl group is bonded to a nitrogen or a halogen. In benzobicyclon, the sulphonyl group has carbons on both sides and it can be expected to be less reactive than the examples in UN-MTC.

After the RAC consultation, the DS provided a study regarding the decomposition energy of benzobicyclon "Benzobicyclon - Determination of the Boiling Point up to 550 °C (823.15 K) following OECD 103 and ASTM E537-07 (2026)". This study has revealed that no boiling point was detected up to a temperature of 550 °K and that the sample of benzobicyclon decomposed at less than 550 °K. The exothermic decomposition energy of benzobicyclon was 180.6±9.94 J/g, which below the CLP screening criteria of 300 J/g (CLP Annex I, part 2.8.2.1(d).

Since the exothermic decomposition energy of benzobicyclon does not meet the above criteria and does not contain chemical groups associated with self-reactive properties, RAC agrees with the DS that benzobicyclon does not warrant classification as a self-reactive substance.

Pyrophoric solid

According to CLP Annex I, section 2.9.4.1, the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture 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)). As experience in use with benzobicyclon over a long period of time shows that it is stable and does not ignite, RAC agrees with the DS that benzobicyclon does not warrant classification as a pyrophoric solid.

Self-heating substances

According to CLP Annex I, section 2.11.1.2, self-heating of a substance is a process where the gradual reaction of that substance with oxygen (in the air) generates heat. If the rate of heat production exceeds the rate of heat loss, then the temperature of the substance or mixture will rise which, after an induction time, may lead to self-ignition and combustion.

A screening test (EU test method A.16 as described in Regulation (EC) No 440/2008) can be used to determine whether self-heating occurs or not. Results of the EC method A.16 performed on benzobicyclon exclude self-heating of the substance up to the melting point. Taking those results into account, RAC agrees with the DS that benzobicyclon does not warrant classification as a self-heating substance.

Substances which in contact with water emit flammable gases

According to CLP Annex I, section 2.12.4.1, the classification procedure for this hazard class need not be applied if the any of the listed screening criteria (a, b, or c) are met.

These criteria are met (a and c), since benzobicyclon does not contain metals/metalloids in its chemicals structure and experience shows that it does not react with water. Consequently, RAC

agrees with the DS that benzobicyclon does not warrant classification as substances which in contact with water emit flammable gases.

Oxidising solids

Oxidising solid means a solid substance or mixture which, while in itself is not necessarily combustible, may, generally by yielding oxygen, cause, or contribute to, the combustion of other material. According to CLP Annex I, section 2.14.4.1, the classification procedure for an organic substance for this class shall not apply if the any of the listed screening criteria (a or b) are met.

Benzobicyclon does contain oxygen bonded to atoms different from carbon and hydrogen, therefore the screening criteria according to the Annex I.2.14.4.1 of Regulation (EC) No. 1272/2008 are not met.

It is noted RAC has recently agreed (RAC-67) to not classify flazasulfuron as oxidising substance on the basis that oxygen and fluorine atoms in the flazasulfuron molecule are bonded only to carbon except the sulphonyl group, where the oxygen atoms are bonded to sulphur. Since, sulphonyl group is generally not oxidising, RAC concludes that benzobicyclon does not warrant classification an oxidising solid.

Organic Peroxides

According to the CLP Annex I, section 2.15.1.1, organic peroxides are substances containing the –O–O– moiety. Consequently, no classification to this hazard class can be concluded if the chemical peroxide group (–O–O–) is absent. Since benzobicylcon does not contain –O–O– and is therefore not a peroxide, RAC agrees with the DS that no classification is warranted.

Corrosive to metals

A substance or a mixture that is corrosive to metals means a substance or a mixture which by chemical action will materially damage, or even destroy, metals. A substance is classified in category 1 if the corrosion rate on either steel or aluminium surfaces exceeds 6.25 mm per year at a test temperature of 55 °C when tested on both materials.

According to CLP, the only substances and mixtures that need to be considered for classification to this hazard class, are those for which the application of the UN Test C.1 (described in part III, Section 37.4.1.1 of the UN-MTC) is relevant. It is considered that solids are considered as not relevant for this hazard class as the UN Test C.1 was designed for liquids. Consequently, only solids having a melting point lower than 55 °C (which is the test temperature required in UN Test C.1) must then be taken into. As benzobicyclon is a solid with a melting point of 187 °C, it will be not liquified at temperatures below 55 °C and therefore does not meet the criteria for classification.

As benzobicyclon has a melting point above 55 °C, RAC agrees with the DS that classification as corrosive to metals is not warranted.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The Dossier Submitter provided the results of two acute oral toxicity studies, one in rats (DAR for Benzobicyclon, Vol. 3 B.6.2.1 - CA 5.2.1/01) and one in mice (DAR for Benzobicyclon, Vol. 3. B.6.2.1 - CA 5.2.1/01). The studies were considered valid, scientifically acceptable and appropriate for the assessment of acute oral toxicity of Benzobicyclon. The oral LD_{50} value of benzobicyclon was > 5000 mg/kg bw in male and female rats as well as in male and female mice.

According to the DS since the oral LD_{50} values of benzobicyclon in rats and mice are > 2000 mg/kg bw, classification for acute oral toxicity according to Regulation (EC) No 1272/2008 is not required.

Acute dermal toxicity

The Dossier Submitter provided the results of two acute dermal toxicity studies in rats, one performed in 1995 (DAR for Benzobicyclon, Vol. 3 Vol. 3 B.6.2.2 - CA 5.2.2/01) and one in 2015 (DAR for Benzobicyclon, Vol. 3. B.6.2.2 - CA 5.2.2/02). The studies were considered valid, scientifically acceptable and appropriate for the assessment of acute dermal toxicity of benzobicyclon. The dermal LD_{50} value of benzobicyclon in male and female rats in both studies was > 2000 mg/kg bw.

According to the DS, since the dermal LD_{50} values of benzobicyclon in rats are > 2000 mg/kg bw, classification for acute dermal toxicity according to Regulation (EC) No 1272/2008 is not required.

Acute inhalation toxicity

The Dossier Submitter provided the results of one acute inhalation toxicity study in rats (DAR for Benzobicyclon, Vol. 3, B.6.2.3 - CA 5.2.3/01). The study is considered valid, scientifically acceptable and appropriate for the assessment of acute inhalation toxicity of benzobicyclon. The acute inhalation LC_{50} value of benzobicyclon in male and female rats in was > 2.72 mg/L. This was the highest technically achievable concentration. According to the DS since no mortality was observed up to the highest attainable dust aerosol concentration of 2.72 mg/L, classification of benzobicyclon for acute inhalation toxicity according to Regulation (EC) No 1272/2008 is not required.

Comments received during consultation

No specific comments were provided regarding acute oral, dermal and inhalation toxicity, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

Since the median lethal doses of benzobicyclon in the acceptable acute oral and dermal toxicity studies in rats and mice were above 2000 mg/kg bw, RAC concludes that the **substance does**

not warrant classification for acute oral toxicity and acute dermal toxicity (in agreement with the DS proposal).

The 4-h LC_{50} of benzobicyclon aerosol for rats of both sexes amounted to > 2.72 mg/L, since no mortality was observed among animals exposed for 4 hours at this highest technically achievable concentration. RAC concludes that the **substance does not warrant classification for for acute inhalation toxicity** (in agreement with the DS proposal)**.**

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

Summary of the Dossier Submitter's proposal

For assessment of STOT SE, the Dossier Submitter referred to studies on acute toxicity and acute neurotoxicity described in the Draft Assessment Report, Volume 1 Level 2, section 2.6.2 and 2.6.7 and to Volume 3, section B.6.2 and B.6.7.

According to the DS no specific target organ toxicity, even in the absence of mortality, was observed after single dosing of animals thus a classification of benzobicyclon for STOT SE according to Regulation (EC) No 1272/2008 is not required.

Comments received during consultation

No specific comments were provided regarding specific target organ toxicity- single exposure, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

In the acute oral, dermal and inhalation toxicity studies (DAR, Volume 3, section B.6.2) neither mortality nor any clinical sign of toxicity were observed in rats and mice at the oral dose of 5000 mg/kg bw, in rats exposed dermally to 2000 mg/kg bw or in rats exposed by inhalation at the highest technically achievable concentration of benzobicyclon dust equal 2.72 mg/L.

Benzobicyclon at oral doses of 78.1, 312.5, 1250 and 5000 mg/kg bw produced no significant changes in the gross behaviour of the mice on the day of dosing or during the subsequent 7-day post-dose observation period (DAR, Volume 3, section B.6.7.1.1.).

Since neither significant toxic effects nor narcotic effects and respiratory tract irritation were observed in the appropriate studies in experimental animals, RAC concludes that benzobicyclon **does not warrant classification for specific target organ toxicity – single exposure.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The potential of benzobicyclon to induce skin irritation was investigated in an acceptable rabbit study performed according to OECD TG 404 and in GLP conditions. Benzobicyclon (0.5 g), moistened with water, was applied to the clipped left dorsal skin area of 6 female rabbits and

covered with oil-paper and then with an elastic bandage (DAR, Volume 3, section B.6.2.4.). The right dorsal skin area remained untreated and served as a control. After a 4-hour exposure period, the application site was wiped with water to remove any residual substance. No irritation reactions were observed in the skin of any animal at any observation time. The mean scores for erythema and oedema at 1, 24, 48 and 72 hours were 0 for all animals.

