European Commission

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

February 2023

Rapporteur Member State: Malta
Malta Competition and Consumer Affairs Authority (MCCAA)
Technical Regulations Division
Regulatory Affairs Directorate

Co-Rapporteur Member State: Greece

Version History

When	What
March-June 2022	Initial DAR to Co-RMS Greece
October 2022	Final DAR
February 2023	Revised Vol 1 after ECHA accordance Check.

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Level 1

BENZOBICYCLON

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

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

This draft assessment report has been prepared to evaluate the dossier for the new active substance Benzobicyclon (ISO) and its formulated product GWN-10235. The dossier was submitted for the first active approval under Regulation (EC) No 1107/2009 with Malta carrying out the assessment as the Rapporteur Member State.

So far, neither EU nor CODEX MRLs exist for Benzobicyclon at this point in time. However, alongside this application Gowan Crop Protection Limited has submitted an application to set specific maximum residue levels (MRLs) as the new active substance does not satisfy the requirements of Annex II/III/IV of Regulation (EC) No 396/2005.

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

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

1.1.3 EU Regulatory history for use in Plant Protection Products

Benzobicyclon is a New Active Substance and products containing have not been previously authorised at EU level before.

1.1.4 Evaluations carried out under other regulatory contexts

Benzobicyclon is the common name of the active substance 3-(2-chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[3.2.1]oct-2-en-4-one, that was discovered and first developed by SDS Biotech K.K. in 2001 in Japan, where certain herbicide activity had been demonstrated under specific conditions, it was authorized to market. It is currently marketed in various Plant Protection Products that are commonly used to control sedges and grasses in paddy rice crop.
Plant protection products containing Benzobicyclon are currently marketed in various other regions of the world where rice is intensively grown, mainly Eastern Asia, but also in the US, in Southern America and in non-EU Mediterranean countries.

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant for approval of the active substance

Name	Gowan Crop Protection Limited
Address	Highlands House Basingstoke Road Spencers Wood Reading Berkshire England, RG7 1NT UK
Contact person	[redacted]
Telephone	
Mobile	
e-Mail	

1.2.2 Producer or producers of the active substance

CONFIDENTIAL information - data provided separately (Volume 4)

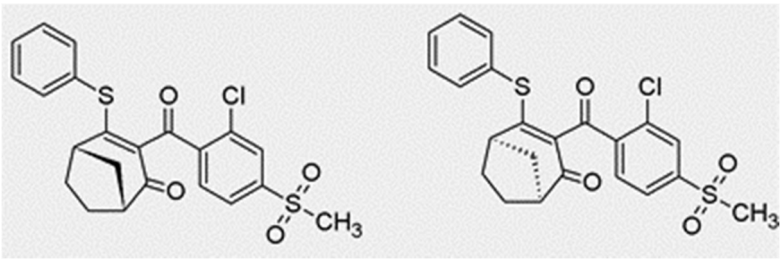
1.2.3 Information relating to the collective provision of dossiers

Gowan Crop Protection Limited is the sole notifier for the active substance Benzobicyclon which will be assessed within the framework of Commission Regulation (EC) No 1107/2009 and for which an application of the first approval for a new active substance in accordance with Article 7 (1) of said Commission Regulation has been assessed and accepted.

Therefore, it was not necessary to undertake steps to present the dossier collectively.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Benzobicyclon
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	(1*RS*,5*RS*)-3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one
CA	Bicyclo[3.2.1]oct-3-en-2-one, 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)-

1.3.3 Producer's development code number	SB-500 and SAN 1315 H
1.3.4 CAS, EEC and CIPAC numbers	
CAS	156963-66-5
EEC	-
CIPAC	None allocated
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{22}H_{19}ClO_4S_2$
Structural formula	
Molecular mass	447.0 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.7 Specification of purity of the active substance in g/kg	980 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.8.2 Significant impurities	CONFIDENTIAL information - data provided separately (Volume 4)
1.3.8.3 Relevant impurities	None. Please refer to Vol. 4.
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately (Volume 4)

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant

Name	Gowan Crop Protection Limited
Address	Highlands House Basingstoke Road Spencers Wood Reading Berkshire England, RG7 1NT UK
Contact person	[redacted]
Telephone	
Mobile	
e-Mail	

1.4.2 Producer of the plant protection product

CONFIDENTIAL information - data provided separately (Volume 4)

1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product

Code number: GWN-10235

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product

1.4.4.1 Composition of the plant protection product

CONFIDENTIAL information - data provided separately (Volume 4)

Pure active substance

content	**400 g/L**	**34.7 (% w / w)****
limits *:	380 – 420	33.0 – 36.4

Technical active substance

content	**408.2 g/L**	**35.4 % w/w****
limits *:	387.79 – 428.61	33.63 – 37.17

* according to Manual on development and use of FAO and WHO specifications for pesticides, prepared by the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS), Rome, 2010.
** based on a density of 1.1544 g/cm^3

1.4.4.2 Information on the active substances

Type	**Name/Code Number**
ISO common name	Benzobicyclon
CAS No	156963-66-5
EC No	-
CIPAC No	Not allocated
Salt, ester anion or cation present	Not relevant

1.4.4.3 Information on safeners, synergists and co-formulants	CONFIDENTIAL information - data provided separately (Volume 4)
1.4.5 Type and code of the plant protection product	Suspension concentrate [Code: SC]
1.4.6 Function	Herbicide
1.4.7 Field of use envisaged	A single yearly application for control of monocotyledonous (grass and non-grass) weeds in paddy rice is envisaged to be made at a maximum application rate of 0.3 kg a.s./ha. A Benzobicyclon containing product should be applied as broadcast application. The product should be applied on flooded paddy rice fields with weeds being in stage BBCH 00-12. Additionally, the application timing is envisaged during BBCH stage 00-21 of the rice crop.
1.4.8 Effects on harmful organisms	Benzobicyclon acts as a highly selective herbicide envisaged to be used to control monocotyledonous (grass and non-grass) weed species. It appears to be absorbed mainly by the rooting system of the plants, and then transported to cells at the level of chloroplasts, where the synthesis of lipidic pigments occurs. Action on these pigments through inhibition of their synthetic process determines the bleaching symptoms in the target weeds.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

The Benzobicyclon containing product GWN-10235 is a suspension concentrate (SC) formulation containing 400 g/L Benzobicyclon and is envisaged to be used to control monocotyledonous (grass and non-grass) weed species in rice. It is a systemic herbicide, absorbed principally by the roots.
GWN-10235 is to be applied as a single yearly application at a maximum application rate of 0.3 kg a.s./ha (0.75 L product/ha). It is applied as broadcast application in flooded rice fields via ground directed tractor mounted sprayer.
Applications must be done on flooded paddy in stable water from pre-emergence to early post-emergence of weeds, until 1 leaf stage (BBCH 00 to 12), with a crop stage of BBCH 00-21.
Applications at early crop stages (around sowing) have shown better efficacy than later applications.
Water has to be maintained five days minimum after application.

1.5.1 Details of representative uses

Crop and/or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Rice (*Oryza sativa L. ssp. indica, ssp. japonica* and related hybrids, 0500060, GC 0649)	SEU	GWN-10235 (Avanza)	F	*Bolboschoenus maritimus* (SCPMA) *(seeds only)* *Cyperus difformis* (CYPDI) *Echinochloa spp.* (ECHSS) *Heteranthera reniformis* (HETRE) *Leptochloa spp.* (DPCFU) *Alisma plantago-aquatica* (ALSPA)	SC	400 g/L	Broadcast in flooded paddy	BBCH 00-21 (rice) BBCH 00-12 (target)	1	-	0.10 - 0.15	200 – 300	0.3	PHI is covered by the vegetation period between application and harvest	hold water 5 days minimum after application

(a) For crops, the EU and Codex classification (both) should be taken into account ; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes – GIFAP Technical Monograph N° 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant – type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

Not needed.

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

Not applicable.

1.5.4 Overview on authorisations in EU Member States

Not applicable as compound is a New Active Substance in EU.

Level 2

BENZOBICYCLON

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

2.1 IDENTITY

2.1.1 Summary or identity

All points of the data requirements regarding Section 1 have been addressed and the information supplied is acceptable. The technical specification is provided in the confidential part (Volume 4).
The active substance Benzobicyclon has a minimum purity of 980 g/kg and is a racemate (1:1 mixture of two enantiomers, confirmed by analytical results). There are no relevant impurities.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [*EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE*]

2.2.1 Summary of physical and chemical properties of the active substance

Benzobicyclon technical is a slightly yellow solid. It has a melting point of 187°C and decomposes at 281°C before reaching a boiling point. Its vapour pressure is lower than 5×10^{-9} Pa at 20°C. The UV-VIS, NMR-, IR- and mass spectra are in agreement with the molecular structure of Benzobicyclon. The water solubility of Benzobicylon at 20°C is 51.8 µg/L and it hydrolyses quickly in aqueous solutions. The solubility in organic solvents was generally low in the tested solvents, the highest solubility was found in dichloroethane (144 g/L). The surface tension of a 90% solution is 70.6 mN/m. Benzobicyclon technical does not self-ignite and is not flammable. It has no explosive or oxidizing properties.

Table 1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid	[redacted] (2016), Doc. No. 116-001	Visual inspection
Melting/freezing point	187 °C (99.0%)	[redacted] (2016), Doc. No. 112-001	Measured
Boiling point	Degradation occurs before boiling (99.0%)	[redacted] (2016), Doc. No. 112-001	Measured
Relative density	Not applicable	-	-
Vapour pressure	Less than5×10^{-9} Pa at 20°C (99.2%) Less than 1×10^{-8} Pa at 25°C.	[redacted] (2021), Doc. No. 115-003	Measured
Surface tension	70.6 mN/m at 20°C (90 % saturated solution)	[redacted] (2016), Doc. No. 116-001	Measured
Water solubility	51.76 µg at 20°C (pH 5.7 – 6.1) (99.2%) (As Benzobicyclon is not expected to dissociate the influence of the pH was not determined.)	[redacted] (1999); Doc. No. 181-001	Measured
Partition coefficient n-octanol/water	log P_{OW} = 3.1 at 20°C (As Benzobicyclon is not expected to dissociate the influence of the pH was not determined.) (20°C)	[redacted] (1999); Doc. No. 181-001	Measured
Henry's law constant	4.31×10^{-5} Pa m^3 mol^{-1} (20°C)	-	Calculated

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	Not required because the melting point is above 40ºC.	-	-
Flammability	not flammable	[redacted] (2016), Doc. No. 142-002	Measured
Explosive properties	Not explosive	[redacted] (2019), Doc. No. 181-002	Statement
Self-ignition temperature	No self-ignition of Benzobicyclon was observed prior to melting point.	[redacted] (2016), Doc. No. 142-001	Measured
Oxidising properties	Data required		
Granulometry	No data. Not a requirement of Reg. (EU) 283/2013	-	-
Solubility in organic solvents and identity of relevant degradation products	Solubility at 20°C (99.2%) Ethanol: 0.19 g/L Methanol: 0.39 g/L Acetone: 9.3 g/L Ethyl acetate: 2.6 g/L n-octanol: 0.05 g/L Xylene: 0.53 g/L Toluene: 1.2 g/L Hexane: < 0.12 g/L n-heptane: < 0.24 g/L 1,2-dichloroethane: 144.0 g/L	[redacted] (1998), Doc. No. 145-001	Measured
Dissociation constant	No dissociation constant could be determined by spectrophotometric method (99.3%)	[redacted] (2018), Doc. No. 115-002	Measured
Viscosity	Not applicable to solids	-	-
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	UV/VIS, IR, NMR and MS spectra were recorded and are in agreement with the structure of Benzobicyclon. The molar extinction coefficients were determined as follows: Methanol: λ_{max} = 322.8 nm; ε = 18900 L mol^{-1} cm^{-1} λ_{max} = 314 nm ; ε = 16400 L mol^{-1} cm^{-1} λ_{max} = 304 nm ; ε = 11700 L mol^{-1} cm^{-1} λ_{max} = 290 nm ; ε = 8200 L mol^{-1} cm^{-1} 1M HCl in methanol : λ_{max} = 323.6nm ; ε = 18900 L mol^{-1} cm^{-1} λ_{max} = 314 nm ; ε = 16100 L mol^{-1} cm^{-1} λ_{max} = 304 nm ; ε = 11400 L mol^{-1} cm^{-1} λ_{max} = 290 nm ; ε = 8100 L mol^{-1} cm^{-1} 1M NaOH in methanol : λ_{max} = 314 nm ; ε = 5200 L mol^{-1} cm^{-1} λ_{max} = 304 nm ; ε = 9100 L mol^{-1} cm^{-1} λ_{max} = 290 nm ; ε = 16100 L mol^{-1} cm^{-1} λ_{max} = 261.8 nm ; ε = 36400 L mol^{-1} cm^{-1}	[redacted] (1999); Doc. No. 181-001	Measured

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives [*equivalent to section 8.1 of the CLH report template*]

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.14	Not explosive	No test has been performed with the substance. Information on the substance is provided as set out in 1.1 of method A.14	[redacted] (2019), Doc. No. 181-002

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

The chemical structure of Benzobicyclon shows that no hazardous groups (like nitro-groups) are present in the molecule.

2.2.1.1.1.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.1.4.3 of Regulation (EC) No. 1272/2008: "*A substance or mixture shall not be classified as explosive if:*

(a) There are no chemical groups associated with explosive properties present in the molecule. Examples of groups which may indicate explosive properties are given in Table A6.1 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria".

As there are no functional groups associated with explosive properties in the molecule no classification is warranted.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Not explosive – data conclusive but not sufficient for classification.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Hazard class not applicable.

2.2.1.1.3 Oxidising gases [*equivalent to section 8.3 of the CLH report template*]

Hazard class not applicable.

2.2.1.1.4 Gases under pressure [*equivalent to section 8.4 of the CLH report template*]

Hazard class not applicable.

2.2.1.1.5 Flammable liquids [*equivalent to section 8.5 of the CLH report template*]

Hazard class not applicable.

2.2.1.1.6 Flammable solids [*equivalent to section 8.6 of the CLH report template*]

Table 3: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.10	Not flammable	-	[redacted] (2016), Doc. No. 142-002

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

No independent burning or glowing over the length of the pile was observed in the preliminary test. The test substance was found to be not flammable

2.2.1.1.6.2 Comparison with the CLP criteria

If available data from an A.10 test method indicate that a classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary. (Guidance on Information requirement and Chemical Safety Assessment, ECHA)

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Not flammable. – data conclusive but not sufficient for classification.

2.2.1.1.7 Self-reactive substances [*equivalent to section 8.7 of the CLH report template*]

Table 4: Summary table of studies on self-reactive substances

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.16	No self-ignition was observed at temperatures up to the melting point.	-	[redacted] (2016), Doc. No. 142-001

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

Benzobicyclon does not self-ignite prior to the melting point.

2.2.1.1.7.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.8.4.2 of Regulation (EC) No. 1272/2008: *"The classification procedures for self-reactive substances and mixtures need not be applied if:*

(a) There are no chemical groups present in the molecule associated with explosive or self reactive properties. Examples of such groups are given in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG Manual of Tests and Criteria.

As there are no functional group associated with self-reactive properties in the molecule, no classification procedure shall be applied.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Not a self-reactive substance. Data conclusive but not sufficient for classification.

2.2.1.1.8 Pyrophoric liquids [*equivalent to section 8.8 of the CLH report template*]

Hazard class not applicable.

2.2.1.1.9 Pyrophoric solids [*equivalent to section 8.9 of the CLH report template*]

Table 5: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.16	No self-ignition was observed at temperatures up to the melting point.	-	[redacted] (2016), Doc. No. 142-001

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

Benzobicyclon does not self-ignite prior to the melting point. Experience in use with Benzobicyclon over a long period of time demonstrates that it is not pyrophoric.

2.2.1.1.9.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.10.4.1 of Regulation (EC) No. 1272/2008: "*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*."
As there was no concern arised from experience in use with Benzobicyclon no classification procedure shall be applied.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Not a pyrophoric solid. Data conclusive but not sufficient for classification.

2.2.1.1.10 Self-heating substances [*equivalent to section 8.10 of the CLH report template*]

Table 6: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.16	No self-ignition was observed at temperatures up to the melting point.	-	[redacted] (2016), Doc. No. 142-001

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

No self-ignition was observed. The substance had melted during the temperature raise. The barometric pressure was 766.2 mm Hg at the beginning and ending of the test.

2.2.1.1.10.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.11.4.2 of Regulation (EC) No. 1272/2008: *"The classification procedure for self-heating substances or mixtures need not be applied if the results of a screening test can be adequately correlated with the classification test and an appropriate safety margin is applied."*
EU test method A.16 as described in Regulation (EC) No 440/2008 checks for self-heating properties.
Results of the EEC method A16 performed on Benzobicyclon exclude self-heating of the substance up to the melting point. Therefore, no classification procedure shall be applied.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Not self-heating. – data conclusive but not sufficient for classification.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

No studies have been submitted.

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

Benzobicyclon does not contain metals or metalloids.

2.2.1.1.11.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.12.4.1 of Regulation (EC) No. 1272/2008: "*The classification procedure for this class need not be applied if:*
(a) the chemical structure of the substance or mixture does not contain metals or metalloids"
As there are no metals or metalloids present in the molecule, Benzobicyclon is not to be considered for classification in this hazard class.

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

Not a substance which in contact with water emit flammable gases. Data conclusive but not sufficient for classification.

2.2.1.1.12 Oxidising liquids [equivalent to section 8.12 of the CLH report template]

Hazard class not applicable.

2.2.1.1.13 Oxidising solids [equivalent to section 8.13 of the CLH report template]

Table 7: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Regulation (EC) No 440; A.17	Not oxidizing	No test has been performed with the substance. Information on the substance is provided as set out in 1.1 of method A.17	[redacted] (2019), Doc. No. 181-002

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

Considering the structural formula and the negative oxygen balance Benzobicyclon is considered to be non-oxidizing.

2.2.1.1.13.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.14.4.1 of Regulation (EC) No. 1272/2008:
"For organic substances or mixtures the classification procedure for this class shall not apply if:
(a) the substance or mixture does not contain oxygen, fluorine or chlorine; or
(b) the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen."
Benzobicyclon does contains oxygen bonded to atoms different from 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. Furthermore, Benzobicyclon is a new active substance and no record of use data to confirm that the molecule is not oxidising are available. Benzobicyclon should be tested for oxidizing properties. **A new study has been requested in the framework of pesticide active substance authorisation and might be available before the RAC opinion finalization.**

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

No classification due to Data lacking.

2.2.1.1.14 Organic peroxides [*equivalent to section 8.14 of the CLH report template*]

No studies have been submitted.

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

Benzobicyclon does not contain the bivalent organic peroxide –O-O structure.

2.2.1.1.14.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.15.1.1 of Regulation (EC) No. 1272/2008: *"Organic peroxides means liquid or solid organic substances which contain the bivalent -O-O- structure"*
As Benzobicyclon does not contain the bivalent O-O- structure, it is not to be considered for classification in this hazard class.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

Data conclusive but not sufficient for classification.

2.2.1.1.15 Corrosive to metals [*equivalent to section 8.15 of the CLH report template*]

No studies have been submitted.

2.2.1.1.15.1 Short summary and overall relevance of the provided information on corrosive to metals

Benzobicyclon has a melting point of 187 °C (99.0%)

2.2.1.1.15.2 Comparison with the CLP criteria

According to the Annex I part 2 point 2.16.2.1. of Regulation (EC) No. 1272/2008: *"A substance or a mixture which is corrosive to metals is classified in a single category for this class, using the test in Part III, subsection 37.4 of the UN RTDG, Manual of Tests and Criteria"*
According to CLP Guidance (2.16.4.1): "*only substances and mixtures 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 and needs to be considered. [..]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 consideration."*
As Benzobicyclon shows a melting point higher than 55 °C, it is not to be considered for classification in this hazard class.

2.2.1.1.15.3 Conclusion on classification and labelling for organic peroxides

Data conclusive but not sufficient for classification.

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

The appearance of GWN-10235 is that of a viscous, beige, untransparent liquid. It has no explosive or oxidising properties and is not flammable. GWN-10235 has not to be classified in respect of physico-chemical hazards. In aqueous solution, it has a pH value of 7.1. The pH of the neat formulation is 8.1. The relative density of GWN-10235 is 1.1544. The technical properties of GWN-10235, suspensibility, persistence of foaming, spontaneity of dispersion, particle size, pourability meet the requirements of the FAO/WHO specifications for plant protection products and are acceptable for a SC formulation.
GWN-10235 is stable when stored for two weeks at 54°C, 7 days at 0°C and is stable when stored for 2 years at ambient temperature in HDPE packaging.

2.3 DATA ON APPLICATION AND EFFICACY

Benzobicyclon is an herbicide active substance belonging to the chemical family of 4-HPPD inhibitors (HRAC Code F2 – inhibition of 4-HPPD). Inhibition of 4-HPPD (4-hydroxyphenyl-pyruvate-dioxygenase) is a new mode of action to control infesting weed species in paddy rice.
The herbicidal activity of Benzobicyclon is induced by hydrolysis of the molecule in the flooded rice fields. After being absorbed mainly by the rooting system of the plants, it inhibits 4-HPPD enzyme which is involved in the synthesis of chlorophyll pigments. Benzobicyclon effect results in weed bleaching.

2.3.1 Summary of effectiveness

Benzobicyclon containing products are envisaged to be used for control of various monocotyledonous grass and non-grass weed species in the representative crop paddy rice.
In the last years an extensive efficacy program was conducted in countries of the Mediterranean EPPO zone to support this use. The field trials data supporting efficacy against the target weed species comprise 43 trials implemented from 2012 to 2015.

The trials were undertaken by Officially Recognised Organisations, all of which follow EPPO guidelines. Trials were conducted in the main countries for rice production in Europe: 21 trials in Italy, 16 trials in Spain, four trials in Spain, one trial in Portugal and two trials in France representing the Mediterranean EPPO climatic zone according to EPPO Standard PP1/241.

GWN-10235 was tested at a range of dose rates from 200 to 300 g a.s./ha (0.5 to 0.75 L product/ha) and compared to approved standard reference products, containing the active substances Oxadiazon and Penoxsulam.
In this program Benzobicyclon has demonstrated a very high level of activity against monocotyledonous weed species, belonging to different families, as reported in the table below.

Table 8: Summary of efficacy Benzobicyclon against important target weeds at different application timings

Level of efficacy GWN-10235 0.75 L/ha (300 g a.s./ha)			Application timing		
Crop	**Family**	**Weed specie**	**Pre-emergence**	**Early post-emergence**	**Post-emergence**
ORYSA in flooded paddies	Cyperaceae	CYPDI	very good 98.97%	very good 99.39%	good 87.03%
	Pontederiaceae	HETRE	very good 97.1%	very good 94.23%	very good 93%
	Poaceae	ECHSS	moderate 60.68%	low 50.49%	low 33.08%
	Poaceae	LEFFA	moderate 75.75%	moderate 73.88%	good 85%
	Cyperaceae	SCPMA	good 79.44%	good 76.46%	low 48.72%
	Alismataceae	ALSPA	moderate 65.58%	low 38.25%	low 42.15%

very good = > 90% efficacy
good = 75 - 90% efficacy
moderate = 60 - 75%
low = < 60%

2.3.2 Summary of information on the development of resistance

No case of resistance was reported following the use of inhibition of 4-HPPD substances.
Unlike insects and pathogens, weeds usually only produce one generation per year and development of resistance is usually a relatively slow process. It is difficult to class any weed species as inherently more or less likely to develop resistance to a particular herbicide. Therefore, general recommendations of HRAC for resistance management should be considered. Key measures are:
- Only one application of a product with Benzobicyclon or another 4-HPPD inhibitor per season
- Combination of herbicide treatments with mechanical weed control.

Considering that the risk associated to the active substance Benzobicyclon is low and that the risk associated to the weed targets is high, and the fact that Benzobicyclon containing products will always be used in sequence with other herbicides with different modes of action, the combined risk of using Benzobicyclon against weeds in paddy rice is low to moderate.

2.3.3 Summary of adverse effects on treated crops

There have been no observations of phytotoxic effects from the use of Benzobicyclon in the representative crop. Neither an effect on crop vigour and crop colour, nor numerically relevant differences in crop yield were observed.

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

It has been established that Benzobicyclon poses an acceptable risk for arthropods other than bees, earthworms and other soil non-target macro-organisms and soil microbial activity.
Considering the subsequent cultivation of paddy rice in mono-rotational cropping systems the evaluation of effects on succeeding crops is not relevant.
Benzobicyclon has been widely approved and used on rice crops in non-EU countries for many years with no apparent problems associated concerning effects on adjacent crops, when applied according to label recommendations.

2.4 FURTHER INFORMATION

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

Please refer to safety data sheet.

2.4.2 Summary of procedures for destruction or decontamination

Please refer to safety data sheet.

2.4.3 Summary of emergency measures in case of an accident

Please refer to safety data sheet.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1 Analysis of the active substance as manufactured

Benzobicyclon content in technical substance can be adequately analysed by HPLC-UV (270 nm), using internal standardization (Diphenylphthalate). The isomeric ratio in the technical material can be determined by HPLC-TOF/MS using Phenomenex Lux Amylose-1, 150x2.0mm, 3µm column. Note that the specific isomers are not specifically identified with this method.

2.5.1.2 Formulation analysis

Benzobicyclon content in GWN-10235 is analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 325 nm. Quantification is performed using external standard solutions. The method is validated in terms of linearity, accuracy and precision in accordance with the requirements of SANCO/3030/99 rev. 5.

2.5.1.3 Methods for Risk Assessment

Plants and plant products

The analytical method Boatwright, M.T. (2018) is being proposed for analysis Benzobicyclon and relevant metabolites in agriculture commodities (wet crops, dry crops, oily crops and acidic crops) for purposes of risk assessment and regulatory enforcement. Residues of benzobicyclon are extracted from high water, acidic and dry samples by shaking with an acetonitrile / 0.55M citric acid (aq.) solution (80:20 v/v).. For oil samples, extraction with hexane and partitioning into acetonitrile was used. The acetonitrile phase is diluted with 0.55M citric acid (aq.) and analysed. For detection of 1315P-966, a clean-up step using SPE cartridge is necessary after the extraction procedures. The final solutions are analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The method limit of quantitation is 0.01 mg/kg. The method can be considered adequate to determine benzobicyclon and its metabolites 1315P-070, 1315P-570 and 1315P-966 for pre-registration purposes in rice grain, rice straw, lettuce, orange juice and olive oil in accordance to the requirements of guideline SANCO/3029/99 rev. 4. See Volume 3 CA B5 Section 4.1.2 for details.

Food of animal origin

No residues in matrices of animal origin are expected as all the calculated intakes are below the trigger value of 0.004 mg/kg bw/d (see CA 6.2.2.1). Therefore, livestock metabolism data are not needed. Furthermore, a residue definition in animal products is not required and no methods for food and feed of animal origin are necessary.

Soil

Methods for the determination of residues in soil have been submitted in support of pre-registration studies. The methods are based on HPLC-MS/MS after extraction with acetonitrile / 0.55M citric acid (aq.) (80:20 v/v). The methods are sufficiently validated according to the criteria of the EU guideline SANCO/3029/99 rev. 4. See Volume 3 CA B5 Section 4.1.2 for details.

Water

HPLC-UV or HPLC-MS/MS analytical methods are applied for the determination of benzobicyclon and metabolites in water in support of risk assessment studies and are adequately validated. For analyses the samples are diluted with acetonitrile and directly injected in HPLC system. See Volume 3 CA B5 Section 4.1.2 for details.

Air

No analytical methods are included in the submitted studies.

Diet and feeding solutions

Adequate methods, based on HPLC-UV for the determination of residues in diet and feeding solutions have been submitted in support of pre-registration studies. See Volume 3 CA B5 Sections 4.1.2(c) and 4.1.2(f).

Body fluids

No analytical methods are included in the submitted studies.

2.5.2 Methods for post control and monitoring purposes

2.5.2.1 Analytical methods for residue determination in food of plant origin:

The LC-MS/MS method GPL-MTH-092 is validated in study by [redacted] (2018) for determination of benzobicyclon and its metabolites 1315P-070, 1315P-570, and 1315P-966 in crops. ILV studies are presented to validate the study for monitoring purposes. Study by [redacted] (2018), report 432 – 004, is adequate to independent validate the primary method for benzobicyclone and 1315P-070 determination in dry commodities LOQ of 0.01 mg/kg. Study [redacted] (2018), report 432-005, is not considered adequate to independently validate the primary method for determination of benzobicyclon, metabolites 1315-070, 1315P-570 and 1315P-966 in rice straw, radish root, lettuce, radish leaves, wheat plants, wheat grain and wheat straw and for 1315P-570 and 1315P-966 in rice grain.
A ILV method is not available to monitor benzobicyclon and metabolites in oily commodities.

No QuEChERS method is presented for enforcement purposes.

2.5.2.2 Analytical methods for residue determination in food of animal origin:

A residue definition in animal products is not required and no methods for food and feed of animal origin are necessary.

2.5.2.3 Analytical method for residue determination in soil and sediment:

Adequate HPLC-MS/MS method is provided to monitor Benzobyciclon and metabolites 1315P-070, 1315P-076, 1315P-570, 1315P-683, 1315P-960, 1315P-966 in soil. The method LOQ is 0.005 mg/kg for all the analytes.

2.5.2.4 Analytical methods for residue determination in water:

Adequate method (report 435-003) is presented to Benzobyciclon and metabolites 1315P-070, 1315P-076, 1315P-570, 1313P-683, 1315P-960, 1315P-962 and 1315P-966 in water at LOQ of 0.1 µg/L in accordance to the requirements of SANCO/3029/99 rev 4 for risk assessment purposes.
Confirmatory validation data are required to validate the method for determination of 1315P-070 for enforcement purposes. Additional information on the independent validation method for drinking water monitoring are also required (relevant for all the analytes of interest). See Volume 3 CA B5 Sections 4.2 for details.

2.5.2.5 Analytical methods for residue determination in air:

Adequate LC-MS/MS method is available to monitor benzobicyclon in air. The method is fully validated in accordance with the requirements of SANCO/3029/99 and SANCO/825/00 rev. 8.1 at LOQ of 0.5 µg/m^3.

2.5.2.6 Analytical method for the determination in Body Fluids

Residues of Benzobicyclon and 1315P-070 in blood, urine, meat and liver are extracted with a modified QuEChERS method (DIN EN 15662:2009- 02) and analysed by high performance liquid chromatography coupled with tandem mass spectrometry. The method fulfils the requirements of SANCO/825/00 rev. 8.1 for selectivity, linearity, accuracy and precision and results adequate to determine the analytes at LOQ of 0.05 mg/L for blood and urine and 0.1 mg/kg for meat and liver.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

More detailed results of the studies are presented in Volume 3, section B.6.

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Absorption, distribution and excretion

The absorption, distribution and excretion of Benzobicyclon was investigated in rats after single oral doses (10 and 500 mg/kg bw), single intravenous dose (10 mg/kg bw) or 7 consecutive daily oral doses of Benzobicyclon (10 mg/kg bw).
Maximum plasma concentrations (C_{max}) were reached between 3 and 6 hours after dosing, showing no relevant differences between sexes. Following single treatment with the low dose, the time to reach the maximum plasma concentration (T_{max}) values was 6 hours at the low dose, 3 (males) to 6 hours (females) at the high dose and 3 (males) to 4 hours (females) after repeated dosing. The mean area under the plasma concentration-time curve at 168 hours (AUC_{168}) was slightly greater for females than males after single oral dosing with 10 mg/kg bw, whereas AUC_{168} was comparable between sexes after single oral dosing with 500 mg/kg bw. Biliary excretion experiments showed low oral absorption of Benzobicyclon, with 11 - 28% of the administered dose absorbed after treatment with the low dose. For all dosing regimen, excretion was rapid, with most of the administered dose excreted within 48 hours after dosing. Almost all of the administered radioactivity was excreted in the faeces (>91% of the dose). A lesser proportion was recovered from urine after 4 days (2 – 3%).
Benzobicyclon was widely distributed in all tissues at the time of the peak plasma concentration in both sexes, with the highest concentrations found in the liver and kidneys. After single oral dosing of 500 mg/kg bw the tissue concentrations were approximately 3 – 11 fold greater than after dosing with 10 mg/kg bw. Seven repeated daily doses of the low dose revealed an approximate doubling of tissue concentrations when compared to the single low dose, indicative of a systemic plateau concentration not being reached after a single dose yet. This is a consequence of binding of Benzobicyclon to red blood cells and thus a blood retention >24 hours, reflected also in tissue levels specifically of the highly perfused organs. Tissue concentrations generally declined more slowly in female animals.

Metabolism

Benzobicyclon, is a pro-pesticide that requires hydrolysis of the thiophenyl group to generate the anticipated pesticidal active moiety, metabolite B (also referred to as 1315P-070). While benzobicyclon is classified as a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, HPPD inhibiting properties were not observed in its toxicological database. However, metabolite B (a metabolite of benzobicyclon and residue of concern in drinking water) does act as an HPPD inhibitor. This human health risk assessment evaluates risks to both benzobicyclon (parent) and metabolite B.

The active moiety of benzobicyclon, metabolite B, has been shown to be more toxic than the parent compound. Therefore, studies were conducted with metabolite B, including a developmental toxicity study in mice. For the US EPA assessment, 2-generation reproduction toxicity studies are available from other HPPD inhibitors were used for bridging and evaluation assessment (please note that such data was not provided for the EU benzobicyclone assessment under EC Reg 1107/2009) (US EPA 2021a and b).

For more details on the toxicity studies available for metabolite B refer to paragraph 2.6.8.1.

Absorbed Benzobicyclon was extensively metabolised by the rat and at least 12 metabolites were present in urine and at least 8 metabolites were present in bile (some were present in both matrices). The parent substance was not present in urine or bile. The most prominent metabolite in urine after intravenous dosing was 1315P-966 (2-chloro-4-methylsulfonylbenzoic acid; CMBA), generated via hydrolytic cleavage of the keto linkage. Three other metabolites present in urine and bile (< 1.2% of administered dose), 1315P-070 (3-(2-chloro-4-methylsulfonylbenzoyl) bicyclo[3.2.1]octane-2,4-dione), 1315P-570 (4-amino-3-[2-chloro-4-(methylsulfonyl)benzoyl]bicyclo[3.2.1]oct-3-en-2-one) and 1315P-570-OH (hydroxylated derivative of 1315P-570), resulted from replacement of the thiophenyl moiety. None of the metabolites in urine or bile were glucuronic acid or sulphate conjugates. Metabolites in faeces were the same as those in bile.

A proposed metabolic pathway is given below in Figure 1.

A comparative *in vitro* metabolism study with cryopreserved mixed-gender human and rat hepatocytes confirmed the main metabolic pathways and revealed no relevant species differences in metabolism. In addition to metabolite 1315P-070 three further major metabolites (> 5%, not identified) were found in both species. Two major metabolites identified in rat metabolism *in vivo*, 1315P-966 and 1315P-570, were not found in relevant amounts under the test conditions *in vitro*. There was no metabolite unique to human, and thus no new toxicological concern is raised by the outcome of this study.