Based on these data, DS concluded that benzobicyclon does not require classification for skin corrosion/irritation.

Comments received during consultation

No specific comments were provided regarding skin corrosion/irritation, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

In the acceptable skin irritation/corrosion study in rabbits, the CLH criteria for skin irritation of a mean score of ≥ 2.3 for erythema/eschar or for oedema were not observed in any of the tested animals, therefore RAC concludes that benzobicyclon **does not warrant classification for skin corrosion/irritation.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritancy of benzobicyclon was investigated in an acceptable rabbit study performed according to OECD TG 405 and in GLP conditions. Benzobicyclon was administered as a 0.1 g dose into the conjunctival sac in one eye each of 6 female rabbits (DAR, Volume 3, section B.6.2.5.). The treated eyes of the animals remained unwashed after application. The untreated eye of all animals served as a negative control. A second group of 3 animals was treated in the same manner and the treated eyes were washed with water 2-3 minutes after application. All animals were subjected to observations at 1, 24, 48 and 72 hours after application to verify the ocular response in the cornea, iris and conjunctiva.

Without washing, the mean irritation score (24, 48, 72 hours) in 3 of 6 rabbits was 0.67, 0.33, 0.33 for conjunctional redness and in 1 of 6 rabbits was 0.33 from chemosis. With washing, the mean irritation score (24, 48, 72 hours) in all rabbits was 0.

Based on results of this study the DS concluded that benzobicyclon does not require classification for skin corrosion/irritation.

Comments received during consultation

No specific comments were provided regarding eye damage/irritation, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

RAC notes that benzobicyclon caused slight reversible eye irritation in an acceptable *in vivo* study in the rabbit.

Mean scores for specific ocular effects are not exceeding the CLP criteria for classification in Category 2. Only slight conjunctival redness and chemosis were observed, but the average scores were < 2 (i.e., the relevant average score for conjunctival redness and oedema). Therefore, RAC concludes that benzobicyclon **does not warrant classification for eye damage/irritation** (in agreement with the DS proposal).

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS noticed that no study on respiratory sensitisation is available for benzobicyclon. There was no evidence for respiratory irritation in the acute inhalation toxicity study in rats and there is no indication of skin sensitisation (see below). There was no reported evidence of respiratory sensitisation in humans.

Taking into account the lack of relevant data, the DS concluded that benzobicyclon does not warrant classification as respiratory sensitiser.

Comments received during consultation

No specific comments were provided regarding respiratory sensitisation, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

As there are not data suggesting that benzobicyclon may cause the respiratory sensitisation, RAC concludes that the substance **does not warrant classification for respiratory sensitisation** (in agreement with the DS proposal).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS provided results of two available skin sensitisation studies with benzobicyclon, *i.e.* a Local Lymph Node Assay (LLNA) in mice and a Guinea Pig Maximisation Test.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Skin sensitisation LLNA OECD 429 GLP Acceptable	Mouse, CBA/J, females, 5/group	Benzobicyclon technical Batch No.: 1A0110 Purity: > 99.9 %	0, 10, 25, 50 %, 3 days	No mortality, no clinical signs Stimulation index (SI) was 1.0, 1.2 and 1.0 for the test substance concentrations of 10, 25 and 50 %, respectively, while in	(Vol. 3 B.6.2.6 - CA 5.2.6/01)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
				positive control SI was 3.7 No sensitising potential	
Skin sensitisation Guinea Pig Maximization Test OECD 406 GLP Supportive information	Guinea pig, Hartley White, females, 25/group	SAN1315H (Benzobicyclon) Batch No.: 6F0502 Purity: 99 %	Intradermal induction: 1 % Topical induction: 50 % Challenge: 50 %	No mortality, no clinical signs No skin reactions at the challenged sites Sensitisation rate treatment groups: 0 % Sensitisation rate positive control group: 100 %	(Vol. 3 B.6.2.6 - CA 5.2.6/02)

While the LLNA was considered as fully acceptable, the GPMT was acceptable only, according to the DS, as supportive information due to the following guideline deviations: limited test substance concentration of 1 % and application of only one concentration (50 %) at topical induction and challenge. The DS noted that testing of higher concentrations should have been possible when moistening the test substance with water.

Taking into account that the stimulation index in the LLNA in mice was < 3 and the sensitisation rate in the Guinea Pig Maximisation Test was 0 %, the DS concluded that classification of benzobicyclon for skin sensitisation according to Regulation (EC) No 1272/2008 is not required.

Comments received during consultation

No specific comments were provided regarding skin sensitisation, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

Analysing the two submitted studies RAC noted that the highest skin non-irritating concentration of the substance in LLNA (as recommended in OECD 429) and the highest concentration to cause mild-to-moderate skin irritation in Guinea Pig Maximization Test (as recommended in OECD 406) were not determined. Benzobicyclon did not cause a skin irritation in any of the performed studies, including the rabbit study performed according to OECD TG 404 at the highest technically achievable concentration, up to approximately 50 %. Benzobicyclon is a slightly yellow solid with a melting point of 187 °C, which has very low solubility in water (51.8 µg/L at 20 °C) and it hydrolyses quickly in aqueous solutions. The solubility in organic solvents is generally low in the tested solvents, the highest solubility was found in dichloroethane (144 g/L).

Taking into account lack of skin irritation and low solubility in water and in organic solvents, the concentration to be used for topical and intradermal application were chosen in the preliminary tests performed in both studies. In the main tests, the highest concentration of 50 % achieved as suspension of benzobicyclon in propylene glycol was used in the LLNA and in the Guinea Pig

Maximization Test a 50 % suspension of benzobicyclon in water was used for topical application and 1 % for intradermal induction. RAC considers that the 50 % suspension were the highest possible concentrations of benzobicyclon in propylene glycol or in water for topical application on skin, and both studies are considered as acceptable since no deviation for the test guidelines were found.

Noting that the criteria for classification in the Local Lymph Node Assay (stimulation index ≥ 3) and in the Guinea Pig Maximisation test (positive response in ≥ 30 % of animals) were not met, RAC concludes that the substance **does not warrant a classification as a skin sensitiser** (in agreement with the DS proposal).

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

Summary of the Dossier Submitter's proposal

There are no data indicating that benzobicyclon has induced adverse effects in humans as a result of long-term exposure. The Dossier Submitter provided in Volume 1 of the Draft Assessment Report (CLH report) results of several relevant animal studies described more in detail in Volume 3, part B.6 (AS) of the Draft Assessment Report of Benzobicyclon.

Oral route

In a **4-week repeated dose toxicity dietary dose selection study in rats** (Final Report, Study No: IET95-0155) with doses 0, 80, 400, 2000, 10000, and 50000 ppm. Hyaline droplet deposition in the renal proximal tubules in the kidneys was observed in male animals. This finding is likely attributable to α2u-globulin deposition specific to male rats.

In a **13-week repeated dose toxicity study** (OECD TG 408 with deviations, GLP) **in rats** (Vol. 3, B.6.3.2- CA 5.3.2/01), the following dietary concentration were used: males (12/dose) 0, 20, 100, or 400 ppm (equivalent to 0, 1.13, 5.73 and 22.74 mg/kg bw/d), females (12/dose) 0, 100, 400, 2000, or 10000 ppm (equivalent to 0, 6.29, 25.17, 125.9, 630 mg/kg bw/d). The deviations to the OECD TG were noted: T3, T4, TSH were not measured, organ weight lacking: epididymides, prostate, seminal vesicles with coagulating gland, uterus, pituitary and thyroid, no sperm parameters, no information on oestrus cycle, no assessment of sensor activity and grip strength, no motor activity assessment or functional observational battery. In males, adverse effects were observed at 100 ppm (5.73 mg/kg bw/d) with clear signs of renal toxicity based on increase in urinary volume and aggravation of tubular basophilic change both in cortex and medulla. In the 400 ppm (22.74 mg/kg bw/d) group, males showed deposition of hyaline droplets in proximal tubular cells in cortex of the kidney in 6/12 animals. Although hyaline droplets could be seen in proximal tubular cells in males of the control group, the droplets observed in the 400 ppm (22.74 mg/kg bw/d) group were large in size and irregular in contour compared with those in the control. There were granular casts in cystically dilated tubules in medulla of all males in the 400 ppm (22.74 mg/kg bw/d) group. The lesions consisted of tubular dilatation with accumulation of eosinophilic granular materials at outer and inner stripe of outer medulla in association with flattened epithelial cells. Immuno-histochemical staining confirmed that the major component of the hyaline droplets in the proximal tubular cells was alpha-2μ-globulin and this MoA is not considered of human relevance.

In females, the increases in liver and kidney weights (less than 10 % with respect to the control) are not considered adverse. A decrease in urinary pH was observed for females treated with 125.9 mg/kg bw/d and above. The effect was reversible and not considered in isolation as adverse.

In a **13-week oral repeated dose toxicity study** (OECD 409, GLP) **the Beagle dogs** (4/sex/dose), the following doses via encapsulated gelatine capsules were used: 0, 20, 200 or 2000 mg/kg bw/d ((Vol. 3 B.6.3.2 - CA 5.3.2/02). Loose and watery stools and vomiting were observed during the administration period, but there was no evidence to attribute their occurrence to administration of the test substance. Slightly increased incidence of the following effects was observed (within the HC upper limit): pituitary gland cyst in males at 200 and 2000 mg/kg bw/day and in females at all dose levels, dilated gland in duodenum in males at 2000 mg/kg bw/day, and Kuersteiner's cysts of the parathyroid glands in females at 2000 mg/kg bw/day. The severity grade of all these findings did not increase when compared to controls or with increasing of doses. In the 1-year dog study Kuersteiner's cysts of the parathyroid glands were reported in females at 2000 mg/kg bw/day and in two females in the 1000 mg/kg bw/d group (Kitajima, S. 1999). Based on HCD, cysts of the parathyroid glands are not an uncommon finding in this laboratory (90 d mean: 10.6 %; 0-50 % range; 1 year mean: 16.7 %, 0.0-66.7 %). Overall, considering that after 1-year treatment cysts of the parathyroid glands was reported in 1, 2, 0 and 1 males of the 0, 10, 100 and 1000 mg/kg bw/day, respectively, the observed finding in females was considered of doubtful relation to treatment.

In a **52-week repeated dose toxicity study the Beagle dogs** (4/sex/dose) (OECD TG 452, GLP) the following doses via encapsulated gelatine capsules were used: 0, 10, 100 or 1000 mg/kg bw/d ((Vol. 3 B.6.3.2 - CA 5.3.2/03). Loose and watery stools and vomiting were observed during the administration period, but there was no evidence to attribute their occurrence to administration of the test substance. Impurities consisting of white material considered to be the test substance were observed in the stools in the 100 and 1000 mg/kg bw/d groups. Declines in the potassium values in males in the 100 and 1000 mg/kg bw/d groups were observed without toxicological significance.

Dermal route

In a 21-day study (Vol. 3 B.6.3.3 - CA 5.3.3/01), New Zealand White (NZW) rabbits were dermally dosed with water moistened benzobicyclon at 100, 300, and 1000 mg/kg bw/d (6 hours/day). Gross macroscopic and microscopic examinations showed some non-adverse alterations in the deep dermis, subcutis, and panniculus muscle that were pressure-related from the wrapping of the animal and not considered as a result of toxicity.

In **the combined chronic toxicity/carcinogenicity study** (Vol. 3 B.6.5.1 - CA 5.5/01) (OECD 453, GLP) male/female Fisher rats (50/sex/dose) were administered benzobicyclon in diet at concentrations of 0, 10, 20, 50, and 100 ppm in males (equivalent to 0, 0.334, 0.667, 1.696 and 3.43 mg/kg bw/d) and 0, 100, 1000, and 10000 ppm in females (equivalent to 0, 4.19, 42.2, 427 mg/kg bw/d) for a period of 24 months (104 weeks).

In males, effects were seen only at top dose. Increases in incidence of hyaline droplet deposition in the proximal tubular cell in the kidney and increases in incidence of nephropathy, chronic with aggravated severity were considered rat male specific effects. Increase in thyroid and pituitary masses were considered unrelated to treatment.

Also in females, effects were seen only at top dose. In urinalysis, pH was decreased at Week 103 without toxicological relevance. In clinical chemistry, increases were noted for total cholesterol after Week 26, total protein after Week 52, and globulin after Weeks 52 with a doubtful relation

to treatment and of not toxicological relevance as these changes did not progress with exposure duration. Regarding increases for mean absolute and relative weight of the liver, animal numbers 582, 601 and 603, were affected by systemic mononuclear cell leukaemia (animals 601 and 603) and severe extramedullary haematopoiesis (animal 582), showed the highest liver weights of the top dose group, thus the liver weights of these animals were influenced by the underlying conditions that were not determined by the treatment. Increases for absolute and relative weight of the kidney in the absence of treatment related physiopathological correlated changes were considered of no toxicological significance. An increase in the absolute mean spleen weight were noted in the 1000 (42.2 mg/kg bw/d) and 10000 ppm (427 mg/kg bw/d) female groups, caused by some specified individual animals. For the 1000 ppm group, animal 565 was reported to be affected by spleen enlargement and mononuclear cell leukaemia also at spleen level. Other abnormal spleen weights were noted in the 10000 ppm group, for animals number 582, 601 and 603 as the liver weight increases. This effect was considered likely a chance finding since the individual animal data does not report any pathological adverse condition at spleen that might have been caused by the treatment.

In a 78 week carcinogenicity study in mice (Vol. 3 B.6.5.1 - CA 5.5/02) (OECD 452, GLP) male /female CrI:CD-l™ (ICR) BR mice (50/sex/dose) were administered benzobicyclon in diet at a concentration of 0, 300, 3000 and 30000 ppm in males (equivalent to 0, 37, 373, 3817 mg/kg bw/d) and 0, 300, 3000 and 30000 ppm in females (equivalent to 0, 45, 473, 4807 mg/kg bw/d). Adaptive changes in the liver (increased organ weight in females and increased centrilobular hepatocellular hypertrophy in both sexes) and increased periportal hepatocyte vacuolation in females were observed at the highest dose applied (30000 ppm). The DS has considered these effects as adaptive, however, the lack of the clinical biochemistry analyses does not allow a sound assessment of the liver findings. A statistically significant increase of relative heart and kidney weights in females at 30000 ppm (4807 mg/kg bw/d) and decreased relative prostate weight in males at 30000 ppm (3817 mg/kg bw/d) were considered of no toxicological relevance.

In the 91 day oral (diet) subchronic neurotoxicity study in rats ((Vol. 3 B.6.7.1.2 - CA 5.7.1/04) (OECD 424, GLP) male /female Crl:CD(SD) rats (10/sex/dose) concentrations of 0, 1000, 5000 and 20000 ppm in males (equivalent to 0, 61.8, 306.5, 1290.0 mg/kg bw/d) and 0, 1000, 5000 and 20000 ppm in females (equivalent to 0, 72.3, 373.6 and 1499 mg/kg bw/d) were administered in diet without any observed treatment related effects.

In a two-generation reproductive toxicity study (Vol. 3 B.6.6.1- CA 5.6.1/01) groups of 24 males and 24 females rats (Crj:CD (SD) concentrations of 0, 100, 1000, or 20000 ppm (equivalent to F0 males/females 0; 5.65/8.44; 56.1/85.4 and 1176/1741 mg/kg bw/d; and to F1 males/females 0, 6.46/8.76; 62.8/89.0 and 1324/1817 mg/kg bw/d) were administered. For females increased organ weights (liver, kidney, adrenals) in F0 and F1 were observed at top dose (for study results details refer to Vol. 3 B.6.6.1 - CA 5.6.1/01). In males renal toxicity (α-2μ-globulin nephropathy) was observed from mid dose onwards. At top dose increased hydropic degeneration cells (basophilic cells) in pituitary in the F0 (in 2 out of 24 examined males - not significant increase) and in pituitary of males in F1 generation (6/24), all accompanied with decrease in absolute and relative pituitary weights. Also increase of relative testes and epididymis weights were observed. The applicant considered the male-rat specific α2μ-globulin nephropathy causes a perturbation of the homeostasis in the hypothalamus and/or the pituitary. Subsequently, LH and FSH levels are affected, as a result of this different peripheral findings such as effects on testosterone levels, testis weight, epididymis weight and spermatogenesis can be observed.

The DS concluded that no relevant treatment related effects were observed after repeated exposure to doses at or below the reference values for STOT RE classification assigned in

Regulation (EC) No 1272/2008, therefore benzobicyclon should not subject to classification for specific target organ toxicity after repeated exposure (STOT RE) according to Regulation (EC) No 1272/2008. The existing data are conclusive but not sufficient for classification.

Comments received during consultation

No comments were provided regarding of specific target organ toxicity-repeated exposure, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

Using Haber's rule for comparison with CLP classification criteria for STOT RE 2 the following guidance values were adopted by RAC's calculation

for oral exposure:

10 < C ≤ 100 mg/kg bw/day, duration 90 days/3 months/13 weeks
7.7 < C ≤ 77 mg/kg bw/day, duration 17 weeks
2.5 < C ≤ 25 mg/kg bw/day, duration 52 weeks
1.7 < C ≤ 17 mg/kg bw/day, duration 78 weeks
1.25 < C ≤ 12.5 mg/kg bw/day, duration 104 weeks
0.05 < C ≤ 0.25 mg/L/6 h/day, duration 365 days
0.03 < C ≤ 0.13 mg/L/6 h/day, duration 730 days

for dermal route

C ≤ 20 mg/kg bw/day, duration 90 days
C ≤ 86 mg/kg bw/day, duration 21 days

Since there are no data on specific target organ toxicity due to repeated exposure in humans, this class of toxicity has to be assessed based on data derived from the acceptable animal studies.