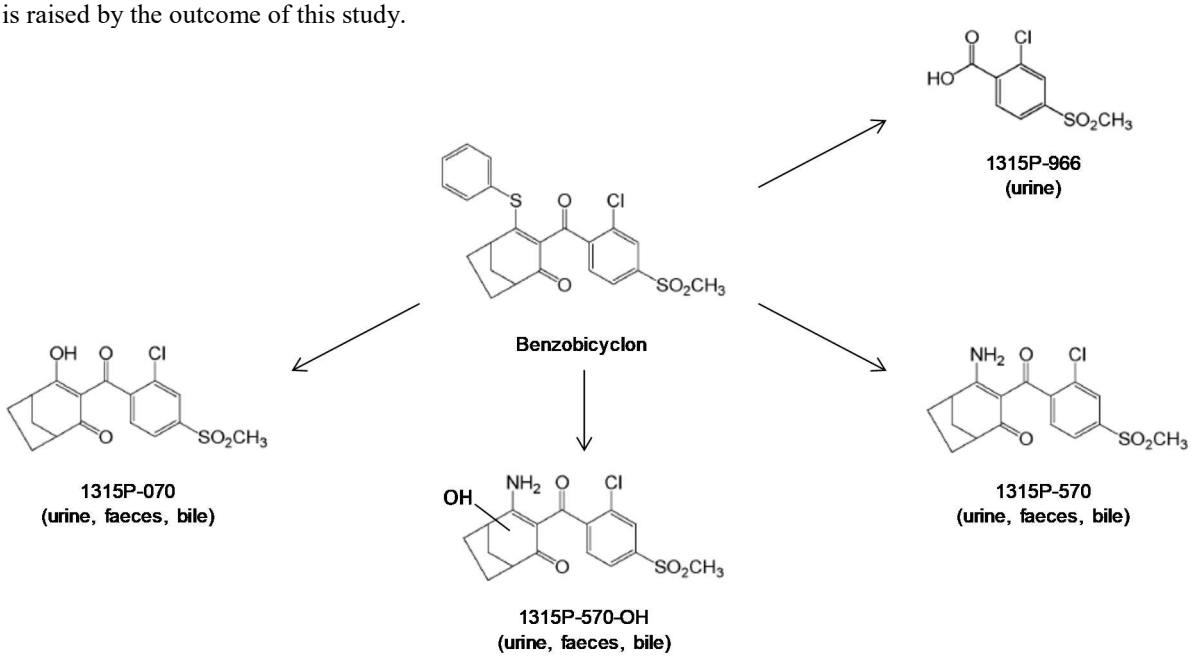


Figure 1: Proposed metabolic pathway of Benzobicyclon ([redacted] (1999))

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Toxicokinetics: Absorption, distribution, metabolism and excretion by oral exposure (single and repeated dosing)	Poorly absorbed following oral administration 10 mg/kg bw: >90% excreted via faeces as parent substance after 4 days, excretion in urine 2 – 3%, low biliary excretion: 6 - 14% (10 mg/kg bw) and 1 - 2% (500 mg/kg bw) Tissue concentrations: generally lower than in plasma, except for liver and kidneys after single dosing with 10 mg/kg bw, doubled after repeated dosing (10 mg/kg bw/d, 7 days) when compared to single dosing, 3 – 11 × higher after single dose of 500 mg/kg bw when compared to low dose Metabolites: large number was excreted in urine and bile, major routes: loss of thiophenyl moiety and cleavage of keto linkage, no glucuronic acid or sulphate conjugated metabolites	OECD 417 GLP Acceptable	[redacted] 1999 (Vol. 3 B.6.1.1 - CA 5.1.1/01) Doc. No.: 512-001
In vitro comparative metabolism study using rat and human cryopreserved hepatocytes	Metabolism of Benzobicyclon was rapid and extensive, rat represented the higher turnover In addition to 1315P-070, metabolites assigned M7, M10 and M17 were other main constituents in both species There was no unique human metabolite	No guideline available (OCSPP 870.7485 was used as a general guidance) GLP Acceptable	[redacted] (2019) (Vol. 3 B.6.1.2 - CA 5.1.2/01) Doc. No.: 514-002

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The studies are considered valid, scientifically acceptable and appropriate for the assessment of *in vivo* absorption, distribution, metabolism (in compliance with OECD 417) and *in vitro* species comparison (no guideline available) of Benzobicyclon. The toxicokinetic studies on Benzobicyclon support the classification proposal.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Acute oral toxicity studies in rats and mice are available for Benzobicyclon and are summarised in Table 10. In addition, an acute oral toxicity in rats with the SC formulation GWN-10235 (containing 400 g/kg of Benzobicyclon) is available and summarised in Table 8. For details on the product study please refer to Volume 3 CP, section B.6.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 10: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity OECD 401 GLP Acceptable	Rat, [redacted]CD (SD), male/female, 5/group	SAN1315H (Benzobicyclon) Batch No.: KK62-135/94 Purity: not reported	0, 5000 mg/kg bw Oral (gavage)	> 5000 mg/kg bw	[redacted] (1995a) (Vol. 3 B.6.2.1 - CA 5.2.1/01) Doc. No.: 521-006
Acute oral toxicity OECD 401 GLP Acceptable	Mouse, [redacted] ([redacted]CD-1), male/female, 5/group	SAN1315H (Benzobicyclon) Batch No.: KK62-135/94 Purity: not reported	0, 5000 mg/kg bw Oral (gavage)	> 5000 mg/kg bw	[redacted] (1995b) (Vol. 3 B.6.2.1 - CA 5.2.1/01) Doc. No.: 521-007

Table 11: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute oral toxicity (rat) OECD 425 GLP Acceptable	GWN-10235[a]	Product study	LD_{50} > 5000 mg/kg bw	[redacted] (2014) (Vol. 3 B.6.1.1 - CP 7.1.1/01) Doc. No.: 526-001

[a] Product containing 400 g/kg of Benzobicyclon

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

In acute oral toxicity studies with Benzobicyclon the LD_{50} values were found to be similar among species (rat and mouse). Based on the results of the first study, the oral LD_{50} value of Benzobicyclon was considered to be > 5000 mg/kg bw in male and female rats. Based on the results of the second study, the oral LD_{50} value of Benzobicyclon in male and female mice was considered to be > 5000 mg/kg bw. Similar to the studies with the active substance Benzobicyclon the acute oral toxicity study with its SC formulation GWN-10235 in rats revealed a LD_{50} value of > 5000 mg/kg bw. The studies are considered valid, scientifically acceptable and appropriate for the assessment of acute oral toxicity of Benzobicyclon.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

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

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Not classified (conclusive but not sufficient for classification).

2.6.2.2 *Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]*

Two acute dermal toxicity studies with Benzobicyclon are available in rats. The results of these studies are summarised in Table 12.
Further, an acute dermal toxicity study in rats with the SC formulation GWN-10235 (containing 400 g/kg of Benzobicyclon) and a 21-day repeated dose dermal toxicity study with the active substance Benzobicyclon in rats is available (Table 13). For details on the product study please refer to Volume 3 CP, section B.6.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 12: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity OECD 402 GLP Acceptable	Rat, [redacted]CD (SD), male/female, 5/group	SAN1315H (Benzobicyclon) Batch No.: KK62-135/94 Purity: not specified	0, 2000 mg/kg bw, 24 hours	> 2000 mg/kg bw	[redacted]. (1995c) (Vol. 3 B.6.2.2 - CA 5.2.2/01) Doc. No.: 522-001
Acute dermal toxicity OECD 402 GLP Acceptable	Rat, [redacted]CD (SD), male/female, 5/group	Benzobicyclon Batch No.: 1A0110 Purity: > 99.9%	2000 mg/kg bw, 24 hours	> 2000 mg/kg bw	[redacted] (2015) (Vol. 3 B.6.2.2 - CA 5.2.2/02) Doc. No.: 522-002

Table 13: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
21-day dermal toxicity study (rabbit) OECD 410 GLP Acceptable	Benzobicyclon Batch No.: 1L0108 Purity: 98%	Topical application once daily (6 h exposure) 0, 100, 300 and 1000 mg/kg bw/d for 21 days to New Zealand White rabbits (10/sex/group)	$NOEL_{systemic/dermal}$: 1000 mg/kg bw/d No mortality or clinical signs, no dermal irritation	[redacted] (2012) (Vol. 3 B.6.3.3 - CA 5.3.3/01) Doc. No.: 532-001
Acute dermal toxicity (rat) OECD 402 GLP Acceptable	GWN-10235[a]	Product study	LD_{50} > 5000 mg/kg bw No skin reactions	[redacted] (2014) (Vol. 3 B.6.1.2 - CP 7.1.2/01) Doc. No.: 527-001

[a] Product containing 400 g/kg of Benzobicyclon

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

Based on the results of both acute dermal toxicity studies, the dermal LD_{50} value of Benzobicyclon in male and female rats is considered to be > 2000 mg/kg bw. The acute dermal toxicity study with the SC formulation GWN-10235 in rats revealed a LD_{50} value of > 5000 mg/kg bw. The studies are considered valid, scientifically acceptable and appropriate for the assessment of acute dermal toxicity of Benzobicyclon. In a valid and scientifically

acceptable 21-day dermal toxicity study neither death of animals, clinical signs of systemic toxicity nor dermal irritation was observed.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

Since the acute dermal toxicity studies in rats revealed LD_{50} values > 2000 mg/kg bw, classification of Benzobicyclon for acute dermal toxicity according to Regulation (EC) No 1272/2008 is not required.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Not classified (conclusive but not sufficient for classification).

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

The acute toxicity via the inhalation route was determined for Benzobicyclon (Table 14) and the SC formulation GWN-10235 (containing 400 g/kg of Benzobicyclon) in rats (Table 15). For details on the product study please refer to Volume 3 CP, section B.6.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 14: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form, particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity OECD 403 GLP Minor guideline deviation: whole body exposure, slight exceedance of the acceptable MMAD range (1 – 4 µm) Acceptable	Rat, SD, male/ female, 5/group	SAN1315H (Benzobicyclon) Batch No.: 960108N Purity: 99.9% Dust aerosol MMAD: 4.3 µm GSD: 2.18	0, 2.72 mg/L (highest technically achievable concentration), 4 hours	> 2.72 mg/L	[redacted] (1997) (Vol. 3 B.6.2.3 - CA 5.2.3/01) Doc. No.: 523-001

MMAD: mass median aerodynamic diameter
GSD: geometric standard deviation

Table 15: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute inhalation toxicity (rat) OECD 403 GLP Acceptable	GWN-10235[a]	Product study nose-only application	LC_{50}: >5.1 mg/L	[redacted] (2014) (Vol. 3 B.6.1.3 - CP 7.1.3/01) Doc. No.: 528-001

[a] Product containing 400 g/kg of Benzobicyclon

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

In conclusion, the acute inhalation LC_{50} for Benzobicyclon is > 2.72 mg/L for male and for female rats. In the product study in rats, a LC_{50} of > 5.1 mg/L was determined. The studies are considered valid, scientifically acceptable and appropriate for the assessment of acute inhalation toxicity of Benzobicyclon.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

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.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Not classified (conclusive but not sufficient for classification).

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

The potential of Benzobicyclon to induce skin irritation was investigated in a rabbit study (Table 16).
Similar to the study with the active substance, a skin irritation study in rabbits was performed for the SC formulation GWN-10235 (containing 400 g/kg of Benzobicyclon) (Table 17). For details on the product study please refer to Volume 3 CP, section B.6.
A 21-day repeated dose dermal toxicity study with the active substance Benzobicyclon in rats is available (Table 17).
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 16: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
Skin irritation OECD 404 GLP Acceptable	Rabbit, Japanese White, female, 6/group	SAN1315H (Benzobicyclon) Batch No.: KK62-135/94 Purity: not reported	0.5 g, 4 hours	No signs of toxicity, no irritation reactions in the skin Mean and individual scores for erythema/oedema: 0	[redacted] (1995a) (Vol. 3 B.6.2.4 - CA 5.2.4/01) Doc. No.: 565-001

Table 17: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
21-day dermal toxicity study (rabbit) OECD 410 GLP Acceptable	Benzobicyclon Batch No.: 1L0108 Purity: 98%	Topical application once daily (6 h exposure) 0, 100, 300 and 1000 mg/kg bw/d for 21 days to New Zealand	$NOEL_{systemic/dermal}$: 1000 mg/kg bw/d No mortality or clinical signs, no dermal irritation	[redacted] (2012) (Vol. 3 B.6.3.3 - CA 5.3.3/01) Doc. No.: 532-001

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		White rabbits (10/sex/group)		
Skin irritation (rabbit) OECD 404 GLP Acceptable	GWN-10235[a]	Product study	Non-irritant Individual animal mean scores for erythema at 24, 48 and 72 hours: were 1/ 0.67/ 0.67. Individual animal mean scores for oedema at 24, 48 and 72 hours were 0.33/0.33/0.33	[redacted] (2014) (Vol. 3 B.6.1.4 - CP 7.1.4/01) Doc. No.: 565-002

[a] Product containing 400 g/kg of Benzobicyclon

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

Based on the evaluation of skin reactions, Benzobicyclon is considered to be non-irritant when applied to the rabbit skin. 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. In the study conducted with the representative product the individual animal mean scores for erythema/oedema at 24, 48 and 72 hours were 1/0.33, 0.67/0.33 and 0.67/0.33. All animals were free of erythema and oedema by 72 hours.
The product study revealed no irritating potential to the skin of rabbits either. The studies are considered valid, scientifically acceptable and appropriate for the assessment of the acute skin irritation potential of Benzobicyclon. In a valid and scientifically acceptable 21-day dermal toxicity study neither death of animals, clinical signs of systemic toxicity nor dermal irritation was observed.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

Since no irritation was observed after application of Benzobicyclon to the rabbit skin and the mean score for erythema/oedema was 0, classification of Benzobicyclon for skin irritation according to Regulation (EC) No 1272/2008 is not required.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Not classified (conclusive but not sufficient for classification).

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

The potential of Benzobicyclon to induce eye irritation was investigated in a rabbit study (Table 18).
Similar to the study with the active substance, an eye irritation study in rabbits was performed for the SC formulation GWN-10235 (containing 400 g/kg of Benzobicyclon) (Table 19). For details on the product study please refer to Volume 3 CP, section B.6.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 18: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
Eye irritation OECD 405 GLP Acceptable	Rabbit, Japanese White, female, 9	SAN1315H (Benzobicyclon) Batch No.: KK62-135/94 Purity: not reported	0.1 g, infinite (6 animals)	Redness of the conjunctiva (score 1): 6/6 animals up to 48 hours Chemosis (score 1): 3/6 animals up to 24 hours Mean scores redness: 0.67|0.33|0.33|0|0|0 Mean scores chemosis: 0.33|0|0|0|0|0	[redacted] (1995b) (Vol. 3 B.6.2.5 - CA 5.2.5/01) Doc. No.: 566-001
			0.1 g, 2 – 3 minutes (3 animals)	Chemosis (score 1): 2/3 animals up to 1 hour Mean scores: 0|0|0	

Table 19: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Eye irritation (rabbit) OECD 405 GLP Acceptable	GWN-10235[a]	Product study	Non-irritant	[redacted] (2014) (Vol. 3 B.6.1.5 - CP 7.1.5/01) Doc. No.: 566-002

[a] Product containing 400 g/kg of Benzobicyclon

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

Based on the evaluation of eye reactions, Benzobicyclon is considered to be non-irritant to the rabbit eye (mean score conjunctival redness/animal (24, 48, 72 hours): 0.67|0.33|0.33|0|0|0, mean score conjunctival chemosis/animal (24, 48, 72 hours): 0.33|0|0|0|0|0). There was no corneal opacity or iritis observed in any treated eye during this study. The product study revealed no irritating potential to the eye of rabbits. The studies are considered valid, scientifically acceptable and appropriate for the assessment of the acute eye irritation potential of Benzobicyclon.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Since no irritation was observed after application of Benzobicyclon to the rabbit eye (cornea, iris) and the mean scores for conjunctival redness/chemosis (24, 48, 72 hours) were below a value of 2, classification of Benzobicyclon for eye irritation according to Regulation (EC) No 1272/2008 is not required.

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

Not classified (conclusive but not sufficient for classification).

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

No study data on respiratory sensitisation are available for Benzobicyclon (there is no accepted and validated model available for this endpoint). The available toxicity study data do not indicate the potential of Benzobicyclon to act as a respiratory sensitiser.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

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

No formally recognised and validated tests currently exist for this endpoint. There was no evidence for respiratory irritation in the acute inhalation toxicity study in rats and there was no indication of sensitisation. There was no reported evidence of respiratory sensitisation in humans.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

As there are no specific study data and there is no evidence from the available toxicological data set and adverse effects in humans, classification is not possible.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not classified (data lacking).

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Two skin sensitisation studies with Benzobicyclon are available, *i.e.* a Local Lymph Node Assay (LLNA) in mice and a Guinea Pig Maximisation Test. The results of these studies are summarised in Table 20.
In addition, a LLNA in mice with the SC formulation GWN-10235 (containing 400 g/kg of Benzobicyclon) is available and summarised in Table 21. For details on the product study please refer to Volume 3 CP, section B.6.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 20: Summary table of animal studies on skin sensitisation

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) for the test substance concentrations 10, 25 and 50%: 1.0, 1.2 and 1.0, respectively (positive control 3.7) No sensitising potential	[redacted] (2015) (Vol. 3 B.6.2.6 - CA 5.2.6/01) Doc. No.: 567-003
Skin sensitisation Guinea Pig Maximization Test OECD 406 GLP	Guinea pig, Hartley White, females, 25/group	SAN1315H (Benzobicyclon) Batch No.: 6F0502 Purity: 99%	intradermal induction: 1, 2 % topical induction: 50 %	No mortality, no clinical signs No skin reactions at the challenged sites Sensitisation rate treatment groups: 0% Sensitisation rate positive	[redacted] (1998) (Vol. 3 B.6.2.6 - CA 5.2.6/02)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
Guideline deviations: limited test substance concentration of 1%, application of only one concentration (50%) at topical induction and challenge, (testing of higher concentrations should have been possible when moistening the test substance with water). Acceptable only as supportive information.			challenge: 50 %	control group: 100%	Doc. No.: 567-002

LLNA: Local lymph node assay

Table 21: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Skin sensitisation (mouse) OECD 429 GLP Acceptable	GWN-10235[a]	Product study LLNA (mouse)	Non-sensitising	[redacted] (2014) (Vol. 3 B.6.1.6 - CP 7.1.6/01) Doc. No.: 567-001

LLNA Local lymph node assay
[a] Product containing 400 g/kg of Benzobicyclon

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

The Local lymph node assay (LLNA) revealed that there was no indication that the test substance elicited a SI ≥ 3 under the conditions of this assay. The study was considered valid, scientifically acceptable and appropriate for the assessment of the skin sensitisation potential of Benzobicyclon. In a second skin sensitisation study (Maximisation test according to Magnusson and Kligman) Benzobicyclon was found not to be a skin sensitiser. The study is considered acceptable only as supportive information due to deviations to OECD guideline 406 (see Table 20). In conclusion, Benzobicyclon is not a skin sensitizer in mice or guinea pigs, neither at induction phase (LLNA) nor in a full skin sensitization assessment (guinea pig maximization test).

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

Since the stimulation index in the LLNA in mice was < 3 after treatment with Benzobicyclon and the sensitisation rate in the Guinea Pig Maximisation Test was 0%, classification of Benzobicyclon for skin sensitisation according to Regulation (EC) No 1272/2008 is not required.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Not classified (conclusive but not sufficient for classification).

2.6.2.8 Phototoxicity

A phototoxicity study was triggered for Benzobicyclon due to a molar extinction coefficient of > 10 L × mol^{-1} × cm^{-1}. The results of the phototoxicity assay in Balb/c 3T3 cells are briefly summarised below in Table 22.

Table 22: Summary table of studies on phototoxicity

Method, guideline, deviations if any	Test substance	Dose levels duration of exposure	Results	Reference
Phototoxicity assay (Neutral red assay) Balb/c 3T3 cells OECD 432 Minor guideline deviation: higher cell number seeded for treatment GLP Acceptable	Benzobicyclon Batch No.: 1L0108 Purity: 99.3%	Pre-experiment: 0, 0.1, 0.2, 0.5, 1, 2, 3.9, 7.8 and 15.6 µg/mL Main experiment: 0, 1.5, 2.1, 2.9, 4.1, 5.7, 8.0, 11.1, 15.6 µg/mL	IC_{50} with/without irradiation: 7.16 µg/mL/12.35 µg/mL PIF: 1.73 MPE: 0.016 IC_{50} with/without irradiation: 9.65 µg/mL/13.84 µg/mL PIF: 1.44 MPE: 0.006 No evidence for a phototoxic potential	[redacted] (2016) (Vol. 3 B.6.2.7 - CA 5.2.7/01) Doc. No.: 547-001

PIF: Photo-Irritancy-Factor
MPE: Mean Phototoxic Effect

Based on the outcome of the phototoxicity assay there was no evidence for a phototoxic potential of Benzobicyclon. The study was considered valid, scientifically acceptable and appropriate for the assessment of the phototoxic potential of Benzobicyclon. No further data from studies performed with Benzobicyclon relevant for this endpoint are available.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Not applicable. Benzobicyclon is a solid.

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

Not applicable.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Not applicable.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Not classified (conclusive but not sufficient for classification).

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

For studies on acute toxicity and acute neurotoxicity please refer to Volume 1 Level 2, section 2.6.2 and 2.6.7 and to Volume 3, section B.6.2 and B.6.7

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

No specific target organ toxicity was observed after a single dose/exposure concentration of Benzobicyclon.
No clinical or other sings of toxicity were noted across the acute toxicity studies. Only in the acute inhalation toxicity study partial closing of the eyes was seen in the Benzobicyclon treated rats. The sign was a non-specific reaction to exposure to a high concentration of dust. Residues of the test substance were visible on the body fur during exposure and immediately thereafter. The rate of body weight gain was marginally reduced in Benzobicyclon treated rats on the day following exposure. The rate of body weight gain was otherwise similar to that of the control rats.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

Based on the outcome of the acute toxicity and acute neurotoxicity studies, where no specific target organ toxicity even in the absence of mortality was observed after single dosing of animals classification of Benzobicyclon for STOT SE according to Regulation (EC) No 1272/2008 is not required.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Not classified (conclusive but not sufficient for classification).

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

The outcomes of the toxicologically relevant and scientifically valid oral and dermal short-term toxicity studies with Benzobicyclon are summarized in Table 23. No repeated dose inhalation toxicity studies were submitted as Benzobicyclon is not regarded to be a volatile substance and reliable oral toxicity studies are available.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 23: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Oral			
90-day subchronic oral toxicity study OECD 409 Minor guideline deviations: T3, T4, TSH were not	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (diet) Males: 0, 20, 100,	NOAEL males: 400 ppm (22.74 mg/kg bw/d), the highest dose tested NOAEL females: 10000 ppm (630 mg/kg bw/d), the highest dose tested	[redacted] (1999) (Vol. 3 B.6.3.2 - CA 5.3.2/01) Doc. No.: 533-002

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
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 GLP Rat, Fischer (F344/[redacted] male/female, 12/group Acceptable	or 400 ppm ales (equivalent to 0, 1.13, 5.73, 22.74 mg/kg bw/d); Females: 0, 100, 400, 2000, or 10000 ppm (equivalent to 0, 6.29, 25.17, 125.9, 630 mg/kg bw/d) 90 days	Major target organs: kidney and liver Renal toxicity in male rats is a consequence of deposition of α-2μ-globulin in renal tubular cells and not relevant to humans	
13-week subchronic toxicity study OECD 409 GLP Dog, Beagle, male/female, 4/group Acceptable	Benzobicyclon Batch No.: 6F0502 Purity: 99% Oral (capsules) 0, 20, 200, 2000 mg/kg bw/d 13 weeks	NOAEL males/females > 2000 mg/kg bw/d No findings clearly attributable to administration of the test substance	[redacted] (1998) (Vol. 3 B.6.3.2 - CA 5.3.2/02) Doc. No.: 533-001
52-week chronic toxicity study OECD 452 GLP Dog, Beagle, male/female, 4/group Acceptable	Benzobicyclon Batch No.: 6F0502 Purity: 99% Oral (capsules) 0, 10, 100, 1000 mg/kg bw/d 52 weeks	NOAEL males/females > 1000 mg/kg bw/d No findings clearly attributable to administration of the test substance	[redacted] (1999) (Vol. 3 B.6.3.2 - CA 5.3.2/03) Doc. No.: 537-001
Dermal			
21-day repeated dose toxicity study OECD 410 GLP Rabbit, NZW ([redacted]:(NZW)SPF), male/female, 10/group Acceptable	Benzobicyclon Batch No.: 1L0108 Purity: 98% Dermal 0, 100, 300, 1000 mg/kg bw/d 21 days	$NOAEL_{systemic/dermal}$ males/females > 1000 mg/kg bw/d No findings clearly attributable to administration of the test substance	[redacted] (2012) (Vol. 3 B.6.3.3 - CA 5.3.3/01) Doc. No.: 532-001

Table 24: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Combined chronic toxicity/carcinogenicity study OECD 453 GLP Rat, Fischer (F344/[redacted] male/female, 50/group Acceptable	Benzobicyclon Batch No.: 6F0502 Purity: 99%	Oral (diet) Males: 0, 0.334, 0.667, 1.696, 3.43 mg/kg bw/d Females: 0, 4.19, 42.2, 427 mg/kg bw/d 24 months	NOAEL for males: 3.4 mg/kg bw/d, the highes dose tested NOAEL for females: 427 mg/kg bw/d, the highest dose tested Major target organs: kidney and liver Renal toxicity in male rats is a consequence of deposition of α-2μ-globulin in renal tubular cells and not relevant to humans. Reported effects in females (decreases in urinary pH, increases in total cholesterol, total protein and globulin associated with increased liver and kidney weights and increases of spleen weights) were not considered toxicologically relevant as they did not progress with exposure duration or were the consequence of a confounding pathological condition not attributable to treatment. No carcinogenic potential was evident in this study	[redacted] (1999) (Vol. 3 B.6.5.1 - CA 5.5/01) Doc. No.: 537-002
Carcinogenicity study OECD 452 Minor deviations: no hematology, clinical biochemistry, urinalysis and ophthalmology GLP Mouse, [redacted]:CD-l, male/female, 50/group Acceptable	Benzobicyclon Batch No.: 6F0502 Purity: 99%	Oral (diet) Males: 0, 37, 373, 3817 mg/kg bw/d Females: 0, 45, 473, 4807 mg/kg bw/d 78 weeks	NOAEL males: 373 mg/kg bw/d NOAEL females: 473 mg/kg bw/d Major target organ: liver (increases both in organ weight in females and in centrilobular hepatocellular hypertrophy in both sexes) and statistical increase in periportal hepatocyte vacuolation in females) No carcinogenic potential was evident in this study	[redacted] (1999) (Vol. 3 B.6.5.1 - CA 5.5/02) Doc. No.: 555-001
Subchronic neurotoxicity OECD 424 GLP Rat, [redacted]CD(SD), male/female, 10/group Acceptable	Benzobicyclon Batch No.: 960108N Purity: 99%	Oral (diet) Males: 0, 61.8, 306.5, 1290.0 mg/kg bw/d Females: 0, 72.3, 373.6, 1498.8 mg/kg bw/d 91 days	NOAEL males: 1290 mg/kg bw/d NOAEL females: 1499 mg/kg bw/d No neurotoxicity as shown by the lack of neurobehavioral and neuropathological effects	[redacted] (2012) (Vol. 3 B.6.7.1.2 - CA 5.7.1/04) Doc. No.: 542-001

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

The available subchronic toxicity studies show that male rats are most sensitive. Renal toxicity observed in male rats after repeated dosing for 90 days (dosed with 0, 20, 100, or 400 ppm for males; 0, 100, 400, 2000, or 10000 ppm for females) is a consequence of deposition of α-2μ-globulin in renal tubular cells. Based on renal toxicity, the NOAEL for male rats was determined to be 20 ppm (equivalent to 1.13 mg/kg bw/d) (see Vol. 3 B6 - Table 2.6.3-25 for incidences and severity grades). It is most likely that the sex difference in renal toxicity is ascribed to α2μ-globulin deposition in the tubular cells which is a sex-specific phenomenon in males. Immuno-histochemical staining confirmed that the major component of the hyaline droplets in the proximal tubular cells was alpha-2μ-globulin. Although the number of samples subject to immune-histochemical staining was low (2 animals from control and high dose males and females) the pattern of kidney lesion (hyaline droplets in proximal tubular cells and severity of granular casts in cystically dilated tubules and papillary mineralisation) is concordant with α2μ-globulin induced nephropathy, specifically in males. This is further supported by the lack of kidney lesions and positive α2μ-globulin staining in females. Overall, the available evidence is in line with the proposed IARC criteria (Swenberg and Lehman-McKeeman, 1999) for rat male specific α2μ-globulin nephropathy and that this MoA is not of human relevance. Considering that the initiating event for this MoA is the ability of a toxicant to bind to the males specific alpha-2μ-globulin (Chaudhuri et al., 1999; Borghoff et al., 1991), leading ultimately to its accumulation, and following a commenting phase with the Co-RMS, the applicant is requested to provide evidence (e.g.: in silico) on potential binding of benzobicyclone and it smajor metabolite(s) to α2μ-globulin.
Focusing on effects unrelated to nephropathy, a no effect level could be established at the highest dose level of 400 ppm (equivalent to 22.74 mg/kg bw/d). The NOAEL for female rats is the highest dose level tested (10000 ppm, equivalent to 630 mg/kg bw/d) since the changes in urinary pH and increases in kidney and liver weight at a lower dose were found to be reversible and were not accompanied by histophathological changes. α-2μ-globulin nephropathy is a species and sex specific finding and not relevant to humans.
A 13-week (dosed with 0, 20, 200 and 2000 mg/kg bw/d) and 52-week (dosed with 0, 10, 100 and 1000 mg/kg bw/d) repeated dose toxicity study in dogs revealed no relevant effects attributable to Benzobicyclon up to the top dose applied and the NOAEL is 2000 or 1000 mg/kg bw/d, respectively.
The dermal NOAEL is 1000 mg/kg bw/d, the highest dose tested in a reliable 21 day dermal toxicity study in rabbits (dosed with 0, 100, 300 and 1000 mg/kg bw/d), showing no test substance related findings.

In a two-generation reproductive toxicity study in rats (dosed with 0, 100, 1000, 20000 ppm). A NOAEL of 100 ppm (7 mg/kg bw/d) for parental toxicity in males was derived based on renal toxicity (α-2μ-globulin nephropathy). α-2μ-globulin nephropathy is a species and sex specific finding and not relevant to humans. Therefore, the relevant parental NOAEL of this 2-generation reproductive toxicity study is considered to be 1000 ppm for males (equivalent to 59.5 mg/kg bw/d; mean of the intake from week 1 to 17), based on the findings in pituitary in the F0 (not significant increase) and F1 generation (significantly increased hydropic degeneration basophilic cells) accompanied with decrease in absolute and relative pituitary weights of F0 and F1. In addition, statistical increase of relative testes and epididymis weights were observed. The applicant performed a non-formal scientific literature search in order to provide information on the postulated MoA. Based on the relevant information retrievied 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. Thereupon, different peripheral findings such as effects on testosterone levels, testis weight, epididymis weight and spermatogenesis can be observed. The RMS is of the opinion that the provided literarure evidence supports the postulated MoA for effects on testes and epididymides (for more details see Vol.1-2.10). The retrived evidence partially/inderectly supports the effect observed in the pituitary (hydropic degeneration basophilic cells), which is anyhow considered plausible. Therefore, since the α2μ-globulin nephropathy is a male rat specific effect without human relevance (ECHA, 2017; IARC, 1999), the effect observed on testis and epididymides weights were not considered for the derivation of the NOAEL for males.
Following a commenting phase with the Co-RMS it is recommended that the applicant further elaborates on the statement: *"increase in the blood α2μ-globulin level in male rats causes increases in blood LH and FSH levels, in testicular and blood testosterone levels, and in the testicular weight"*. It is noted that there are no measurements of α2μ-globulin levels in blood of male rats in the study and this statement should be further substantiated.
The MoA of the male-rat specific α2μ-globulin and its putative contribution to the perturbation of the homeostasis in the hypothalamus and/or the pituitary and how this is related with the observed effects of the study (decreased absolute pituitary weight, increased testicular and epididymal weight) needs to be elucidated. More literature, up to date data are needed to fully address human relevance of observed effects.
For females a NOAEL of 1000 ppm (87.2 mg/kg bw/d) was derived based on increased organ weights (liver, kidney, adrenals) in F0 and F1 (for study results details refere to Vol. 3 B.6.6.1 - CA 5.6.1/01).

In prenatal developmental toxicity studies conducted in rats (dosed with 0, 40, 200, 2000 ppm) and rabbits (dosed with 0, 40, 200, 1000 ppm) and in rabbits (dosed with 0, 111, 333, 1000 mg/kg bw/d) the NOAELs for parental toxicity are 1000 mg/kg bw/d, the highest dose tested

The 2-year combined dietary chronic toxicity/carcinogenicity study in rats (dosed with 0, 10, 20, 50 and 100 ppm in males; 0, 100, 1000 and 10000 ppm in females) revealed renal toxicity manifested as α-2μ-globulin nephropathy in male animals (not relevant for humans) (see Vol. 3 B6 - Table 2.6.3-26 for incidences and severity grades). Focussing on effects unrelated to nephropathy, a no effect level could be established at 100 ppm (3.43 mg/kg bw/d), the highest dose applied to male rats without toxicologically significant findings other than α-2μ-globulin nephropathy. In females, decreases in urinary pH, increases in total cholesterol, total protein and globulin associated with increased liver and kidney weights and increases of spleen weights were observed (see Vol. 3 B6 - Table 2.6.3-27 and Table 2.6.3-28 for details). However, as these changes did not progresss with exposure duration and/or were the consequence of a confounding condition not attributable to treatment, they were not concsedered of toxicological significance. Therefore, the NOAEL for females was set at the highest dose tested of 10000 ppm (427 mg/kg bw/d).

In the carcinogenicity study in mice (dosed with 0, 300, 3000 and 30000 ppm) benzobicyclon also did not show any carcinogenic potential. 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 (see Vol. 3 B6 - Table 2.6.3-12 for incidences and severity grades). The absence of clinical biochemistry analyses, effects on liver were considered as toxicologically relevant. Therefore, a NOAEL of 3000 ppm (dietary concentrations: 373 mg/kg bw/d for males, 473 mg/kg bw/d for females) was derived.
No carcinogenic potential was observed for Benzobicyclon in both species.

In a subchronic neurotoxicity study in rats no neurobehavioral and neuropathological effects were observed up to 20000 ppm (equivalent to 1290 and 1499 mg/kg bw/d in males and females, respectively).

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Besides species and sex specific renal toxicity, related to deposition of α-2μ-globulin in renal tubular cells in male rats, and adaptive changes in liver tissue without evidence of functional hepatic impairment, 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.
Based upon these data, Benzobicyclon is not subject to classification for danger of serious damage to health by prolonged exposure or specific target organ toxicity after repeated exposure (STOT RE) according to Regulation (EC) No 1272/2008.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Not classified (conclusive but not sufficient for classification).

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

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 are summarised in Table 29 and Table 30.
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).
In the chromosomal aberration assay, 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 RMS does not support the Applicant’s view who attributes to cytotoxicity the likely cause of the chromosomal

aberrations. 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) there was no clear evidence of bone marrow exposure, however, information in the ADME study conducted in rats ([redacted] 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 benzobicylone is low. Although the present MNT study was conducted in mice, ADME comparative investigations (B.6.9: [redacted], 2017a, b) on metabolite 1315P-070 (mesotriketone containing the bicycle group obtained after hydrolysis of the phenothio group of benzobicyclone) did not show substantial differences occurring between rat and mouse aspecies. Therefore, the available evidence sustain that a read-across between rats and mice metabolism is possible. In the second study (B.6.4.2/02) evidence of bone marrow exposure was provided by a significant decrease of PCE% when compare to the control group. Although some limitations were observed, none of these were considered severe enought to invalidate the results of this supplementary study.
Overall, based on a weight of evidence approach the two in vivo MNT test in mice were considered adequate to conlude on the negative outcome for structural and numerical chromosomal aberrations potential of benxzobicyclone.

There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 29: 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	[redacted] (1994a) (Vol. 3 B.6.4.1 - CA 5.4.1/01) Doc. No.: 557-008
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	[redacted] (1994b) (Vol. 3 B.6.4.1 - CA 5.4.1/02) Doc. No.: 557-007
Chromosomal aberration assay OECD 473 Minor guideline deviations: lower metaphase number (200 vs 300)	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:	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).	[redacted] (1996) (Vol. 3 B.6.4.1 - CA 5.4.1/03) Doc. No.: 557-009

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
evaluated, different method of cytotoxicity determination, lack of cytotoxicity data in the cytogenetic experiments, lack of historical control data GLP Acceptable		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		
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	[redacted] (2016) (Vol. 3 B.6.4.1 - CA 5.4.1/04) Doc. No.: 557-011

Table 30: 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	Benzobicyclon Batch No.: 1L0108 Purity: 99.3%	Single oral application (gavage) Doses selected based on dose range finding	Negative Exposure of target tissue was demonstrated in the study by [redacted] (1999; KCA 5.1.1/01, Doc. No. 512-001)	[redacted] (2016) (Vol. 3 B.6.4.2 - CA 5.4.2/01) Doc. No.: 557-012

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Mouse (NMRI), male, 7/group Acceptable		study (maximum tolerable dose 2000 mg/kg bw) 24 hours: 0, 500, 1000, 2000 mg/kg bw oral 48 hours: 2000 mg/kg bw		
Micronucleus assay in bone marrow OECD 474 GLP Mouse (CD-1 [redacted] male, 6/group Supplementary to [redacted] (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 Exposure of target tissue was demonstrated by decrease in PCE%	[redacted] (1996b) (Vol. 3 B.6.4.2 - CA 5.4.2/02) Doc. No.: 557-010

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

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 are summarised in the table below.

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).

In the chromosomal aberration assay, 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 RMS does not support the Applicant's view who attributes to cytotoxicity the likely cause of the chromosomal aberrations. 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) 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 ([redacted] 1999; CA 5.1.1/01, Doc No 512-001), showed that radioactivity was widely sistributed and reached the bone marrow, although the bioavailability of benzobicylone is low. Although the present MNT study was conducted in mice, ADME comparative investigations (B.6.9: [redacted], 2017a, b) on metabolite 1315P-070 (mesotriketone containing the bicycle group obtained after hydrolysis of the phenothio group of benzobicyclone) did not show substantial differences occurring between rat and mouse aspecies. Therefore, the available evidence sustain that a read-across between rats and mice metabolism is possible. In the second study (B.6.4.2/02) 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 2.6.4-31). Although some limitations were observed (1000 PCE/animal examined and no HC data), none of these were considered severe enought to invalidate the results of this supplementary study.
Overall, based on a weight of evidence approach the two in vivo MNT test in mice were considered adequate to conlude on the negative outcome for structural and numerical chromosomal aberrations potential of benxzobicyclone.
A photomutagenicity study would be triggered since the molar extinction coefficient of Benzobicyclon is > 1000 $L \times mol^{-1} \times cm^{-1}$. However, based on the outcome of the phototoxicity study, which showed that Benzobicyclon did not possess any phototoxic potential, the Applicant states that Benzobicyclon does not need to be tested for photomutagenicity, since phototoxicity is known to correlate with photomutagenicity. Therefore, only phototoxic substances display photomutagenicity. Furthermore, the RMS relies also on the EFSA "Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology" (EFSA supporting publication 2016:EN-1074. 24 pp.) where it is reported the following "The UK Committee on Mutagenicity (2013) recommends that if there is a negative response from the phototoxicity test, no photomutagenicity test is required; in the case the test is positive, no specific guidance is provided." Therefore, no photomutagenicity testing is considered necessary at this time.
In conclusion, Benzobicyclon shows clastogenic and aneugenic effects only in one *in vitro* test system (chromosomal aberration assay), while other *in vitro* test systems showed no genotoxic/mutagenic potential of Benzobicyclon. Furthermore, there is no evidence for a clastogenic and/or aneugenic potential of Benzobicyclon *in vivo* based on a study using somatic cells of mice with proof of target cell exposure.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

Benzobicyclon showed clastogenic and aneugenic effects only in one *in vitro* test system (chromosomal aberration assay under cytotoxic conditions), while other *in vitro* test systems showed no genotoxic/mutagenic potential of Benzobicyclon. Furthermore, there is no evidence for a clastogenic and/or aneugenic potential of Benzobicyclon *in vivo* based on a study using somatic cells of mice with proof of target cell exposure. Considering the weight of evidence from *in vitro* and *in vivo* tests, Benzobicyclon is not genotoxic and not considered to trigger classification for genotoxicity according to Regulation (EC) No 1272/2008.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Not classified (conclusive but not sufficient for classification).

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

The outcomes of the toxicologically relevant and scientifically valid long-term toxicity and carcinogenicity studies with Benzobicyclon in rats and mice are summarized in Table 32.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 32: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Combined chronic toxicity/carcinogenicity study OECD 453	Benzobicyclon Batch No.: 6F0502 Purity: 99%	NOAEL for males: 3.4 mg/kg bw/d, the highes dose tested NOAEL for females: 427 mg/kg bw/d, the highes dose tested	[redacted] (1999) (Vol. 3 B.6.5.1 - CA 5.5/01) Doc. No.: 537-002

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
GLP Rat, Fischer (F344/[redacted] male/female, 50/group Acceptable	Oral (diet) Males: 0, 0.334, 0.667, 1.696, 3.43 mg/kg bw/d Females: 0, 4.19, 42.2, 427 mg/kg bw/d 24 months	Major target organs: kidney and liver (see Vol. 3 B6 - Table 6.5.1 18 for incidences and severity grades) Renal toxicity in male rats is a consequence of deposition of α-2μ-globulin in renal tubular cells and not relevant to humans. Reported effects in females (decreases in urinary pH, increases in total cholesterol, total protein and globulin associated with increased liver and kidney weights and increases of spleen weights) were not considered toxicologically relevant as they did not progress with exposure duration or were the consequence of a confounding pathological condition not attributable to treatment. No carcinogenic potential was evident in this study	
Carcinogenicity study OECD 452 Deviations: no hematology, clinical biochemistry, urinalysis and ophthalmology GLP Mouse, [redacted]CD-1, male/female, 50/group Acceptable	Benzobicyclon Batch No.: 6F0502 Purity: 99% Oral (diet) Males: 0, 37, 373, 3817 mg/kg bw/d Females: 0, 45, 473, 4807 mg/kg bw/d 78 weeks	NOAEL males: 373 mg/kg bw/d NOAEL females: 473 mg/kg bw/d Major target organ: liver (increases both in organ weight in females and in centrilobular hepatocellular hypertrophy in both sexes) and statistical increase in periportal hepatocyte vacuolation in females) (see Vol. 3 B6 - Table 6.5.2-4 and 12 for incidences and severity grades) No carcinogenic potential was evident in this study	[redacted] (1999) (Vol. 3 B.6.5.1 - CA 5.5/02) Doc. No.: 555-001

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

The 2-year combined dietary chronic toxicity/carcinogenicity study in rats (dosed with 0, 10, 20, 50 and 100 ppm in males; 0, 100, 1000 and 10000 ppm in females) revealed renal toxicity in males (hyaline droplet deposition in the proximal tubular cel and chronic nephropathy).
Benzobicyclone was recognized to produce α2μ-globulin nephropathy in male rats in the previous 90-day subchronic toxicity study in which immuno-histochemical staining confirmed that the major component of the hyaline droplets in the proximal tubular cells was alpha-2μ-globulin. Although the number of samples subject to immune-histochemical staining was low (2 animals from control and high dose males and females) the pattern of kidney lesion (hyaline droplets in proximal tubular cells and severity of granular casts in cystically dilated tubules and papillary mineralisation) is concordant with α2μ-globulin induced nephropathy, specifically in males. This is further supported by the lack of kidney lesions and positive α2μ-globulin staining in females. Overall, the avialable evidence is in line with the proposed IARC criteria (Swenberg and Lehman-McKeeman, 1999) for rat male specific α2μ-globulin nephropathy and that this MoA is not of human relevance. Therefore, it is most likely that renal toxicity observed only in males in the two year rat study is ascribed to α2μ-globulin deposition in the tubular cells which is a sex-specific phenomenon in males. Considering that the initiating event for this MoA is the ability of a

toxicant to bind to the males specific alpha-2µ-globulin (Chaudhuri et al., 1999; Borghoff et al., 1991), leading ultimately to its accumulation, and following a commenting phase with the Co-RMS the applicant is requested to provide evidence (e.g.: in silico) on potential binding of benzobicyclone and it smajor metabolite(s) to α2µ-globulin.
Focussing on effects unrelated to nephropathy, a no effect level could be established at 100 ppm (3.43 mg/kg bw/d), the highest dose applied to male rats without toxicologically significant findings other than α-2µ-globulin nephropathy. In females, decreases in urinary pH, increases in total cholesterol, total protein and globulin associated with increased liver and kidney weights and increases of spleen weights were observed (see Vol. 3 B6 - Table 2.6.5-33 and Table 2.6.5-34 for details). However, as these changes did not progresss with exposure duration and/or were the consequence of a confounding condition not attributable to treatment, they were not concsedered of toxicological significance. Therefore, the NOAEL for females was set at the highest dose tested of 10000 ppm (427 mg/kg bw/d).
In the carcinogenicity study in mice (dosed with 0, 300, 3000 and 30000 ppm) benzobicyclon also did not show any carcinogenic potential. 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 (see Vol. 3 B6 - Table 6.5.2 12 for incidences and severity grades). The absence of clinical biochemistry analyses, effects on liver were considered as toxicologically relevant. Therefore, a NOAEL of 3000 ppm (dietary concentrations: 373 mg/kg bw/d for males, 473 mg/kg bw/d for females) was derived.
No carcinogenic potential was observed for Benzobicyclon in both species.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

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.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Not classified (conclusive but not sufficient for classification).

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

The outcomes of the toxicologically relevant and scientifically valid reproductive toxicity studies with Benzobicyclon are summarized in Table 35.

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

The outcome of the toxicologically relevant and scientifically valid two-generation reproductive toxicity study with Benzobicyclon is summarized in Table 35.

The outcomes of the toxicologically relevant and scientifically valid reproductive toxicity studies with Benzobicyclon are summarized in the table below.
The potential toxicity of Benzobicyclon on reproduction was investigated in a two-generation reproductive toxicity study in rats (dosed with 0, 100, 1000, 20000 ppm). A NOAEL of 100 ppm (7 mg/kg bw/d) for parental toxicity in males was derived based on renal toxicity (α-2µ-globulin nephropathy). α-2µ-globulin nephropathy is a species and sex specific finding and not relevant to humans. Therefore, the relevant parental NOAEL of this 2-generation reproductive toxicity study is considered to be 1000 ppm for males (equivalent to 59.5 mg/kg bw/d; mean of the intake from week 1 to 17), based on the findings in pituitary in the F0 (not significant increase) and F1 generation (significantly increased hydropic degeneration basophilic cells) accompanied with decrease in absolute and relative pituitary weights of F0 and F1. In addition, statistical increase of relative testes and epididymis weights were observed. The applicant performed a non-formal scientific literature search in order to provide information on the postulated MoA. Based on the relevant information retrievied 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. Thereupon, different peripheral findings such as effects on testosterone levels, testis weight, epididymis weight and spermatogenesis can be observed. The RMS is of the opinion that the provided literarure evidence supports the postulated MoA for effects on testes and epididymides (for more details see Vol.1-2.10). The retrived evidence partially/inderectly supports the effect observed in the pituitary (hydropic degeneration basophilic cells), which is anyhow considered plausible. Therefore, since the α2μ-globulin nephropathy is a male rat specific effect without human relevance (ECHA, 2017; IARC, 1999), the effect observed on testis and epididymides weights were not considered for the derivation of the NOAEL for males.
Following a commenting phase with the Co-RMS it is recommended that the applicant further elaborates on the statement: *"increase in the blood α2μ-globulin level in male rats causes increases in blood LH and FSH levels, in testicular and blood testosterone levels, and in the testicular weight"*. It is noted that there are no measurements of α2μ-globulin levels in blood of male rats in the study and this statement should be further substantiated.
The MoA of the male-rat specific α2μ-globulin and its putative contribution to the perturbation of the homeostasis in the hypothalamus and/or the pituitary and how this is related with the observed effects of the study (decreased absolute pituitary weight, increased testicular and epididymal weight) needs to be elucidated. More literature, up to date data are needed to fully address human relevance of observed effects.
For females a NOAEL of 1000 ppm (87.2 mg/kg bw/d) was derived based on increased organ weights (liver, kidney, adrenals) in F0 and F1. The reproductive performance and fertility were not affected by the treatment with the test substance (NOAEL 20000 ppm, equivalent to 1250 mg/kg bw/d in males and 1779 mg/kg bw/d in females. No toxicity to offspring was observed in this study NOAEL 20000 ppm (equivalent to 1779 mg/kg bw/d) (for study results details refer to Vol. 3 B.6.6.1 - CA 5.6.1/01).

Benzobicyclon is not considered to trigger classification for reproductive toxicity according to Regulation (EC) No 1272/2008.

There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 35: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
Two-generation reproductive toxicity OECD 416 Minor deviation: dose setting, for the dietary studies the dose interval should be not more than 3 fold GLP Rat, [redacted]CD (SD), male/female, 24/group Acceptable	Benzobicyclon Batch No.: 6F0502 Purity: 99% Oral (diet) F0 males/ females: 0, 5.65/8.44, 56.1/85.4, 1176/1741 mg/kg bw/d F1 males/ females: 0, 6.46/8.76, 62.8/89.0, 1324/1817 mg/kg bw/d	Parental toxicity: NOAEL F0/F1 males: 59.5 mg/kg bw/d (mean of the intake from week 1 to 17) based on slight effects on the pituitary reaching statistical significance in F1 males (microscopic findings pituitary) NOAEL F0/F1 females: 87.2 mg/kg bw/d (mean of the intake from week 1 to LD 21) based on increased organ weights (liver, kidney, adrenals) in F0 and F1. Reproduction (fertility) toxicity: NOAEL F0/F1 males: 1250 mg/kg bw/d NOAEL F0/F1 females: 1779 mg/kg bw/d reproductive performance and fertility were not affected Offspring toxicity: NOAEL 1515 mg/kg bw/d no relevant toxicity to offspring	[redacted] (1999) (Vol. 3 B.6.6.1 - CA 5.6.1/01) Doc. No.: 553-001

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

The potential toxicity of Benzobicyclon on reproduction was investigated in a two-generation reproductive toxicity study in rats (dosed with 0, 100, 1000, 10000 ppm). No reproductive effects were also observed. The reproductive NOAEL is 20000 ppm for males and females, equivalent to 1515 mg/kg bw/d.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

Benzobicyclon is considered not to trigger classification for reproductive toxicity/effects on fertility according to Regulation (EC) No 1272/2008 since neither effects on sexual function nor on fertility were observed in a two-generation reproductive toxicity study.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

The outcome of the toxicologically relevant and scientifically valid developmental toxicity studies with Benzobicyclon in rats and rabbits are summarized in Table 36; for more details on studies and their results see Vol. 3 B.6.6.2.

The prenatal developmental toxicity was investigated in rats (dosed with 0, 40, 200, 2000 ppm) and rabbits (dosed with 0, 40, 200, 1000 ppm). In rat species, neither maternal toxicity nor embryotoxicity or fetotoxicity were observed. In addition, no teratogenicity was observed. Therefore, the NOAEL (maternal/offspring/teratogenicity) is 1000 mg/kg bw/d for rat species (for more details refer to Vol. 3 B.6.6.2. - CA 5.6.2/01).
In a teratogenicity study in rabbits (dosed with 0, 40, 200 and 1000 mg/kg bw/d) incidental deaths or unscheduled sacrifices for human reasons were observed in all study groups including the control. The maternal mortality in the study was greater than 10 % and was more represented in control does. The high mortality from unknown causes questions the health conditions of the rabbits. A number of skeletal abnormalities apparently ascribable to HPPD inhitor MOA were reported in all groups including control, althought incidences were higher in treated groups. However, 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.
Therefore, this developmental toxicity study conducted with rabbits is considered unacceptable (for more details refer to Vol. 3 B.6.6.2. - CA 5.6.2/02).
In a subsequent ~~an~~ adequate teratogenicity study in rabbits (dosed with 0, 111, 333, 1000 mg/kg bw/d) was performed showed neither maternal toxicity nor embryotoxicity/fetotoxicity and no teratogenicity up to the highest applied dose. An apparent dose related non statistically significant increased number of litters with foetuses with the minor abnormality astragalus, uni-or bilateral not ossified was observed. 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.
Consequently, the NOAEL for maternal toxicity is 1000 mg/kg bw/d, the highest dose tested. The NOAEL for embryo-foetal toxicity is 1000 mg/kg bw/d, the highest dose tested (for more details refer to Vol. 3 B.6.6.2. - CA 5.6.2/04).

There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 36: Summary table of animal studies on adverse effects on development

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
Developmental toxicity OECD 414 Minor deviations: no endocrine related measurements, exposure from gestation day 6 to 15 GLP Rat, [redacted]CD (SD)[redacted] female, 25/group Acceptable	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (gavage) 0, 40, 200, 1000 mg/kg bw/d 10 days	$NOAEL_{maternal}$: 1000 mg/kg bw/d no maternal toxicity $NOAEL_{developmental}$: 1000 mg/kg bw/d no developmental toxicity	[redacted] (1997) Doc. No.: 551-002 Vol. 3 B.6.6.2. - CA 5.6.2/01
Developmental toxicity OECD 414 Deviations: 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.GLP Rabbit, New Zealand White, female, 18 - 21/group Not acceptable	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (gavage) 0, 40, 200, 1000 mg/kg bw/d 13 days	Not determined	[redacted] (1998) Doc. No.: 551-001 Vol. 3 B.6.6.2. - CA 5.6.2/02
Developmental toxicity OECD 414 GLP Rabbit, New Zealand White, female, 22/group Acceptable	Benzobicyclon Batch No.: 1A0709 Purity: 99.2% Oral (gavage) 0, 111, 333, 1000 mg/kg bw/d 13 days	$NOAEL_{maternal}$: 1000 mg/kg bw/d no maternal toxicity $NOAEL_{developmental}$: 1000 mg/kg bw/d	[redacted] (2022) Doc. No.: 551-006 Vol. 3 B.6.6.2. - CA 5.6.2/04

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

The prenatal developmental toxicity was investigated in rats and rabbits dosed with 0, 40, 200 or 1000 mg/kg bw/d. In both species, neither maternal toxicity nor embryotoxicity or fetotoxicity were observed. In addition, no teratogenicity was observed in both species. Therefore, the NOAEL (maternal/offspring/teratogenicity) is 1000 mg/kg bw/d.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

There were no effects developmental toxicity and thus Benzobicyclon is considered not to trigger classification for reproductive toxicity/effects on development according to Regulation (EC) No 1272/2008.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

For summary on two-generation reproductive toxicity study please refer to section 2.6.6.1.

2.6.6.3.1 2.6.6.3.1 Short summary and overall relevance of the provided information on 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.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

There were no effects to warrant classification of Benzobicyclon for effects on or via lactation according to Regulation (EC) No 1272/2008.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Not classified (conclusive but not sufficient for classification).

2.6.7 Summary of neurotoxicity

Benzobicyclon was assessed for its neurotoxic potential in three non-guideline acute neurotoxicity studies in rats or cats and a subchronic neurotoxicity study in rats. The results are summarised in Table 37.
There are no data indicative of Benzobicyclon related adverse effects (health problems) regarding this endpoint in humans (see section 2.6.9).

Table 37: Summary table of animal studies on neurotoxicity

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 at LOAEL	Reference
Acute			
Irwin dose range finding study No guideline available GLP Mouse, [redacted] CD-1, male, 4/group Acceptable for supportive information	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (gavage) 0, 78.1, 312.5, 1250, 5000 mg/kg bw	NOAEL > 5000 mg/kg bw No functional changes (Irwin profile) The reported decreased rectal temperature was not considered of particular toxicological/biological significance.	[redacted] (1997a) (Vol. 3 B.6.7.1.1 - CA 5.7.1/01) Doc. No.: 531-001
Slant test No guideline available GLP Mouse, [redacted] CD-1, male, 5/group Acceptable for	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (gavage) 0, 200, 1000, 5000 mg/kg bw	NOAEL > 5000 mg/kg bw No effect on muscle atonic activity	[redacted]. (1997b) (Vol. 3 B.6.7.1.1 - CA 5.7.1/02) Doc. No.: 541-

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 at LOAEL	Reference
supportive information			002
Effects on autonomic nervous system No guideline available GLP Cat, female, 3/group. Acceptable for supportive information	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (gavage) 0, 2000 mg/kg bw	NOAEL > 2000 mg/kg bw No effect on autonomic functions	[redacted] (1997) (Vol. 3 B.6.7.1.1 - CA 5.7.1/03) Doc. No.: 541-001
Subchronic			
Subchronic neurotoxicity OECD 424 GLP Rat, [redacted]CD(SD), male/female, 10/group Acceptable	Benzobicyclon Batch No.: 960108N Purity: 99% Oral (diet) Males: 0, 61.8, 306.5, 1290.0 mg/kg bw/d Females: 0, 72.3, 373.6, 1498.8 mg/kg bw/d 91 days	NOAEL males: 1290 mg/kg bw/d NOAEL females: 1499 mg/kg bw/d No neurotoxicity as shown by the lack of neurobehavioral and neuropathological effects	[redacted] (2012) (Vol. 3 B.6.7.1.2 - CA 5.7.1/04) Doc. No.: 542-001 ~~KCA 5.7.1/04~~

Three non-guideline studies which assessed selected endpoints of neurotoxicological relevance after single bolus administration of Benzobicyclon are summarised above. These studies showed no statistically significant changes in gross behaviour, but a statistically significant decrease in rectal temperatures in doses between 312.5 - 5000 mg/kg bw ([redacted] (1997a); CA 5.7.1/01) was reported. The variations observed for body temperature were not considered of particular toxicological significance. No statistically significant effect on muscle tone ([redacted] (1997b)) and no effect in autonomic functions in the anaesthetised cat, as determined by arterial blood pressure and heart rate ([redacted] (1997)) were observed. None of the studies revealed an indication for Benzobicyclon-related neurotoxicity.
Benzobicyclon administered in the diet for 13 weeks to rats at concentrations up to 20000 ppm did not result in neurotoxicity as shown by the lack of neurobehavioral and neuropathological effects.
Therefore, Benzobicyclon is not neurotoxic and not subject to classification and labelling according to Regulation (EC) No 1272/2008.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Metabolite 1315P-070

1315P-070 occurs as a metabolite in rat and plant metabolism. It is formed by cleavage of the thiophenyl group from the bicyclooctene moiety and is an inhibitor of 4-hydroxyphenylpyruvate dioxygenase (HPPD). HPPD is a key enzyme in the catabolism of tyrosine in mammals. Inhibition of HPPD results in elevated blood tyrosine levels (tyrosinemia) and associated secondary toxic effects, such as ocular opacity and keratitis, liver and kidney findings, and skeletal abnormalities in developmental toxicity studies with marked differences in response among different species. The rat has been shown to be unusually sensitive compared to other species including humans for toxic effects related to HPPD inhibition. Unlike humans (and mice), rats (as well as dogs and rabbits) have a very low activity of tyrosine aminotransferase (TAT), an efficient alternative metabolic process to HPPD-mediated catabolism for clearing excess tyrosine.

An *in vitro* HPPD enzyme inhibition assay was performed with 1315P-070 and submitted to EPA for evaluation. It was shown that 1315P-070 is a HPPD enzyme inhibitor with a median IC_{50} value of 0.348 µM. The study report is provided (see KCA 5.8.1/29) but no detailed study summary was prepared.
In an *in vivo* comparative plasma tyrosine study higher plasma tyrosine levels were observed in rats than in mice after oral administration of the metabolite, in agreement with the greater toxicity observed in rats versus mice after dosing with the metabolite.
The limited toxicity of 1315P-070 in mice compared to rats is not a consequence of significantly different ADME in the species. Comparative data on absorption, distribution and excretion in rats and mice show a rapid absorption of the metabolite. Elimination half-life was longer for mice than for rats and C_{max} and AUC were higher in mice than in rats. High oral bioavailability of 1315P-070 was seen in both species and some evidence of enterohepatic recirculation was shown in mice but not in rats. Tissue distribution was comparable in rats and mice. 1315P-070 was extensively excreted via the urine in both species. 1315P-070 was poorly metabolised in both species with higher metabolite amounts in mouse urine than in rat urine.
A comparative mechanistic *in vitro* study was conducted in hepatocytes of different species ([redacted] 2019b; CA 5.8.1/19). The aim was to assess the ability of the different species to excrete tyrosine (which is constantly coming in via the diet *in vivo*) when HPPD as the normal pathway is inhibited. When HPPD is inhibited, the liver cells convert tyrosine to 4-hydroxyphenyl pyruvic acid (HPPA) via TAT, so HPPA in cell culture media is a direct marker of TAT activity. TAT activity is the rate limiting step, as HPPA can be excreted *in vivo* via the urine and is the key to the different capacities of species to deal with tyrosine (Lewis and Botham, 2013; CA 5.8.1/35, Doc. No. 592-011[1]).
Hepatocytes from rats, dogs, rabbits, mice and humans were treated with 1315P-070 (and the strong pharmacological HPPD-inhibitor nitisinone as a positive control). Substantially higher levels of HPPA in this *in vitro* study demonstrated that rats, dogs and rabbits had low capacity to deal with excess tyrosine, whereas mice and humans showed high capacity.
An acute toxicity study in rats shows that 1315P-070 is not acutely toxic (LD_{50} > 5000 mg/kg bw). In clinical observation, decrease in spontaneous motor activity was observed in both sexes. This sign began to appear 1 hour after administration and disappeared by Day 1. During the 14-day observation period, no deaths occurred in either sex. All males and females favourably gained body weight. No macroscopic abnormalities were observed in any animal of either sex at the final necropsy (Day 14 after administration).
In a 90-repeated dose toxicity study in rats, dosed at 0, 5, 20, or 400 ppm for males (0.3005, 1.205 and 24.47 mg/kg bw/d) and 0, 20, 400, or 2000 ppm for females (1.345, 27.79 and 137.8 mg/kg bw/d) typical tyrosinemia-related ocular effects (corneal opacity, vascularisation) were observed. The NOAEL was determined at 5 ppm (0.3005 mg/kg bw/d) for males based on corneal opacity, increased albumin and A/G ratio, increased absolute and relative liver weight, increased incidence of centrilobular hepatocellular swelling and of tubular basophilic change. The NOAEL for females is 20 ppm (1.345 mg/kg bw/d) based on opacity of the eye, opthalmology findings, soiled fur, urinalysis, haemathology and blood chemistry observations, increased absolute and relative liver weight, and relative kidney weight, and histopathological adverse findings in the eyes (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/04).
Whereas, in a ~~mouse~~ 90-day repeated dose toxicity study in mouse dosed at 0, 175, 1050 and 6300 ppm (males: 25.81, 152.16 and 894.12 mg/kg bw/d; females: 29.05, 178.63 and 1026.58 mg/kg bw/d) no evidence of ocular effects was found up to the guideline recommended limit dose. The NOAEL was 6300 ppm (894.12 mg/kg bw/d) for male mice and 1050 ppm (178.63 mg/kg bw/d) for female mice, based on the statistical significant marked increases of absolute and relative liver weights observed in the female mice of the top dose group (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/05).
This confirms the picture seen in the comparative *in vitro* mechanistic study (Vol. 3 B.6.8.1.2 - CA 5.8.1/19) and supports that TAT activity is the key to species sensitivity. In this study, significant increases in HPPA were observed in all species of hepatocytes after treatment with 200 µM of the test substance or positive control (NTBC). Moreover, quantitative differences across hepatocytes from different species were observed. The greatest of HPPA were observed in mice and human hepatocytes, either with 1315P-070 or the positive control, while the other species showed substantially lower levels, thus, indicating high TAT activity in mice and humans. The elevated levels of HPPA in human and mouse hepatocytes suggests their comparatively greater ability to clear excess of tyrosine as compared to the other tested species (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/19).

In a bacterial mutation assay in Salmonella typhimurium TA 100, TA 1535, TA 98, TA 1537 and Escherichia coli WP2 uvrA, 1315P-070 was tested up to concentrations of 5000 µg/plate with and without metabolic activation (S9 mix). No relevant increase in the number of revertanat colonies was observed in the pre-test and main test in any

[1] Lewis R.W. & Botham J.W. (2013). A review of the mode of toxicity and relevance to humans of the triketone herbicide 2-(4-methylsulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione. Critical Reviews in Toxicology, 43:3, 185-199

strain at any concentration with or without S9 mix. The positive controls showed marked mutagenic effects, thus demonstrating the functioning of the metabolic activation system and the validity of the assay.
It is concluded that 1315P-070 is non-mutagenic to bacteria under the conditions used in this experiment in the absence and presence of metabolic activation (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/06).

In clastogenicity assay in human lymphocytes *in vitro* in the absence of metabolic activation 1315P-070 was tested up to 2000 µg/mL with metabolic activation for an exposure period 4 hours and without metabolic activation for an exposure period of 4 and 22 hours. 1315P-070 induced structural chromosomal aberrations in human lymphocytes *in vitro* in the absence of metabolic activation when tested up to cytotoxic and/or the highest required concentration (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/07).

In a mammalian gene mutation assay on mouse lymphoma cells (L5178Y) 1315P-070 was tested up to 2000 µg/mL with and without metabolic activation for an exposure period 4 hours and without metabolic activation for an exposure period of 4 and 24 hours. the test item did not induce gene mutations at the mouse lymphoma thymidine kinase locus using the cell line L5178Y (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/08).

In an in vivo MNT, the potential of 1315P-070 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse [redacted] (CD-1®) at doses of 0, 250, 500 and 1000 mg/kg bw at the 24 hour preparation interval, and at the high dose of 1000 mg/kg bw at the 48 hour preparation interval. In the range finding test bioanalysis of bone marrow showed evidence of test substance absorption and therefore justification to proceed with the main test. Furthermore, in the comparative ADME studies of [redacted] (2017a and 2018) it was shown that 1315P-070 is extensively and efficiently absorbed in both rat and mouse species. There was no evidence of any significant increases in the incidence of micronucleated PCE in animals dosed with 1315P-070 when compared to the vehicle control group. The positive control group showed a marked increase in the incidence of micronucleated PCE hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test. It is concluded that 1315P-070 did not induce micronuclei as determined by the *in vivo* micronucleus test with bone marrow cells of the mouse (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/09).
Overall, comprehensive genotoxicity data package revealed no mutagenic potential in bacteria and mammalian cells *in vitro*, but clastogenic *in vitro* in the chromosome aberration assay. At higher tier in vivo, no clastogenic or aneugenic potential was observed (mouse bone marrow micronucleus assay with proof of exposure of the target cell). Therefore, 1315P-070 is considered non-genotoxic.

A developmental toxicity study was performed in mice and showed no treatment-related fetal malformations or fetal lethality. In this study, 1315P-070 was administered by gavage to four groups of 24 sexually mature and pregnant [redacted]CD1([redacted] female mice at dose levels of 0, 100, 300 and 1000 mg/kg bw/d once daily for 12 consecutive days from day 6 to 17 of presumed gestation (DGs 6 through 17). The NOAEL for maternal toxicity was 300 mg/kg bw/d based on the reductions in maternal body weight gains and gravid uterine weights. The developmental NOAEL is also 300 mg/kg bw/d. Developmental delays, consisting of reduced fetal body weights, an increase in the incidence of bipartite ossification of the sternebrae, asymmetric sternebrae, skull misshapen occipital and frontal incomplete ossification and delays in ossification, occurred at the 1000 mg/kg bw/d dose level. There were no treatment-related fetal malformations or embryo/fetal lethality at doses as high as 1000 mg/kg bw/d (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/10).

Due to the investigations on species sensitivity, this study should be considered as key study in the safety evaluation of 1315P-070, since mouse was demonstrated to be the species most relevant for human safety.

A guideline compliant developmental toxicity study with 1315P-070 in rabbits (Vol. 3 B.6.8.1.2 - CA 5.8.1/33) has been performed and showed only transient decreases in food consumption of does. The treatment with 1315P-070 revealed a substantial increase in plasma tyrosine levels at the highest dose applied (400 mg/kg bw/d) indicative of test substance-related tyrosinemia. A dose of 800 mg/kg bw/d led to substantial maternal toxicity in a preceding dose-range finding study. Tyrosine, as a consequence of HPPD-inhibition, is the actual toxicant in sensitive species such as rat, dog and rabbit (Lewis and Botham, 2013; CA 5.8.1/35, Doc. No. 592-011). Hence, in the case of rabbit developmental toxicity, tyrosinemia is the key marker for maternal toxicity. The maternal NOAEL was 150 mg/kg bw/d based on elevated tyrosine plasma levels and increased minimal histopathology finidings in the kidney and liver. The NOAEL for embryo-foetal developmental toxicity was also 150 mg/kg bw/d based on low incidences (limited to 2 fetuses in total in 2 litters only) of skeletal malformations (fused costal cartilage, branched costal cartilage, fused rib, branched rib, detached rib and thoracic vertebra absent) (for study results details refer to Vol. 3 B.6.8.1.2 - CA 5.8.1/33). It is important to note that the total incidence of

malformations and variations did not increase with dose. Developmental skeletal effects can be observed in rats, rabbits and mice after treatment with HPPD inhibitors with rats and rabbits being sensitive, but not mice (Yozzo and Perron, 2020[2]). This aligns with the mechanistic results on TAT activity, as well as the general toxicological findings across repeated dose toxicity studies. The evaluation of the available developmental toxicity data of HPPD inhibiting substances by Yozzo and Perron (2020) showed that fetal skeletal changes in rabbits were correlated with elevated plasma tyrosine concentrations. The observed effects are strictly limited to concentrations proven to elevate tyrosine levels, *i.e.* to cause clinical tyrosinemia; hence they are considered secondary in nature and only the data from dose levels not overloading the capacity to clear tyrosine should be considered relevant in the context of classification and labelling.

Table 38: Summary table of toxicity studies with metabolite 1315P-070

Parameter [Reference]		Species/ Model	Outcome
ADME			
Comparative ADME	[redacted] (2017a) Doc. No. 035712-1 (563-001) (Vol. 3 B.6.8.1.2 - CA 5.8.1/01) Acceptable	Rats and mice	Rapid absorption in mice and rats. Elimination half-life was longer and bioavailability was higher in mice. Tissue distribution was comparable in rats amd mice.
Acute toxicity			
Oral	[redacted (1996) Doc. No. 521-008] (Vol. 3 B.6.8.1.2 - CA 5.8.1/03) Acceptable	Rat	LD_{50}: > 5000 mg/kg bw
Subchronic toxicity			
Oral	[redacted (1999c) Doc. No. 533-003] (Vol. 3 B.6.8.1.2 - CA 5.8.1/04) Acceptable	Rat	NOAEL: m: 5 ppm 0.3 mg/kg bw/d f: 20 ppm 1.3 mg/kg bw/d LOAEL: m: 20 ppm 1.2 mg/kg bw/d corneal opacity, increased albumin and A/G ratio, increased absolute and relative liver weight, increased incidence of centrilobular hepatocellular swelling and of tubular basophilic change. f: 400 ppm 27.8 mg/kg bw/d opacity of the eye, opthalmology findings, soiled fur, urinalysis, haemathology and blood chemistry observations, increased absolute and relative liver weight, and relative kidney weight, and histopathological adverse findings in the eyes.
Oral	[redacted (2018) Doc. No. 563-003] (Vol. 3 B.6.8.1.2 - CA 5.8.1/05) Acceptable	Mouse	NOAEL: 6300 ppm m: 894.12 mg/kg bw/d f: 178.63 mg/kg bw/d LOAEL: f: 1026.58 mg/kg bw/d statistical significant increases of absolute and relative liver weights
Genotoxicity			
Mutagenicity	[redacted (1996a) Doc. No. 557-001]	Ames	Negative (with and without metabolic activation)

[2] Yozzo, K. and Perron, M. (2020). Memorandum – HPPD Inhibiting Herbicides: State of the Science. US EPA DP Barcode D439367

Parameter [Reference]		Species/ Model	Outcome
	(Vol. 3 B.6.8.1.2 - CA 5.8.1/06) Acceptable		
Mutagenicity	[redacted (2016) Doc. No. 557-014] (Vol. 3 B.6.8.1.2 - CA 5.8.1/08) Acceptable	MLA	Negative (with and without metabolic activation)
Clastogenicity	[redacted (2018) Doc. No. 557-015] (Vol. 3 B.6.8.1.2 - CA 5.8.1/07) Acceptable	CA	Positive (without metabolic activation)
Clastogenicity/ Aneugenicity	[redacted (2019) Doc. No. 563-007] (Vol. 3 B.6.8.1.2 - CA 5.8.1/09) Acceptable	MNT Mouse bone marrow cells	Negative
Reproduction toxicity			
Developmental toxicity	[redacted (2017) Doc. No. 563-004] (Vol. 3 B.6.8.1.2 - CA 5.8.1/10) Acceptable	Mouse	$NOAEL_{maternal}$: 300 mg/kg bw/d $NOAEL_{developmental}$: 300 mg/kg bw/d $LOAEL_{maternal}$: 1000 mg/kg bw/d based on maternal body weight gains and gravid uterine weights $LOAEL_{developmental}$: 1000 mg/kg bw/d based on reduced fetal body weights, an increase in the incidence of bipartite ossification of the sternebrae and delays in ossification
Developmental toxicity – Tollerability study	[redacted (2022) Doc. No. 563-014] (Vol. 3 B.6.8.1.2 - CA 5.8.1/31) Acceptable as tolerability study	Rabbit	Not determined
Developmental toxicity – Dose-range finder	[redacted (2022) Doc. No. 563-016] (Vol. 3 B.6.8.1.2 - CA 5.8.1/32) Acceptable as dose range finding study	Rabbit	Not determined
Main developmental toxicity	[redacted (2021) Doc. No. 563-015] (Vol. 3 B.6.8.1.2 - CA 5.8.1/33) Acceptable	Rabbit	$NOAEL_{maternal}$: 150 mg/kg bw/d $NOAEL_{developmental}$: 150 mg/kg bw/d $LOAEL_{maternal}$: 400 mg/kg bw/d increased tyrosin and increased minimal histopathology finidings in the kidney and liver $LOAEL_{developmental}$: 400 mg/kg bw/d increased visceral and skeletal malformations
Other toxicity studies			
Mechanistic investigation	[redacted (2017b) Doc. No. 563-005] (Vol. 3 B.6.8.1.2 - CA 5.8.1/11) Acceptable	Mouse and Rat	Measurement of plasma tyrosine concentrations
Mechanistic investigation	[redacted (2019a) Doc. No. 563-012] (Vol. 3 B.6.8.1.2 - CA 5.8.1/18)	Rat, mouse, dog, rabbit and human hepatocytes *in vitro*	Measurement of HPPA and HPLA concentrations

Parameter [Reference]		Species/ Model	Outcome
	Limited value (method optimization)		
Mechanistic investigation	[redacted (2019b) Doc. No. 563-013] (Vol. 3 B.6.8.1.2 - CA 5.8.1/19) Acceptable	Rat, mouse, dog, rabbit and human hepatocytes *in vitro*	Measurement of HPPA concentrations
Mechanistic investigation	[redacted (2016) Doc. No. 344-001] (Vol. 3 B.6.8.1.2 - CA 5.8.1/29) Acceptable	Carrot *in vitro*	Measurement of HPPD enzyme inhibition IC_{50} 0.348 µM

CA: chromosome aberration, MNT: micronucleus test, MLA: mouse lymphoma assay, m: males, f: females, HPPA: 4-hydroxyphenylpyruvic acid, HPLA: 4-hydroxyphenyllactic acid

Metabolite 1315P-966

The metabolite 1315P-966 (2-chloro-4-methylsulfonyl benzoic acid) is a minor metabolite in rat metabolism. According to the PECgw calculations the concentrations are > 0.1 µg/L and < 0.75 µg/L. The abbreviations CMBA and CMSBA are used in the study reports and refer to the same molecule, i.e. 1315P-966.
Metabolite 1315P-966 is not acutely toxic and was shown to be non-genotoxic in *in vitro* test systems. The substance is also not eye nor a skin irritant or sensitizer. Following repeated oral exposure in sub-chronic toxicity studies, no relevant adverse effects have been reported at the maximal tested doses. In a 28-days inhalation study, reversible squamous metaplasia of the ventromedial epithelium of the larynx was noted in rat species. No reproductive effects were observed in a one-generation reproduction toxicity study conducted with rats.