It is noted that benzobicyclon in the repeated dose toxicity studies at relatively low dose levels induces nephropathy only in male rats, but not in female rats, in mice and in dogs of both sexes. As pointed out by the Dossier Submitter, it is likely that the species and sex difference in renal toxicity is ascribed to α2μ-globulin deposition in the tubular cells which is a sex-specific phenomenon in male rats. Immuno-histochemical staining carried out in 13-week repeated dose toxicity study in rats (Vol. 3, B.6.3.2- CA 5.3.2/01) confirmed that the major component of the hyaline droplets in the proximal tubular cells was alpha-2μ-globulin. As indicated in Guidance on the Application of the CLP Criteria, Version 5.0 – July 2017 the protein α-2-μ globulin, which is primarily synthesized in male rats, has the capability to bind to certain chemicals. The resultant adducts accumulate as droplets in the kidneys and causes progressive renal toxicity within a few weeks which can ultimately lead to kidney tumours. This specific mechanism is unique to male rats and has no relevance for humans. In line with Regulation 1272/2008 (CLP Annex I, 3.9.2.8.1. (e): *substance-induced species-specific mechanisms of toxicity, i.e. demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification.* Taking this provision into account, RAC agrees with the DS that this effect, as not relevant for humans, should not be considered for classification of benzobicyclon for specific target organ toxicity-repeated exposure.

Studies in rats

No human-relevant adverse effects were observed in the following studies :

- 13-week dietary repeated dose toxicity study (Vol. 3, B.6.3.2- CA 5.3.2/01) in male rats at dose level of 400 ppm (22.74 mg/kg bw/d), which was the highest dose tested and in female rats at dose level of 10000 ppm (630 mg/kg bw/d), the highest dose tested;
- the 2-year chronic oral toxicity and carcinogenicity study (Vol. 3 B.6.5.1 - CA 5.5/01) in female rats at the repeated dose of 427 mg/kg bw/d, the highest dose tested and in male rats at a the repeated dose of 3.43 mg/kg bw/d), the highest dose applied to male rats;

- the 91-day oral (diet) subchronic neurotoxicity study (Vol. 3 B.6.7.1.2 - CA 5.7.1/04) in female rats at the repeated dose of 1499 mg/kg bw/d, the highest dose tested and in male rats at the repeated dose of 1290 mg/kg bw/d), the highest dose applied to male rats.

Additionally, no human-relevant systemic, other than reproductive, adverse effects were observed in a two-generation reproductive toxicity study (Vol. 3 B.6.6.1 - CA 5.6.1/01) in female rats at the repeated dose of 87.2 mg/kg bw/d and in male rats at the repeated dose of 59.5 mg/kg bw/d). The potential adverse effects were found in female rats at the repeated dose of 1741-1817 mg/kg bw/d, and in male rats at dose level of 1176-1324 mg/kg bw/d, well above reference values for category 2 of 7.7 < C ≤ 77 mg/kg bw/day. Overall, RAC concluded that the criteria for classification to STOT RE 2 are not met on rats studies.

Studies in mice

No adverse systemic effect were observed in 78-week carcinogenicity study (Vol. 3 B.6.5.1 - CA 5.5/02) in male mice at dose levels of 37, 373, 3817 mg/kg bw/d and in female mice exposed at dose levels 45, 473, 4807 mg/kg bw/d, thus the criteria for classification to STOT RE 2 are not met.

Studies in dogs

No adverse effect were observed in 13-week repeated dose toxicity study (Vol. 3 B.6.3.2 - CA 5.3.2/02) in male and female Beagle dogs at dose levels of 20, 200 or 2000 mg/kg bw/d, thus the criteria for classification to STOT RE 2 are not met.

No adverse effects were observed in 52-week repeated dose toxicity study (Vol. 3 B.6.3.2 - CA 5.3.2/03) in male and female Beagle dogs at dose levels of 10, 100 or 1000 mg/kg bw/d, thus the criteria for classification to STOT RE 2 are not met.

Studies in rabbits

No adverse effect were observed in 21-day repeated dose dermal toxicity study (Vol. 3 B.6.3.3 - CA 5.3.3/01) in New Zealand White (NZW) rabbits at dose levels of 100, 300, and 1000 mg/kg bw/d (6 hours/day) administered by topical application to the shaved intact dorsal surface (covering approximately 10 % of the body surface area), thus the criteria for classification to STOT RE 2 are not met.

Summing up, taking into account that in the repeated dose toxicity study in rats, mice, dogs and rabbits, no significant toxic effects of relevance to human health were produced below the guidance values relevant for STOT RE 2, RAC concludes that benzobicyclon **does not warrant classification for specific target organ toxicity-repeated exposure.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Benzobicyclon was evaluated for possible genotoxic effects in *in vitro* test systems using bacterial and mammalian cells and in *in vivo* test systems using somatic cells of mice. The results of the available genotoxicity tests *in vitro* and *in vivo* submitted by the DS are summarised below.

Summary table of genotoxicity/germ cell mutagenicity tests in vitro

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Reverse mutation in bacteria (Ames) OECD 471 Minor guideline deviation: duplicate plates, no historical control data reported GLP Reliable with limitations	Benzobicyclon (Technical) Batch No.: KS-2-146 Purity: 100 % (it seems a considerations of the study author)	*Salmonella typhimurium* TA 100, TA 1535, TA 98, TA 1537 *Escherichia coli* WP2 *uvrA* Concentrations +/- metabolic activation: 0, 156, 313, 625, 1250, 2500, 5000 µg/ plate as determined in a pre-test (no toxicity, precipitation at 1000 µg/plate and above)	Negative +/- metabolic activation	Anonymous (1994a) (Vol. 3 B.6.4.1 - CA 5.4.1/01) Doc. No.: 557-008
Salmonella typhimurium and Escherichia coli reverse mutation assay OECD No. 471 (June 2020) EC No. 440/ 2008 (May 2008) US EPA Guideline OCSPP 870.5100 August 1998 GLP Reliable with limitations	Benzobicyclon (GWN-8001) Batch No.: 1A0709 Purity: 98.0 % (w/w) by HPLC	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 and the *Escherichia coli* strain WP2 *uvrA* Concentrations +/- metabolic activation: Pre-Experiment/ Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate Experiment II: 33; 100; 333; 1000; 2500; and 5000 µg/plate *Reduced background growth of* TA 1537 and TA 100 without S9 mix *at* 1000-5000 µg/plate *Precipitation of the test item in the overlay agar on the incubated agar plates was observed from 1000 to 5000 µg/plate in both experiments.*	Negative +/- metabolic activation	Chang S. (November, 2023) Report: GWN-8001: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay. EFSA-2023-00023648
DNA repair in bacteria (REC) No guideline available GLP Supplementary	Benzobicyclon (Technical) Batch No.: KS-2-146 Purity: 100 %	*Bacillus subtilis* H17, M45 Concentrations +/- metabolic activation: 0, 20, 50, 100, 200, 500, 1000 µg/mL	Negative +/- metabolic activation	Anonymous (1994b) (Vol. 3 B.6.4.1 - CA 5.4.1/02) Doc. No.: 557-007

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Chromosomal aberration assay OECD 473 Minor guideline deviations: lower metaphase number (200 vs 300) evaluated, different method of cytotoxicity determination, lack of cytotoxicity data in the cytogenetic experiments, lack of historical control data GLP Acceptable	Benzobicyclon Batch No.: 941208 Purity: 99 %	Chinese hamster lung fibroblasts Concentrations as determined in a pre-test (toxicity at 50 µg/mL and above) 6 hours treatment: 0, 5, 10, 20 and 40 µg/mL +/- metabolic activation 24 hours treatment: 0, 5, 10, 20 and 40 µg/mL without metabolic activation 48 hours treatment: 0, 2.5, 5, 10, 20 µg/mL without metabolic activation	Positive +/- metabolic activation Dose related increase in numerical and structural chromosomal aberrations. The frequency of aberrant cells was greater in the presence of metabolic activation (3.5-9.5 % -S9; 5-23 % +S9).	Anonymous (Vol. 3 B.6.4.1 - CA 5.4.1/03) Doc. No.: 557-009
Gene mutation assay in mammalian cells (Mouse lymphoma) OECD 490 GLP Acceptable	Benzobicyclon Batch No.: 1L0108 Purity: 99.3 %	L5178Y cells Concentrations as determined in a pre-test (toxicity starting at 15.6 µg/mL) Experiment 1, 4 hours treatment: 0, 1, 2, 4, 8, 12 µg/mL without metabolic activation 0, 2, 4, 8, 12, 16 µg/mL with metabolic activation Experiment 2, 24 hours treatment: 0, 2, 4, 8, 12, 16, 24 µg/mL without metabolic activation Experiment 3, 4 hours treatment: 0, 8, 10, 12, 14 and 16 µg/mL with metabolic activation	Negative +/- metabolic activation	Anonymous (2016) (Vol. 3 B.6.4.1 - CA 5.4.1/04) Doc. No.: 557-011

The bacterial reverse mutation assay (Ames test) in different bacterial strains did not show any mutagenic potential of benzobicyclon up to the highest requested dose in the absence and presence of a mammalian metabolic activation system. This result was supported by a negative outcome of the DNA-repair test using bacterial strains (Rec-assay). Benzobicyclon did not exhibit any mutagenic potential in a gene mutation assay in mammalian cells (mouse lymphoma assay). On the other hand, in the in vitro chromosomal aberration assay (Vol. 3 B.6.4.1 - CA 5.4.1/03), benzobicyclon induced increases in structural and numerical aberration frequencies in mammalian cells. The evaluation of the cytotoxicity was not performed in the main assay; therefore, the DS did not support the Applicant's view who attributes to cytotoxicity the likely cause of the chromosomal aberrations. Thus in vivo test became necessary for assessment of chromosomal aberrations.

Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Micronucleus assay in bone marrow OECD 474 GLP Mouse (NMRI), male, 7/group Acceptable	Benzobicyclon Batch No.: 1L0108 Purity: 99.3 %	Single oral application (gavage) Doses selected based on dose range finding study (maximum tolerable dose 2000 mg/kg bw) 24 hours: 0, 500, 1000, 2000 mg/kg bw oral 48 hours: 2000 mg/kg bw	Negative Exposure of target tissue was demonstrated in the study by Shaw (1999; KCA 5.1.1/01, Doc. No. 512-001)	Anonymous (2016) (Vol. 3 B.6.4.2 - CA 5.4.2/01) Doc. No.: 557-012
Micronucleus assay in bone marrow OECD 474 GLP Mouse (CD-1 (ICR)), male, 6/group Supplementary to Dony, E. (2016)	Benzobicyclon Batch No.: 960108N Purity: 99 %	Single oral application (gavage) 500, 1000, and 2000 mg/kg. The incidence of micronucleated polychromatic erythrocytes relative to total erythrocytes in femoral bone marrow 48 hours after administration was investigated.	Negative The frequency of micronucleated polychromatic erythrocytes (MNPCE) in treatment groups were 0.10 % to 0.15 % at 500, 1000, and 2000 mg/kg, and in the negative control group (0.10%). Historical control data for examination period: Mar/1989 ~ Dec/1997, MNPCE%, Mean ± 3S.D.% - 0.135 % ± 0.257 % Exposure of target tissue was demonstrated by decrease in PCE%	Anonymous (1996b) (Vol. 3 B.6.4.2 - CA 5.4.2/02) Doc. No.: 557-010

At higher tier *in vivo*, no relevant increase in micronucleus frequencies indicative of clastogenic and/or aneugenic potential were observed in the bone marrow of mice in two micronucleus tests *in vivo*. In the first study (B.6.4.2/01 - CA 5.4.2/01) there was no clear evidence of bone marrow exposure, as the mean number of PCE was not substantially decreased after treatment with the test substance as compared to the mean value of PCEs of the vehicle control. However, information in the ADME study conducted in rats (Shaw 1999; CA 5.1.1/01, Doc No 512-001), showed that radioactivity was widely distributed and reached the bone marrow, although the bioavailability of benzobicyclon is low. Although this micronucleus assay was conducted in mice, ADME comparative investigations (B.6.9: McClanahan RH, 2017a, b) on metabolite 1315P-070 (mesotriketone containing the bicycle group obtained after hydrolysis of the phenothio group of benzobicyclon) did not show substantial differences occurring between rat and mouse species. Therefore, the available evidence sustains that a read-across between rats and mice metabolism is possible.

In the second study (B.6.4.2/02- CA 5.4.2/02) the evidence of bone marrow exposure was provided by a significant decrease of PCE% when compared to the control group (for details refer to Vol. 3 B6, Table 6.4.2-5:). Although some limitations were observed (1000 PCE/animal examined and no HC data), none of these were considered severe enough to invalidate the results of this supplementary study.

Overall, based on a weight of evidence approach the two *in vivo* micronucleus assays in mice were considered adequate to conclude on the negative outcome for structural and numerical chromosomal aberrations potential of benzobicyclon.

The Dossier Submitter concluded that considering the weight of evidence from *in vitro* and *in vivo* tests, benzobicyclon is not genotoxic and does not require classification for genotoxicity according to Regulation (EC) No 1272/2008.

Comments received during consultation

One MSCA noted that based on a weight-of-evidence approach, it is concluded, that the positive findings in one *in vitro* chromosomal aberration assay are false positive, as both *in vivo* micronucleus tests were found to be negative. No mutagenicity, neither in mammalian, nor in bacterial cells was reported. No classification required.

Assessment and comparison with the classification criteria

The germ cell mutagenicity potential of benzobicyclon has been assessed in relevant *in vitro* and *in vivo* tests.

Benzobicyclon was negative in all tested assays *in vitro* (see four studies in a table above) and *in vivo* (see two studies in a table above), except in one *in-vitro* chromosomal aberration assay (Anonymous (Vol. 3 B.6.4.1 - CA 5.4.1/03) Doc. No.: 557-009) showing a dose related increase in numerical and structural chromosomal aberrations, however this was not confirmed in two *in vivo* micronucleus assays in mice.

RAC therefore concludes that **no classification for germ cell mutagenicity is warranted for** benzobicyclon (in agreement with the DS proposal).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Dossier Submitter provided results of carcinogenicity studies in rats and mice exposed to benzobicyclon in the diet.

In **the combined chronic toxicity/carcinogenicity study** (Vol. 3 B.6.5.1 - CA 5.5/01) (OECD 453, GLP) male/female Fisher rats (50/sex/dose) were administered benzobicyclon in diet at a concentration of 0, 10, 20, 50, and 100 ppm in males (equivalent to 0, 0.334, 0.667, 1.696 and 3.43 mg/kg bw/d) and 0, 100, 1000, and 10000 ppm in females (equivalent to 0, 4.19, 42.2, 427 mg/kg bw/d) for a period of 24 months (104 weeks).

Statistically significant increase in incidence of fibroadenoma of the mammary gland was found in females of the 1000 ppm (42.2 mg/kg bw/d) group at terminal kill after Week 104 and killed in extremis/found dead during study, but there was no increase in incidence of this neoplasm in females exposed at higher concentration (427 mg/kg bw/d). In females of the same group

(42.2 mg/kg bw/d) found dead or killed in extremis during the treatment there was a decrease in incidence of endometrial stromal polyp. The Dossier Submitter considered these changes to be incidental without toxicological significance because of no dose dependency or decreasing incidences. Moreover, the incidences of neoplastic lesions observed in the present study were considered to be within the historical control limits of the test facility or those of Fischer rats available in literature, including mammary fibroadenoma described above. In addition, no mammary adenocarcinoma and no increase in the incidence of the pre-neoplastic lesions for mammary gland was found. No carcinogenic effects were found in other organs of both male and female rats. As there were neither statistically significant increases in incidence nor in earlier occurrence of neoplastic lesions in the treated groups of both sexes, the Dossier Submitter concluded that no carcinogenic potential of the test substance was shown in this study (carcinogenic NOAEL above 3.43 mg/kg bw/d for males and 427 mg/kg bw/d for females).

In the carcinogenicity study in mice (Vol. 3 B.6.5.1 - CA 5.5/02) (OECD 452, GLP) male /female CrI:CD-l™ (ICR) BR mice (50/sex/dose) were administered benzobicyclon in diet at a concentration of 0, 300, 3000 and 30000 ppm in males (equivalent to 0, 37, 373, 3817 mg/kg bw/d) and 0, 300, 3000 and 30000 ppm in females (equivalent to 0, 45, 473, 4807 mg/kg bw/d) for a period of 78 weeks.

There was no evidence of an increase in incidence of any tumour type in mice receiving benzobicyclon. Full statistical analysis was performed on tumour types where at least two tumours were observed across the treatment groups, and for groups where either all or the vast majority (approximately 95 %) of animals were examined. Although mammary adenocarcinoma were reported in 4 of 50 females receiving 30000 ppm (where they were recorded clinically and macroscopically as masses), compared with 0 of 50 control females, this incidence was not statistically significant in one-tailed pairwise comparison against the control group, and they were considered unlikely to be related to treatment. This incidence (8 %) was only just outside the historical control incidence of 6.7 % in a first set of HCD (11 studies between March 1993 and May 1996) of the laboratory. The current study was reported in 1999. On request of the DS more recent HCD of 39 studies centred around the time of the study (August 1995 and October 2002) were provided by the applicant. The incidence of mammary adenocarcinomas of 8 % was within the range and the 95 % confidence interval of this latter HCD (0-8.9 %). In addition, the DS retrieved HCD from the breeder containing data of 51 studies initiated between January 1987 and December of 1996. It is acknowledged that such HCD also contains information on studies conducted more than 5 years from the current carcinogenicity assay (CA 5.5/02), however, they are considered supportive for the interpretation of the findings on mammary gland. In fact, the HCD of Charles River confirm that mammary gland adenocarcinoma is a tumour occurring with frequencies ranging from 0.73 to 8.33 % in CrI:CD-l™ (ICR) BR mice, in line with the observations of the current study (CA 5.5/02). No increase of preneoplastic lesions in the mammary gland was reported for any of the treatment groups. In addition, the intergroup comparison of tumour incidences showed that there was no increase in the general pattern of tumours' occurrence between control and treatment groups. Overall, it is concluded that there was no evidence of an increased incidence of any tumour type. In the opinion of the DS benzobicyclon did not show any tumorigenic potential in mice when administered at concentrations up to 30000 ppm over 78 weeks. The NOAEL for carcinogenicity is set at 3817 mg/kg bw/d for males, 4807 mg/kg bw/d for females.

Based on results of these two studies the Dossier Submitter concluded that benzobicyclon does not possess a carcinogenic potential in rats and mice after life-time treatment and is not triggering classification for repeated dose effects or carcinogenicity according to Regulation (EC) No 1272/2008.