Table 39: Summary table of toxicity studies with metabolite 1315P-966

Parameter [Reference]		Species/ Model	Outcome
Acute toxicity studies			
Oral	[redacted (1999b) Doc. No. 521-002] (Vol. 3 B.6.8.1.3 - CA 5.8.1/12)	Mouse	LD_{50}: > 5000 mg/kg bw
Oral	[redacted (1991)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/13)	Rat	LD_{50}: > 2000 mg/kg bw
Oral	[redacted (1989)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/14)	Rat	LD_{50}: > 2000 mg/kg bw
Dermal	[redacted (1991)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/15)	Rat	LD_{50}: > 2000 mg/kg bw
Inhalation	[redacted (1995)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/16)	Rat	LC_{50}: > 4.31 mg/L
Irritation and sensitisation studies			
Skin irritation	[redacted (1991)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/17)	Rabbit	Slightly irritant but not sufficient for classification
Eye irritation	[redacted (1991)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/18)	Rabbit	Eye irritant Category 1, "H318: Causes serious eye damage"
Skin sensitisation, maximisation	[redacted (1991)] (Vol. 3 B.6.8.1.3 - CA 5.8.1/19)	Guinea pig	Non sensitizer
Genotoxicity			
Mutagenicity	[redacted (1990) Doc. No. 994-05001]	Ames[1]	Negative (with and without metabolic activation) (supplemental study)

Parameter [Reference]		Species/ Model	Outcome
	(Vol. 3 B.6.8.1.3 - CA 5.8.1/20)		
Mutagenicity	[redacted (1991) Doc. No. 994-05002] (Vol. 3 B.6.8.1.3 - CA 5.8.1/21)	Ames[2]	Negative (with and without metabolic activation)
Mutagenicity	[redacted (1997) Doc. No. 994-05003] (Vol. 3 B.6.8.1.3 - CA 5.8.1/22)	MLA	Negative (with and without metabolic activation)
Clastogenicity	[redacted (1991) Doc. No. 994-05004] (Vol. 3 B.6.8.1.3 - CA 5.8.1/23)	CA	Negative (with and without metabolic activation)
Mutagenicity	[redacted (1999a) Doc. No. 563-011] (Vol. 3 B.6.8.1.3 - CA 5.8.1/24)	Ames[2]	Negative (with and without metabolic activation)
Repeated dose studies			
28-day oral 0-50-250-1000 mg/kg bw/ day	[redacted] (Vol. 3 B.6.8.1.3 - CA 5.8.1/25)	Rat	NOAEL: 1000 mg/kg bw/d LOAEL > 1000 mg/kg bw/d
90-day dietary 0-500-2500-10000 ppm	[redacted] (Vol. 3 B.6.8.1.3 - CA 5.8.1/26)	Rat	NOAEL: 10000 ppm (763.4 mg/kg bw/d, males; 901.7 mg/kg bw/d, females) LOAEL > 10000 ppm
28-day inhalation 0-0.17-0.42-1.2-11.84 µg/L	[redacted] (Vol. 3 B.6.8.1.3 - CA 5.8.1/27)	Rat	NOAEL: 1.20 µg/L LOAEL:11.84 µg/L (reversible squamous metaplasia of the ventromedial epithelium of the larynx)
One-generation reproduction study 0-500-2500-10000 ppm	[redacted] (Vol. 3 B.6.8.1.3 - CA 5.8.1/28)	Rat	$NOAEL_{parental}$: 2500 ppm (247.6 mg/kg bw/d, males; 267.0 mg/kg bw/d, females) $NOAEL_{reproductive}$ and $NOAEL_{offspring}$: 10000 ppm (969.2 mg/kg bw/day, M; 1035.4 mg/kg bw/day, F) $LOAEL_{parental}$: 2500 ppm (969.2 mg/kg bw/day, M; 1035.4 mg/kg bw/day, F) based on decreased food consumption $LOAEL_{reproductive}$ > 10000 ppm

[1] *Salmonella typhimurium*, [2] *Salmonella typhimurium* and *Escherichia coli,* CA: chromosome aberration, MLA: mouse lymphoma assay

The Notifier referred to studies evaluated in the Sulcotrione Assessment Report, therefore, already evaluated. The RMS, but not the Notifier, has obtained the original study reports for the two Ames test of [redacted] the MLA of [redacted] and the Chromosomal Aberration assay of [redacted] All the studies shaded in grey in Table 39 have been evaluated and summarized in the Sulcotrione Assessment Report. Studies not shaded are owned by the Notifier and summarized in this Assessment Report.
However, the Notifier provided or referred to an acute oral toxicity study ([redacted] 1999b) and to genotoxicity studies only for the toxicity assessment of the stages 2 and 3 of step 3 as per the Guidance Document on the assessment of the relevance of metabolites in groundwater of substances regulated under council Directive 91/414/EEC.
At this stage the RMS is only able to provide a summary of available studies and to refer to the conclusions of the Sulcotrione Assessment Report.

Anyway, considering that the metabolite is not genotoxic and does not derive from a parental compound having carcinogenic, reproductive or acute toxicity properties, the application of the TTC value of 0.02 µg/kg bw/d (Cramer Class III, high toxicity compounds), as foreseen by the step 4 of the Groundwater metabolites guidance

document, is considered sufficient to conclude that any contamination of groundwater will not lead to unacceptable exposure of consumers via their drinking water.
More studies are available for the metabolite 1315P-966 (2-chloro-4-methylsulfonyl benzoic acid). The following studies on the metabolite have been summarized and evaluated by MS-DE in the Assessment Report of Sulcotrione (DAR, 2006). The metabolite of Benzobicyclone is the same of that produced during the metabolism of Sulcotrione. In the Sulcotrione Assessment Report, the metabolite is called CMBA or CMSBA.
The Notifier has not access to the original studies and intends to refer to the conclusions of that Assessment Report for the following studies, considered no more data protected, in order to exclude the toxicological relevance of the metabolite for the groundwater:

- Ames test, [redacted] 1990;
- Ames test, [redacted] 1991;
- Mouse lymphoma assay, [redacted] 1991;
- Chromosome aberration assay, [redacted] 1991.

The RMS is aware that more studies have been conducted for the metabolite, therefore, the summaries of the other studies (i.e. acute toxicity, irritation/corrosion, sensitisation, repeated dose, reproductive studies) are reported. The RMS has obtained the access to the original study reports and provided more information with respect that summarised in the Sulcotrione Assessment Report when retained necessary. Overall, the conclusions reported in the Sulcotrione assessment are agreed upon.

Metabolite 1315P-076

Metabolite 1315P-076 is not acutely toxic (LD_{50} > 5000 mg/kg bw) and was shown to be non-mutagenic in the Ames test.

Table 40: Summary table of toxicity studies with metabolite 1315P-076

Parameter [Reference]		Species/ Model	Outcome
Acute toxicity			
Oral	[redacted (1999) Doc. No. 521-005] (Vol. 3 B.6.8.1.4 - CA 5.8.1/14)	Mouse	LD_{50}: > 5000 mg/kg bw
Genotoxicity			
Mutagenicity	[redacted (1999b) Doc. No. 557-006] (Vol. 3 B.6.8.1.4 - CA 5.8.1/15)	Ames	Negative (with and without metabolic activation)

Metabolite 1315P-570

Metabolite 1315P-570 is not acutely toxic (LD_{50} > 5000 mg/kg bw) and was shown to be non-mutagenic in the Ames test.

Table 41: Summary table of toxicity studies with metabolite 1315P-570

Parameter [Reference]		Species/ Model	Outcome
Acute toxicity			
Oral	[redacted (1999a) Doc. No. 521-001] (Vol. 3 B.6.8.1.5 - CA 5.8.1/16)	Mouse	LD_{50}: > 5000 mg/kg bw
Genotoxicity			
Mutagenicity	[redacted (1999c) Doc. No. 557-003] (Vol. 3 B.6.8.1.5 - CA 5.8.1/17)	Ames	Negative (with and without metabolic activation)

Metabolite 1315P-683

Metabolite 1315P-683 is not acutely toxic (LD_{50} > 5000 mg/kg bw) and was shown to be non-mutagenic in the Ames test.

Table 42: Summary table of toxicity studies with metabolite 1315P-683

Parameter [Reference]		Species/ Model	Outcome
Acute toxicity			
Oral	[redacted (1999d) Doc. No. 521-004] (Vol. 3 B.6.8.1.6 - CA 5.8.1/20)	Mouse	LD_{50}: > 5000 mg/kg bw
Genotoxicity			
Mutagenicity	[redacted (1999d) Doc. No. 557-005] (Vol. 3 B.6.8.1.6 - CA 5.8.1/21)	Ames	Negative (with and without metabolic activation)

Metabolite 1315P-960

Metabolite 1315P-960 is not acutely toxic (LD_{50} > 5000 mg/kg bw) and was shown to be non-mutagenic in the Ames test.

Table 43: Summary table of toxicity studies with metabolite 1315P-960

Parameter [Reference]		Species/ Model	Outcome
Acute toxicity			
Oral	[redacted (1999c) Doc. No. 521-003] (Vol. 3 B.6.8.1.7 - CA 5.8.1/22)	Mouse	LD_{50}: > 5000 mg/kg bw
Genotoxicity			
Mutagenicity	[redacted (1999e) Doc. No. 557-004] (Vol. 3 B.6.8.1.7 - CA 5.8.1/23)	Ames	Negative (with and without metabolic activation)

Metabolite 1315P-DAC

Metabolite 1315P-DAC was shown to be non-mutagenic in the Ames test.

Table 44: Summary table of toxicity studies with metabolite 1315P-DAC

Parameter [Reference]		Species/ Model	Outcome
Genotoxicity			
Mutagenicity	[redacted (1996b) Doc. No. 557-002] (Vol. 3 B.6.8.1.8 - CA 5.8.1/24)	Ames	Negative (with and without metabolic activation)

Impurities

An assessment of the genotoxic potential of Benzobicyclon impurities was conducted ([redacted] 2019, Doc. No.: 581-0013). Based on the 5-batch analysis of the active substance [redacted] of the Benzobicyclon impurities was present in significant (≥ 1 g/kg) amounts. [redacted] Benzobicyclon impurities were found to be below the Limit Of Quantification (LOQ) and are thus not considered in the toxicological hazard assessment. [redacted] Benzobicyclon impurities showed values at the LOQ (0.1 g/kg) in some batches. Their genotoxic potential was evaluated in accordance with the Guidance Document on Equivalence of Technical Materials (SANCO/10597/2003 -rev 10.1; EC, 2012).

The comprehensive genotoxicity data package for Benzobicyclon provides sufficient evidence that this active substance is not genotoxic. For [redacted] impurities experimental data were available and considered sufficient to conclude on the non-genotoxicity of [redacted] impurities. For the [redacted] impurity no experimental data were available and therefore, genotoxicity endpoints were assessed *in silico* using the software tools Toxtree, VEGA, T.E.S.T, the Danish (Q)SAR Database, Derek Nexus and the OECD QSAR Toolbox. No additional alerts in comparison to Benzobicyclon were predicted for this substance.
The weight of evidence strongly suggests that all three impurities raise no additional concern for genotoxicity when compared to Benzobicyclon.

2.6.8.2 Supplementary studies on the active substance

The majority of plant protection products do not possess an immunotoxic potential or in case of immunotoxic effects this endpoint is less sensitive than other toxicity endpoints, as established by systematic review of 170 immunotoxicity studies by United States Environmental Protection Agency (US EPA, 2013).
There is no indication of an immunotoxic potential of Benzobicyclon in the available toxicological database. A 28-day immunotoxicity study (sheep red blood cell assay) was conducted in rats. No effects on splenic anti-sRBC (IgM) response were observed and no effect on organs of the immune system (weight, histopathology) or on white blood cells in any study of the standard toxicology dataset.

Table 45: Summary table of animal studies on immunitoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results:	Reference
Immunotoxicity study OPPTS 870.7800 (no OECD guideline available) GLP Rat, [redacted]CD(SD), male, 10/group Acceptable	Benzobicyclon Batch No.: 1A0110 Purity: 99.2% 0, 1182 mg/kg bw/d Oral (diet) 28 days	No immunotoxicity as shown by lack of effects on splenic anti-sRBC (IgM) response.	[redacted] (2012) (Vol. 3 B.6.8.2 - CA 5.8.2/01) Doc. No.: 546-001

2.6.9 Summary of medical data and information

No cases of health effects were observed at regular medical examinations on workers who were employed in the manufacturing of Benzobicyclon under the normal safety precautions. There are no detrimental effects on health of participating personnel in manufacturing of Benzobicyclon. Records on clinical cases or poisoning incidents are not known. There is no evidence of adverse effects of Benzobicyclon to agricultural workers and consumers. For details see Vol. 3 Section 6.9 ([redacted] 2019, Doc. No.: 574-001, KCA 5.9.1/01; [redacted], 2019, Doc. No.: 574-002, KCA 5.9.1/02).

2.6.10 Toxicological end points for risk assessment (reference values)

Table 46: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat	Two-generation reproductive toxicity Oral (diet)	Benzobicyclon F0 males/ females: 0, 5.65/8.44,	Parental toxicity: slight effects on the	F0/F1 males: 59.5 mg/kg bw/d (mean of the	F0/F1 males: 1250 mg/kg bw/d (mean of the	[redacted] (1999) (Vol. 3 B.6.6.1 -

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
		56.1/85.4, 1176/1741 mg/kg bw/d F1 males/ females: 0, 6.46/8.76, 62.8/89.0, 1324/1817 mg/kg bw/d	pituitary reaching statistical significance in F1 males (microscopic findings pituitary)	intake from week 1 to 17)	intake from week 1 to 17)	CA 5.6.1/01) Doc. No.: 553-001
Rat	Combined chronic and carcinogenicity study Oral (diet)	Benzobicyclon Males: 0, 0.334, 0.667, 1.696, 3.43 mg/kg bw/d Females: 0, 4.19, 42.2, 427 mg/kg bw/d 24 months	Highest dose applied in male animals, no relevant effect observed besides renal toxicity (sex/species specific and not relevant to humans)	3.4 mg/kg bw/d	-	[redacted] (1999) (Vol. 3 B.6.5.1 - CA 5.5/01) Doc. No.: 537-002

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The kidney was demonstrated to be the primary target organ of Benzobicyclon treatment. Since renal toxicity in male rats is a species and sex specific effect related to α-2μ-globulin nephropathy without any relevance to humans, the most relevant NOAEL for derivation of the ADI was considered to be the highest dose applied to male rats (3.4 mg/kg bw/d) in a 2-year chronic toxicity/carcinogenicity study. At this dose level there were no toxicologically significant effects observed besides α-2μ-globulin nephropathy.

The ADI of Benzobicyclon is 0.034 mg/kg bw/d based on the critical NOAEL with relevance to humans determined in a 2-year chronic toxicity/carcinogenicity study in rats (NOAEL: 3.4 mg/kg bw/d) and a safety factor of 100.

This ADI is considered to cover toxicological effects reported for metabolite 1315P-070 (HPPD inhibitor). The available *in vivo* and *in vitro* experimental evidence indicated that the mouse is an appropriate experimental model for human risk assessment of HPPD inhibitors, based on the similar TAT activity between mouse and human, when compared to much more sensitive species such as rats, rabbits and dogs, that showed substantial lower TAT activity.

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

An ARfD was not derived for Benzobicyclon, in accordance with the criteria for not setting an ARfD by Solecki *et al.* (2005)[3] and OECD Guidance for derivation of ARfD (2010)[4]:

[3] Solecki R., Davies, L., Dellarco, V., Dewhurst, I., van Raaij, M. and Tritscher, A. (2015). Guidance on setting of acute reference dose (ARfD) for pesticides. Food and Chemical Toxicology 43, 1569-1593.
[4] OECD. Environment, Health and Safety Publications. Series on Testing and Assessment, No. 124. GUIDANCE FOR THE DERIVATION OF AN ACUTE REFERENCE DOSE, ENV/JM/MONO(2010)15. Paris. 2010.

- No findings indicative of effects elicited by an acute exposure up to 500 mg/kg bw.
- No substance related mortality up to 1000 mg/kg bw in the acute oral toxicity study.

The toxicological data set available for Benzobicyclon demonstrates that the setting of an ARfD is not needed.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The lowest relevant NOAEL for AOEL setting (59.5 mg/kg bw/d) was derived from a 2-generation reproductive toxicity study in rats and is based on pituitary findings in the F1 generation (increased organ weight and hydropic degeneration basophilic cells seen at highest applied dose). The finding of α-2μ-globulin nephropathy in male rats was considered not relevant since this is a species and sex specific effect without human relevance.

The AOEL of Benzobicyclon is 0.060 mg/kg bw/d based on the relevant NOAEL determined in a 2-generation reproductive toxicity study in rats (NOAEL: 59.5 mg/kg bw/d), a safety factor of 100 and an oral absorption of 10%.

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

An ARfD was not derived for Benzobicyclon since no relevant effects/mortality were observed by acute exposure. Therefore, no AAOEL setting for Benzobicyclon is needed in accordance with SANTE-10832-2015 rev. 1.7 (2017)[5].

2.6.11 Summary of product exposure and risk assessment

Usage information pertinent to exposure is summarised in Table 47.

Table 47: Summary of representative use according to GAP

Crop	Application rate [g a.s./ha]	Volume rate [L/ha]	Application equipment	Number of applications
Outdoor or field use (downward spraying)				
Rice	300	200	Tractor-mounted/trailed boom sprayer (hydraulic nozzles)[a]	1

[a] The outdoor application by means of tractor-mounted equipment is foreseen to be done in flooded rice paddy.

Estimations of potential non-dietary exposure have been undertaken for Benzobicyclon considering the intended use of the representative formulation GWN-10235 and the following predictive model:
Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (EFSA Journal 2014;12(10):3874)[6] - including an exposure calculation spreadsheet ("**EFSA model**"). Calculator version of 30 March 2015.
In the EFSA model, risk assessments must be carried out for all scenarios of exposure of operators, workers, residents and bystanders that can be expected to occur as a consequence of the proposed uses of a plant protection product.
Long-term risk assessments for operator, worker, and resident are presented with the AOEL of Benzobicyclon as appropriate 'reference value non-acutely toxic active substance' (RVNAS).
Acute risk assessments are only necessary in case of derivation of a 'reference value acutely toxic active substance' (RVAAS) for the active substance. No acute reference value was set for Benzobicyclon since no substance-related

http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282010%2915&doclanguage=en

[5] EUROPEAN COMMISSION. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. COMMISSION GUIDANCE DOCUMENT SANTE-10832-2015 rev. 1.7. 24 January 2017.

[6] EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp., doi:10.2903/j.efsa.2014.3874

mortality up to 1000 mg/kg bw in the acute oral toxicity study or any finding indicative of effects elicited by acute exposure in the early phase of repeated dose studies. Therefore, acute risk assessments for operator and bystander are not presented. The long-term risk assessment for the bystander is considered covered by the resident exposure assessment (EFSA Guidance on exposure assessment, 2014).
The exposure estimations were conducted using the following dermal absorption values determined for Benzobicyclon in the representative formulation GWN-10235 and compared to the AOEL.

End-Point	Value for the Active Substance
Dermal absorption	Concentrate: 0.66% Spray dilution: 16%
AOEL	0.06 mg/kg bw/d

2.6.11.1 Operator exposure - Risk assessment for operator

A summary of the estimated operator exposure is presented in the following table.

Table 48: Summary of estimated operator exposure to Benzobicyclon according to EFSA model

Exposure scenario[1]		Application rate Minimum volume rate [g a.s./ha] [L/ha]	In-use concen-tration [g a.s./L]	Total systemic exposure		PPE
				[mg a.s./kg bw/d]	[% AOEL][2]	
OUTDOOR						
Spraying – mixing/loading and application						
FCTM (Rice)	tractor, downward spraying	300 200	1.5	0.011	18	workwear

[1] FCTM = Field crop tractor-mounted
[2] AOEL = systemic AOEL of 0.06 mg/kg bw/d = RVNAS (Reference value non acutely toxic active substance)

Conclusion

The long-term EFSA model estimates for the proposed use of SC formulation GWN-10235 in the flooded rice paddy show that the Benzobicyclon AOEL of 0.06 mg/kg bw/d is not exceeded for the operator using standard work wear (arms, body and legs covered).

2.6.11.2 Bystander and resident exposure - Risk assessment for bystander and resident

Long-term risk assessments for the resident are presented in relation to the AOEL of Benzobicyclon as appropriate 'reference value non acutely toxic active substance' (RVNAS).
According to the EFSA Guidance on exposure estimation in the EFSA model (2014), the bystander risk assessment is covered by the resident risk assessment (long-term) for substances that do not show an acute hazard potential like Benzobicyclon. In the EFSA model 4 pathways of resident exposure are considered: spray drift, vapour, surface deposit, and entry into treated crops. Resident exposure is based on the 75th percentile estimates. However, total exposure via all pathways is derived by summing the means. On this basis, both the 75th percentile and mean values were calculated for each resident exposure assessment; the 75th percentiles were assessed per pathway and the means per pathway were summed up. The dermal absorption percentage resulting from contact with the spray dilution is used for resident exposure assessment, *i.e.* 16%.
A summary of the estimated bystander/resident exposure is presented in Table 49

Table 49: Summary of estimated bystander/resident exposure to Benzobicyclon according to the EFSA model (long-term)

Exposure scenario[1,2]	Application rate [g a.s./ha]	Person	Total systemic exposure[3]	
			[mg a.s./kg bw/d]	[% AOEL]
OUTDOOR				
FCTM	300	Adult	0.0048	8
Rice		Child	0.0118	19.64

[1] FCTM = Field crop tractor-mounted
[2] Buffer strip: 2-3 m, DFR = 3.0 µg/cm^2
[3] Assuming 16 % dermal absorption and 100 % absorption for inhalation; body weight: 60 kg and 10 kg for adults and children (1-3 year old), respectively; AOEL = systemic AOEL of 0.06 mg/kg bw/d = RVNAS.

Conclusion

The predicted level of bystander/resident exposure using the EFSA model is well below the AOEL of Benzobicyclon (0.06 mg/kg bw/d) for tractor mounted application to the rice paddy with and without drift reduction nozzles. Thus, there is no undue health risk to any bystander or resident after accidental exposure to SC formulation GWN-10235.

2.6.11.3 Worker exposure - Risk assessment for worker

In the case of Benzobicyclon, entering the rice paddy shortly after spraying is not necessary. The paddy remains flooded for a minimum of 5 days and the presence of workers in the drained field is not envisaged. The crop harvest is done mechanically and thus, workers will not be in close contact to the crop and determination of a worker re-entry period is considered not necessary. However, worker exposure estimates are presented in case a worker re-enters the treated crop for inspection and/or irrigation activities. The potential major route of exposure on re-entry is contact with residues via the skin. Inhalation does not need to be considered in the exposure assessment due to the low vapour pressure of Benzobicyclon (< 5.6×10^{-5} Pa at 25°C). Two scenarios are presented following an accepted approach for the use in rice paddy in EU review of a.s.:

Scenario 1 re-entry into a drained field immediately after application (EFSA model)
Scenario 2 re-entry into a flooded field immediately after application (using dermal exposure factors for complete immersion of hands in water according to US EPA[7]).

A summary of the estimated worker exposure is presented in Table 50.

Table 50: Summary of intended use according to GAP

Crop	Application rate [g a.s./ha]	Number of applications (interval)	PHI [d]	Application mode	Transfer coefficient (TC) [cm²/h]	Re-entry activities
Outdoor use						
Rice	300	1	-[c]	Tractor-mounted / trailed boom sprayer	1400[b]	Inspection, irrigation

[a] Dermal transfer coefficient (no PPE) - arms, body and legs covered
[b] Dermal transfer coefficient (with PPE) – hands, arms, body and legs covered
[c] PHI (pre-harvest interval) is covered by the vegetation period between application and harvest

[7] U.S. EPA. Exposure Factors Handbook 2011 Edition (Final Report). Chapter 7 – Dermal Exposure Factors. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F, 2011

Table 51: Estimated worker exposure to Benzobicyclon and % of the AOEL in the drained rice paddy (EFSA model) and flooded rice paddy (US EPA Exposure Factors Handbook, 2011)

Scenario / Crop	Application rate [g a.s./ha]	DFR [µg a.s./cm² per kg a.s./ha]	Total systemic exposure	
			[mg/kg bw/d]	% AOEL[1]
Drained paddy: Work clothing[2], direct re-entry – Scenario 1				
Inspection, irrigation rice paddy	1 × 300	3	0.0067	11.2
Flooded paddy: Potential exposure direct re-entry (feet, legs, hands) – Scenario 2				
Inspection, irrigation rice paddy	1 × 300	-	0.0000927	0.15

[1] AOEL = systemic AOEL of 0.06 mg/kg bw/d (AOEL = RVNAS)
[2] Work clothing: arms, body and legs covered

Conclusion

No worker re-entry activities are foreseen in the case of the use. On the basis of exposure estimates calculated according to the EFSA model (drained paddy, scenario 1) and based on the dermal exposure factors according to US EPA (2011; flooded paddy, scenario 2) there is no undue health risk for a worker, when re-entering areas treated with SC formulation GWN-10235 for inspection or irrigation purposes.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Plants

Two studies are available on storage stability. In the study [redacted] (2015) the stability of Benzobicyclon and 1315P-070 was investigated in rice grain, straw and whole plant at 0 and 299 days. A slight decline of Benzobicyclon was observed in rice grain after 299 days (64%) but since storage stability was tested on two sampling intervals (0 and 299 days), the rate of decline cannot be established. A request to perform another storage stability study to clarify the lower recovery for Benzobicyclon with additional storage stability points was submitted to the applicant.
Referring to this request, a new storage stability study was submitted. In the study [redacted] (2021), the stability of Benzobicyclon and metabolites 1315P-070, 1315P-570, 1315P-966 was investigated in various plant matrices after a storage interval of 0, 364-369, 814-824 days.
The available storage stability data, were reported in the table below.

Table 52: Storage stability results.

Commodity category	Commodities	Storage stability period (days)	Reference
Benzobicyclon			
Starch content	Rice grain	814	KCA 6.1/02 (645-001)
Starch content	Radish root	818	KCA 6.1/02 (645-001)
Water content	Rice whole plant	299	KCA 6.1/01 (634-46001)
Water content	Radish leaves with tops	818	KCA 6.1/02 (645-001)
Water content	Lettuce leaves	820	KCA 6.1/02 (645-001)
Others	Rice Straw	817	KCA 6.1/02 (645-001)
1315P-070			
Starch content	Rice grain	814	KCA 6.1/02 (645-001)
Starch content	Radish root	818	KCA 6.1/02 (645-001)
Water content	Rice whole plant	299	KCA 6.1/01 (634-46001)
Water content	Radish leaves with tops	818	KCA 6.1/02 (645-001)
Water content	Lettuce leaves	820	KCA 6.1/02 (645-001)
Others	Rice Straw	817	KCA 6.1/02 (645-001)

Commodity category	Commodities	Storage stability period (days)	Reference
1315P-570			
Starch content	Rice grain	820	KCA 6.1/02 (645-001)
Starch content	Radish root	820	KCA 6.1/02 (645-001)
Water content	Radish leaves with tops	820	KCA 6.1/02 (645-001)
Water content	Lettuce leaves	818	KCA 6.1/02 (645-001)
Others	Rice straw	820	KCA 6.1/02 (645-001)
1315P-966			
Starch content	Rice grain	820	KCA 6.1/02 (645-001)
Starch content	Radish root	819	KCA 6.1/02 (645-001)
Water content	Radish leaves with tops	822	KCA 6.1/02 (645-001)
Water content	Lettuce leaves	824	KCA 6.1/02 (645-001)
Others	Rice straw	819	KCA 6.1/02 (645-001)

It was shown that Benzobicyclon and 1315P-070 residues are stable in rice grain (high starch content), rice straw (high water content) and rice whole plant (high water content) for at least 10 months when the crops are stored under frozen (below - 18 °C) conditions.

Animal products

No studies are submitted. Calculated dietary burden is < 0.004 mg/kg bw. No metabolism studies or feeding studies are necessary.

Storage stability of residues in sample extracts

The storage stability of Benzobicyclon and 1315P-070 in sample extracts was shown by procedural recoveries handled and stored in the same way and for the same time period as field samples within the residue studies.

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

The metabolism of Benzobicyclon was investigated on primary crop (water flooded treatment to rice). No studies on livestock, rotational crops and processing were submitted.

2.7.2.1 Plant Metabolism

Rice.

Two metabolism studies on rice were conducted with Benzobicylon ([bic-^{14}C]-benzobicyclon label and [ben-^{14}C]-benzobicyclon label) was either applied as a granular or an SC formulation in water flooded plot.

In the study [redacted] (2013) Benzobicyclon was applied once as a granular formulation directly over the water flooded plot. The application was made at early tillering stage (BBCH 21-24) and at a nominal rate of 390 g a.s./ha (429 g a.s./ha for the [bic-^{14}C]-Benzobicyclon label and 411 g a.s./ha for the [ben-^{14}C]-Benzobicyclon label. Sampling was 30 days after application (immature whole plants) and at maturity at 145 days (brown rice, hulls and straw).
The TRR in brown rice was 0.005 - 0.009 mg/kg, in hulls 0.030 - 0.051 mg/kg, in straw 0.072 - 0.143 mg/kg and in immature plants 0.058 - 0.078 mg/kg. The extractability with acetonitrile : water (twice, 1:1, v/v) followed by acetonitrile was 20 - 22 % of TRR for brown rice, 73 - 77 % of TRR for hulls, 64 - 79 % of TRR for straw and 43 - 60 % of the TRR for immature plants. TRR remaining in PES after all extractions was <0.015 mg/kg in all matrices.
Benzobicyclon was found below the limit of quantification in brown rice, hulls, straw and immature plants (<0.001 mg/kg). Identified metabolites were 1315P-570, 1315P-070, and a conjugate of 1315P-966. The highest level of 1315P-070 was in straw (0.005 - 0.009 mg/kg, 4 - 13 % TRR) and the highest level of 1315P-570 was in straw (0.014 - 0.027 mg/kg, 19 % TRR) and hulls (0.012 - 0.018 mg/kg, 35 - 40 % TRR). The highest level of the 1315P-966 conjugate was in immature plants (0.019 mg/kg, 24.4 % TRR). Generally, similar low levels of the

same metabolites were found in immature and mature plants. No single metabolite in brown rice was >0.001 mg/kg.

In second study [redacted] (1999), Benzobicyclon (SAN 1315H, [bic-^{14}C]-Benzobicyclon label and [ben-^{14}C]-Benzobicyclon label) was applied once as a SC formulation directly to the surface of a water flooded pot. The application was made 1 week after transplanting rice seedlings at the 2.5 leaf stage and at a target rate of 300 g a.s./ha. Sampling was 42 days after treatment (foliage, roots) and at maturity at 119 days (foliage, roots, brown rice and hulls).
The TRR in brown rice was 0.039 - 0.045 mg/kg, in hulls 0.130 - 0.131 mg/kg, in foliage 0.289 - 0.545 mg/kg and in immature foliage 0.505 - 0.640 mg/kg. The extractability with acetonitrile : water (8:2, v/v) was 23.4 - 28.8 % of TRR for brown rice, 63.1 - 70.6 % of TRR for hulls, 70.2 - 72.0 % of TRR for foliage and 73.4 - 78.8 % of the TRR for immature foliage. TRR remaining in PES after all extractions was <0.013 mg/kg in brown rice and <0.040 mg/kg in mature rice foliage.
Benzobicyclon was below the limit of quantification in brown rice, hulls and foliage from the mature sampling and <0.0045 mg/kg (0.89% TRR) in immature foliage from 42 DAA harvest. Identified metabolites were 1315P-570, 1315P-070, 1315P-076 and a conjugate of 1315P-966.
The highest level of 1315P-570 was found in immature foliage (0.0261 mg/kg, 4.1% TRR), for 1315P-070 the highest level was found in mature foliage after acid hydrolysis (0.0132 mg/kg, 2.7% TRR), for 1315P-076 in foliage from 42 DAA sampling (0.0188 mg/kg, 3.7% TRR), and for 1315P-966 the highest level was found in mature rice foliage after enzyme hydrolysis (0.0279 mg/kg, 5.8% TRR). In brown rice, no single metabolite was >0.0016 mg/kg or >4.16% TRR.

In both studies no consideration was given to the quality of benzobicyclone to act as a pro-herbicide i.e. featuring high lipophilic character for ready uptake via root and shoot tissue to form the herbicidal active triketone (metabolite 1315P-070). Thanks to the achieved ionisability (keto-enol tautomerism) and increased phloem mobility, the metabolite exhibits a strong pH-dependent water solubility (<0.1 mg/L at low to >6 g/L at high pH). This is a relevant physical/chemical property of cyclohexanedione herbicides, to be taken into consideration prior to any investigation. Similar ionisabilities could be anticipated for all enamine metabolites identified 1315P-570, 1315P-076 and 1315P-960 (imine-enamine tautomerism).
Also, in both studies the HPLC analysis of reference materials evidenced partial co-elution between the signals of 1315P-570 (enamine) and of 1315P-960 (carboxymethyl-enamine) on one hand and between the signals of 1315P-076 (hydroxyethyl-enamine) and of 1315P-683 (xanthene) on the other. In the Gowan study however, a clear difference in the TLC-elution is shown between 1315P-570 (enamine) and of 1315P-960 (carboxymethyl-enamine), confirming the radioactivity to be associated with the enamine (1315P-570), the principal metabolite observed in all matrices (0.001 mg/kg in brown rice).
In the SDS study ([redacted] (1999), HPLC analyses were preceded by SPE (C18) absorption of the acetonitrile/water and separate elution with water, benzene or methanol. The benzene and methanol fractions were separately submitted to HPLC analysis, all of them conducted on the same C18 column at 13 different elution conditions (all of them containing (A) 5mM phosphoric acid and (B) acetonitrile), both gradient and isocratic. A UV detector, installed in series with the radioactivity detector(s), was not used, i.e. no UV chromatograms were presented. Mass spectrometry was useful for only the identification of metabolites 1315P-076 (hydroxyethyl-enamine) and 1315P-966 (chlorobenzoic acid). Since all significant results in the SDS study were founded on co-chromatography by utilizing the same stationary phase, the approach was not considered in line with requirements of guideline OECD 501. Moreover, according to OECD 501, at least 90% of TRR should be identified/characterised. In brown rice, no reasonable attempt was done to identify >20% TRR residues, e.g. by concentrating the ACN/water extract and analysing with LC-MS, instead of further diluting the extract by SPE purification and threefold elution. Also, 10% TRR in the buffer rinse of the PES further demonstrates the inadequacy of the method.
Overall, it could reliably be concluded that the metabolic degradation of benzobicyclone is essentially focused to the cyclohexanedione ring and the hydrolytic cleavage of the benzoyl moiety. The enamine (1315P-570) and a conjugated benzoyl moiety (1315P-966) are the only significant metabolites formed and only occurred at significant levels in feed items (hulls, straw and immature plant). Benzobicyclone and the triketone (1315P-070) were always at insignificant levels following an application rate as high as N=1,4 in brown rice or at low level in the straw (0.009 mg/kg; 12.5% for 1315-070).

Based on the available data, the residue definition for enforcement and risk assessment is proposed as sum of benzobicyclon and 1315P-070 expressed as benzobicyclon. For feed commodities, the potential inclusion of the predominant enamine metabolite (1315P-570) in the residue definition for risk assessment should be discussed

taking into account the negligible contribution of this metabolite on the animal diet demonstrated in the dietary burden calculation (see section B.7.4, Vol.3).

2.7.2.2 Animal Metabolism

According to Commission Regulation (EU) No. 283/2013 "Metabolism studies on poultry and lactating ruminants shall be provided where the plant protection product is to be used in crops whose parts of products, also after processing, are fed and where the intake is expected to exceed 0.004 mg/kg bw/day."
Rice bran/pollard and straw are potential feed items according to the EU animal model (EFSA, 2017). Based on the animal dietary burden calculated for ruminants, pig and poultry, the estimated intakes are below the trigger value of 0.004 mg/kg bw and thus, no livestock metabolism studies are triggered and the setting of MRLs in commodities of animal origin is not necessary.

Fish

Benzobicyclon has a Log POW 3.1 thus, an estimation of the fish dietary burden for Benzobicyclon and metabolite 1315P-070 has been carried out. Rice and processed products broken grain and hulls are potential fish feed according to the SANCO guideline (SANCO/11187/2013) however, Benzobicyclon and 1315P-070 residues are below the LOQ in all potential feed items, and the dietary burden is <0.1 mg/kg of the total diet (dry weight basis). Therefore, neither a dietary fish metabolism study nor a feeding study is triggered.

2.7.3 Definition of the residue

2.7.3.1 Proposed Residue definitions (Food of plant origin)

1315P-070 (active portion of Benzobyciclon) is a major metabolite in straw and hull (metabolism study [redacted] 2013) moreover it was detected in the straw in residue trials. As reported in the section 2.6.10.1 the toxicological properties can be considered covered by the studies performed for the active substance Benzobyciclon.
Taking into account the information reported above the residue definition proposed are as follows:

Definition for risk assessment: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon.
Definition for monitoring: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon.

Co-RMS EL suggests that should be restricted to rice only (food and feed commodities).

2.7.3.2 Proposed Residue definitions (Food of animal origin)

As all calculated intakes for livestock are below the respective trigger values, livestock metabolism studies and livestock feeding studies are not needed. Hence, no residue definition for food of animal origin is required and the setting of MRLs in commodities of animal origin is not necessary.

Definition for risk assessment: not required
Definition for monitoring: not required

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

Table 53: Summary of the critical GAP.

Crop	Country/ Region	Indoor/ outdoor	Max. rate kg as/ha	Growth stage at latest application (BBCH)	Number of applications (minimum interval in days)	PHI (days)
Rice	SEU	outdoor	0.30	BBCH 00-21 (rice) BBCH 00-12 (target)	1	Not relevant. PHI is covered by the vegetation period between application and harvest

The representative formulated product for this evaluation is GWN-10235 SC containing 400 g/l of Benzobicyclon. A total of eight trials on rice in Southern Europe were performed. Trials were not previously submitted or assessed. Locations and detailed use patterns for the trials are provided below.

Table 54: Summary of the number of residue trials with GWN-10235 SC.

Year	Crop	Zone	Study Type		Total Number of Trials
			Decline	At-Harvest	
2013	Rice	SEU	1	3	4
2014	Rice	SEU	0	4	4

In all trials the application was carried out in flooded condition with approximately 4 cm of water level. The water in the paddy field was held for 5 days after application.
Samples of rice grain (paddy rice), straw and whole plants were analysed for Benzobicyclon and its metabolite 1315P-070 with the validated analytical method (LOQ of 0.01 mg/kg). Recoveries are within acceptable limits.
No quantifiable residues of Benzobicyclon and 1315P-070 were detected in rice grain. These results are further supported by residue levels of Benzobicyclon and 1315P-070 below the LOQ in the trials performed with an exaggerated application rate of 573.3 - 616.0 g a.s./ha.
The maximum storage interval for samples from harvest to analysis is 245 days in line with the storage stability data.

Rice is a major crop in Europe therefore eight trials compliant to the proposed GAP are required.
Some trials are conducted in the same location (or <20 km far from one another) and under identical conditions. Nevertheless a sufficient independent trials on rice grain (paddy rice) are available (4 trials <LOQ) (SANCO 7525/VI/95, Rev. 10.3_2019).
In conclusion, four residue trials below the LOQ are considered sufficient to propose an MRL for Benzobicyclon in rice grain at LOQ level.
No MRL is proposed for rice straw.

Table 55: Locations and detailed use patterns for the trials on rice.

Location: City, state/province; year (Trial ID)	End-use product/ formulation (nominal a.s./L)	Method of application/ timing of application	Rate per application (g a.s./ha)	Surfactant/ Adjuvant	Comments
1119.H.SAG13/r Olcenengo, Vercelli, Piemonte, Italy 2013	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	296.2	None	Not independent trial
		Foliar/ BBCH 21	586.7	None	Overdosed trial, not considered in the MRL calculation
1120.H.SAG13/r Borgo Vercelli, Piemonte, Italy 2013	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	328.3	None	Considered in the MRL calculation.
		Foliar/ BBCH 21	573.3	None	Overdosed trial, not considered in the MRL calculation
1121.H.SAG13/r Borgo Vercelli, Piemonte, Italy 2013	GWN-10235 SC 400 g/L	Foliar/ BBCH 19-21	310.0	None	Not independent trial
		Foliar/ BBCH 19-21	576.7	None	Overdosed trial, not considered in the MRL calculation

Location: City, state/province; year (Trial ID)	End-use product/ formulation (nominal a.s./L)	Method of application/ timing of application	Rate per application (g a.s./ha)	Surfactant/ Adjuvant	Comments
1122.H.SAG13/r La Puebla del Rio, Seville, Spain 2013	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	324.0	None	Considered in the MRL calculation.
		Foliar/ BBCH 21	616.0	None	Overdosed trial, not considered in the MRL calculation
1113.H.SAG14/r Olcenengo, Vercelli, Piemonte, Italy 2014	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	288.4	None	Not independent trial
1114.H.SAG14/r Borgo Vercelli, Piemonte, Italy 2014	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	278.4	None	Considered in the MRL calculation.
1115.H.SAG14/r Des Hermanas, Seville, Spain 2014	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	317.6	None	Considered in the MRL calculation.
1116.H.SAG14/r La Puebla del Rio, Seville, Spain 2014	GWN-10235 SC 400 g/L	Foliar/ BBCH 21	521.7	None	Overdosed trial, not considered in the MRL calculation

Table 56: Overview of the available residues trials data and MRL calculations.

Crop	Region/ Indoor	Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	Recommendations/comments (OECD calculations)	MRL proposal (mg/kg)	HR (mg/kg)	STMR (mg/kg)
RA: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon RMo: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon						
Rice paddy (with hull)	SEU (4)	**0.30 kgas/ha; BBCH: 19-21; PHI: 93-120 days** 4 × <0.02	MRL corresponding to LOQ	0.02*	0.02*	0.02*
Straw	SEU (4)	**0.30 kgas/ha ; BBCH: 19-21; PHI: 93-120 days** 3 × <0.02; 0.10	No MRL required for feed item	-	0.10	0.02*

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

Rice grain and straw are potential feed items according to the EU animal model (EFSA, 2017). Livestock dietary burden calculations were therefore performed for different groups of livestock.
The use of Benzobicyclon in rice, does not lead to significant residues (>0.004 mg/kg body weight) in potential livestock feed. Thus, livestock feeding studies are not required.

Table 57: Input values for the dietary burden calculation.

Feed Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon				
Rice straw	0.02	STMR (default PF not applied)	0.10	HR (default PF not applied)
Rice bran/pollard	0.02	STMR (default PF not applied)	-	-

Table 58: Results of the dietary burden calculation (EFSA animal model_2017).

Revelant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0,000	0,000	0,01	0,01	Dairy cattle	Rice	straw	No
Cattle (dairy only)	0,000	0,000	0,01	0,01	Dairy cattle	Rice	straw	No
Sheep (all diets)	0,000	0,001	0,01	0,02	Lamb	Rice	straw	No
Sheep (ewe only)	0,000	0,001	0,01	0,02	Ram/Ewe	Rice	straw	No
Swine (all diets)	0,000	0,000	0,00	0,00	Swine (breeding)	Rice	bran/pollard	No
Poultry (all diets)	0,000	0,000	0,00	0,00	Poultry broiler	Rice	bran/pollard	No
Poultry (layer only)	0,000	0,000	0,00	0,00	Poultry layer	Rice	bran/pollard	No

Fish

Based on the representative use in rice, only residues in rice grain need to be considered. Using the STMR in rice grain of 0.02 mg/kg and hulls results in a fish dietary burden of 0.012 mg/kg dry feed for common carp and 0.002 mg/kg for rainbow trout which is well below the trigger of 0.1 mg/kg dry feed. Thus, no feeding studies are triggered.

2.7.6 Summary of effects of processing

2.7.6.1 Processing nature of the residue

According to Commission Regulation (EU) No. 283/2013 studies on the nature of residues in processing shall be provided where residues in products of plant or animal origin subject to processing may occur at a level of or higher than 0.01 mg/kg (based on the residue definition for risk assessment for the raw commodity).
As a sufficient residue data package is available showing that residues of Benzobicyclon and its metabolite 1315P-070 are below the limit of quantification (LOQ) in rice grain, no simulated processing study is required.

2.7.6.2 Distribution of the residue in inedible peel and pulp

For the representative use in rice, the distribution in inedible peel and pulp is not required.

2.7.6.3 Magnitude of residues in processed commodities

As the residue levels in the presented supervised residue trials supporting the representative crop rice are below the limit of quantification and the total contribution to the theoretical maximum daily intake (TMDI) is < 10 % of the ADI, no processing study is needed.

2.7.7 Summary of residues in rotational crops

Although rice is mostly considered a semipermanent crop, rice can be rotated with soybean, but also sorghum and maize. No data were submitted on rotational crop.
As the DT_{90} for Benzobicyclon and its relevant soil metabolites is >100 days, and RMS considers the studies on rotational crops necessary. **Data gap**.

2.7.7.1 Metabolism in rotational crops

See above.

2.7.7.2 Magnitude of residues in rotational crops

See above.

2.7.8 Summary of other studies

Effect on the residue level in pollen and bee products

No data were provided by the applicant on the residue level in pollen or bee products.
Rice is not considered a melliferous crop (SANTE/11956/2016 rev. 9); nevertheless, the attractiveness of the rice pollen for bees cannot be excluded (EFSA Journal 2013;11(7):3295). Considering that the *"Consumption of pollen (including pollen present in honey), royal jelly, propolis, bee wax and honeycomb is negligible* there is no need to generate experimental residue data for these commodities."Thus a residue study on honey for rice which is not attractive for bees is not needed.

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

The input data for calculating the dietary risk are based on supervised residue trials:

Code number	Crop	Country / Region (SEU / NEU)	STMR [mg/kg]	HR [mg/kg]	Proposed MRL [mg/kg]
0500060	Rice	SEU	< 0.02	< 0.02	0.02*

SEU = Southern Europe
* Indicates lower limit of analytical determination

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

TMDI calculation

According to the assessment of the available toxicological data, the following toxicological reference value for the calculation of the chronic dietary risk assessment was derived:

Endpoint	Value	Study	Safety Factor
Acceptable Daily Intake (ADI)	0.034 mg/kg bw/day	Rat, 2 year study	100

The calculation of the TMDI was performed taking into account the proposed MRL for the representative crop to which Benzobicyclon may be applied.
With the current EFSA PRIMo model rev. 3.1, the chronic risk assessment ranges from 0.004 to 0.091 % of the ADI. The diet with the highest TMDI is “GEMS/Food G06” with 0.091 % of the ADI.
These results show that there is no chronic risk for consumer for the active substance.

NEDI calculation

Due to the fact that the highest residue levels of Benzobicyclon and 1315P-70 in treated crops are below the proposed MRL based on the residue trials presented in this dossier, and the calculated TMDI using this MRL is shown to be acceptable, no further NEDI calculation is presented.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

IESTI calculation

The setting of an ARfD is not needed and consequently, acute dietary exposure calculations are not needed.

Table 2.7.9- 1 TMDI for Benzobicyclon using the EFSA PRIMo Model Rev. 3.1

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

Benzobicyclon + 1315P-070

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	0,034	ARfD (mg/kg bw):	not necessary
Source of ADI:		Source of ARfD:	
Year of evaluation:		Year of evaluation:	

Input values

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

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

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)

Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
0,091%	GEMS/Food G06	**0,03**	0,091%	Rice						**0,1%**
0,074%	GEMS/Food G10	**0,03**	0,074%	Rice		FRUIT AND TREE NUTS				0,1%
0,046%	PT general	**0,02**	0,046%	Rice		FRUIT AND TREE NUTS				0,0%
0,037%	NL toddler	**0,01**	0,037%	Rice		FRUIT AND TREE NUTS				0,0%
0,037%	UK infant	**0,01**	0,037%	Rice		FRUIT AND TREE NUTS				0,0%
0,035%	FR toddler 2 3 yr	**0,01**	0,035%	Rice		FRUIT AND TREE NUTS				0,0%
0,034%	UK toddler	**0,01**	0,034%	Rice		FRUIT AND TREE NUTS				0,0%
0,033%	FI 3 yr	**0,01**	0,033%	Rice		FRUIT AND TREE NUTS				0,0%
0,028%	ES child	**0,01**	0,028%	Rice		FRUIT AND TREE NUTS				0,0%
0,026%	FR child 3 15 yr	**0,01**	0,026%	Rice		FRUIT AND TREE NUTS				0,0%
0,025%	FI 6 yr	**0,01**	0,025%	Rice		FRUIT AND TREE NUTS				0,0%
0,024%	SE general	**0,01**	0,024%	Rice		FRUIT AND TREE NUTS				0,0%
0,022%	UK vegetarian	**0,01**	0,022%	Rice		FRUIT AND TREE NUTS				0,0%
0,022%	UK adult	**0,01**	0,022%	Rice		FRUIT AND TREE NUTS				0,0%
0,021%	GEMS/Food G07	**0,01**	0,021%	Rice		FRUIT AND TREE NUTS				0,0%
0,018%	GEMS/Food G15	**0,01**	0,018%	Rice		FRUIT AND TREE NUTS				0,0%
0,018%	IE child	**0,01**	0,018%	Rice		FRUIT AND TREE NUTS				0,0%
0,017%	DK child	**0,01**	0,017%	Rice		FRUIT AND TREE NUTS				0,0%
0,017%	GEMS/Food G11	**0,01**	0,017%	Rice		FRUIT AND TREE NUTS				0,0%
0,016%	DE child	**0,01**	0,016%	Rice		FRUIT AND TREE NUTS				0,0%
0,016%	GEMS/Food G08	**0,01**	0,016%	Rice		FRUIT AND TREE NUTS				0,0%
0,015%	RO general	**0,01**	0,015%	Rice		FRUIT AND TREE NUTS				0,0%
0,014%	ES adult	**0,00**	0,014%	Rice		FRUIT AND TREE NUTS				0,0%
0,013%	IE adult	**0,00**	0,013%	Rice		FRUIT AND TREE NUTS				0,0%
0,013%	LT adult	**0,00**	0,013%	Rice		FRUIT AND TREE NUTS				0,0%
0,011%	IT toddler	**0,00**	0,011%	Rice		FRUIT AND TREE NUTS				0,0%
0,011%	IT adult	**0,00**	0,011%	Rice		FRUIT AND TREE NUTS				0,0%
0,010%	NL child	**0,00**	0,010%	Rice		FRUIT AND TREE NUTS				0,0%
0,009%	FR adult	**0,00**	0,009%	Rice		FRUIT AND TREE NUTS				0,0%
0,008%	NL general	**0,00**	0,008%	Rice		FRUIT AND TREE NUTS				0,0%
0,008%	FI adult	**0,00**	0,008%	Rice		FRUIT AND TREE NUTS				0,0%
0,005%	DK adult	**0,00**	0,005%	Rice		FRUIT AND TREE NUTS				0,0%
0,004%	FR infant	**0,00**	0,004%	Rice		FRUIT AND TREE NUTS				0,0%
	Column7			FRUIT AND TREE NUTS		FRUIT AND TREE NUTS				
	Column7			FRUIT AND TREE NUTS		FRUIT AND TREE NUTS				
	Column7			FRUIT AND TREE NUTS		FRUIT AND TREE NUTS				

Conclusion:

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

The long-term intake of residues of Benzobicyclon + 1315P-070 is unlikely to present a public health concern.

2.7.10 Proposed MRLs and compliance with existing MRLs

MRLs for rice grain is set at 0.02 mg/kg, the lowest validated levels of the analytical method. For rice straw, a feed item, an MRL is not required.

2.7.11 Proposed import tolerances and compliance with existing import tolerances

No import tolerance MRLs for Benzobicyclon are included with this submission.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

The route and rate of degradation of [bic-^{14}C] or [ben-^{14}C]-benzobicyclon was investigated in flooded and non-flooded soils under aerobic conditions in the dark (OECD 307 guideline). Further, degradation was assessed under anaerobic conditions in one flooded soil. Under both aerobic and anaerobic conditions Benzobicyclon ([bicyclooctane ring-2,4-^{14}C]-Benzobicyclon-bic label and [chlorophenyl-^{14}C(U)]-Benzobicyclon - ben label) (radiochemical purity 100%) degraded rapidly in flooded soils with DT_{50} values of 1.2 to 18.0 days in the total system to form several metabolites, namely, 1315P-070 (40.1 % AR and 48.9 % AR under aerobic and anaerobic conditions), 1315P-570 (aerobic : 56.7 % AR, anaerobic: 21.7 % AR). Transformation of Benzobicyclon is proposed to proceed via hydroxylation of the thioenol ester group resulting in the formation of 1315P-070 or alternatively by substitution of the thioenol ester by ammonia, forming 1315P-570. Further degradation products are 1315P-960 (up to 13.6 % AR under aerobic conditions) and 1315P-076 (7.4 % AR at the end of the study period under anaerobic conditions). Non-extractable residues amounted to 11.0 % AR to 19.6 AR under aerobic conditions and were slightly higher under anaerobic conditions (26.2 % AR). Maximum mineralization of 5.3 % AR was encountered for aerobic samples and was negligible under anaerobic conditions.

In non-flooded aerobic soil, Benzobicyclon ([bicyclooctane ring-2,4-^{14}C]-Benzobicyclon-bic label and [chlorophenyl-^{14}C(U)]-Benzobicyclon - ben label) (radiochemical purity 99.2%) was similarly transformed to 1315P-070, 1315P-570 and 1315P-960, but occurrences being generally lower for the different metabolites with maximum values of 25.4 % AR, 13.3 % AR, 5.9 % AR. The secondary degradation product 1315P-966 was additionally identified at 20.8 % AR maximum. Further degradation products including 1315P-683 were encountered at levels < 5 % AR.
Bound residues accounted for 18.2% AR to 33.4 % AR for the different soils and labels. Mineralization amounted to values of 2.0 % AR to 39.8 % AR at the end of the study period.
The degradation of Benzobicyclon in non-flooded aerobic soils was most adequately described with biphasic modes with DT50 values ranging from 8.0 to 38.2 days and DT90 values of 119 to 223 days.

In a laboratory photolysis study, Benzobicyclon degraded slowly on dry soil surface, still accounting for > 74 % AR at study end. One major metabolite was observed in light exposed samples, i.e. 1315P-683 (5.6 % AR).

On overview of transformation processes of Benzobicyclon in non-flooded and flooded soil is given below. In the different laboratory studies with application of the parent substance or respective degradation products, no or only limited degradation of metabolites was observed.

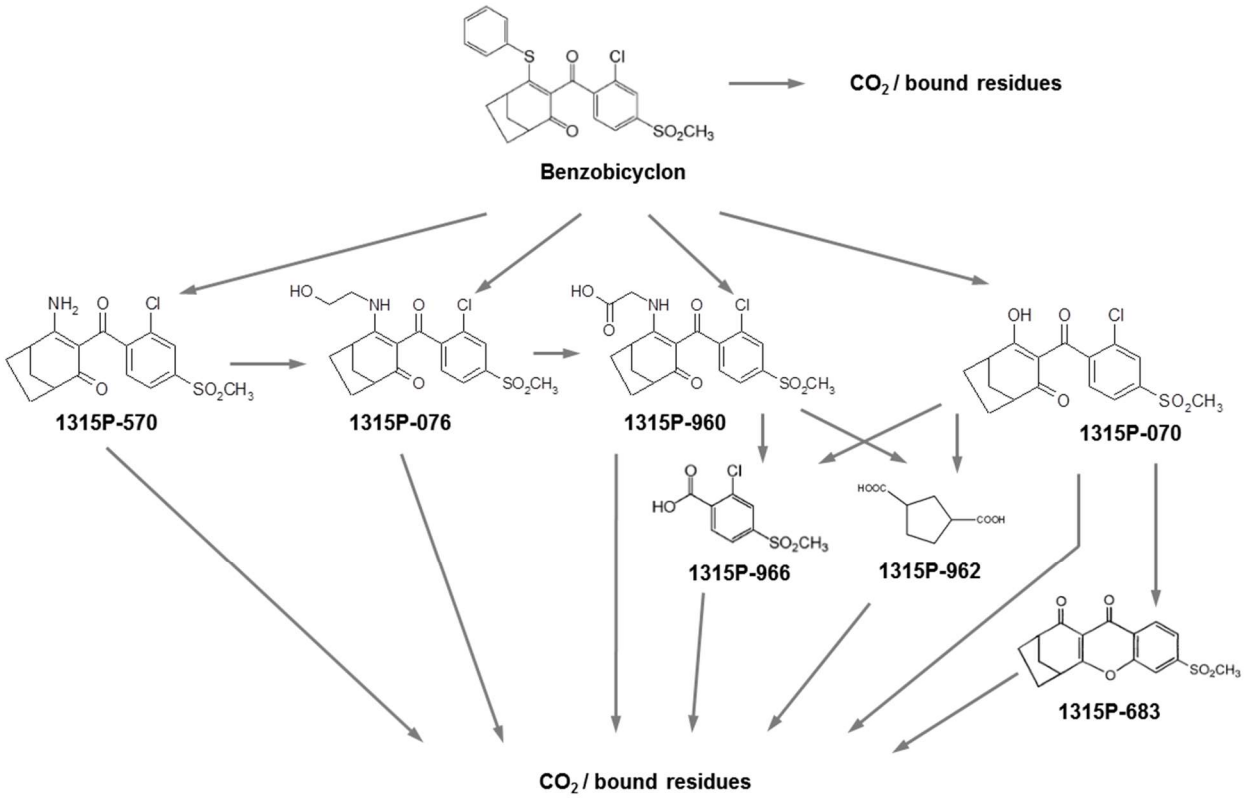


Figure 2 Overview of transformation processes of Benzobicyclon in soil

There are two field dissipation studies available with a total of four test sites situated in Spain, Italy, California and Louisiana. All test sites were flooded prior to application and a water level of a few centimetres was maintained until permanent drainage after 57 to 60 days. At all test sites, Benzobicyclon dissipated fast and transformed to several degradation products. In contrast to laboratory assessments, most of the transformation products were encountered at low levels and degraded well within the study period.
Metabolites 1315P-070, 1315P-570, 1315P-960 and 1315P-966 has been identified as relevant metabolites in soil.
The sorption behaviour of Benzobicyclon, 1315P-070, 1315P-570 and 1315P-966 has been assessed in batch equilibrium experiments. For Benzobicyclon, 1315P-070 and 1315P-966 five soils each were used; for 1315P-570 the study was based on three soils. For Benzobicyclon K_{Foc} values for adsorption in the range of 4351.1 to 24796.5 mL/g (geometric mean 8438.5 mL/g) were determined. 1/n values ranged from 0.766 to 0.882 (arithmetic mean 0.826). The derived adsorption coefficients indicate that Benzobicyclon is strongly adsorbed onto soil. Once adsorbed onto soil, Benzobicyclon is readily desorbed, as the desorption coefficients are at a similar height than the adsorption coefficients.
With adsorption coefficients of 81.5 to 844.1 mL/g (geometric mean 250.9 mL/g) and 1/n values of 0.784 to 0.982 (arithmetic mean 0.886), 1315P-070 is moderately to strongly adsorbed onto soil.
No reliable adsorption studies are available for the metabolite 1315P-966. The default values (Koc 0 and 1/n 0.9) are used for modelling purposes.
For the metabolite 1315P-076 only for two of the three soils investigated a reliable kfoc values can be derived. Nevertheless, considering that the metabolite 1315P-076 is not a relevant metabolite in soil no further information are required.
Due to the uncertain results of the adsorption studies provided, reliable Kfoc values for the metabolites 1315P-570 and 1315P-960 cannot be derived. The default values (Koc 0 and 1/n 0.9) are used for modelling purposes.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

The fate and behaviour of Benzobicyclon in water was investigated under abiotic and biotic conditions.
Two studies were available to investigate the route and rate of degradation of Benzobicyclon in sterile buffered solutions. In one study, (conducted following OCSPP 835.2120 and OECD No. 111 guidelines), Benzobicyclon

([bicyclooctane ring-2,4-^{14}C]-Benzobicyclon-bic label and [chlorophenyl-^{14}C(U)]-Benzobicyclon - ben label) (radiochemical purity 99.2%) was assessed at temperatures of 10, 25 and 50°C at a pH range of 4, 7 and 9 and showed rapid degradation. The sole hydrolysis product, 1315P-070, was hydrolytically stable. The Arrhenius half-life at 25°C, pH 4, 7 and 9, was determined to be 0.6, 0.7 and 0.5 days, respectively.
The second study was not considered acceptable and the kinetic fit results obtained by the applicant are not included in the LoEP.

In an irradiated degradation study (MA FF (Japan), 9 Nosan No. 5089 (1997) and FOCUS 2014 guidelines), Benzobicyclon (purity 100%) hydrolyzed rapidly in distilled water and paddy water, but the photolysis rate could not be determined due to extremely rapid hydrolysis. 1315P-070 rapidly photolysed and production levels of 1315P-683 and 1315P-966 were low. In a separate study, rapid hydrolysis to 1315P-070 was determined to be the primary degradation pathway for Benzobicyclon, with photolysis playing an insignificant role in the degradation of Benzobicyclon. In contrast, 1315P-070 photolysed rapidly (DT_{50} <2 days) in natural water. Major photolytic degradation products of 1315P-070 are 1315P-962, 1315P-966, and 1315P-683, together with mineralization to CO_2.

In two studies, Benzobicyclon was evaluated in four aerobic water/sediment systems, with total system DT_{50} values between 0.76 and 1.03 days. The main degradates, 1315P-070 and 1315P-570, were observed in all test systems. 1315P-070 represented up to 89.6% AR and was present preferentially in the water layers. 1315P-570 represented up to 59.8 % AR and was present in both water and sediment layers. The DT_{50} of 1315P-070 in the total system ranged from 167.5 to 324 days. The DT_{50} of 1315P-570 was calculated for two test systems, and ranged between 40.5 to 88.5 days in the water layer.

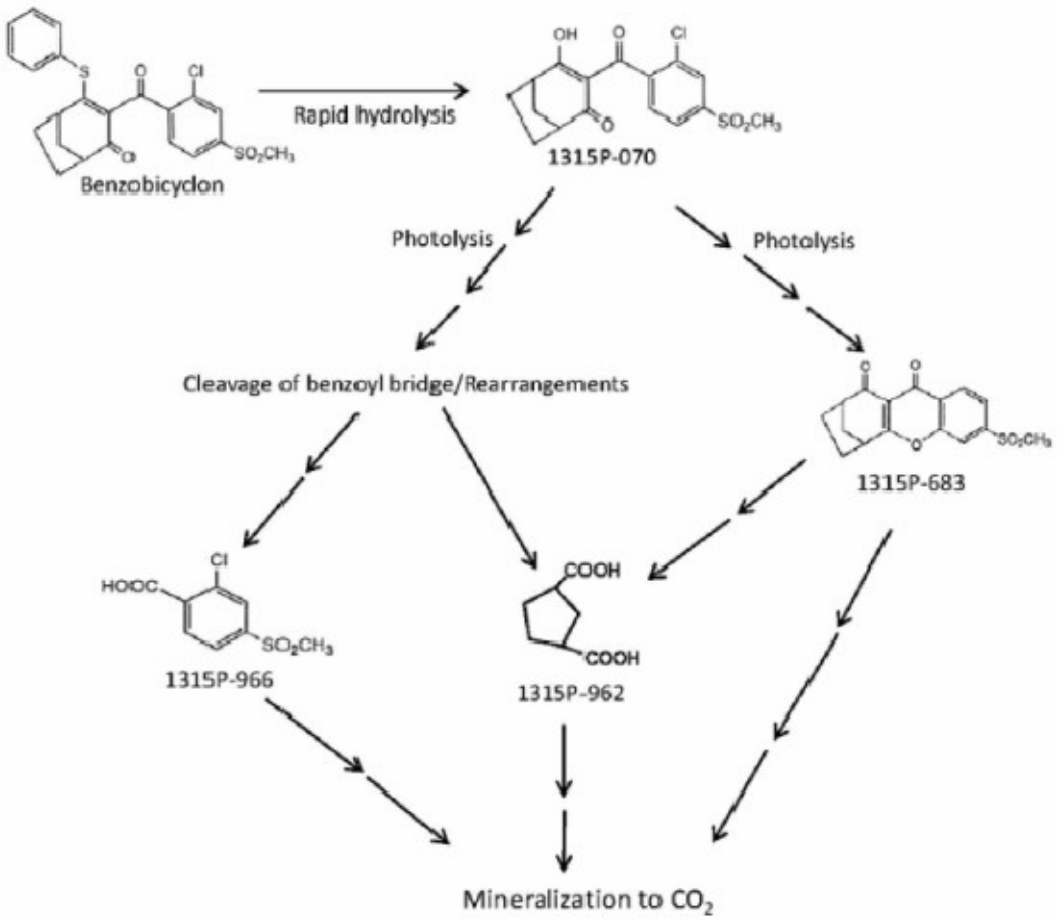


Figure 3: Proposed degradation pathway of Benzobicyclon and 1315P-070 in aqueous environments

2.8.2.1 Rapid degradability of organic substances

Table 59: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks	Reference
Aerobic soil degradation **Guidelines followed**: OECD 307. **Test material:** - [bicyclooctane ring-2,4-^{14}C]-Benzobicyclon (bic label) (radiochemical purity 99.2%); - [chlorophenyl-^{14}C(U)]-Benzobicyclon (ben label) (radiochemical purity 99.2%.) **Test duration**: 120 days	DT_{50} 8.6 d – 37.6 d	Key study	-	[redacted] (2019); Doc. No.: 721-009
Aerobic soil degradation **Guidelines followed**: OECD 307 flooded, FOCUS 2006 and FOCUS 2014. **Test material:** - [bicyclooctane ring-2,4-^{14}C]-Benzobicyclon (bic label) (radiochemical purity 100 %); - [chlorophenyl-^{14}C(U)]-Benzobicyclon (ben label) (radiochemical purity 100 %. **Test duration**: 120 days	DT_{50} 1.2 – 17.8 d	Key study	Although low mineralization was observed, Benzobicyclon degraded rapidly forming degradation products.	[redacted] (2019 Doc. N° 721-007 [redacted] (2020) Doc No 721-014 Anonymous (2021) Doc. N° 781-005
Anaerobic soil degradation **Guidelines followed**: OECD 307 flooded, FOCUS 2006 and FOCUS 2014.	DT_{50} 1.7 – 9.4 d	Key study	Although low mineralization was observed, Benzobicyclon degraded rapidly forming degradation products.	[redacted] (2019 Doc. N° 721-007 [redacted] (2020) Doc No 721-014 Anonymous (2021) Doc. N° 781-005

Method	Results	Key or Supportive study	Remarks	Reference
Test material: - [bicyclooctane ring-2,4-^{14}C]-Benzobicyclon (bic label) (radiochemical purity 100 %); - [chlorophenyl-^{14}C(U)]-Benzobicyclon (ben label) (radiochemical purity 100 %. **Test duration**: 120 days				
Photodegradation in/on soil surface **Guidelines followed**: OCSPP 835-2410 (2008), OECD Guidelines for the Testing of Chemicals, Phototransformation of Chemicals on Soil Surfaces (2002), FOCUS 2006 and FOCUS 2014. **Test material:** - [bicyclooctane ring-2,4-^{14}C]-Benzobicyclon (bic label) (Radiochemical Purity 100 %); - [chlorophenyl-^{14}C(U)]-Benzobicyclon (ben label) (radiochemical Purity >98 %. **Test duration**: 16 days	$DT_{50\ at\ 50°N}$ 41.82 – 52.6 d	Key study	Although low mineralization was observed, Benzobicyclon degraded rapidly forming degradation products.	[redacted] (2012) Report No 724-001 [redacted] (2020) Doc.No 781-002
Hydrolysis **Guidelines followed**: OCSPP 835.2120 (2008), OECD No. 111 (2004). **Test material:**	Degradation rate at 25 °C: pH 4: 0.6 days pH 7: 0.7 days pH 9: 0.5 days	Key study	For the 50.0°C test, temperatures of 49.3°C - 50.7°C were measured. The deviations were transient and only slightly outside the target of	[redacted] (2014); Doc. No.: 711-001

Method	Results	Key or Supportive study	Remarks	Reference
- [^{14}C]benzobicyclon bicyclooctane (bic label) (radiochemical Purity 99.2 %); - [^{14}C]Benzobicyclon chlorophenyl (ben label) (radiochemical Purity 99.2 %. **Test duration**: 61 days			±0.5°C, and so did not impact samples.	
Aqueous photolysis **Guidelines followed**: MAFF (Japan), 9 Nosan No. 5089 (1997), FOCUS 2014. **Test material:** - Benzobicyclon (Purity 100%) **Test duration**: up to 14 days	Benzobicyclon: not relevant 1315P-070: DT_{50} 3.7-5.1 days (distilled and natural water respectively)	Key study	-	[redacted] (1999); Doc. No.: 712-002 [redacted] (2021a) Doc No 781-003
Ready biodegradability **Guidelines followed**: OECD No. 301 B (1992). **Test material:** - Benzobicyclon (Purity >99.9 %) **Test duration**: 36 days **Reliable for use under CLP**	Benzobicyclon cannot be considered readily biodegradable	Key study	-.	[redacted] 2015, Doc. No.: 713-001
Aerobic mineralisation in surface water **Guidelines followed**: OECD No. 309 (2004), OPPTS 835.3190 (2008). **Test material:** - [^{14}C]benzobicyclon bicyclooctane (bic	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.	Key study	-	[redacted] (2019); Doc. No.: 714-003

Method	Results	Key or Supportive study	Remarks	Reference
label) (radiochemical Purity 98.5 %) - [^{14}C]Benzobicyclon chlorophenyl (ben label) (radiochemical Purity 98.6%). **Test duration**: 58 days **Reliable for use under CLP**				
Degradation in water/sediment systems [redacted]. **(2015)** **Guidelines followed**: OECD No. 308 (2002), SANCO/3029/99 rev.4 (2000). **Test material:** - [bic-^{14}C]-Benzobicyclon (bic label) (radiochemical Purity 99.2 %) - [ben-^{14}C]-Benzobicyclon (ben label) (radiochemical Purity 99.2%). **Test duration**: 104 days [redacted] **(2012)** **Guidelines followed**: OCSPP 835.4300 (2008). **Test material:** - [^{14}C]benzobicyclon bicyclooctane (bic label) (radiochemical Purity >98 %) - [^{14}C]benzobicyclon chlorophenyl (ben	<u>Benzobicyclon</u>: DT_{50} whole system 0.76 -1.03 days DT_{50} water 0.5 -0.86 days DT_{50} sediment 4.77 – 16.10 days <u>1315P-070:</u> DT_{50} whole system 167.5 -324 days DT_{50} water 140.5 -214.5 days Sediment: no decline <u>1315P-570:</u> Whole system: no decline DT_{50} water 40.5-88.5 days Sediment: no decline	Key study	-	[redacted] (2015); Doc. No.: 714-001 [redacted] (2012); Doc. No.: 714-002

Method	Results	Key or Supportive study	Remarks	Reference
label) (radiochemical Purity ≥98 %). **Test duration**: 100 days **Reliable for use under CLP**				

2.8.2.1.1 Ready biodegradability

The ready biodegradability of Benzobicyclon (purity >99.9%) was assessed in one study (2015, Doc. No.: 713-001) by measuring carbon dioxide evolution according to the Modified Sturm Test (OECD 301 B, 1992). Benzobicyclon did not show any evidence of significant biodegradation and cannot be considered readily biodegradable. Biodegradation was 0.3 % on Day 35.

2.8.2.1.2 BOD5/COD

The ready biodegradability of Benzobicyclon (purity >99.9%) was assessed in one study (2015, Doc. No.: 713-001) by measuring carbon dioxide evolution according to the Modified Sturm Test (OECD 301 B, 1992). In this study the oxygen demand was not measured, but the amount (%) of the theoretical CO_2 amount ($ThCO_2$) of the test item was determined. Degradation of the test item was determined by measuring the produced CO_2.The amount of CO_2 produced from the test item less the amount derived from the blank inoculum is expressed as percentage of $ThCO_2$. After 35 days of incubation a maximum of 44.2 mg CO_2 was measured. The degradation extent of Benzobicyclon was 0.3% within 35 days; no degradation of the test item was observed. Benzobicyclon did not pass the level for ready biodegradability.

2.8.2.2 Other convincing scientific evidence

No other convincing study was conducted.

2.8.2.2.1 Aquatic simulation tests

Three studies with Benzobicyclone in aquatic systems are available. One surface water study (OECD 309; 2019, Doc. No.: 714-003) and two water/sediment studies (OECD 308; , 2015, Doc. No.: 714-001 and OCSPP 835.4300, 2012, Doc. No.: 714-002). In the surface water study degradation of [^{14}C]benzobicyclon bicyclooctane (bic label) (radiochemical purity 98.5 %) and [^{14}C]Benzobicyclon chlorophenyl (ben label) (radiochemical purity 98.6%) was determined in two suspended sediment systems at a low and a high concentration. Benzobicyclon degraded very fast in the water phase. Benzobicyclon had completely disappeared in all test systems after seven days of incubation, forming degradation products. CO_2 amounted up to 1.4% AR after 58 days of incubation. Degradation of [^{14}C]benzobicyclon bicyclooctane (bic label) (radiochemical purity 99.2 %) and [^{14}C]Benzobicyclon chlorophenyl (ben label) (radiochemical purity 99.2%) was determined in four water/sediment systems, two sampled in Europe and two sampled in the US. Samples were incubated under aerobic conditions. Benzobicyclon degraded rapidly especially in the water phase. A mineralisation of up to 6.9% was observed after 100 days of incubation.

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

Two aquatic field dissipation studies are available (2019, Doc. No.: 723-003 and 2017, Doc. No.: 723-002) with a total of four test sites situated in Spain, Italy, California and Louisiana. All test sites were flooded prior to application and a water level of a few centimetres was maintained until permanent drainage after 57 to 60 days. CO_2 release as indication of total mineralisation cannot be measured in a field study, but at all test sites, Benzobicyclon dissipated fast and transformed to several degradation products. Most of the transformation products were encountered at low levels and all degraded completely within the study period.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

No inherent and enhanced ready biodegradability tests were submitted.

2.8.2.2.4 Soil and sediment degradation data

The route and rate of degradation of [bic-^{14}C] or [ben-^{14}C]-benzobicyclon was investigated in flooded and non-flooded soils under aerobic and anaerobic conditions in the dark (OECD 307). Under both aerobic and anaerobic conditions Benzobicyclon ([bicyclooctane ring-2,4-^{14}C]-Benzobicyclon-bic label and [chlorophenyl-^{14}C(U)]-Benzobicyclon - ben label) (radiochemical purity 100%) degraded rapidly in flooded soils with DT_{50} values of 1.2 to 18.0 days in the total system to form 1315P-070 (up to 48.9 % AR) or 1315P-570 (≤ 56.7 % AR). Further degradation products are 1315P-960 (up to 13.6 % AR) formed primarily under aerobic conditions and 1315P-076 (≤ 7.4 % AR) formed under anaerobic conditions. Mineralization amounted to ≤ 5.3 % AR for aerobic samples and was negligible under anaerobic conditions.

In non-flooded aerobic soil, Benzobicyclon ([bicyclooctane ring-2,4-^{14}C]-Benzobicyclon-bic label and [chlorophenyl-^{14}C(U)]-Benzobicyclon - ben label) (radiochemical purity 99.2%) was similarly transformed to 1315P-070, 1315P-570 and 1315P-960, but occurrences being generally lower for the different metabolites with maximum values of 25.4 % AR, 13.3 % AR, and 5.9 % AR. The secondary degradation product 1315P-966 was additionally identified at 20.8 % AR maximum. Further degradation products including 1315P-683 were encountered at levels < 5 % AR.
Bound residues accounted for 18.2% AR to 33.4 % AR for the different soils and labels. Mineralization amounted to values of 2.0 % AR to 39.8 % AR at the end of the study period.
The degradation of Benzobicyclon in non-flooded aerobic soils was most adequately described with biphasic modes with DT50 values ranging from 8.0 to 38.2 days and DT_{90} values of 119 to 223 days.

However, the behaviour of Benzobicyclon and its degradation products under realistic outdoor conditions is considered to be most adequately reflected by field dissipation data. In aquatic field dissipation studies with a total of four test sites flooded prior to application and maintained flooded until permanent drainage after 57 to 60 days, Benzobicyclon dissipated fast and transformed to several degradation products.
Metabolites 1315P-070, 1315P-570, 1315P-960 and 1315P-966 were qualifying as major metabolites in soil. Accordingly, Benzobicyclon and the degradation products occurring at relevant concentrations under realistic outdoor conditions are considered as relevant residues requiring further assessment.

For Benzobicyclon, DT_{50} values < 1 day and DT_{90} values ranging from 11 to 103 days were encountered in the total system. Dissipation of 1315P-070 in the total systems of three sites is described by DT_{50} values of 17.4 days in one site (Italy). For 1315P-966 the dissipation behaviour in the total system could be described by DT_{50} values between 2.0 and 5.4 at two sites. For 1315P-570 the dissipation behaviour in the total system could be described by DT_{50} values of 22.6 days at one site (Italy). For 1315P-960 the dissipation behaviour in the total system could be described by DT_{50} values of 3.5 days at one site (Italy).

Benzobicyclon was evaluated in four aerobic water/sediment systems, with total system DT_{50} values between 0.76 and 1.03 days. The main degradates, 1315P-070 and 1315P-570, were observed in all test systems. 1315P-070 represented up to 89.6% AR and was present preferentially in the water layers. 1315P-570 represented up to 59.8 % AR and was present in both water and sediment layers. The DT_{50} of 1315P-070 in the total system ranged from 167.5 to 324 days. The DT_{50} of 1315P-570 was calculated for two test systems, and ranged between 40.5 to 88.5 days in the water layer.