Comments received during consultation

No comments were provided regarding of carcinogenicity, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

The DS presented the results of two reliable chronic toxicity/carcinogenicity studies via the oral route of exposure in rats (Vol. 3 B.6.5.1 - CA 5.5/01) and mice (Vol. 3 B.6.5.1 - CA 5.5/02). There was no statistically significant increase in tumour incidences in the rat and mice study (using the concurrent controls) in spite of very high dose levels used in mice: 3817 mg/kg bw/d for males, 4807 mg/kg bw/d for females). Based on two negative and valid carcinogenicity studies, supported by a lack of genotoxicity, RAC concludes that **no classification of benzobicyclon for carcinogenicity is warranted based on conclusive data** (in agreement with the DS).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

The Dossier Submitter evaluated effect of benzobicyclon on sexual function and fertility based on a **two-generation reproductive toxicity study** (OECD 416, GLP) **in rats** (Vol. 3 B.6.6.1- CA 5.6.1/01) with minor deviations from the guideline: the ratio of dose levels was 10 instead of the 3-fold recommended for dietary studies. Groups of 24 males and 24 females rats (Crj:CD (SD) were given for two successive generations diets containing benzobicyclon at concentrations of 0, 100, 1000, or 20000 ppm (equivalent to F0 males/females 0; 5.65/8.44; 56.1/85.4 and 1176/1741 mg/kg bw/d; and to F1 males/females 0, 6.46/8.76; 62.8/89.0 and 1324/1817 mg/kg bw/d).

No treatment related adverse effects on sexual function and fertility were noted in any parameters tested, i.e. sexual development (F1 parental animals only), the incidence of normal oestrous cycle, mating index, fertility index, gestation index, duration of gestation, the mean number of implantation sites, and the number, motility, and morphology of epididymal sperm in any of the treated groups. It was noted that the absolute ovary weight for F1 parental females in the 20000 ppm group was statistically significantly increased by 15 % when compared to controls. This change was considered of doubtful relation to treatment because the relative ovary weight was comparable to that in the control group and a statistically significant increased body weight was observed in these animals. Additionally, it is noted that individual absolute ovary weights of these animals (mean: 60.8 mg; range: 42.6-74.4 mg) fell within the concurrent control individual range (mean: 52.9 mg; range: 40.4-77.0 mg). Therefore, considering that no treatment related effects were observed on reproductive function, oestrus cyclicity or histopathology findings in the ovary the increased absolute ovary weight was considered of no toxicological relevance.

Based on these data the Dossier Submitter concluded that benzobicyclon do not warrant classification for reproductive toxicity/adverse effects on sexual function and fertility according to Regulation (EC) No 1272/2008 since neither effect on sexual function nor on fertility were observed in a two-generation reproductive toxicity study.

Adverse effects on development

One **developmental toxicity study in rabbits (Vol. 3 B.6.6.2. - CA 5.6.2/02)** was considered by the DS as unsuitable for establishing maternal and developmental effects because of the maternal mortality in the study was greater than 10 % at all doses including controls. The high mortality from unknown causes questions the health conditions of the rabbits. In addition, the resulting reduced number of litters available for the evaluation and comparison of effects makes the study unsuitable for establishing maternal and developmental effect levels.

Thus, the DS evaluated the effect of benzobicyclon on development based on a prenatal developmental toxicity study in rats (Vol. 3 B.6.6.2. – CA 5.6.2/01) and in rabbits (Vol. 3 B.6.6.2. – CA 5.6.2/04) and a two-generation reproductive toxicity study in rats (Vol. 3 B.6.6.1 - CA 5.6.1/01).

In the **two-generation reproductive toxicity study (**OECD 416, GLP) **in rats** (Vol. 3 B.6.6.1 - CA 5.6.1/01) no effect of benzobicyclon on the development of F1 and F2 pups was observed in any of the treated groups for any parameters tested: the results of the clinical findings, number of pups delivered, sex ratio, viability indices, body weights, gross pathological findings, and organ weights in the treated groups were comparable to those in the control group.

In **the prenatal developmental toxicity study** (Vol. 3 B.6.6.2. - CA 5.6.2/01) (OECD 414 (1981), GLP) female Sprague Dawley rats received benzobicyclon by oral gavage at dose levels of 0, 40, 200 and 1000 mg/kg bw/d for 10 consecutive days from gestational day (GD) 6 to 15, inclusive. One group of 25 sexually mature and inseminated rats of the same strain that received the vehicle (water + Tween 80, 1 %) over the same period served as the control group. The animals were sacrificed on day GD 20 and the foetuses removed and examined. There were no deaths and no treatment-related clinical signs observed in any of the dose groups. Food consumption and body weight development was similar in all groups and not affected by treatment. Necropsy did not reveal any treatment-related findings. The implantation data did not show any significant differences between the study groups. The pre-implantation loss was comparable in all groups. Post-implantation loss was not affected by treatment. The mean number of foetuses, foetal sex distribution and mean foetal weight were similar in all groups. Foetal examination did not reveal any treatment-related malformations or variations.

In **the prenatal dose range finding study** (Vol. 3 B.6.6.2. - CA 5.6.2/03) four groups of 7-time mated female New Zealand White rabbits received benzobicyclon daily by oral gavage at dose levels of 0, 111, 333 and 1000 mg/kg bw/d from day gestational day (GD) 6 to 27, inclusive, at a dose volume of 5 mL/kg bw. The study included a bioanalysis phase to measure benzobicyclon and the major metabolite 1315P-070 (blood sampling on GD 6 and GD 27). There were no treatment related effects in mortalities, clinical signs, body weight gain or food intake. Circulating blood levels of benzobicyclon were below limit of quantification, however there was measurable exposure to the major metabolite, 1315P-070, on Days 6 and 27 of gestation. All females were pregnant at necropsy and no macroscopic abnormalities were detected. No effects were observed for the pregnancy, uterine data, number of litters, foetal or placenta weight. No major skeletal (visceral compartment not examined) abnormalities were observed (only 1000 mg/kg bw/d examined). According to the study protocol the highest dose tested of 1000 mg/kg bw/d was well tolerated and is considered suitable for the main prenatal developmental toxicity study.

In **the prenatal developmental toxicity study** carried out in 2022 (Vol. 3 B.6.6.2. - CA 5.6.2/04) (OECD 414, GLP) 4 groups of 22 sexually mature, timed mated female New Zealand White rabbits were dosed once daily by gavage, with 0 (0.5 % aqueous methylcellulose as vehicle control) 111, 333 or 1000 mg/kg bw/d benzobicyclon from GD 6 to 27, inclusive. There were no maternal treatment related effects, nor treatment related effects at caesarean section examination and foetal toxicity examination.

Diaphragmatic hernia of liver lobe(s) was reported in one foetus at 333 mg/kg bw/day and in one foetus at 1000 mg/kg bw/day. The litter incidence of diaphragmatic hernia fell within an adequate set of the laboratory historical control data (data generated between 2016 and 2022 from 24 studies on 2162 foetuses and on 507 litters) according to which this finding is occasionally observed [Range N (%): 0-1 (0.0 %-4.8 %)].

Severely reduced lung lobe(s) was reported in one foetus at 111 mg/kg bw/day and in one foetus at 1000 mg/kg bw/day. The occurrence of this finding was not considered to be treatment related as it was not observed at the mid dose of 333 mg/kg bw/day and because the litter incidences fell with the laboratory historical control range [Range N (%): 0-1 (0.0 %-5.0 %)].

The incidence and intergroup distribution of the other major foetal abnormalities did not indicate an adverse effect attributable to the treatment with benzobicyclon.

There were no treatment related changes in the incidence of minor or variant foetal abnormalities, with the numbers of foetuses and litters affected.

An apparent dose related increased number of litters with foetuses with the minor abnormality (variation) astragalus, uni-or bilateral not ossified was observed. The incidences of this variation were not statistically significant when compared to control by pairwise comparison. The litter incidence of this finding at 1000 mg/kg bw/day was above the incidence seen in the historical control data range. Evaluation of the individual litter data highlighted that this minor abnormality was often associated with foetuses of low weight; the weights of the foetuses with this abnormality in the high dose ranged from 9.5 g to 27.5 g (for reference, the mean foetal weight for this strain is 33.09 g to 35.97 g). The absence of ossification in the astragalus for these foetuses was considered likely to indicate a delay in ossification due to low foetal weight, rather than direct disruption/structural change in bone development.

The NOAEL for benzobicyclon for maternal toxicity was 1000 mg/kg bw/day, the highest dose tested. The NOAEL for embryo-foetal toxicity was 1000 mg/kg bw/d, the highest dose tested.

Adverse effects on or via lactation

In a two-generation reproductive toxicity study no relevant effects on growth/ development of offspring were observed. Therefore, the study results do not indicate any direct adverse effect on the offspring due to the transfer of the substance on or via lactation. Based on these data the DS concluded that there were no effects to warrant classification of benzobicyclon for effects on or via lactation according to Regulation (EC) No 1272/2008

Summing up, the DS is of the opinion that benzobicyclon does not warrant classification for reproductive toxicity according to criteria given in Regulation (EC) No 1272/2008.

Comments received during consultation

No comments were provided regarding reproductive toxicity, but one MSCA noted that classification for human health hazards is not required.