2.8.2.2.5 Hydrolysis

Two studies were available to investigate the route and rate of degradation of Benzobicyclon in sterile buffered solutions. In one study (conducted following OCSPP 835.2120 and OECD No. 111 guidelines), Benzobicyclon ([bicyclooctane ring-2,4-^{14}C]-Benzobicyclon-bic label and [chlorophenyl-^{14}C(U)]-Benzobicyclon - ben label) (radiochemical purity 99.2%) was assessed at temperatures of 10, 25 and 50°C at a pH range of 4, 7 and 9 and showed rapid degradation. The sole hydrolysis product, 1315P-070, was hydrolytically stable. The Arrhenius half-life at 25°C, pH 4, 7 and 9, was determined to be 0.6, 0.7 and 0.5 days, respectively.
The second study was not considered acceptable and the kinetic fit results obtained by the applicant are not included in the LoEP.

2.8.2.2.6 Photochemical degradation

In an irradiated degradation study (MA FF (Japan), 9 Nosan No. 5089 (1997) and FOCUS 2014 guidelines) , Benzobicyclon (purity 100%) hydrolyzed rapidly in distilled water and paddy water, but the photolysis rate could not be determined due to extremely rapid hydrolysis. 1315P-070 rapidly photolysed and production levels of 1315P-683 and 1315P-966 were low. In a separate study, rapid hydrolysis to 1315P-070 was determined to be the primary degradation pathway for Benzobicyclon, with photolysis playing an insignificant role in the degradation of Benzobicyclon. In contrast, 1315P-070 photolysed rapidly (DT_{50} <2 days) in natural water. Major photolytic degradation products of 1315P-070 are 1315P-962, 1315P-966, and 1315P-683, together with mineralization to CO_2.

2.8.2.2.7 Other / Weight of evidence

No other data was submitted.

2.8.3 Summary of fate and behaviour in air

The information on vapour pressure (2.91×10^{-5} Pa at 20 °C) and Henry's law constant (0.484 Pa×m^3/mol at 25 C) suggest that Benzobicyclon may only slightly partition from soil, moist surfaces and water to air. The photochemical and oxidative decomposition of Benzobicyclon in air was evaluated on theoretical calculation according to Atkinson using the programme AopWin. Benzobicyclon is estimated to degrade quickly in the atmosphere with a DT_{50} of 2.2 hours.

2.8.3.1 Hazardous to the ozone layer

Table 60: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
No data available. Not required			

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

The vapour pressure of 2.9×10^{-5} Pa at 20 °C of Benzobicyclon is below the trigger for volatilisation of 1×10^{-4} Pa for soil and only marginally above the trigger of 1×10^{-5} Pa for plants. Additionally, 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.

2.8.3.1.2 Comparison with the CLP criteria

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 below the trigger for volatilisation and the quickly degradation in the atmosphere (DT_{50} 2.2 hours), no hazard to the ozone layer is expected.

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

Benzobicyclon is not listed in Appendix I of Regulation No 1005/2009 of the European Parliament and of the council (16 September 2009). Therefore no classification is required according to CLP.

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

No monitoring data in the different environmental compartments of the active ingredient Benzobicyclon have been reported.

2.8.5 Definition of the residues in the environment requiring further assessment

The following residues require risk assessments in different environmental compartments:

	Residue definition
Soil	Benzobicyclon, 1315P-070, 1315P-966, 1315P-570 and 1315P-960
Groundwater	Benzobicyclon, 1315P-070, 1315P-966, 1315P-570 and 1315P-960
Surface water	Benzobicyclon, 1315P-070, 1315P-570, 1315P-966, 1315P-960, 1315P-076, 1315P-683, 1315P-962
Sediment	Benzobicyclon, 1315P-070, 1315P-570, 1315P-966 and 1315P-960
Air	Benzobicyclon

2.8.6 Summary of exposure calculations and product assessment

SOIL

PECsoil calculations were performed for the representative use in rice for an application rate of 300 g a.s./ha. No interception was assumed as the formulation is intended to be applied at an early growth stage. Following application, a minimum water holding period of 5 days was assumed.
PEC_{SOIL} for benzobicyclon and its metabolites has been calculated using the MED-Rice tool and guidance.

Table 61: PEC_{soil}, initial and PEC_{soil} (t_{close}) for Benzobicyclon, 1315P-070, 1315P-570, 1315P-960 and 1315P-966 determined according to MED-Rice Step 1a for a single application of 300 g a.s./ha Benzobicyclon to flooded rice paddy (RMS re-calculations)

Substance	$PEC_{SOIL, initial}$ (mg/kg)		PEC_{SOIL} (t_{close}) (mg/kg)	
	Scenario 1 [a)]	Scenario 2 [a)]	Scenario 1 [a)]	Scenario 2 [a)]
Benzobicyclon	0.397	0.393	0.379	0.376
1315P-070	0.0412	0.0335	0.0395	0.0322
1315P-966	0.000	0.000	0.000	0.000
1315P-570	0.000	0.000	0.000	0.000
1315P-960	0.000	0.000	0.000	0.000

[a)] Scenario 1: clayey soil texture with high OC content
Scenario 2: sandy soil texture with low OC content

At the beginning of January 2018, Italy, the major rice producing country in EU, started a project entitled: "Update and harmonization of rice pesticide risk assessment and revision of European guidelines" (ICPS, 2020) involving other European rice producing countries from the Southern zone, in particular: Spain, Portugal, France, Greece and Bulgaria. This project provides a proposal for environmental risk assessment for pesticide to be used in rice. A revision of scenarios, clarifications on data requirements and equations of STEP 1 evaluation (lower tier MED-Rice) and a proposal for the higher tier to be used to refine exposure in surface water were described.
In the context of the registration of benzobicyclon, the RMS would like to re-calculate Tier 1 PEC values with the new MED-Rice tool developed in the abovementioned project, in order to provide a more reliable assessment.

The main changes on Tier 1 MED-Rice calculations are summarized below:
- MED-Rice representative scenarios characteristics were re-evaluated by ICPS (2020) with data retrieved from the European Soil Data Centre of the Joint Research Centre of the European Commission (ESDAC), from the Agri4cast data resource portal of the JRC and from the Corine Land Cover project. A GIS-based methodology with ESRI ArcGIS 9.3.1 was used.
According to this new analysis, the MED-Rice sandy scenario has been confirmed. On the contrary, for the clayey scenario, OC % and bulk density result quite different from those proposed in MED-Rice (2003) scenarios (OC= 1.8%, bulk density=1.5 g/cm^3). Organic carbon percentage is proposed to be the same for both scenarios (OC=0.9%), instead bulk density is set to 1.4 g/cm^3 for clay scenario. In this way, more conservative results of groundwater and surface water exposure concentrations are obtained.
- Into the new MED-Rice update proposal, a distinction was made between wet and dry application to keep into consideration new and different management practices and different degradation mechanisms that can occur in these two different application types. Multiple application equations were also introduced to avoid overestimation of PEC for not-persistent compounds.

GWN-10235 is intended to be applied on wet paddy fields which are neither dry nor submerged, and therefore it is the RMS' opinion that wet applications only should be considered. For wet applications, a component describing

the degradation of the applied substance occurring between two or more applications is added to the equations for the calculation of the initial concentrations. However, since only one application of GWN-10235 is recommended, the new proposed equations were not reported here. A change in the PEC_{SOIL} values could be expected for clayey scenario only, due to the modified scenario parameters.

The re-calculated PEC_{SOIL} values for benzobicyclon and its metabolites for clayey scenario following wet application on rice are reported in the following table:

Table 62: Maximum PEC_{SOIL} values for benzobicyclon and its metabolites following a single application to flooded rice paddies at 300 g a.s./ha (RMS re-calculation with the revised approach)

Substance	Max PEC_{SOIL} (µg/kg)
	Clayey scenario
Benzobicyclon	0.420
1315P-070	0.035
1315P-966	0.000
1315P-570	0.000
1315P-960	0.000

GROUNDWATER

First-tier PEC_{GW} for Benzobicyclon and its major metabolites 1315P-070, 1315P-570, 1315P-966 and 1315P-960 were calculated by the applicant using the MED-Rice tool for the two scenarios (clay and sandy) developed in the MED-Rice (2003) guidance document.
The RMS has to notice that the calculation method for PEC_{GW} described in the MED-Rice guidance document has to be modified in order to correct a mass balance accounting error in the equations, which was found during the European registration of bensulfuron methyl. This error can result in the mass of active substance leached being higher than the amount applied, due to the fact that the there is no accounting for the dilution that results from inflowing water to replace water lost by infiltration (seepage). This results in an over-estimation of the mass of active substance remaining in the paddy water from which the PEC_{GW} is derived. The problem is addressed by adding a rate constant to equations 4, 5, 8 and 10 in the guidance document to account for the infiltration rate. The infiltration rates of 1 mm/day for clay and 10 mm/day for sand are converted to rate constant k_{leak} of 0.01 and 0.1 d^{-1}, respectively.

Table 63: PEC_{GW} values for benzobicyclon and its metabolites following application to rice (RMS re-calculation with MedRice_Modified version)

Substance	PEC_{GW} (µg/L)	
	Scenario 1	Scenario 2
Benzobicyclon	0.000	0.000
1315P-070	0.000	0.066
1315P-570	0.08	0.04
1315P-960	0.0414	0.0221
1315P-966	**0.524**	**0.278**

Based on both approaches, PEC_{GW} for benzobicyclon and its metabolites are predicted to be less than 0.1 µg/L, except for the metabolite 1315P-966. The available genotoxicity data package for 1315P-966 shows that the metabolite is non-genotoxic and the biological activity is not higher than that of the parent. Thus, 1315P-966 is considered as a non-relevant metabolite.

SURFACE WATER AND SEDIMENT

$PEC_{SW/SED}$ for benzobicyclon and its metabolites has been calculated using the MED-Rice tool and guidance.

Table 64: Summary of PECsw pw /sed of Benzobicyclon at Step 1c (RMS re-calculation)

PECsw pw			
		Scenario 1	Scenario 2
Step 1c	PEC pw, initial (mug/L)	2.661	5.176
	PEC pw (t close) (mug/L)	0.000	0.000
	PEC sw (t close) (mug/L)	0.000	0.000
PEC sed			
Step 1c	PEC sed (t close) (mug/kg)	6.353	6.353

Table 65: Summary of PECsw pw /sed of 1315P-070 at Step 1c (RMS re-calculation)

PECsw pw			
		Scenario 1	**Scenario 2**
Step 1c	PEC pw, initial (mug/L)	9.115	14.846
	PEC pw (t close) (mug/L)	7.467	12.162
	PEC sw (t close) (mug/L)	0.755	1.182
PEC sed			
Step 1c	PEC sed (t close) (mug/kg)	2.645	4.095

Table 66: Summary of PECsw pw /sed of 1315P-966 at Step 1c (RMS re-calculation)

PECsw pw			
		Scenario 1	**Scenario 2**
Step 1c	PEC pw, initial (mug/L)	15.000	15.000
	PEC pw (t close) (mug/L)	13.187	13.187
	PEC sw (t close) (mug/L)	1.236	1.236
PEC sed			
Step 1c	PEC sed (t close) (mug/kg)	0.000	0.000

Table 67: Summary of PECsw pw /sed of 1315P-570 at Step 1c (RMS re-calculation)

PECsw pw			
		Scenario 1	**Scenario 2**
Step 1c	PEC pw, initial (mug/L)	2.00	2.00
	PEC pw (t close) (mug/L)	1.84	1.84
	PEC sw (t close) (mug/L)	0.17	0.17
PEC sed			
Step 1c	PEC sed (t close) (mug/kg)	0.000	0.000

Table 68: Summary of PECsw pw /sed of 1315P-960 at Step 1c (RMS re-calculation)

PECsw pw			
		Scenario 1	**Scenario 2**
Step 1c	PEC pw, initial (mug/L)	1.2	1.2
	PEC pw (t close) (mug/L)	1.05	1.05
	PEC sw (t close) (mug/L)	0.0984	0.0984
PEC sed			
Step 1c	PEC sed (t close) (mug/kg)	0.000	0.000

PEC_{SW} for aqueous photolysis metabolites 1315P-683 and 1315P-962 were calculated by the RMS multiplying the maximum $PEC_{SW\ (drift\ initial)}$ calculated at Step1c for benzobicyclon by the molecular weight ratio and the maximum occurrence of metabolites, due to the lack of reliable degradation and sorption endpoints.

Table 69: PEC_{SW} values for aqueous photolysis metabolites in rice after application to flooded paddy (RMS calculation)

Parameter	**1315P-683**	**1315P-962**
Molecular weight (g/mol)	318.3	158.1
Max. formation in aqueous photolysis (%)	17.7	47.2
PEC_{SW}* – Scenario 1	0.009	0.012
PEC_{SW}* – Scenario 2	0.009	0.012

*PEC_{SW} calculated from $PEC_{SW\ (drift,initial)}$ of benzobicyclon at Step 1c (=0.0747 µg/L)

Updated PEC_{PW}, PEC_{SW} and PEC_{SED} calculations are provided by the RMS based on the new MED-Rice proposal (Italy_ICPS, 2020). See RMS comment to PEC_{SOIL} for further details.
GWN-10235 is intended to be applied on wet paddy fields which are neither dry nor submerged, and therefore it is the RMS' opinion that wet applications only should be considered. For wet applications, a component describing the degradation of the applied substance occurring between two or more applications is added to the equations for the calculation of the initial concentrations. However, since only one application of GWN-10235 is recommended,

the new proposed equations were not reported here. A change in the PEC_{PW} and $PEC_{SW/SED}$ values could be expected for clayey scenario only, due to the modified scenario parameterization.
The re-calculated PEC_{PW} and $PEC_{SW/SED}$ values for benzobicyclon and its metabolites considering the proposed modified clayey scenario following wet application on rice are reported in the following table:

Table 70: PEC_{PW} and $PEC_{SW/SED}$ values for benzobicyclon and its metabolites following a single application to flooded rice paddies at 300 g a.s./ha (RMS re-calculation with the revised approach)

Substance	$PEC_{PW\ (initial)}$ (µg/L)	PEC_{SW} (t_{close}) (µg/L)	PEC_{SED} (t_{close}) (µg/kg)
	Clayey scenario		
Benzobicyclon	5.54	0.0002	6.35
1315P-070	15.5	1.230	4.26
1315P-966	15.0	1.236	0.00
1315P-570	2.0	0.172	0.000
1315P-960	1.2	0.098	0.000

AIR

In view of the low volatility and the low half-life of Benzobicyclon in air, PECair are considered to be negligible. Accordingly, neither local nor global effects as global warming potential, ozone depleting, photochemical ozone creation potential, accumulation in the troposphere, acidification and eutrophication potential are expected

OTHER ROUTES OF EXPOSURE

No other routes of exposure were identified to be necessary for calculation of PEC values and consideration during environmental fate risk assessment.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Birds

Avian acute oral and long-term reproduction studies have been carried out with Benzobicyclon. The acute oral LD_{50} based on the studies with bobwhite quail and mallard duck is greater than 2250 mg a.s./kg bw, and the NOED based on the study with bobwhite quail is 36.2 mg a.s./kg bw/d.
An acute oral toxicity study with the bobwhite quail is also available for the metabolite 1315P-070 with an LD_{50} of > 2000 mg/kg bw.

Table 71: Summary of avian toxicity endpoints for Benzobicyclon used for risk assessment

Test Substance	Test Species	Endpoint	Value	Reference
Acute oral toxicity				
Benzobicyclon	*Colinus virginianus* (Northern bobwhite quail)	LD_{50}	**> 2250 mg a.s./kg bw**	[redacted] (1998a) KCA 8.1.1.1/01
	Anas platyrhynchos (Mallard duck)	LD_{50}	**> 2250 mg a.s./kg bw**	[redacted] (1998b) KCA 8.1.1.1/02
	*Taeniopygia guttata (*Zebra finch)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2012) KCA 8.1.1.1/03
1315P-070	*Colinus virginianus* (Northern bobwhite quail)	LD_{50}	**> 2000 mg/kg bw**	[redacted] (2019) KCA 8.1.1.1/04
Reproductive (long-term) toxicity				
Benzobicyclon	*Colinus virginianus* (Northern bobwhite quail)	NOEC NOED	400 mg a.s./kg diet, corresponding to **36.2 mg a.s./kg bw/d**	[redacted] (2012a) KCA 8.1.1.3/01
	Anas platyrhynchos (Mallard duck)	NOEC NOED	1000 mg a.s./kg diet, corresponding to 124.8 mg a.s./kg bw/d	[redacted] (2012b) KCA 8.1.1.3/03

Values marked in **bold** are used in the risk assessment

Terrestrial vertebrates other than birds

Mammalian acute oral and long-term reproduction studies have been carried out with Benzobicyclon. The acute oral LD_{50} based on the studies with mouse and rat is greater than 5000 mg a.s./kg bw, and the NOAEL based on the 2 generation study with rat is 59.5 mg a.s./kg bw/d. Based on the developmental toxicity studies with rat and rabbit the lowest NOAEL is 1000 mg a.s./kg bw/d.

Acute oral toxicity studies rat and mice are also available for the metabolites 1315P-070, 1315P-70 and 1315P-966 with LD_{50} of > 5000 mg/kg bw, respectively.

Table 72: Summary of mammalian toxicity endpoints for Benzobicyclon used for risk assessment

Test Substance	Test Species	Endpoint	Value	Reference
Acute oral toxicity				
Benzobicyclon	Rat	LD_{50}	**> 5000 mg a.s./kg bw**	[redacted] (1995a) KCA 5.2.1/01
	Mouse	LD_{50}	**> 5000 mg a.s./kg bw**	[redacted] (1995b) KCA 5.2.1/02
1315P-070	Rat	LD_{50}	> 5000 mg a.s./kg bw	[redacted] (1996) KCA 5.8.1/03
1315P-570	Mouse	LD_{50}	> 5000 mg a.s./kg bw	[redacted] (1999a) KCA 5.8.1/22
1315P-966	Mouse	LD_{50}	> 5000 mg a.s./kg bw	[redacted] (1999b) KCA 5.8.1/12
Reproductive (long-term) toxicity				
Benzobicyclon	Rat (2-generation study)	NOAEL	**59.5 mg a.s./kg bw/d**	[redacted] (1999) KCA 5.6.1/01
	Rat (developmental toxicity study)	NOAEL	1000 mg a.s./kg bw/d	[redacted] (1997) KCA 5.6.2/01
	Rabbit (developmental toxicity study)	NOAEL	1000 mg a.s./kg bw/d	[redacted] (2022) KCA 5.6.2/04

Values marked in **bold** are used in the risk assessment

2.9.1 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

2.9.1.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 73: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study	Remarks	Reference
OECD 305, GLP = yes, Rel = 1	*Pimephales promelas*	BCFss = 126 L/kg BCFk = 161 L/kg (lipid corrected, whole fish)	Key	Not bioaccumulative	[redacted] (2015), Doc. No. 872-001

2.9.1.1.1 Estimated bioaccumulation

Not relevant since measured data is available.

2.9.1.1.2 Measured partition coefficient and bioaccumulation test data

[redacted] (2015) has evaluated the bioaccumulation potential on fish of Benzobicyclon according to test guideline OECD 305.

Fathead minnow (*Pimephales promelas*) were exposed to Benzobicyclon under flow-through conditions at one mean measured concentration, 0.00979 mg TRR/L (0.010 mg a.s./L nominal) in a flow-through test system. Fish were exposed to the test concentration for a 10 day period. For the elimination of ^{14}C residues (depuration), the test organisms were placed in clean water for 35 days. The uptake (exposure) and depuration of Benzobicyclon were measured by determining its uptake rate constant (k_1), depuration rate constant (k_2), and steady-state bioconcentration factor (BCF).

Steady state equilibrium tissue residues in whole fish were determined to be 2210 µg/kg, yielding a steady state BCF_{ss} of 226 L/kg. Based on the ratio of the uptake and depuration rate constants (k_1 = 85.29 L/kg/day, and k_2 = 0.297 day^{-1}, respectively), the whole fish BCF_k was calculated to be 288 L/kg. Correction of the BCF values to a 5 % lipid content yielded estimates of the BCF_{ssl} = 126 L/kg and the BCF_{kl} = 161 L/kg.

Following transfer to clean water, the whole body ^{14}C-residue was depurated gradually, reaching 94 % by day 35 of depuration. The calculated time to 95 % depuration of the TRR in whole fish was 10 days. Extrapolation of the data indicated that the time to 95 % depuration is likely between 35 and 42 days.

2.9.1.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 74: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Key or Supportive study	Remarks	Reference
Acute toxicity to fish						
OECD 203 OPPTS 850.1075 GLP = yes	*Oncorhynchus mykiss*	Benzobicyclon (purity >99.9%)	**96 h LC_{50} > 0.489 mg a.s./L (mm)**	Key study. The study is valid and acceptable for regulatory use.	Flow-through, solvent = DMF. Minor deviation on water conductivity.	[redacted] (2014a) CA 8.2.1/01
OECD 203 OPPTS 850.1075 GLP = yes	*Pimephales promelas*	Benzobicyclon (purity >99.9%)	96 h LC_{50} > 0.496 mg a.s./L (mm)	Acceptable	Flow-through, solvent = DMF. Minor deviation on	[redacted] (2014b) CA 8.2.1/02

Method	Species	Test material	Results[1]	Key or Supportive study	Remarks	Reference
					water conductivity.	
OECD 203 OPPTS 850.1075 GLP = yes	*Cyprinodon variegatus*	Benzobicyclon (purity >99.9%)	96 h LC_{50} > 0.506 mg a.s./L (mm)	Acceptable	Flow-through, solvent = DMF. Minor deviation on standard length.	[redacted] (2014c) CA 8.2.1/03
OECD 203 OPPTS 850.1075 GLP = yes	*Oncorhynchus mykiss*	1315P-070 (purity 99.8%)	96 h LC_{50} > 100 mg/L (nom)	Acceptable	Flow-through, no solvent. Minor deviation on water conductivity.	[redacted] (2014a) CA 8.2.1/01
OECD 203 OPPTS 850.1075 GLP = yes	*Cyprinodon variegatus*	1315P-070 (purity 99.7%)	96 h LC_{50} > 120 mg/L (nom)	Key study. The study is valid and acceptable for regulatory use.	Flow-through, no solvent. Minor deviation on water conductivity.	[redacted] (2014c) CA 8.2.1/03
OECD 203 OPPTS 850.1075 GLP = yes	*Oncorhynchus mykiss*	GWN-10235	96 h LC_{50} > 1.3 mg a.s./L (> 3.8 mg GWN-10235/L) (mm)	Acceptable	Flow-through, no solvent. Minor deviations on environmental conditions.	[redacted] (2019a) CP 10.2.1/01
Acute toxicity to aquatic invertebrates						
OECD 202 OPPTS 850.1010 GLP = yes	*Daphnia magna*	Benzobicyclon (purity >99.9%)	**48 h EC_{50} > 0.368 mg a.s./L (mm)**	Key study. The study is valid and acceptable for regulatory use.	Flow-through, solvent = DMF. Minor deviation on number of individuals per replicate.	[redacted] (2014a) CA 8.2.4.1/01
OECD 202 OPPTS 850.1010 GLP = yes	*Daphnia magna*	1315P-070 (purity 99.7%)	48 h EC_{50} > 120 mg/L (nom)	Key study. The study is valid and acceptable for regulatory use.	Flow-through, no solvent. Minor deviation on number of individuals per replicate.	[redacted] (2014a) CA 8.2.4.1/01
OECD 202 GLP = yes	*Daphnia magna*	GWN-10235	96 h LC_{50} > 1.0 mg a.s./L (> 2.9 mg GWN-10235/L) (mm)	Acceptable	Flow-through, no solvent. Minor deviation on number of individuals per replicate.	[redacted] (2019b) CP 10.2.1/02

Method	Species	Test material	Results[1]	Key or Supportive study	Remarks	Reference
Algae						
OECD 201 GLP = yes	*Raphidocelis subcapitata*	Benzobicyclon (purity 99.3%)	72 h E_rC_{50} > 0.45 mg a.s./L (mm)	The study is valid (when considering the solvent control replicates only) and acceptable for regulatory use.	Static solvent = DMF:HCO40. Minor deviation on temperature.	[redacted] (2019a) CA 8.2.6.1/01
OPPTS 850.4550 GLP = yes	*Anabaena flos-aquae*	Benzobicyclon (purity >99.9%)	**72 h E_rC_{50} > 0.184 mg a.s./L (mm)**	Key study. The study is valid (when considering the 72-hour test period) and acceptable for regulatory use.	Static, solvent = DMF. No deviations.	[redacted] (2012) CA 8.2.6.2/01; [redacted] (2019c) CA 8.2.6.2/02 and Anonymous (2022) CA 8.2.6.2/04
OECD 201 GLP = yes	*Raphidocelis subcapitata*	1315P-070 (purity 99.34%)	72 h E_rC_{50} = 44.7 mg/L (nom)	Key study. The study is valid and acceptable for regulatory use.	Static, no solvent. Minor deviation on pH in control.	[redacted] (2019a) CA 8.2.6.1/02 [redacted] (2019b), CA 8.2.6.1/03
OECD 201 GLP = yes	*Anabaena flos-aquae*	1315P-070 (purity 99.34%)	72 h E_rC_{50} = 100 mg/L (nom)	Acceptable	Static, no solvent. No deviations.	[redacted] (2019b), CA 8.2.6.2/03
OECD 201 GLP = yes	*Raphidocelis subcapitata*	GWN-10235	72 h E_rC_{50} > 0.66 mg a.s./L (> 1.9 mg GWN-10235/L) (mm)	Acceptable	Static, no solvent. Minor deviation on temperature.	[redacted] (2019d), CP 10.2.1/03
Aquatic macrophytes						
OECD 221 GLP = yes	*Lemna gibba*	Benzobicyclon (purity 99.4%)	7 d E_rC_{50} = 0.00619 mg a.s./L (twa)	Key study. The study is valid and acceptable for regulatory use.	Semi-static, solvent = DMF. No deviations.	[redacted] (2020), CA 8.2.7/04
OECD 221 GLP = yes	*Lemna gibba*	1315P-570 (purity 99.8%)	7 d E_rC_{50} > 1.0 mg/L (nom)	Key study. The study is valid and acceptable for regulatory use.	Semi-static, solvent = DMF. No deviations.	[redacted] (2019b), CA 8.2.7/01

Method	Species	Test material	Results[1]	Key or Supportive study	Remarks	Reference
OECD 221 GLP = yes	*Lemna gibba*	1315P-070 (purity 99.4%)	7 d E_rC_{50} = 0.0135 mg/L (mm)	Key study. The study is valid and acceptable for regulatory use.	Semi-static, solvent = DMF:HCO4 0. EC_{50} estimated by non-linear regression. No deviations.	[redacted] (2019c), CA 8.2.7/02 and Anonymous (2022) CA 8.2.7/05
OECD 221 GLP = yes	*Lemna gibba*	1315P-966 (purity 99.9%)	7 d E_rC_{50} > 100 mg/L (nom)	Key study. The study is valid and acceptable for regulatory use.	Static, no solvent. No deviations.	[redacted] (2019), CA 8.2.7/03
OECD 221 GLP = yes	*Lemna gibba*	GWN-10235	7 d E_rC_{50} = 0.026 mg a.s./L (0.076 mg GWN-10235/L) (mm)	Acceptable	Semi-static, no solvent. No deviations.	[redacted] (2019e), CP 10.2.1/04

2.9.1.2.1 Acute (short-term) toxicity to fish

Three acute fish toxicity studies with rainbow trout, fathead minnow and sheepshead minnow are available for the active substance Benzobicyclon, showing no mortalities at concentrations up to and including 0.489 to 0.506 mg a.s./L.
For the metabolite 1315P-070 two studies with rainbow trout and sheepshead minnow are available, showing no mortalities at concentrations up to and including 100 to 120 mg/L.

2.9.1.2.2 Acute (short-term) toxicity to aquatic invertebrates

For Benzobicyclon and its metabolite 1315P-070 a study on the acute toxicity to daphnids (*Daphnia magna*) is available with EC_{50} values of > 0.368 mg a.s./L and > 120 mg/L, respectively.

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

Two algal toxicity studies with *Raphidocelis subcapitata* and *Anabaena flos-aquae* are available for the active substance Benzobicyclon. From the study on *R. subcapitata* a E_rC_{50} value of > 0.45 mg a.s./L was determined, while a E_rC_{50} value of > 0.184 mg a.s./L was determined in the study on *A. flos-aquae.*
An algal toxicity study with *Raphidocelis subcapitata* is available for the formulation, showing an E_rC_{50} value of > 0.66 mg a.s./L.
For the metabolite 1315P-070 a study with *Raphidocelis subcapitata* is available with an E_rC_{50} of 44.7 mg/L.

Studies with aquatic macrophytes (*Lemna gibba*) are available for the active substance Benzobicyclon and the metabolites 1315P-070, 1315P-570 and 1315P-966 with E_rC_{50} values of 0.00619, 0.0135, > 1.0 and > 100 mg/L, respectively.

A toxicity study with *Lemna gibba* is available for the formulation, showing an E_rC_{50} value of 0.026 mg a.s./L.

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

No additional acute toxicity studies for other aquatic organisms are available as they are not required.

2.9.1.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 75: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Relevant study	Remarks	Reference
Fish						
OECD No 210, OPPTS 850.1400 GLP	*Pimephales promelas*	Benzobicyclon (purity >99.9%)	28 d NOEC = 0.100 mg a.s./L (mm) 28 d EC_{10} = 0.207 mg a.s./L (mm)	Acceptable	Flow-through, solvent = DMF. No deviations.	[redacted] (2012) CA 8.2.2.1/01; [redacted] (2019b), CA 8.2.2.1/02 and Anonymous (2022) CA 8.2.2.1/05
OECD No 210, OPPTS 850.1400 GLP	*Cyprinodon variegatus*	Benzobicyclon (purity >99.9%)	28 d NOEC = 0.323 mg a.s./L 28 d EC_{10} > 0.323 mg a.s./L (mm)	Acceptable	Flow-through, solvent = DMF. Minor deviation on temperature.	[redacted] (2014d), CA 8.2.2.1/03; [redacted] (2019c), CA 8.2.2.1/04 and Anonymous (2022) CA 8.2.2.1/06
OPPTS 850.1500 GLP	*Pimephales promelas*	Benzobicyclon (purity >99.9%)	146 d NOEC = 0.0403 mg a.s./L **146 d EC_{10} = 0.018 mg a.s./L (mm)**	Key study. The study is considered acceptable for regulatory use.	Full life cycle study. Further information and clarifications should be provided by the applicant.	[redacted] (2014e), CA 8.2.2.2/01 and [redacted] (2019a), CA 8.2.2.2/02 and Anonymous (2022) CA 8.2.2.2/03
OECD No 210, OPPTS 850.1400 GLP	*Pimephales promelas*	1315P-070 (purity 99.8%)	28 d NOEC = 5 mg/L (nom) 28 d EC_{10} > 10 mg/L (nom)	Acceptable	Flow-through, no solvent. No deviations.	[redacted] (2012), CA 8.2.2.1/01 and [redacted] & [redacted] (2019b), CA 8.2.2.1/02
OECD No 210, OPPTS 850.1400 GLP	*Cyprinodon variegatus*	1315P-070 (purity 99.8%)	28 d NOEC = 5 mg/L (nom) **28 d EC_{10} = 3.43 mg/L (nom)**	Key study. The study is considered acceptable for regulatory use.	Flow-through, no solvent. No deviations.	[redacted] (2014d), CA 8.2.2.1/03 and [redacted] (2019c), CA 8.2.2.1/04

Method	Species	Test material	Results[1]	Relevant study	Remarks	Reference
Aquatic invertebrates						
OECD 211, OPPTS 850.1300 GLP	*Daphnia magna*	Benzobicyclon (purity >99.9%)	21 d NOEC = 0.279 mg a.s./L **21 d EC_{10} = 0.272 mg a.s./L (mm)**	Key study	Flow-through, solvent = DMF. No deviations. Biological relevance of dry weight reduction to be further discussed.	[redacted] (2014b), CA 8.2.5.1/01 and Anonymous (2022) CA 8.2.5.1/03
OECD 211, OPPTS 850.1300 GLP	*Daphnia magna*	1315P-070 (purity 99.8%)	21 d NOEC = 60 mg/L (nom)	Key study	Flow-through, no solvent. No deviations.	[redacted] (2014c), CA 8.2.5.1/02 and Anonymous (2022) CA 8.2.5.1/04
Algae						
OECD 201 GLP = yes	*Raphidocelis subcapitata*	Benzobicyclon (purity 99.3%)	72 h NOE_rC = 0.45 mg a.s./L (mm) 72 h E_rC_{10} > 0.45 mg a.s./L (mm)	The study is valid (when considering the solvent control replicates only) and acceptable for regulatory use.	Static solvent = DMF:HCO40. Minor deviation on temperature.	[redacted] (2019a), CA 8.2.6.1/01
OPPTS 850.4550 GLP = yes	*Anabaena flos-aquae*	Benzobicyclon (purity >99.9%)	72 h NOE_rC = 0.0774 mg a.s./L (mm) **72 h E_rC_{10} = 0.150 mg a.s./L (mm)**	Key study. The study is valid (when considering the 72-hour test period) and acceptable for regulatory use.	Static, solvent = DMF. No deviations.	[redacted] (2012), CA 8.2.6.2/01; [redacted] (2019c), CA 8.2.6.2/02 and Anonymous (2022) CA 8.2.6.2/04
OECD 201 GLP = yes	*Raphidocelis subcapitata*	1315P-070 (purity 99.34%)	72 h NOE_rC = 5.0 mg/L (nom) 72 h E_rC_{10} = 15.3 mg/L (nom)	Key study. The study is valid and acceptable for regulatory use.	Static, no solvent. Minor deviation on pH in control.	[redacted] (2019a), CA 8.2.6.1/02 and [redacted] (2019b), CA 8.2.6.1/03

Method	Species	Test material	Results[1]	Relevant study	Remarks	Reference
OECD 201 GLP = yes	*Anabaena flos-aquae*	1315P-070 (purity 99.34%)	72 h NOE_rC = 100 mg/L (nom) 72 h E_rC_{10} > 100 mg/L (nom)	Acceptable	Static, no solvent. No deviations.	[redacted] (2019b), CA 8.2.6.2/03
OECD 201 GLP = yes	*Raphidocelis subcapitata*	GWN-10235	72 h NOE_rC = 0.66 mg a.s./L (mm) 72 h E_rC_{10} > 0.66 mg a.s./L (mm)	Acceptable	Static, no solvent. Minor deviation on temperature.	[redacted] (2019d), CP 10.2.1/03
Aquatic macrophytes						
OECD 221 GLP = yes	*Lemna gibba*	Benzobicyclon (purity 99.4%)	7 d NOE_rC = 0.000167 mg a.s./L (twa) **7 d E_rC_{10} = 0.000447 mg a.s./L (twa)**	Key study. The study is valid and acceptable for regulatory use.	Semi-static, solvent = DMF. No deviations.	[redacted]. & [redacted] P. (2020), CA 8.2.7/04
OECD 221 GLP = yes	*Lemna gibba*	1315P-570 (purity 99.8%)	7 d NOE_rC = 1.0 mg/L (nom) **7 d E_rC_{10} > 1.0 mg/L (nom)**	Key study. The study is valid and acceptable for regulatory use.	Semi-static, solvent = DMF. No deviations.	[redacted] (2019b), CA 8.2.7/01
OECD 221 GLP = yes	*Lemna gibba*	1315P-070 (purity 99.4%)	7 d NOE_rC = 0.00075 mg/L (mm) **7 d E_rC_{10} = 0.0024 mg/L (mm)**	Key study. The study is valid and acceptable for regulatory use.	Semi-static, solvent = DMF:HCO40. No deviations.	[redacted] (2019c), CA 8.2.7/02 and Anonymous (2022) CA 8.2.7/05
OECD 221 GLP = yes	*Lemna gibba*	1315P-966 (purity 99.9%)	7 d NOE_rC = 100 mg/L (nom) **7 d E_rC_{10} >100 mg/L (nom)**	Key study. The study is valid and acceptable for regulatory use.	Static, no solvent. No deviations.	[redacted] (2019), CA 8.2.7/03
OECD 221 GLP = yes	*Lemna gibba*	GWN-10235	7 d NOE_rC = 0.00084 mg a.s./L (mm) 7 d E_rC_{10} = 0.0017 mg a.s./L (mm)	Acceptable	Semi-static, no solvent. No deviations.	[redacted] (2019e), CP 10.2.1/04

[1] mean measured (mm) or nominal concentration (nom)

2.9.1.3.1 Chronic toxicity to fish

Fish early life stage studies with Benzobicyclon are available for fathead minnow and sheepshead minnow with NOEC values of 0.100 mg a.s./L and 0.323 mg a.s./L, respectively. In addition, a fish full life cycle toxicity study with the fathead minnow is also available for Benzobicyclon with an EC_{10} of 0.018 mg a.s./L which is the relevant endpoint used in the risk assessment.

Fish early life stage studies with the metabolite 1315P-070 are available for fathead minnow and sheepshead minnow with NOEC value of 5 mg/L and EC_{10} value of 3.43 mg/L, respectively.

2.9.1.3.2 Chronic toxicity to aquatic invertebrates

For Benzobicyclon and its metabolite 1315P-070, studies on the chronic toxicity to daphnids (*Daphnia magna*) are available with EC_{10} value of 0.272 mg a.s./L and NOEC value of 60 mg/L, respectively.

2.9.1.3.3 Chronic toxicity to algae or aquatic plants

Two algal toxicity studies with *Raphidocelis subcapitata* and *Anabaena flos-aquae* are available for the active substance Benzobicyclon. From the study on *R. subcapitata* a NOE_rC value of 0.45 mg a.s./L was determined, while a NOE_rC value of > 0.0774 mg a.s./L was determined in the study on *A. flos-aquae.*

For the metabolite 1315P-070 a study with *Raphidocelis subcapitata* is available with a NOE_rC of 5.0 mg/L.
An algal toxicity study with *Raphidocelis subcapitata* is available for the formulation, showing a NOE_rC value of 0.66 mg a.s./L.
Studies with aquatic macrophytes (*Lemna gibba*) are available for the active substance Benzobicyclon and the metabolites 1315P-070, 1315P-570 and 1315P-966 with NOE_rC values of 0.000167, 0.00075, 1.0 and 100 mg/L, respectively.
A toxicity study with *Lemna gibba* is available for the formulation, showing a NOE_rC value of 0.00084 mg a.s./L.

2.9.1.3.4 Chronic toxicity to other aquatic organisms

No additional chronic toxicity studies for other aquatic organisms are available as they are not required.

2.9.1.4 Comparison with the CLP criteria

In the test on biodegradation of Benzobicyclon (CA 7.2.2.1/01; [redacted] 2015, Doc. No. 713-001), measured as carbon dioxide evolution, 0.3 % biodegradation occurred until day 36, which is lower than the trigger of 60% biodegradation (criterion (a) of the CLP Guidance, section 4.1.3.2.3.2). In the two water-sediment studies (CA 7.2.2.3/01; [redacted] 2015 and CA 7.2.2.3/02; [redacted] 2012), the DT_{50} total system for Benzobicyclon was determined to be about 1 day, which is lower than the trigger of 16 days (corresponding to a degradation of >70 % within 28 days), indicating a potential rapid degradability. However, 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; NOE_rC = 0.00075 mg/L on *Lemna gibba*). Therefore, Benzobicyclon should be considered as not rapidly degradable for the purpose of classification and labelling, according to the criterion (c) of the CLP Guidance, section 4.1.3.2.3.2.
Benzobicyclon has a log Kow of 3.1. The experimentally derived BCF is 161 L/kg which is below the trigger of 500, indicating that benzobicyclon is not bioaccumulative for classification and labelling purposes.

2.9.1.4.1 Acute aquatic hazard

Table 76: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results[1]	Remarks	Reference
Active substance					
OECD 203, OPPTS 850.1075	*Oncorhynchus mykiss*	Benzobicyclon (purity >99.9%)	96 h LC_{50} > 0.489 mg a.s./L (mm)	Flow-through, solvent = DMF. Minor deviation on water conductivity.	[redacted] (2014a), CA 8.2.1/01
OECD 202 OPPTS 850.1010	*Daphnia magna*	Benzobicyclon (purity >99.9%)	48 h EC_{50} > 0.368 mg a.s./L (mm)	Flow-through, solvent = DMF. Minor deviation on number of individuals per replicate.	[redacted] (2014a), CA 8.2.4.1/01

Method	Species	Test material	Results[1]	Remarks	Reference
OECD 201	*Anabaena flos-aquae*	Benzobicyclon (purity >99.9%)	72 h E_rC_{50} >0.184 mg a.s./L (mm)	Static, solvent = DMF. No deviations.	[redacted] (2012) CA 8.2.6.2/[redacted] & [redacted] (2019c) CA 8.2.6.2/02 and Anonymous (2022) CA 8.2.6.2/04
OECD 221	*Lemna gibba*	Benzobicyclon (purity 99.4%)	7 d E_rC_{50} = 0.00619 mg a.s./L (twa)	Semi-static, solvent = DMF. No deviations.	[redacted] H. & [redacted] P. (2020), CA 8.2.7/04

[1] mean measured (mm) or nominal concentration (nom)

The acute classification of the active substance is based on the endpoint from the toxicity study with *Lemna gibba* as the E_rC_{50} is below 1 mg/L. The M factor is determined to be 100 as the 7d E_rC_{50} of 0.00619 mg/L for *Lemna gibba* is in the range $0.001 < L(E)C_{50} \leq 0.01$ (CLP Table 4.1.3).

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

Table 77: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results[1]	Remarks	Reference
OPPTS 850.1500	*Pimephales promelas*	Benzobicyclon (purity >99.9%)	146 d EC_{10} = 0.018 mg a.s./L (mm)	Full life cycle study. Further information and clarifications should be provided by the applicant.	[redacted] (2014e), CA 8.2.2.2/01
OECD 211, OPPTS 850.1300	*Daphnia magna*	Benzobicyclon (purity >99.9%)	21 d EC_{10} = 0.272 mg a.s./L (mm)	Flow-through, solvent = DMF. No deviations. Biological relevance of dry weight reduction to be further discussed.	[redacted] (2014b), CA 8.2.5.1/01
OECD 201	*Anabaena flos-aquae*	Benzobicyclon (purity >99.9%)	72 h NOE_rC = 0.150 mg a.s./L (mm)	Static, solvent = DMF. No deviations.	[redacted] (2012) CA 8.2.6.2/01; [redacted] (2019c) CA 8.2.6.2/02 and Anonymous (2022) CA 8.2.6.2/04
OECD 221	*Lemna gibba*	Benzobicyclon (purity 99.4%)	EC_{10} = 0.000447 mg a.s./L (twa)	Semi-static, solvent = DMF. No deviations.	[redacted] (2020), CA 8.2.7/04

[1] mean measured (mm) or nominal concentration (nom)

Benzobicyclon is considered not rapidly degradable for the purpose of classification and labelling, according to the criterion (c) of the CLP Guidance, section 4.1.3.2.3.2 (refer to § 2.9.1.4 for details).
Benzobicyclon has a log Kow of 3.1. The experimentally derived BCF is 161 L/kg which is below the trigger of 500 indicating that benzobicyclon is not bioaccumulative for classification and labelling purposes. The log K_{OW} values for all metabolites are below 3.0 (please refer to CA B.2.7). Thus, the potential for bioconcentration for the metabolites is low and studies on bioconcentration are not required for the metabolites.

Chronic toxicity data are available for the active substance for all trophic levels. The chronic classification of the active substance is based on the endpoint from the toxicity study with *Lemna gibba* as the EC_{10} is below 0.1 mg/L.

The M factor is determined to be 100 as the EC_{10} of 0.000447 mg/L for *Lemna gibba* is in the range 0.0001 < $NOE_rC \leq 0.001$ (CLP Table 4.1.3).

2.9.1.5 Conclusion on classification and labelling for environmental hazards

Benzobicyclon fulfils the criteria for classification as Aquatic Acute 1 with an M-factor of 100.
Benzobicyclon fulfils the criteria for classification as Aquatic Chronic 1 with an M-factor of 100.

2.9.2 Summary of effects on arthropods

Effects on bees

The acute oral and contact toxicity LD_{50} values for honey bees based on toxicity studies with Benzobicyclon are both > 200.0 µg a.s./bee. In the chronic adult honey bee toxicity study, the 10 day NOEDD and the 10 day LD_{50} were determined to be ≥ 62 µg a.s./bee/day and > 62 µg a.s./bee/day, respectively. There are two honey bee larval toxicity studies. The 3-d LD_{50} and 3-d NOED were determined to be > 44 µg a.s./larva and 44 µg a.s./larva, respectively. The second study resulted in a 22 day NOED of 1.2 µg a.s./larva per developmental time.
The acute oral and contact toxicity LD_{50} values for honey bees based on toxicity studies with the Benzobicyclon metabolite 1315P-070 are both > 100.4 µg/bee. The chronic adult honey bee toxicity study with 1315P-070 resulted in a 10-d NOED and 10 d LD_{50} of ≥ 79.6 µg/bee/day and > 79.6 µg/bee/day, respectively. The honey bee larval toxicity study with 1315P-070 resulted in a 22 d ED_{10} of 4.8 µg/larva per development period.

Table 78: Summary of honey bee toxicity endpoints of Benzobicyclon and its metabolite 1315P-070 used for the risk assessment

Test Substance	Endpoint	Value	Reference
Acute toxicity to honey bees			
Benzobicyclon	48 h LD_{50} (oral)	**> 200 µg a.s./bee**	[redacted] (1998), CA 8.3.1.1.1/01
1315P-070	48 h LD_{50} (oral)	**> 100.4 µg/bee**	[redacted] (2019), CA 8.3.1.1.1/02
Benzobicyclon	48 h LD_{50} (contact)	**> 200 µg a.s./bee**	[redacted] (1998), CA 8.3.1.1.2/01
1315P-070	48 h LD_{50} (contact)	**> 100.4 µg/bee**	[redacted] (2019), CA 8.3.1.1.2/02
Chronic toxicity to honey bees			
Benzobicyclon	10 d NOED (oral) 10 d LD_{50} (oral)	≥ 62 µg a.s./bee/day **> 62 µg a.s./bee/day**	[redacted] (2019), CA 8.3.1.2/01
1315P-070	10 d NOED (oral) 10 d LD_{10} (oral) 10 d LD_{50} (oral)	≥ 79.6 µg/bee/day 44.9 µg/bee/day **> 79.6 µg/bee/day**	[redacted] (2019), CA 8.3.1.2/02
Larval toxicity to honey bees			
Benzobicyclon	3 d LD_{50} * 3 d NOED *	> 44 µg a.s./larva 44 µg a.s./larva	[redacted] (2014), CA 8.3.1.3/01
Benzobicyclon	22 d NOED 22 d ED_{10}	**1.2 µg a.s./larva per development period** 2.4 µg a.s./larva per development period	[redacted] (2019a), CA 8.3.1.3/02 and Anonymous (2022), CA 8.3.1.3/04
1315P-070	22 d NOED 22 d ED_{10}	11.1 µg/larva per development period **4.8 µg/larva per development period**	[redacted] (2019b), CA 8.3.1.3/03

* total study duration, 72 h of exposure after single application
Values in **bold** are used for the risk assessment

Effects on non-target arthropods other than bees

The toxicity of the formulated product GWN-10235, an SC formulation containing 400 g/L Benzobicyclon to non-target arthropods has been investigated by carrying out laboratory tests (glass plate studies) on both indicator species, i.e. *Typhlodromus pyri* (LR_{50} > 600 g a.s./ha, ER_{50}: < 37.5 g a.s./ha) and *Aphidius rhopalosiphi* (LR_{50}: > 600 g a.s./ha, ER_{50}: 346 g a.s./ha).
Extended laboratory studies (fresh residue) were also conducted with the formulated product GWN-10235 with *Typhlodromus pyri* (LR_{50} and ER_{50} > 623 g a.s./ha), *Chrysoperla carnea* (LR_{50} and ER_{50} > 600 g a.s./ha), and *Orius laevigatus* (LR_{50} and ER_{50} > 623 g a.s./ha).

Table 79: Toxicity of GWN-10235 to non-target arthropods

Species	Exposed life stage	Study duration and type	LR_{50} * [g a.s./ha]	ER_{50} * [g a.s./ha]	Reference
Typhlodromus pyri	Protonymphs	Tier I (glass plate exposure)	> 600 (> 1.5)	< 37.5 (< 0.094)	[redacted] (2016a) KCP 10.3.2.1/01
Aphidius rhopalosiphi	Adult females	Tier I (glass plate exposure)	> 600 (> 1.5)	346# (0.865)	[redacted] (2016b) KCP 10.3.2.1/02
Typhlodromus pyri	Adults	Tier II (extended lab test - fresh residue)	> 623 ** (> 1.5)	> 623 ** (> 1.5)	[redacted] (2019) KCP 10.3.2.2./01
Chrysoperla carnea	Adults	Tier II (extended lab test – fresh residue)	> 600 (> 1.5)	> 600 (> 1.5)	[redacted] (2019) KCP 10.3.2.2/02
Orius laevigatus	Adults	Tier II (extended lab test – fresh residue)	> 623 ** (> 1.5)	> 623 ** (> 1.5)	[redacted] (2019) KCP 10.3.2.2/03

* Values in parentheses are the endpoints for the formulation given in L/ha (calculated from active substance data using active substance content in the formulation as endpoints for the formulation are not given in the report)
** calculated from a rate of 1500 mL/ha using active substance content in the formulation and taking the product density into account

2.9.3 Summary of effects on non-target soil meso- and macrofauna

Summary of effects on earthworms

A sub-lethal laboratory study with earthworms was conducted with Benzobicyclon. The study yielded a NOEC of 1000 mg a.s./kg soil and an EC_{10} of 79 mg a.s./kg soil. These endpoints were corrected by a factor of 2 due to the log Pow of Benzobicyclon which is above the trigger of 2, resulting in a $NOEC_{corr}$ of 500 mg a.s./kg soil and an $EC_{10,\ corr}$ of 39.5 mg a.s./kg soil.
Data on sub-lethal effects to earthworms were also generated with the metabolite 1315P-070 with a NOEC of 17.2 mg/kg soil and an EC_{10} of 6.67 mg/kg soil.

Table 80: Chronic toxicity of Benzobicyclon and its metabolite 1315P-070 to earthworms

Test substance	Endpoints *	Reference
Chronic laboratory studies		
Benzobicyclon	NOEC = 1000 mg a.s./kg soil $NOEC_{corr}$ = 500 mg a.s./kg soil EC_{10} = 79 mg a.s./kg soil **$EC_{10,corr}$ = 39.5 mg a.s./kg soil**	[redacted] (2019a), KCA 8.4.1/01
1315P-070	NOEC = 17.2 mg/kg soil **EC_{10} = 6.67 mg/kg soil**	[redacted] (2019b), KCA 8.4.1/02 and Anonymous (2022), KCA 8.4.1/03

* values in **bold** are used for the risk assessment

Summary of effects on other non-target soil meso- and macrofauna (other than earthworms)

Long-term toxicity studies on *Folsomia candida* and *Hypoaspis aculeifer* have been carried out with Benzobicyclon. Based on reproduction and survival for both studies, the NOEC was determined to be 1000 mg a.s./kg soil and the EC_{10} and EC_{20} values were calculated to be > 1000 mg a.s./kg soil.

Table 81: Chronic toxicity of Benzobicyclon to non-target soil organisms other than earthworms

Test substance	Species	EC_{10} [mg a.s./kg sdw]	EC_{20} [mg a.s./kg sdw]	NOEC [mg a.s./kg sdw]	Reference
Benzobicyclon	*Folsomia candida*	> 1000 (> 500)	> 1000 (> 500)	1000 (500)	[redacted] (2019a), CA 8.4.2.1/01
	Hypoaspis aculeifer	> 1000 (> 500)	> 1000 (> 500)	1000 (500)	[redacted] (2019b), CA 8.4.2.1/02

sdw = soil dry weight
A values in parentheses represent corrected values

2.9.4 Summary of effects on soil nitrogen transformation

The effect of the formulated product GWN-10235, an SC formulation containing 400 g/L Benzobicyclon, on the nitrogen transformation of soil microorganisms was tested in the laboratory. No difference to the untreated control above 25 % was noted at 2.0 mg a.s./kg soil dry weight) after 28 days.

Table 82: Effects of Benzobicyclon to soil micro-organisms (nitrogen transformation)

Test substance	Test design	< 25 % difference to control (NOEC) [mg a.s./kg sdw]	Reference
Benzobicyclon	Nitrogen transformation	2.0 (after 28 days)	[redacted] (2015), CA 8.5/01

sdw = soil dry weight

2.9.5 Summary of effects on terrestrial non-target higher plants

Studies with Benzobicyclon, to investigate the effects of Benzobicyclon to the vegetative vigour and to seedling emergence of non-target plants are available.
The most sensitive species in the seedling emergence study conducted with the formulation were beans (*Phaseolus vulgaris*) with NOER, ER_{25}, and ER_{50} of 75, 110 and > 300 g a.s./ha. The most sensitive species in the vegetative vigour study conducted with the formulation were radishes (*Raphanus sativus*) with NOER, ER_{25}, and ER_{50} of 38, 73, and > 300 g a.s./ha.

Table 83: Summary of toxicity endpoints for non-target plants for GWN-10235 derived from terrestrial plant studies

Test type	Test species	Endpoint	Value [g a.s./ha] **	Reference
Seedling emergence	Bean *	NOER ER_{25} ER_{50}	75 (0.188) 110 (0.275) > 300 (> 0.75)	[redacted] (2017a), KCP 10.6.2/01
Vegetative vigour	Radish *	NOER ER_{25} ER_{50}	38 (0.095) 73 (0.183) > 300 (> 0.75)	[redacted] (2017b), KCP 10.6.2/02

* most sensitive of the ten species tested
** values in parentheses are based on the formulation and are given in L/ha

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

Tests on other non-target species are not required. There is no further data available from preliminary tests used to assess the biological activity and dose range finding.

2.9.7 Summary of effects on biological methods for sewage treatment

Benzobicyclon showed no inhibitory effect on the respiration rate of micro-organisms in the activated sludge. The 3 h EC_{50} was determined to be > 1000 mg a.s./L.

2.9.8 Summary of product exposure and risk assessment

Birds and mammals

The dietary risk assessments were conducted in accordance with the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) with consideration of the recent Pesticide Peer Review Meeting 181 (EFSA, 2018).

The only likely route of exposure of birds and mammals is the uptake of Benzobicyclon when feeding on treated parts of plants, insects or fish and amphibians through contaminated drinking water reservoirs. The risk assessments are based on the limiting endpoints representing a worst-case. A safety factor (trigger value) is

included in the risk assessment scheme to (among others) take account of species possibly more sensitive than the most sensitive tested.

Risk assessment for birds after application of GWN-10235

Dietary risk assessment for birds – Applicant proposal

Screening step

The RMS agrees with the acute and long-term screening step assessments for birds performed by the applicant using the indicator species and the default SV for cereal (surrogate crop for rice), in line with the agreements of the recent Pesticide Peer Review meeting 181 (EFSA, 2018). The screening risk assessments indicate an acceptable acute risk for birds and a potential unacceptable long-term risk for birds.

Tier 1 risk assessment

The Tier 1 long-term risk assessment was conducted using specific focal species potentially occurring in rice paddies for the following feeding guilds: herbivorous, insectivorous, omnivorous and piscivorous. For the selection of the focal species, the applicant has considered the information retrieved from the literature review by Vallon *et al.* (2018) and also referring to the outcome of the PPR meeting 181. The diet compositions were adopted for rice scenario from the cereal scenario of Appendix A of the EFSA GD (2009), replacing the terrestrial food items, such as crop leaves or arthropods with aquatic plants and aquatic invertebrates, in line with the agreements of the PPR meeting 181.

Table 84: Reproductive risk assessment for birds after exposure to the representative formulation of Benzobicyclon (Tier I assessment)

Application Rate [kg a.s./ha]	MAF (mean)	Food type	FIR/bw	ftwa	RUD	PD	DDD [mg a.s./kg bw]	NOAEL [mg a.s./kg bw/d]	TER_{LT}
Mallard duck (large herbivorous bird)									
0.3	1	Aquatic plants	0.597	0.53	54.2	1	5.12	36.2	**7.07** (3.54*)
Black-winged stilt (medium insectivorous bird)									
0.3	1	Aquatic insects	0.370	0.53	21.0	1	1.23	36.2	**29.5 (14.7*)**
Black-headed gull (medium omnivorous bird)									
0.3	1	Aquatic plants	0.191	0.53	54.2	0.25	0.41		
		Weed seeds			40.2	0.25	0.31		
		Aquatic insects			21.0	0.50	1.03		
Sum:							1.83	36.2	**19.8 (9.89*)**
Little bittern (medium piscivorous bird)									
0.3	1	Fish	0.297	0.53	0.470	1	0.022	36.2	**1632 (823*)**

FIR/bw = food intake rate per body weight, PD = Portion of diet, RUD = residue unit dose, MAF = multiple application factor, ftwa = time weighted average factor, DDD = daily dietary dose, TER = toxicity exposure ratio,
Values in **bold** are above the trigger value of 5, indicating an acceptable risk

The outcome of the proposed Tier 1 risk assessment highlighted an acceptable long-term risk for all the selected focal species, with the exception of mallard ducks (representing herbivorous birds) when considering the additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.
In RMS opinion, the Tier 1 risk assessment proposed by the applicant should be intended as an attempt to overcome the lack of specific recommendations in EFSA GD (2009) for addressing the risk to birds foraging in flooded rice fields. However, the selected focal species and the parameters used by the applicant should be further discussed, taking into account the limitations regarding the literature review by Vallon *et al.* (2018) and the uncertainties related to fact that such Tier 1 assessment does not follow a standard scheme.

Higher tier risk assessment for mallard duck

The applicant presented a higher tier risk assessment for mallard ducks using a refined diet composition, which is assumed to consist of 52% plant matter (aquatic plants) and 48% animal matter (aquatic invertebrates). The refined

diet was estimated on the basis of the available data retrieved from the submitted literature studies (refer to CA-B.9.1.1.2 for the study summaries and for an overview of the different dietary compositions collected). It is noted that the submitted studies were conducted in various locations, seasons and with different methodologies. For higher tier risk assessment purposes, the applicant proposed to consider only the data from those studies which focused on feeding habits of mallard ducks during spring, regardless of the habitat. The refined diet composition was therefore estimated by averaging the determined percentages (as dry mass of stomach contents) of plant matter, including seeds, and animal matter in mallard diet from the studies of Tidwell et al. (2013, CP 10.1.1.2/03), Street (1975, CP 10.1.1.2/19), Patterson (1982, CP 10.1.1.2/13) and Swanson et al. (1985, CP 10.1.1.2/18). The applicant's assumption was that the feeding habits of mallard ducks are not expected to be significantly different in rice fields during spring, following the application of GWN-10235, in comparison with other habitat, as wetlands or water reservoirs, and other regions.

Table 85: Refined reproductive risk assessment for mallard ducks after exposure to the representative formulation of Benzobicyclon

Application Rate [kg a.s./ha]	MAF (mean)	Food type	FIR/bw	ftwa	RUD	PD *	DDD [mg a.s./kg bw]	NOAEL [mg a.s./kg bw/d]	TER_{LT}
Mallard duck (large herbivorous bird)									
0.3	1	Aquatic plants	0.257	0.53	54.2	0.52	1.15		
		Aquatic insects			21.0	0.48	0.411		
Sum:							1.56	36.2	**23.2**
Sum **:							0.781		**11.6**

FIR/bw = food intake rate per body weight, PD = Portion of diet, RUD = residue unit dose, MAF = multiple application factor, ftwa = time weighted average factor, DDD = daily dietary dose, TER = toxicity exposure ratio,
* based on the mean dietary composition from Tidwell et al. (2013, CP 10.1.1.2/03), Street (1975, CP 10.1.1.2/19), Patterson (1982, CP 10.1.1.2/13) and Swanson et al. (1985, CP 10.1.1.2/18)
** with an additional uncertainty factor of 2
Values in **bold** are above the trigger value of 5, indicating an acceptable risk

By using the refined PD values, an acceptable long-term risk for mallard ducks has been indicated, even considering an additional uncertainty factor of 2 to account for the toxicity of the stereoisomers.
The RMS is of the opinion that all the available data on feeding habits of mallard ducks retrieved from the submitted literature studies could be taken into account in a qualitative way for higher tier risk assessment purposes. In agreement with the applicant, the RMS considers as not suitable the use of the data on diet composition of mallard ducks collected during fall/winter or late summer, even if the studies were conducted in rice fields, since the prey availability and the water management could be completely different compared to the potential conditions in early growth stages of rice, when the product is intended to be applied (BBCH 00-21), which means during spring (April-May). On the other hand, in the lack of robust data addressing the diet of mallard duck under such specific conditions, it could be reasonable to consider, at least in a qualitative way, the data collected in spring from other habitat and locations, which provides information on the potential dietary preferences of mallard ducks during this time period (i.e. breeding season). For instance, various literature studies showed an increasing content of aquatic invertebrates in the diet of mallard ducks in spring and summer, in contrast with the typical diet during fall and winter, which is dominated by seeds. According to the available data, it is unlikely that mallard ducks have a strictly herbivorous diet during spring, even when foraging in flooded rice fields, following the application of GWN-10235. The RMS therefore considers the refined diet composition proposed by the applicant for higher tier risk assessment as more realistic, compared to that assumed in Tier 1 risk assessment. Beyond that, it should be noted that the fraction of plant and animal matter in the diet is expected to be variable in relation to the specific water management of rice paddies in different countries, which can influence the prey availability. Different percentages of plant and animal matter can be assumed, bearing in mind none of them would be supported by robust data. In that regard, the co-RMS EL has suggested to perform the calculations by considering a theoretical worst-case diet of 90 % aquatic plants and 10 % aquatic invertebrates (food items with the highest RUD values).
The additional line of evidence based on the results from a residue decline study ([redacted] (2013), CA 6.3.1/01) is not considered as sufficiently robust, since no suitable estimations of foliar DT_{50} values can be derived from a study which consisted only of two trials and with residue levels measured only at two time points (DAT 0 and 30).

Overall, the higher tier risk assessment for mallard ducks based on a refined diet composition is deemed reasonable from a qualitative point of view. However, in RMS opinion, further discussions are deemed necessary to have an agreement between Member States and for drawing a definitive conclusion.

Dietary risk assessment for birds – RMS proposal based on specific guidance developed by Italy (ICPS, 2020)

A dietary risk assessment for birds in rice fields was proposed in the specific guidance entitled "Update and harmonization of rice pesticide risk assessment and revision of European guidelines" developed by Italy (ICPS, 2020), involving other European rice producing countries from the Southern zone (i.e. Spain, Portugal, France, Greece and Bulgaria).
Based on the information reported in this guidance, a dietary risk assessment for herbivorous and insectivorous birds exposed to benzobicyclon in rice fields is proposed below by the RMS.

Table 86: Acute and long-term risk assessment for birds following the use of benzobicyclon in rice

Intended use	Rice					
Active substance/product	Benzobicyclon/GWN-10235					
Application rate (g a.s./ha)	1 × 300					
Acute toxicity (mg a.s./kg bw)	>2250					
TER criterion	10					
Feeding guild (food item) BBCH	**Representative species**	**FIR/bw**	**90th percentile RUD value**	**SV for acute assessment**	**DDD_{acute} (mg/kg bw/d)**	**TER**
Herbivorous (duckweeds) BBCH from 10 to 75	Common moorhen *Gallinula chloropus*	0.66	200	99[a]	29.70	75.8 (37.9*)
Insectivorous (foliar insects) BBCH from 00 to 75	Barn swallow *Hirundo rustica*	0.47	54.1	25.4	7.63	295 (147.5*)

[a] 132 × (1 – 25/100)
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Reprod. toxicity (mg/kg bw/d)	36.2						
TER criterion	5						
Feeding guild (food item) BBCH	**Representative species**	**FIR/bw**	**Mean RUD value**	**SV for chronic assessment**	**TWA**	**DDD_{repro} (mg/kg bw/d)**	**TER**
Herbivorous (duckweeds) BBCH from 10 to 75	Common moorhen *Gallinula chloropus*	0.66	100	49.5[a]	0.53	7.87	**4.60 (2.3*)**
Insectivorous (foliar insects) BBCH from 00 to 75	Barn swallow *Hirundo rustica*	0.47	21	9.9	0.53	1.57	23.1 (11.5*)

[a] 66 × (1 – 25/100)
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

For benthophagous birds, the RMS has calculated the residue in benthic organisms (C_A) following the equations reported in the guidance and using the maximum benzobicyclon concentration in paddy soil calculated by the RMS with MED-RICE model (revised approach): max PEC_{soil} = 0.420 mg a.s./kg soil (clay). In detail:

$C_W = PEC_{Sed} / (f_{oc} \times K_{ow}) = 0.420 / (0.02 * 1259) = 0.016$
$LogBCF = 0.9172 \times logK_{ow} + 0.8953 = 0.9172 \times (3.1) + 0.8953 = 3.738$
$BCF = 5470$

$C_A = BCF \times C_W = 2.204 \times 10.719 = 91.25$

The risk assessment for the representative benthophagous species black-winged stilt (*Himantopus himantopus*) is reported below:

$DDD = C_A \times FIR/bw \times TWA = 91.25 \times 0.38 \times 0.53 = 18.38$ mg/kg bw/d

TER = NOEC / DDD = 36.2 / 18.38 = 1.97 (0.98*) < trigger of 5

Overall, the TER values calculated in the risk assessment for birds proposed by the RMS indicate an acceptable acute risk for herbivorous and insectivorous birds and an acceptable long-term risk for insectivorous birds. A potential high long-term risk has been identified for herbivorous and benthophagous birds. The RMS would like to remark that the presented risk assessment should be intended as a preliminary proposal and has been reported for illustrative purposes only, since the mentioned guidance (ICPS, 2020) is not officially adopted yet.

Drinking water risk assessment

An acceptable acute and long-term risk for birds from exposure to contaminated water can be expected, based on the preliminary assessments performed by the applicant (ratios of effective application rate to relevant endpoint), in accordance with EFSA GD (2009).

Food chain behaviour

The risk assessment for birds from secondary poisoning is triggered only for the active substance benzobicyclon, due the fact that its log Kow exceeds the trigger of 3 (actual 3.1).

Earthworm-eating birds

The RMS has updated the calculation of $BCF_{earthworm}$ using the geometric mean Koc value of 8438.5 mL/g reported in CP B.8, as follows:

$BCF_{earthworm} = (0.84 + (0.012 \times K_{ow})) / (f_{oc} \times K_{oc}) = (0.84 + (0.012 \times 1259) / (0.02 \times 8438.5) = 0.094$

The TER calculations for earthworm-eating birds have been updated in the following table, using the revised $BCF_{earthworm}$ and the worst-case maximum PEC_{SOIL} value re-calculated by the RMS in CP B.8 (using both standard and revised approach).

Table 87: Exposure of earthworm-eating birds to Benzobicyclon (dry soil approach)

Use/crop	PEC_{soil} [mg a.s./kg]	$BCF_{earthworm}$	$PEC_{earthworm}$ [mg a.s./kg]	Daily dose [mg a.s./kg bw/d]	NOEL [mg a.s./kg bw/d]	TER
Rice	0.210[1]	0.094	0.0198	0.0208	36.2	1740 (870*)
	0.223[2]	0.094	0.0209	0.0219	36.2	1653 (826*)

[1] Maximum PEC_{SOIL} value of 0.397 mg a.s./kg soil (standard approach) multiplied by 0.53
[2] Maximum PEC_{SOIL} value of 0.420 mg a.s./kg soil (revised approach) multiplied by 0.53
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Fish-eating birds

The TER calculations for fish-eating birds have been updated in the following table, using the revised the maximum PEC_{SW} value re-calculated by the RMS in CP B.8 (using both standard and revised approach).

Table 88: Exposure of fish-eating birds to Benzobicyclon

Use/crop	PECsw [1] [µg a.s./L]	BCF_{fish}	PEC_{fish} [mg a.s./kg]	Daily dose [mg a.s./kg bw/d]	NOEL [mg a.s./kg bw/d]	TER
Rice	0.000[1]	126	0.000	0.000	36.2	-
	0.000106[2]	126	0.0133	0.0021	36.2	17238 (8619*)

[1] Maximum PEC_{SW} value of 0.0002 mg a.s./kg soil (revised approach) multiplied by 0.53
[2] Maximum PEC_{SW} value of 0.0002 mg a.s./kg soil (revised approach) multiplied by 0.53
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Overall, the re-calculated TER values for earthworm-eating and fish-eating birds far exceed the trigger of 5, indicating an acceptable risk for birds from secondary poisoning.

Risk assessment for mammals after application of GWN-10235

Dietary risk assessment for mammals – Applicant proposal

Screening step

The acute and long-term screening step assessments for mammals were performed using the indicator species and the default SV for cereal (surrogate crop for rice), as agreed in the recent Pesticide Peer Review meeting 181. The

screening risk assessments indicate an acceptable acute risk for mammals and a potential unacceptable long-term risk for mammals, when considering the additional uncertainty factor of 2 to to account for the composition of benzobicyclon as a racemate.

Tier 1 risk assessment

The Tier 1 long-term risk assessment was conducted using specific focal species potentially occurring in rice paddies for the following feeding guilds: herbivorous, insectivorous and omnivorous. For the selection of the focal species, the applicant considered the information retrieved from the literature review by Vallon *et al.* (2018) and also referring to the outcome of the PPR meeting 181. The diet compositions were adopted for rice scenario from the cereal scenario of Appendix A of the EFSA GD (2009), replacing the terrestrial food items, such as crop leaves or arthropods, with aquatic plants and aquatic invertebrates.

Table 89: Reproductive risk assessment for mammals after exposure to the representative formulation of Benzobicyclon (Tier I assessment to address the risk for the stereoisomers)

Application rate [kg a.s./ha]	MAF (mean)	Food type	FIR/bw	ftwa	RUD	PD	DDD [mg a.s./kg bw]	NOAEL [mg a.s./kg bw/d]	TER_{LT}*
European water vole (small herbivorous mammal)									
0.3	1	Aquatic plants	0.622	0.53	54.2	1	5.36	59.5	**11.1 (5.55)**
Brown rat (medium insectivorous mammal)									
0.3	1	Aquatic insects	0.300	0.53	21.0	1	1.00	59.5	**59.5 (29.7)**
Brown rat (medium omnivorous mammal)									
0.3	1	Aquatic plants	0.132	0.53	54.2	0.25	0.284		
		Weed seeds			40.2	0.50	0.110		
		Aquatic insects			21.0	0.25	0.421		
Sum:							0.816	59.5	**72.9 (36.4)**

FIR/bw = food intake rate per body weight, PD = Portion of diet, RUD = residue unit dose, MAF = multiple application factor, ftwa = time weighted average factor, DDD = daily dietary dose, TER = toxicity exposure ratio,
* values in parentheses are based on the consideration of an additional uncertainty factor of 2 to account for the stereoisomers of Benzobicyclon.
Values in **bold** are above the trigger value of 5, indicating an acceptable risk

The outcome of the proposed Tier 1 risk assessment highlighted an acceptable long-term risk for all the selected focal species.
In RMS opinion, the Tier 1 risk assessment proposed by the applicant should be intended as an attempt to overcome the lack of specific recommendations in EFSA GD (2009) for addressing the risk to mammals foraging in flooded rice fields. However, the selected focal species and the parameters used by the applicant should be further discussed, taking into account the limitations regarding the literature review by Vallon *et al.* (2018) and the uncertainties related to fact that such Tier 1 assessment does not follow a standard scheme.

Dietary risk assessment for mammals – RMS proposal based on specific guidance developed by Italy (ICPS, 2020)

A dietary risk assessment for mammals in rice fields was proposed in the specific guidance entitled "Update and harmonization of rice pesticide risk assessment and revision of European guidelines" developed by Italy (ICPS, 2020), involving other European rice producing countries from the Southern zone (i.e. Spain, Portugal, France, Greece and Bulgaria).

Based on the information reported in this guidance, a dietary risk assessment for herbivorous and insectivorous mammals exposed to benzobicyclon in rice fields is proposed by the RMS in the following tables.

Table 90: Acute and long-term risk assessment for mammals following the use of benzobicyclon in rice

Intended use	Rice
Active substance/product	Benzobicyclon/GWN-10235
Application rate (g a.s./ha)	1 × 300
Acute toxicity (mg a.s./kg bw)	>5000
TER criterion	10

Feeding guild (food item) BBCH	**Representative species**	**FIR/bw**	**90th percentile RUD value**	**SV for acute assessment**	**DDD_{acute} (mg/kg bw/d)**	**TER**
Herbivorous (duckweeds) BBCH from 10 to 75	European water vole *Arvicola amphibius*	0.74	200	111[a]	33.30	150 (75*)
Insectivorous (foliar insects) BBCH from 00 to 75	Common pipistrelle *Pipistrellus pipistrellus*	0.52	54.1	28.1	8.44	178 (89*)

[a] 148 × (1 – 25/100)
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Reprod. toxicity (mg/kg bw/d)	59.5
TER criterion	5

Feeding guild (food item) BBCH	**Representative species**	**FIR/bw**	**Mean RUD value**	**SV for chronic assessment**	**TWA**	**DDD_{repro} (mg/kg bw/d)**	**TER**
Herbivorous (duckweeds) BBCH from 10 to 75	European water vole *Arvicola amphibius*	0.74	100	55.5[a]	0.53	8.82	6.7 **(3.4*)**
Insectivorous (foliar insects) BBCH from 00 to 75	Common pipistrelle *Pipistrellus pipistrellus*	0.52	21	10.9	0.53	1.74	34.2 (17.1*)

[a] 74 × (1 – 25/100)
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Overall, the TER values calculated in the risk assessment for mammals proposed by the RMS indicate an acceptable acute risk for herbivorous and insectivorous mammals and an acceptable long-term risk for insectivorous mammals. A potential high long-term risk has been identified for herbivorous mammals, when considering the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate. The RMS would like to remark that the presented risk assessment should be intended as a preliminary proposal and has been reported for illustrative purposes only, since the mentioned guidance (ICPS, 2020) s not officially adopted yet.

Drinking water risk assessment

An acceptable acute and long-term risk for mammals from exposure to contaminated water can be expected, based on the preliminary assessments performed by the applicant (ratios of effective application rate to relevant endpoint), in accordance with EFSA GD (2009).

Food chain behaviour

The risk assessment for mammals from secondary poisoning is triggered only for the active substance benzobicyclon, due the fact that its log Kow exceeds the trigger of 3 (actual 3.1).

Earthworm-eating mammals

The RMS has updated the calculation of $BCF_{earthworm}$ using the geometric mean Koc value of 8438.5 mL/g reported in CP-B.8, as follows:

$BCF_{earthworm} = (0.84 + (0.012 * K_{ow})) / (f_{oc} * K_{oc}) = (0.84 + (0.012 * 1259) / (0.02 * 8438.5) = 0.094$

The TER calculations for earthworm-eating mammals have been updated in the following table, using the revised $BCF_{earthworm}$ and the worst-case maximum PEC_{SOIL} value re-calculated by the RMS in CP B.8 (using both standard and revised approach).

Table 91: Exposure of earthworm-eating mammals to Benzobicyclon (dry soil approach)

Use/crop	PEC_{soil} [mg a.s./kg]	$BCF_{earthworm}$	$PEC_{earthworm}$ [mg a.s./kg]	Daily dose [mg a.s./kg bw/d]	NOAEL [mg a.s./kg bw/d]	TER
Rice	0.210[1]	0.094	0.0198	0.0253	59.5	2352 (1176*)
	0.2226[2]	0.094	0.0209	0.0267	59.5	2228 (1114*)

[1] Maximum PEC_{SOIL} value of 0.420 mg a.s./kg soil (revised approach) multiplied by 0.53
[2] Maximum PEC_{SOIL} value of 0.420 mg a.s./kg soil (revised approach) multiplied by 0.53
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Fish-eating mammals

The TER calculations for fish-eating mammals have been updated in the following table, using the revised the maximum PEC_{SW} value re-calculated by the RMS in CP B.8 (using both standard and revised approach).