Assessment and comparison with the classification criteria

The DS presented the results of one two-generation reproductive toxicity study in rats (Vol. 3 B.6.6.1 - CA 5.6.1/01), two developmental toxicity studies in rabbits (Vol. 3 B.6.6.2. - CA 5.6.2/04 and Vol. 3 B.6.6.2. - CA 5.6.2/02), one in rats (Vol. 3 B.6.6.2. - CA 5.6.2/01) and one prenatal range finding study in rabbits (Vol. 3 B.6.6.2. - CA 5.6.2/03). Since none of the studies reveal data indicating that benzobicyclon is causing reproductive toxicity, RAC concludes that **no**

classification of benzobicyclon for reproductive toxicity is warranted based on conclusive data (in agreement with the DS).

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

In the opinion of the DS, classification of benzobicyclon for aspiration toxicity is not applicable, because benzobicyclon is a solid.

Comments received during consultation

One MSCA considered that since benzobicyclon is a solid, this field is not applicable.

Assessment and comparison with the classification criteria

According to Regulation (EC) No 1272/2008 point 3.10.1.2 'Aspiration' means the entry of a liquid or solid substance or mixture directly through the oral or nasal cavity, or indirectly from vomiting, into the trachea and lower respiratory system. Substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard.

In line with Regulation (EC) No 1272/2008: A substance is classified in Category 1:

(a) based on reliable and good quality human evidence or

(b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40 ^oC.

Since there are no human data on aspiration toxicity of benzobicyclon and it is not possible to determine dynamic viscosity by the measurement of the fluid's internal resistance to flow, because the substance is a solid in temperatures up to melting point of 187 °C , RAC agrees with the conclusion of the DS that that a **classification for aspiration toxicity is not applicable for benzobicyclon.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as:

- Aquatic Acute 1, H400, M=100 based on 7-d E_rC_{50} value of 0.00619 mg/L for *Lemna gibba* and
- Aquatic Chronic 1, H410, M=100 based on 7-d EC_{10} value of 0.000447 mg/L for *Lemna gibba* and a lack of rapid degradation.

Degradation

One study carried out according to OCSPP 835.2120 and OECD TG 111 was provided for the hydrolysis of benzobicyclon. Benzobicyclon ([bicyclooctane ring-2,4-^{14}C]-benzobicyclon-bic label

and [chlorophenyl-^{14}C(U)]-benzobicyclon - ben label) was assessed at 10, 25 and 50 °C, and at a pH 4, 7 and 9. In the study, rapid degradation of the benzobicyclon was observed. The half-life calculated by the Arrhenius equation at 25 °C and pH 4, 7, and 9 was determined to be 0.6, 0.7, and 0.5 days, respectively. The sole hydrolysis product, 1315P-070, was hydrolytically stable.

A ready biodegradation study for benzobicyclon following OECD TG 301B (CO_2 Evolution (Modified Sturm Test)) over 36 days was available. Benzobicyclon did not show any evidence of significant biodegradation (0.3 % on Day 35) and therefore cannot be considered readily biodegradable.

In an aerobic mineralisation in surface water study carried out in line with OECD TG 309 and OPPTS 835.3190, the degradation of [^{14}C]benzobicyclon bicyclooctane (bic label) and [^{14}C]benzobicyclon chlorophenyl (ben label) was determined after 58 days in two suspended sediment systems at a low and a high concentrations. Benzobicyclon degraded very fast in the water phase and had completely disappeared in all test systems after 7 days of incubation, forming various degradation products. CO_2 accounted for up to 1.4 % AR after 58 days of incubation. Due to the very fast dissipation of benzobicyclon, no kinetics could be calculated. Likewise, no kinetics could be derived for the metabolites 1315P-070 and 1315P-570 due to their stability after formation.

Two water/sediment simulation studies carried out according to OECD TG 308 and OCSPP 835.4300 were run for 104 days (Class and Heinz, 2015) and 100 days (Schick, 2012) using four water/sediment systems, two sampled in Europe and two sampled in the US. Benzobicyclon degraded rapidly especially in the water phase. A mineralisation of up to 6.9 % was observed after 100 days of incubation. The following half-lives (DT_{50}) were determined for benzobicyclon: 0.76-1.03 days (whole system; indicating a potential rapid degradability), 0.5-0.86 days (water) and 4.77-16.10 days (sediment). Two main degradation products were determined, namely 1315P-070 and 1315P-570. For the first degradation product (1315P-070), the half-lives (DT_{50}) were 167.5-324 days (whole system), 140.5-214.5 days (water). No decline was observed in sediment, while for the second degradation product (1315P-570) the half-lives (DT_{50}) were 40.5-88.5 days (water) and no decline (whole system and sediment).

The available aquatic toxicity data clearly indicate that the metabolite 1315P-070 fulfils the criteria for classification as hazardous to the aquatic environment (7-d E_rC_{50} = 0.0135 mg/L and 7-d NOE_rC = 0.00075 mg/L on *Lemna gibba*).

The DS concluded that benzobicyclon should be considered as not rapidly degradable for the purpose of classification according to CLP Regulation.

Bioaccumulation

An experimental aquatic BCF study in fathead minnow (*Pimephales promelas*) following OECD TG 305 showed a lipid normalised whole fish BCF_{ssl} value of 126 L/kg and BCF_{kl} value of 161 L/kg.

Benzobicyclon has a measured octanol-water partition coefficient (log K_{OW}) of 3.1 at 20 °C. As benzobicyclon is not expected to dissociate the influence of the pH was not determined.

The log K_{OW} values for all metabolites are below 3.0.

The DS concluded that benzobicyclon does not have a potential to bioaccumulate in aquatic organisms.

Aquatic toxicity

Reliable aquatic toxicity data are available for benzobicyclon, its metabolites and the formulation GWN-10235. A summary of the relevant information on aquatic toxicity for benzobicyclon are provided in the following Table (the key endpoints used in hazard classification are highlighted in bold). Data provided in the CLH report indicate that toxicity studies with different metabolites

and formulation GWN-10235 derive effect values higher than for benzobicyclon and are, therefore, not further considered for classification purposes. Reliable acute and chronic aquatic toxicity data on benzobicyclon are available for fish, invertebrates, algae, and aquatic plants.

Table: *Summary of relevant information on aquatic toxicity of benzobicyclon*

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
Short term toxicity				
OECD TG 203 OPPTS 850.1075	*Oncorhynchus mykiss*	96-h LC_{50}	> 0.489 (mm)	Anonymous (2014a) CA 8.2.1/01
OECD TG 203 OPPTS 850.1075	*Pimephales promelas*	96-h LC_{50}	> 0.496 (mm)	Anonymous (2014b) CA 8.2.1/02
OECD TG 203 OPPTS 850.1075	*Cyprinodon variegatus*	96-h LC_{50}	> 0.506 (mm)	Anonymous (2014c), CA 8.2.1/03
OECD TG 202 OPPTS 850.1010	*Daphnia magna*	48-h EC_{50}	> 0.368 (mm)	Anonymous (2014a) CA 8.2.4.1/01
OECD TG 201	*Raphidocelis subcapitata*	72-h E_rC_{50}	> 0.45 (mm)	Anonymous (2019a) CA 8.2.6.1/01
OPPTS 850.4550	*Anabaena flos-aquae*	72-h E_rC_{50}	> 0.184 (mm)	Anonymous (2012) CA 8.2.6.2/01; Anonymous (2019c) CA 8.2.6.2/02 and Anonymous (2022) CA 8.2.6.2/04
OECD TG 221	*Lemna gibba*	7-d E_rC_{50}	**0.00619 (twa)**	Anonymous (2020), CA 8.2.7/04
Long term toxicity				
OECD TG 210, OPPTS 850.1400	*Pimephales promelas*	28-d NOEC 28-d EC_{10}	0.100 (mm) 0.207 (mm)	Anonymous (2012) CA 8.2.2.1/01; Anonymous (2019b), CA 8.2.2.1/02 and Anonymous (2022) CA 8.2.2.1/05
OECD TG 210, OPPTS 850.1400	*Cyprinodon variegatus*	28-d NOEC 28-d EC_{10}	0.323 (mm) > 0.323 (mm)	Anonymous (2014d), CA 8.2.2.1/03; Anonymous (2019c), CA 8.2.2.1/04 and Anonymous (2022) CA 8.2.2.1/06
OPPTS 850.1500	*Pimephales promelas*	146-d NOEC 146-d EC_{10}	0.0403 (mm) 0.018 (mm)	Anonymous (2014e), CA 8.2.2.2/01 and Anonymous (2019a), CA 8.2.2.2/02 and Anonymous (2022) CA 8.2.2.2/03
OECD TG 211, OPPTS 850.1300	*Daphnia magna*	21-d NOEC 21-d EC_{10}	0.279 (mm) 0.272 (mm)	Anonymous (2014b), CA 8.2.5.1/01 and Anonymous (2022) CA 8.2.5.1/03

Method	Species	Endpoint	Toxicity value (mg/L)	Reference
OECD TG 201	*Raphidocelis subcapitata*	72-h NOE_rC 72-h E_rC_{10}	0.45 (mm) > 0.45 (mm)	Anonymous (2019a), CA 8.2.6.1/01
OPPTS 850.4550	*Anabaena flos-aquae*	72-h NOE_rC 72-h E_rC_{10}	0.0774 (mm) 0.150 (mm)	Anonymous (2012), CA 8.2.6.2/01; Anonymous (2019c), CA 8.2.6.2/02 and Anonymous (2022) CA 8.2.6.2/04
OECD TG 221	*Lemna gibba*	7-d NOE_rC **7-d E_rC_{10}**	0.000167 (twa) **0.000447 (twa)**	Anonymous (2020), CA 8.2.7/04

mm - mean measured; nom - nominal concentration; twa - time-weighted average;

Acute Aquatic toxicity

Three acute toxicity studies with three different fish species (*Oncorhynchus mykiss, Pimephales promelas and Cyprinodon variegatus)* were available. All the studies followed OECD TG 203 and OPPTS 850.1075. No mortality was observed up to 0.506 mg/L.

There was one study available for *Daphnia magna*, conducted according to OECD TG 202 and OPPTS 850.1010, with a mean measured 48h EC_{50} value of > 0.368 mg/L.

Two acute toxicity studies with two different algae (*Raphidocelis subcapitata* and *Anabaena flos-aquae*) were available. The lowest acute endpoint for algae is mean measured 72-h E_rC_{50} of > 0.184 mg/L for *A. flos-aquae* from the study conducted according to OPPTS 850.4550.

There was only one study following OECD TG 221 available for aquatic plants, with a 7-d E_rC_{50} value of 0.00619 mg/L (frond number), based on time-weighted average (TWA) for *Lemna gibba*.

Based on the available information for aquatic acute toxicity, the DS concluded that aquatic plants are the most acutely sensitive taxonomic group. The lowest acute toxicity value is the 7-d E_rC_{50} value of 0.00619 mg/L for *Lemna gibba.* The value is below the classification threshold value of 1 mg/L, therefore the classification of benzobicyclon as Aquatic Acute 1 (H400) with an M-factor of 100 ($0.001 < L(E)C_{50} \leq 0.01$ mg/L) is warranted.

Chronic Aquatic toxicity

Two chronic toxicity studies with fathead minnow (*Pimephales promelas*) and one study with sheepshead minnow (*Cyprinodon variegatus*) were available. The studies were conducted according to OECD TG 210 and OPPTS 850.1400 or OPPTS 850.1500. The lowest chronic endpoint for fish is a mean measured 146d EC_{10} value of 0.018 mg/L for *Pimephales promelas*.

There was one study available for *Daphnia magna*, conducted according to OECD TG 211 and OPPTS 850.1300, with a mean measured 21d EC_{10} value of 0.272 mg/L.

Two toxicity studies with two different algae (*Raphidocelis subcapitata* and *Anabaena flos-aquae*) were available, conducted according to OECD TG 201 or OPPTS 850.4550. The lowest chronic endpoint for algae is a mean measured 72-h E_rC_{10} value of 0.150 mg/L for *A. flos-aquae*.

There was one study available for aquatic macrophytes (*Lemna gibba*), conducted according to OECD TG 211, with a 7-d E_rC_{10} value of 0.000447 mg/L (frond number) based on time-weighted averages (TWA).

Based on the available information for aquatic chronic toxicity data, the DS concluded that aquatic plants are the most chronically sensitive taxonomic group. The lowest chronic toxicity value is 7-d E_rC_{10} value of 0.000447 mg/L for *Lemna gibba*. The 7-d E_rC_{10} value of 0.000447 mg/L is

below the classification threshold value of ≤ 0.1 mg/L for a not rapidly degradable substance, therefore classification of benzobicyclon as Aquatic Chronic 1 (H410) with an M-factor of 100 (0.0001 < NOEC ≤ 0.001 mg/L) is warranted.

Comments received during public consultation

Comments were received from three Member States (MS). Two MSs agreed with the DS's proposal for the aquatic acute and chronic classification of the substance, while one MS provisionally agreed, pending studies on aquatic macrophytes.

One MS provided some suggestions for improvement of the degradation and ecotoxicity part of the CLH report.

The same MS pointed out that further studies in addition to the current study with *Lemna gibba*, e.g., with *Myriophyllum spicatum*, are required to finalise the assessment of the substance since aquatic macrophytes are the most sensitive taxon. Therefore, the current classification proposal should be considered provisional. The DS agreed.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider benzobicyclon as not rapidly degradable based on:

- The substance is not hydrolytically stable at environmentally relevant pHs (pH 5-9) and undergoes hydrolysis. The hydrolysis DT_{50} values at 25 °C are 0.6 (pH 4), 0.7 (pH 7) and 0.5 (pH 9) days. The sole hydrolysis product (1315P-070) was hydrolytically stable.
- No significant biodegradation is observed in a ready biodegradability study (0.3 % after 35 days). Accordingly, benzobicyclon is considered not readily biodegradable.
- In the aerobic mineralisation study, benzobicyclon degrades very fast in the water phase and has completely disappeared in all test systems after 7 days, forming stable degradation products (1315P-070 and 1315P-570). Low mineralisation is observed (1.4 % after 58 days).
- The results of water/sediment simulation studies show that benzobicyclon degrades rapidly, especially in the water phase. The whole system half-lives (DT_{50}) for benzobicyclon were 0.76-1.03 days, indicating a potential for rapid degradability. Mineralisation is low (6.9 % after 100 days). Two main degradation products are determined, namely 1315P-070 and 1315P-570. Whole system DT_{50} for 1315P-070 are 167.5-324 days, while no decline of the 1315P-570 is observed in the whole system.

Bioaccumulation

RAC agrees with the DS that benzobicyclon has a low potential for bioaccumulation in the aquatic environment. The basis for this is that the measured BCF value of 161 L/kg is below the CLP criterion of 500. This is supported by a log K_{ow} value of 3.1, which is below the CLP criterion of 4.

Aquatic toxicity

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

For both acute and chronic toxicity, aquatic plants are the most sensitive group, with the most sensitive species being *Lemna gibba*.

RAC notes that based on the ecotoxicity data for all trophic levels presented in the CLH report, the metabolite 1315P-070 would be classified as hazardous to the aquatic environment based on data for *Lemna gibba* (Aquatic Acute 1 based on 7-d E_rC_{50} = 0.0135 mg/L and Aquatic Chronic 1 based on 7-d E_rC_{50} = 0.0024 mg/L, log K_{OW} < 3.0 and independent on the conclusion on degradation, although for both acute and chronic classification the outcomes are less stringent than for the parent substance). For metabolite 1315P-570, only toxicity data for *Lemna gibba* (7-d E_rC_{50} > 1 mg/L and 7-d E_rC_{10} > 1 mg/L) and bioaccumulation (log K_{OW} < 3.0) are available, thus no classification is derived from the limited dataset.

Based on a 7-d E_rC_{50} value of 0.00619 mg/L (*Lemna gibba*), which is below the E_rC_{50} ≤ 1 mg/L criterion, RAC supports the classification of benzobicyclon as Aquatic Acute 1 (H400). RAC also supports an M-factor of 100 for aquatic acute toxicity since the E_rC_{50} value falls within the range of 0.001 < $L(E)C_{50}$ ≤ 0.01 mg/L.

RAC is of the opinion that EC_{10} should take precedence over NOEC as this is in line with the current CLP Guidance (Version 6.0, Jan 2024). As discussed previously, benzobicyclon is not rapidly degradable and based on the chronic endpoint 7-d E_rC_{10} value of 0.000447 mg/L (*Lemna gibba*), which is below the NOEC or EC_x of ≤ 0.1 mg/L criterion, RAC concludes on classification of benzobicyclon as Aquatic Chronic 1, H410. RAC also concludes on an M-factor of 100 for aquatic chronic toxicity since the relevant EC_{10} value falls within the range 0.0001 < NOEC ≤ 0.001 mg/L.

In summary, RAC concludes that **benzobicyclon warrants classification as Aquatic Acute 1 (H400), M factor of 100** and **Aquatic Chronic 1 (H410), M-factor of 100,** as proposed by the DS.

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The vapour pressure of benzobicyclon is 2.9 × 10^{-5} Pa at 20 °C. Benzobicyclon is estimated to degrade quickly in the atmosphere with a DT_{50} of 2.2 hours. Therefore, hazards to the ozone layer are not expected.

Benzobicyclon is not listed in Appendix I of Regulation No 1005/2009 of the European Parliament and of the council (16 September 2009).

Considering the vapour pressure of benzobicyclon is below the trigger for volatilisation and the quick degradation in the atmosphere (DT_{50} 2.2 hours), no hazard to the ozone layer is expected.

Comments received during consultation in the ECHA website

No comments were received for this hazard class.

Assessment and comparison with the classification criteria

Benzobicyclon is not expected to remain stable in the air based on half-life of 2.2 hours (estimated by the software AOPWIN). Due to its low half-life in the atmosphere combined with a low vapour pressure (less than 5 × 10^{-9} Pa at 20 °C and less than 1 × 10^{-8} Pa at 25 °C) indicating non-volatility and resulting in a low value for the Henry's Law constant (4.31 × 10^{-5} Pa m^3/mol at 20 °C), benzobicyclon is considered not to be subject to transport via air or cause hazard to ozone layer. In addition, benzobicyclon is not listed in Appendix I of Regulation No 1005/2009 of the European Parliament and of the council (16 September 2009).

Therefore, RAC concludes that benzobicyclon **does not warrant classification as hazardous to the ozone layer**, as proposed by the DS.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. It is the combined Draft Assessment Report prepared according to Regulation (EC) N° 1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008. BENZOBICYCLON. Volume 1 and Volume 3 – B.6 (AS). February 2023

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