Table 92: Exposure of fish-eating birds to Benzobicyclon

Use/crop	PECsw [µg a.s./L]	BCF_{fish}	PEC_{fish} [mg a.s./kg]	Daily dose [mg a.s./kg bw/d]	NOAEL [mg a.s./kg bw/d]	TER
Rice	0.000[1]	126	0.000	0.000	59.5	-
	0.000106[2]	126	0.0133	0.0019	59.5	31315 (15657*)

[1] Maximum PEC_{SW} value of 0.0002 mg a.s./kg soil (revised approach) multiplied by 0.53
[2] Maximum PEC_{SW} value of 0.0002 mg a.s./kg soil (revised approach) multiplied by 0.53
* applying the additional uncertainty factor of 2 to account for the composition of Benzobicyclon as a racemate

Overall, the re-calculated TER values for earthworm-eating and fish-eating mammals far exceed the trigger of 5, indicating an acceptable risk for mammals from secondary poisoning.

Risk assessment for aquatic organisms after application of GWN-10235

The aquatic risk assessments were conducted in accordance with the EFSA Guidance Document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013).

Benzobicyclon

The risk assessment for aquatic organisms was performed using the lowest relevant endpoints determined in the available studies conducted with benzobicyclon. The PEC/RAC calculations for benzobicyclon have been updated in the following table using the PEC_{SW} values re-calculated by the RMS in CP B.8, according to the standard and the revised MED-RICE model.

Table 93: PEC/RAC ratios for Benzobicyclon for each organism group based on calculations according to standard and revised MED-Rice model for the use of GWN-10235 in rice

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		*O. mykiss*	*P. promelas*	*D. magna*	*D. magna*	*A. flos-aquae*	*L. gibba*
Endpoint [µg a.s./L]		LC_{50} > 489	EC_{10} 18.0	EC_{50} > 368	EC_{10} 272	E_rC_{50} > 184	E_rC_{50} 6.19
AF		100	10	100	10	10	10
RAC [µg a.s./L]		4.89	1.80	3.68	27.2	18.4	0.619

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Scenario 2	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Revised MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.0002	4.09E-05	1.11E-04	5.43E-05	7.35E-06	1.09E-05	3.23E-04

Using both the standard and revised MED-RICE model re-calculations, an acceptable risk is indicated for all the aquatic organisms from exposure to benzobicyclon, even due to its negligible exposure in surface waters.
The RMS agrees with the applicant's consideration that an additional uncertainty factor of 2 is not necessary in this case to account for the composition of benzobicyclon as a racemate, since the study by [redacted] (2020), summarized under paragraph 2.13.7 shows a comparable toxicity between the two enantiomers of benzobicyclon and the racemate towards the most sensitive specie *Lemna gibba*.

Benzobicyclon metabolites

Metabolite 1315P-070
The risk assessment for aquatic organisms was performed using the lowest relevant endpoints determined in the available studies conducted with metabolite 1315P-070. The PEC/RAC calculations for metabolite 1315P-070 have been updated in the following table using the PEC_{SW} values re-calculated by the RMS in Vol.3 CP B.8, according to the standard and the revised MED-RICE model.

Table 94: PEC/RAC ratios for metabolite 1315P-070 for each organism group based on calculations according to standard and revised MED-Rice model for the use of GWN-10235 in rice

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		***C. variegatus***	***C. variegatus***	***D. magna***	***D. magna***	***R. subcapitata***	***L. gibba***
Endpoint [µg a.s./L]		LC_{50} > 120000	EC_{10} 3430	EC_{50} > 120000	NOEC 60000	E_rC_{50} 44700	E_rC_{50} 13.5
AF		100	10	100	10	10	10
RAC [µg a.s./L]		1200	343	1200	6000	4470	1.35
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.755	0.0006	0.0022	0.0006	0.0001	0.0002	0.5033
Scenario 2	1.182	0.0010	0.0034	0.0010	0.0001	0.0003	0.7880
Revised MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	1.230	0.0010	0.0036	0.0010	0.0002	0.0003	0.9111

Using both the standard and revised MED-RICE model re-calculations, an acceptable risk is indicated for all the aquatic organisms from exposure to metabolite 1315P-070.

Metabolites 1315P-570 and 1315P-966
The aquatic risk assessment for the metabolites 1315P-570 and 1315P-966 was performed assuming the same toxicity of the parent, since it was demonstrated that for both these metabolites the toxophore is lost, having more than 10 times lower toxicity (on a molar basis) compared to the parent for the most sensitive species *Lemna gibba*. Instead, for aquatic macrophytes, the risk assessment was performed using the relevant endpoints determined in the available studies conducted with metabolites 1315P-570 and 1315P-966.

The PEC/RAC calculations for metabolites 1315P-570 and 1315P-966 have been updated in the following tables using the PEC_{SW} values re-calculated by the RMS in CP B.8, according to the standard and the revised MED-RICE model.

Table 95: PEC/RAC ratios for metabolite 1315P-570 for each organism group based on calculations according to standard and revised MED-Rice model for the use of GWN-10235 in rice

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint [µg a.s./L]		LC_{50} > 489*	EC_{10} 18.0*	EC_{50} > 368*	EC_{10} 272*	E_rC_{50} > 184*	E_rC_{50} > 1000
AF		100	10	100	10	10	10
RAC [µg a.s./L]		4.89	1.80	3.68	27.2	18.4	100
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.17	0.0348	0.0944	0.0462	0.0063	0.0092	0.0017
Scenario 2	0.17	0.0348	0.0944	0.0462	0.0063	0.0092	0.0017
Revised MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.172	0.0352	0.0956	0.0467	0.0063	0.0093	0.0017

*assuming the same toxicity of the parent

Table 96: PEC/RAC ratios for 1315P-966 for each organism group based on calculations according standard and revised MED-Rice model for the use of GWN-10235 in rice

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint [µg a.s./L]		LC_{50} > 489*	EC_{10} 18.0*	EC_{50} > 368*	EC_{10} 272*	E_rC_{50} > 184*	E_rC_{50} > 100000
AF		100	10	100	10	10	10
RAC [µg a.s./L]		4.89	1.80	3.68	27.2	18.4	10000
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	1.236	0.2528	0.6867	0.3359	0.0454	0.0672	0.0001
Scenario 2	1.236	0.2528	0.6867	0.3359	0.0454	0.0672	0.0001
Revised MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	1.236	0.2528	0.6867	0.3359	0.0454	0.0672	0.0001

*assuming the same toxicity of the parent

Using both the standard and revised MED-RICE model re-calculations, an acceptable risk is indicated for all the aquatic organisms from exposure to metabolites 1315P-570 and 1315P-966.

Metabolites 1315P-076, 1315P-960, 1315P-683 and 1315P-962

The metabolite 1315P-076 was not considered as a major metabolite by the RMS in the E-fate section. As a consequence, no PEC_{SW} re-calculations were performed and no aquatic risk assessment is deemed necessary.

On the other hand, the two aqueous photolysis metabolites 1315P-683 and 1315P-962 have been included in the surface water residue definition by the RMS. PEC_{SW} values have been therefore calculated by the RMS in CP B.8, accordingly. Since for the metabolites 1315P-960, 1315P-683 and 1315P-962 no toxicity endpoints are available, the aquatic risk assessment has to be performed assuming that the toxicity of these metabolites is 10 times higher than that of the parent.

The PEC/RAC calculations for metabolites 1315P-960, 1315P-683 and 1315P-962 have been updated in the following tables using the PEC_{SW} values re-calculated by the RMS in CP B.8, according to the standard and the revised MED-RICE model.

Table 97: PEC/RAC ratios for 1315P-960 for each organism group based on calculations according to standard and revised MED-Rice model for the use of GWN-10235 in rice assuming a 10x higher toxicity than the parent

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint [µg a.s./L]		LC_{50} > 48.9 *	EC_{10} 1.80*	EC_{50} > 36.8 *	EC_{10} 27.2 *	E_rC_{50} > 18.4*	E_rC_{50} 0.619 *
AF		100	10	100	10	10	10
RAC [µg a.s./L]		0.489	0.180	0.368	2.72	1.84	0.062
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.098	0.2004	0.5444	0.2663	0.0360	0.0533	**1.5806**
Scenario 2	0.098	0.2004	0.5444	0.2663	0.0360	0.0533	**1.5806**
Revised MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.098	0.2004	0.5444	0.2663	0.0360	0.0533	**1.5806**

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

Table 98: PEC/RAC ratios for 1315P-683 for each organism group based on calculations according to standard and revised MED-Rice model for the use of GWN-10235 in rice assuming a 10x higher toxicity than the parent

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint [µg a.s./L]		LC_{50} > 48.9 *	EC_{10} 1.80*	EC_{50} > 36.8 *	EC_{10} 27.2 *	E_rC_{50} > 18.4*	E_rC_{50} 0.619 *
AF		100	10	100	10	10	10
RAC [µg a.s./L]		0.489	0.180	0.368	2.72	1.84	0.062
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.009	0.0184	0.0500	0.0245	0.0033	0.0049	0.1452
Scenario 2	0.009	0.0184	0.0500	0.0245	0.0033	0.0049	0.1452

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

Table 99: PEC/RAC ratios for 1315P-962 for each organism group based on calculations according to standard and revised MED-Rice model for the use of GWN-10235 in rice assuming a 10x higher toxicity than the parent

Group		Fish		Invertebrates		Algae	Plants
		Acute	Chronic	Acute	Chronic		
Test species		***O. mykiss***	***P. promelas***	***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint [µg a.s./L]		LC_{50} > 48.9 *	EC_{10} 1.80*	EC_{50} > 36.8 *	EC_{10} 27.2 *	E_rC_{50} > 18.4*	E_rC_{50} 0.619 *
AF		100	10	100	10	10	10
RAC [µg a.s./L]		0.489	0.180	0.368	2.72	1.84	0.062
Standard MED-Rice	**PECsw [µg a.s./L]**						
Scenario 1	0.012	0.0245	0.0667	0.0326	0.0044	0.0065	0.1935
Scenario 2	0.012	0.0245	0.0667	0.0326	0.0044	0.0065	0.1935

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

As regard the metabolite 1315P-960, an acceptable risk is indicated for all the aquatic organisms with the exception of *Lemna gibba*. However, considering the overly conservative assessment based on 10 times higher toxicity of the parent, resulting in PEC/RAC values slightly higher than the trigger of 1, an acceptable risk can be expected

even for aquatic macrophytes, in RMS opinion. The conclusion for the metabolite 1315P-960 can be further discussed.
For the aqueous photolysis metabolites 1315P-683 and 1315P-962, an acceptable risk for all the aquatic organisms can be concluded.

Aquatic risk assessment in paddy water - RMS proposal based on the specific guidance developed by Italy (ICPS, 2020)

In the specific guidance entitled "Update and harmonization of rice pesticide risk assessment and revision of European guidelines" developed by Italy (ICPS, 2020), an in-field risk assessment in paddy water is proposed for aquatic organisms.

Based on the information reported in this guidance, an in-field paddy water risk assessment for aquatic organisms is proposed by the RMS and reported in the following tables. For the time being, such assessment was performed for benzobicyclon and its metabolites 1315P-070, 1315P-570 and 1315P-966**,** using the available toxicity endpoints (the use of the surrogate endpoints for the metabolites has been disregarded for this specific assessment) and the $PEC_{PW\ (initial)}$ values re-calculated by the RMS in CP B.8. Since benzobicyclon is intended to be applied under flooded conditions, the use of the initial $PEC_{PW\ (initial)}$ is deemed justified.

Table 100: PEC/RAC ratios for Benzobicyclon for each organism group in paddy water for the use of GWN-10235 in rice

Group		**Invertebrates**		**Algae**	**Plants**
		Acute	**Chronic**		
Test species		***D. magna***	***D. magna***	***A. flos-aquae***	***L. gibba***
Endpoint [µg a.s./L]		EC_{50} > 368	EC_{10} 272	E_rC_{50} > 184	E_rC_{50} 6.19
AF		50	5	5	5
RAC [µg a.s./L]		>7.36	54.4	>36.8	1.238
Standard MED-Rice	**PEC_{PW} [µg a.s./L]**				
Scenario 1	2.661	0.362	0.049	0.072	**2.15**
Scenario 2	5.176	0.703	0.095	0.141	**4.18**
Revised MED-Rice	**PEC_{PW} [µg a.s./L]**				
Scenario 1	5.54	0.753	0.102	0.151	**4.47**

Table 101: PEC/RAC ratios for metabolite 1315P-070 for each organism group in paddy water for the use of GWN-10235 in rice

Group		**Invertebrates**		**Algae**	**Plants**
		Acute	**Chronic**		
Test species		***D. magna***	***D. magna***	***R. subcapitata***	***L. gibba***
Endpoint [µg a.s./L]		EC_{50} > 120000	NOEC 60000	E_rC_{50} 44700	E_rC_{50} 13.5
AF		50	5	5	5
RAC [µg a.s./L]		>2400	12000	8940	2.7
Standard MED-Rice	**PEC_{PW} [µg a.s./L]**				
Scenario 1	9.115	0.0038	0.0008	0.0010	**3.38**
Scenario 2	14.846	0.0062	0.0012	0.0017	**5.50**
Revised MED-Rice	**PEC_{PW} [µg a.s./L]**				
Scenario 1	15.5	0.0065	0.0013	0.0017	**5.74**

Table 102: PEC/RAC ratios for metabolites 1315P-570 and 1315P-966 for aquatic plants in paddy water for the use of GWN-10235 in rice

Group		**1315P-570**	**1315P-966**
Test species		***L. gibba***	***L. gibba***
Endpoint [µg a.s./L]		E_rC_{50} > 1000	E_rC_{50} > 100000

Group		**1315P-570**	**1315P-966**
AF		5	5
RAC [µg a.s./L]		200	20000
Standard MED-Rice – Scenario 2	**PEC_{PW} [µg a.s./L]**	2.0	15.0
	PEC/RAC ratio	0.01	0.0007
Revised MED-Rice – Scenario 1	**PEC_{PW} [µg a.s./L]**	2.0	15.0
	PEC/RAC ratio	0.01	0.0007

Based on the PEC/RAC calculated by the RMS, an unacceptable in-field risk is highlighted only for aquatic macrophytes (*L.gibba*) for both benzobicyclon and metabolite 1315P-070.
The RMS would like to remark that the presented in-field risk assessment should be intended as a preliminary proposal and has been reported for illustrative purposes only, since the mentioned guidance (ICPS, 2020) is not officially adopted yet..

Risk assessment for bees after application of GWN-10235

Acute risk assessment for benzobicyclon

The acute risk assessments for honeybees were performed according to SANCO/10329/2002 rev. 2 final and EFSA Guidance (2013) and using the available LD_{50} values from the acute oral and contact toxicity studies with the active substance benzobicyclon. Acceptable acute oral and contact risks for honeybees are indicated following both the risk assessment approaches, even applying an additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.

Chronic adult and larval risk assessment for benzobicyclon

The chronic risk assessments for larvae and adult honeybees were performed according to EPPO scheme (2010) and EFSA Guidance (2013) and using the available toxicity endpoints from the chronic adult and larval studies with the active substance benzobicyclon. In RMS opinion, the presented risk assessments based on EPPO scheme (2010) can be considered as supplementary information only, since it is not an agreed approach at EU level. As correctly noted by the co-RMS EL, the maximum residue level of 1 mg a.s./kg, which is considered in the calculations, is not a sufficiently conservative value for spray applications.
Instead, the additional Tier I risk assessment for honeybees according to EFSA Guidance (2013) is considered the relevant assessment for drawing a conclusion on the chronic risk to adult and larval honeybees, as it was also recommended in the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA Supporting publication 2015:EN-924), in absence of alternative approaches.

As regard the adult honeybees, the calculated ETR values were below than the trigger of 0.03, indicating an acceptable chronic risk, even applying the additional uncertainty factor of 2.

As regard honeybee larvae, a potential unacceptable risk has been shown at Tier I assessment for the "weeds" scenario only. In order to address the risk for this specific scenario, the applicant submitted a position paper ([redacted] (2021); CP 10.3.1/01), which documented the results of the available efficacy studies conducted with benzobicyclon containing formulations to obtain information on the presence of weeds in their flowering stage in rice fields. This kind of investigation is deemed in line with the Appendix N of the EFSA Guidance (2013), where it is stated that *it may be checked whether it is likely that a significant fraction of the surface area of treated fields is covered by weeds at the application time. If this will happen at less than 10% of the area of use of the substance, no weeds will occur in a 90th percentile case and thus their exposure can be ignored*. According to the reported data, the incidence of attractive flowering weeds in rice fields is low (7 %) and the incidence of attractive flowering weeds with > 10 % ground cover is even lower (3 %) in the European rice fields.
In RMS opinion, the information included in the submitted paper provides sufficient evidence to assume a very low potential for exposure to bees via flowering weeds in rice fields after the application of benzobyciclon. In addition, the following sentence is reported in the proposal developed by Italy (ICPS, 2020) regarding the consumption of pollen from weeds in the field: *no exposure is expected due to the low attractiveness for bees and since weeds are usually controlled by application of herbicides and agricultural practices*. Thus, the RMS believes that an acceptable chronic risk for honeybee larvae can also be expected for "weeds" scenario.
Regarding such refinement, it should be pointed out that co-RMS EL considers that the results presented by the applicant are insufficient to establish the absence of effects on honeybees from exposure to flowering weeds after application of the active substance in rice. According to co-RMS, additional information should be collected and reported by the applicant to facilitate the decision making, including:

- The % of recording where weeds exceeded 3 and 5% of ground coverage (separately for monocots and dicots; a threshold lower than 10% may be considered as more relevant by the experts).

- Information regarding the time necessary for the active substance to affect susceptible monocot weeds to a point that renders them unattractive to bees.
- Any other information that can be valued as suitable lines of evidence.

In light of the concerns raised by the co-RMS EL, the acceptability of the chronic risk for honeybee larvae and/or the need to include a mitigation phrase as "*do not apply when flowering weeds are present"* can be further discussed.

Assessment of risk from exposure to contaminated water

The risk assessment from exposure to guttation water performed by the applicant according to EFSA Guidance (2013) highlighted an acceptable acute and chronic risk for honeybees following the intended uses of GWN-10235 in rice fields.
As regard the exposure to surface water and puddle water, the RMS has updated the ETR calculations using the worst-case maximum PEC_{PW} (paddy water) re-calculated by the RMS in CP-B8, which is considered to cover the assessment for both surface water and puddle water scenarios. This approach is also suggested in the proposal developed by Italy (ICPS, 2020).
The calculated ETR values are lower than the trigger values, indicating an acceptable acute and chronic risk for adult and larval honeybees from exposure to paddy water (covering surface water and puddle water).

Risk assessment for metabolite 1315P-070

The acute risk assessment for the metabolite 1315P-070 was performed according to both SANCO/10329/2002 rev. 2 final and EFSA Guidance (2013). An acceptable acute risk has been identified following both the risk assessment approaches.
The chronic adult and larval risk assessments were performed according to both EPPO scheme (2010) and EFSA Guidance (2013). As mentioned above, only the ETR calculations performed in accordance to EFSA, 2013 has been considered relevant. An acceptable chronic risk for adult and larval honeybees from exposure to metabolite 1315P-070 has been indicated.

Risk assessment for other metabolites

The acute and chronic risk assessments for the other two relevant plant metabolites 1315P-570 and 1315P-966 were performed according to EFSA Guidance (2013) and assuming a 10 times higher toxicity than the parent benzobicyclon, since no toxicity studies with such metabolites were conducted.
The RMS has updated the EXP_{met} and ETR calculations for the metabolite 1315P-570 using the maximum Ftrr value from the available plant metabolism studies, according to CA B.7 document (i.e. 40.0 % TRR in hulls), instead of 41.1% originally used by the applicant. For the metabolite 1315P-966, the Ftrr used by the applicant is in line with the maximum TRR of 24.4% in immature plants reported in CA B.7, but not with the values reported in Table 9-2 and Table 9.1.1-2 in CP-B.9 (i.e. maximum TRR of 41.1 % after 24/25 d (foliage)). The applicant should therefore clarify such discrepancy.
Based on the current Tier I risk assessments, a potential high chronic risk is indicated for "weeds" scenario from exposure to both the metabolites and for the "next crop" scenario for honeybee larvae exposed to the metabolite 1315P-570. It should be noted that such conclusions are due to the assumption that these metabolites have a 10 times higher toxicity than the parent, which is deemed as overly conservative, in RMS opinion. Overall, the outcome of the risk assessment for bees from exposure to the plant metabolites 1315P-570 and 1315P-966 should be further discussed.

Risk assessment for non-target arthropods other than bees after application of GWN-10235

For the risk assessment of non-target arthropods (NTA) after spray application of GWN-10235, the approach outlined in ESCORT 2 (2001) is followed, which is also in line with the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final) and ESCORT 3 (2012).

Non-target arthropods living in and around the crop can be exposed to residues from GWN-10235 by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items.

The risk assessment for non-target arthropods was performed using the lowest endpoints determined from the available laboratory and extended laboratory studies conducted with the formulation GWN 10235. The RMS supports the use of the endpoint in terms of active substance, instead of the corresponding endpoint in terms of formulation, also taking into account that the total deposition after volatilization, which is included in the calculation of the overall drift rate, refers only to the active substance.

The Tier I risk assessment was performed using the relevant ER_{50} values determined for the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* in the standard laboratory tests. As already highlighted, the estimated ER_{50} for *Aphidius rhopalosiphi* should not be considered as fully reliable, since the results on fecundity were available only for the two highest treatment groups.

Table 103: Tier I risk assessment for non-target arthropods

Crop	Application rate [g a.s./ha]	ER_{50} [g a.s./ha]	Field rate [g a.s./ha]	In-field hazard quotient	Drift rate* [g a.s./ha]	Off-field hazard quotient
Typhlodromus pyri						
Rice	300	< 37.5	300	> 8.00	8.36	**> 0.223**
Aphidius rhopalosiphi						
Rice	300	346[#]	300	**0.867**	8.36	**0.024**

Values in **bold** are below the trigger of 2

***** including safety factor and deposition after volatilisation

[#] ER_{50} not considered as fully reliable by the RMS, since the results on fecundity were available only for the two highest treatment groups.

According to the presented risk assessment, a potential unacceptable in-field and off-field risk is indicated for *Typhlodromus pyri*, triggering the submission of further extended laboratory tests on this indicator species and two additional species (*Chrysoperla carnea* and *Orius laevigatus*). The toxicity endpoints determined from the available extended lab studies were used in Tier II risk assessment.

Table 104: Tier II risk assessment for non-target arthropods

Crop	LR_{50} [g a.s./ha]	ER_{50} [g a.s./ha] (Effects [%])	Field rate [g a.s./ha]	In-field risk indicated?	Drift rate [g a.s./ha]	Off-field risk indicated?
Typhlodromus pyri						
Rice	> 623	> 623 (42.5)	300	No	4.21 *	No
Chrysoperla carnea						
Rice	> 600	> 600 (15.8)	300	No	4.21 *	No
Orius laevigatus						
Rice	> 623	> 623 (2.6)	300	No	4.21 *	No

***** including safety factor of 5 and deposition after volatilisation

Since the calculated field and drift rates do not exceed the relevant LR_{50}/ER_{50} values for the three tested species, an acceptable in-field on off-field risk can be concluded for non-target arthropods, following the intended uses of GWN-10235 in rice, without the need of mitigation measures.
The same outcome has been achieved applying an additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.

Risk assessment for earthworms after application of GWN-10235 (Volume 3CP B9.8)

The exposure of soil organisms was estimated by calculating the maximum predicted environmental concentrations in soil (PEC_{soil}) for the representative use. The resulting maximum initial PEC_{soil} value is used to perform the risk assessment for earthworms in accordance with the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).
The applicant performed the risk assessment for earthworms only for benzobicyclon and its major metabolite 1315P-070 (which covers the risk assessment for the metabolite 1315P-966). According to the RMS assessment in the E-fate section, the metabolites 1315P-570 and 1315P-960 should also be considered as major in soil. In the absence of toxicity studies on earthworms for these metabolites, the risk assessment has been performed assuming a 10 times higher toxicity compared to the parent, to cover a worst-case situation.
The TER calculations for earthworms have been updated in the following tables using the PEC_{SOIL} values re-calculated by the RMS in CP B.8, according to the standard and the revised MED-RICE model.

Table 105: Chronic TER values for earthworms exposed to Benzobicyclon and its metabolites (standard MED-RICE model)

Test substance	Crop	$NOEC/EC_{10}$ [mg/kg soil]	PEC_{SOIL} (initial) [mg/kg soil]	TER_{LT}
			Scenario 1	
Benzobicyclon	Rice	39.5	0.397	99
1315P-070		6.67	0.0412	162

1315P-966		3.95*	0.000	-
1315P-570		3.95*	0.000	-
1315P-960		3.95*	0.000	-

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

Table 106: Chronic TER values for earthworms exposed to Benzobicyclon and its metabolites (revised MED-RICE model)

Test substance	Crop	NOEC/EC_{10} [mg/kg soil]	$PEC_{SOIL\ (initial)}$ [mg/kg soil]	TER_{LT}
			Scenario 1	
Benzobicyclon	Rice	39.5	0.420	94
1315P-070		6.67	0.035	190
1315P-966		3.95*	0.000	-
1315P-570		3.95*	0.000	-
1315P-960		3.95*	0.000	-

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

The re-calculated TER values for benzobicyclon and its major soil metabolites exceed the trigger of 5, indicating an acceptable chronic risk for earthworms following the intended uses of GWN-10235 in rice.
For the parent benzobicyclon, the same outcome is achieved applying an additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.

Risk assessment for non-target soil meso- and macrofauna other than earthworms after application of GWN-10235

The exposure to soil organisms was estimated by calculating the maximum initial predicted environmental concentrations in soil (PEC_{soil}) and comparing these concentrations with the toxicity endpoints obtained from studies with *Folsomia candida* and *Hypoaspis aculeifer* in accordance with the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).
The applicant performed the risk assessment for soil macro-organisms only for the parent benzobicyclon. According to the RMS assessment in the E-fate section, the metabolites 1315P-070, 1315P-966, 1315P-570 and 1315P-960 should be considered as major in soil. In the absence of toxicity studies on *Folsomia candida* and *Hypoaspis aculeifer* for these metabolites, the risk assessment has been performed assuming a 10 times higher toxicity compared to the parent, to cover a worst-case situation.
The TER calculations for soil macro-organisms have been updated in the following tables using the PEC_{SOIL} values re-calculated by the RMS in CP B.8, according to the standard and the revised MED-RICE model.

Table 107: Chronic TER values for soil non-target organisms other than earthworms exposed to Benzobicyclon and its metabolites (standard MED-RICE model)

Test substance	Species	$NOEC_{corr}$ [mg a.s./kg soil]	$PEC_{SOIL\ (initial)}$ [mg/kg soil]	TER_{LT}
			Scenario 1	
Benzobicyclon	*Folsomia candida*	> 500	0.397	>1259
	Hypoaspis aculeifer	> 500		>1259
1315P-070	*Folsomia candida*	> 50*	0.0412	>1214
	Hypoaspis aculeifer	> 50*		>1214
1315P-966	*Folsomia candida*	> 50*	0.000	-
	Hypoaspis aculeifer	> 50*		-
1315P-570	*Folsomia candida*	> 50*	0.000	-
	Hypoaspis aculeifer	> 50*		-
1315P-960	*Folsomia candida*	> 50*	0.000	-
	Hypoaspis aculeifer	> 50*		-

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

Table 108: Chronic TER values for soil non-target organisms other than earthworms exposed to Benzobicyclon and its metabolites (revised MED-RICE model)

Test substance	Species	$NOEC_{corr}$ [mg a.s./kg soil]	$PEC_{SOIL\ (initial)}$ [mg/kg soil]	TER_{LT}
			Scenario 1	

Benzobicyclon	*Folsomia candida*	> 500	0.420	>1190
	Hypoaspis aculeifer	> 500		>1190
1315P-070	*Folsomia candida*	> 50*	0.035	>1429
	Hypoaspis aculeifer	> 50*		>1429
1315P-966	*Folsomia candida*	> 50*	0.000	-
	Hypoaspis aculeifer	> 50*		-
1315P-570	*Folsomia candida*	> 50*	0.000	-
	Hypoaspis aculeifer	> 50*		-
1315P-960	*Folsomia candida*	> 50*	0.000	-
	Hypoaspis aculeifer	> 50*		-

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

The re-calculated TER values for benzobicyclon and its major soil metabolites exceed the trigger of 5, indicating an acceptable chronic risk for soil macro-organisms following the intended uses of GWN-10235 in rice.
For the parent benzobicyclon, the same outcome is achieved applying an additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.

Risk assessment for soil nitrogen transformation after application of GWN-10235

The risk assessment for soil nitrogen transformation was done in accordance with the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002). In a soil nitrogen transformation study with Benzobicyclon no difference to the untreated control above 25 % was noted at 2.0 mg a.s./kg soil dry weight after 28 days. Thus, the NOEC is at least 2.0 mg a.s./kg soil dry weight.
The applicant performed the risk assessment for soil micro-organisms only for the parent benzobicyclon. According to the RMS assessment in the E-fate section, the metabolites 1315P-070, 1315P-966, 1315P-570 and 1315P-960 should be considered as major in soil. In the absence of nitrogen transformation studies for these metabolites, the risk assessment has been performed assuming a 10 times higher toxicity compared to the parent, to cover a worst-case situation.
The risk assessment for soil micro-organisms has been updated in the following tables using the PEC_{SOIL} values re-calculated by the RMS in Vol.3 CP B.8, according to the standard and the revised MED-RICE model.

Table 109: Risk assessment for soil microflora exposed to Benzobicyclon and its metabolites (standard MED-RICE model)

Test substance	Crop	NOEC [mg a.s./kg sdw]	$PEC_{SOIL\ (initial)}$ [mg/kg soil] Scenario 1	Risk indicated?
Benzobicyclon	Rice	2.0	0.397	No
1315P-070		0.2*	0.0412	No
1315P-966		0.2*	0.000	No
1315P-570		0.2*	0.000	No
1315P-960		0.2*	0.000	No

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

Table 110: Risk assessment for soil microflora exposed to Benzobicyclon and its metabolites (revised MED-RICE model)

Test substance	Crop	NOEC [mg a.s./kg sdw]	$PEC_{SOIL\ (initial)}$ [mg/kg soil] Scenario 1	Risk indicated?
Benzobicyclon	Rice	2.0	0.420	No
1315P-070		0.2*	0.035	No
1315P-966		0.2*	0.000	No
1315P-570		0.2*	0.000	No
1315P-960		0.2*	0.000	No

* assuming that the toxicity of the metabolite is 10x higher than that of the parent

The updated risk assessment for benzobicyclon and its major soil metabolites indicates an acceptable risk for soil micro-organisms following the intended uses of GWN-10235 in rice.
For the parent benzobicyclon, the same outcome is achieved applying an additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.

Risk assessment for terrestrial non-target higher plants after application of GWN-10235

The Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) recommends a tiered approach towards assessing the risk to non-target plants. For the first tier, a preliminary assessment is conducted using available information. Preference is given to screening data, using at least 6 species from different taxa tested at the highest nominal single application rate.

Benzobicyclon is an herbicide and therefore, the risk to non-target plants needs to be addressed.

The risk assessment for non-target plants was performed using the available ER_{50} > 300 g a.s./ha determined in the seedling emergence and vegetative vigour studies conducted with the formulation GWN 10235. The RMS supports the use of the endpoint in terms of active substance, instead of the corresponding endpoint in terms of formulation, also taking into account that the total deposition after volatilization, which is included in the calculation of the overall drift rate, refers only to the active substance.

Table 111: Risk assessment for terrestrial non-target plants exposed to GWN-10235

Crop	Application rate [g a.s./ha]	MAF	Drift value [%]	Drift rate * [g a.s./ha]	ER_{50} [g a.s./ha]	TER
Rice	300	1	2.77	8.36	> 300	**> 35.9**

MAF = multiple application factor, TER = toxicity exposure ratio
* including deposition after volatilisation
Values in **bold** are above the trigger of 5

Overall, the calculated TER value exceeds the trigger of 5, indicating an acceptable risk for non-target plants following the intended uses of GWN-10235 in rice, without the need of mitigation measures.
The same outcome has been achieved applying an additional uncertainty factor of 2 to account for the composition of benzobicyclon as a racemate.
The RMS points out that the presented risk assessment should be intended as provisional, pending on further clarifications to address the concerns raised on the adequacy of the available seedling emergence and vegetative vigour studies.

2.10 ENDOCRINE DISRUPTING PROPERTIES

Benzobicyclon was assessed against the scientific criteria for the determination of endocrine disrupting properties (Commission Regulation (EU) 2018/605) according to the EFSA/ECHA Guidance for the identification of endocrine disruptors.
An assessment of the putative ED properties of Benzobicyclon was submitted in July 2019 as part of the Dossier in support of the approval of Benzobicyclon in the context of Regulation (EU) No 1107/2009. The RMS for the evaluation of the approval was Malta and the Co-RMS was Greece. The present document is a comprehensive update of the assessment of the putative ED properties of Benzobicyclon (2019) and includes newly conducted studies (aromatase (human recombinant) assay, H295R steroidogenesis assay, Hershberger assay, uterotrophic assay, prenatal developmental toxicity study in rabbits, peripubertal male and female assay and respective dose range-finding studies) and additional information on non-endocrine MoA regarding α2μ-globulin due to Malta's request for a major description of this MoA and the conduction of EAS-specific OECD CF level 2 and 3 studies. The prenatal developmental toxicity study in rabbits was performed since the available study was not in line with the current test guideline protocol and considered not acceptable by the RMS.
For the purpose of data gathering, all existing relevant data were identified in accordance with the revised GD 150 (OECD, 2018) such as "*structural; physico-chemical information; in vivo and in vitro guideline and non-guideline testing; QSAR models; computational and other non-testing assays; toxicokinetic, pharmacokinetic and toxicodynamic information; category and read-across assessment methodologies*".
For the gathering of toxicological and ecotoxicological data, all available relevant and reliable information was summarized for Benzobicyclon in the table (Annex 1) prepared in line with Appendix E of the ED GD (ECHA and EFSA, 2018) (hereinafter the table is referred to as 'Appendix E'), where applicable.

Toxicological studies

A total of 15 *in vivo* toxicity studies (including 3 dose range-finding studies) performed according to or similar to current OECD test guidelines were included in the present assessment of standard studies, *i.e.* all parameters which are necessary for the ED assessment (including parameters indicative of target organ toxicity as well as general adversity) identified in each relevant and reliable study were reported. Detailed information on the studies listed

below can be found in Vol.3 CA B6. Table 112 summarises the 15 *in vivo* toxicity studies included in the present assessment.

With regards to toxicity, the available database of OECF CF level 4 and 5 *in vivo* studies on Benzobicyclon includes the whole range of standard repeated dose toxicity studies covering all necessary endpoints for the elucidation of the complete toxicological profile. These studies spanned subchronic up to chronic exposure duration including carcinogenicity testing. Subacute dermal exposure was also investigated. Prenatal developmental toxicity as well as reproductive toxicity over two generations including fertility and postnatal development was sufficiently investigated. The requested species for each study type were used and comprised rats, rabbits, mice and dogs.

Table 112: Summary of the in vivo toxicity studies included in the present assessment

Study Type[a]	Spe-cies/Strain	Test substance	Dose levels or dietary concentrations	Reference (year and Doc. No.)	Study ID[a]
Repeated dose 90-day oral toxicity study in rodents	Rat/ Fischer (F344/[redacted]	Batch no: 960108N purity: 99 %	M: 0; 20; 100; 400 ppm F: 0; 100; 400; 2000; 10000 ppm corresponding to M: 0; 1.13; 5.73 and 22.74 mg/kg bw/day F: 0; 6.29; 25.17; 125.9 and 630 mg/kg bw/day	1999a, Doc. No. 533-002	1
Repeated dose 90-day oral toxicity study in non-rodents	Dog/ Beagle	Batch no: 6F0502 purity: 99 %	0; 20; 200; 2000 mg/kg bw/day	1998, Doc. No. 533-001	2
Chronic toxicity study	Dog/ Beagle	Batch no: 6F0502 purity: 99 %	0; 10; 100; 1000 mg/kg bw/day	1999, Doc. No. 537-001	3
Repeated dose dermal toxicity	Rabbit/ NZW	Batch no: 1L0108 purity: 98 %	0; 100; 300; 1000 mg/kg bw/day	2012, Doc. No. 532-001	4
Combined chronic toxicity/carcinogenicity study	Rat/ Fischer (F344/ [redacted]	Batch no: 6F0502 purity: 99 %	M: 0; 10; 20; 50; 100 ppm F: 0; 100; 1000; 10000 ppm corresponding to M: 0; 0.3; 0.7; 1.7 and 3.4 mg/kg bw/day F: 0; 4.2; 42 and 427 mg/kg bw/day	1999b, Doc. No. 537-002	5
Combined chronic toxicity/carcinogenicity study	Mouse/ [redacted]:CD-1™ ([redacted]	Batch no: 6F0502 purity: 99 %	0; 300; 3000; 30000 ppm corresponding to M: 0; 37; 373; 3817 mg/kg bw/day F: 0; 45; 473; 4807 mg/kg bw/day	1999, Doc. No. 555-001	6
Two-generation reproduction toxicity study	Rat/ [redacted]CD (SD)	Batch no: 6F0502 purity: 99 %	0; 100; 1000; 20000 ppm corresponding to M: 0; 5.7; 56 and 1176 mg/kg bw/day (F0), 0; 6.5; 63 and 1324 mg/kg bw/day (F1), F: 0; 8.4; 85 and 1741 mg/kg bw/day (F0), 0; 8.8; 89 and 1817 mg/kg bw/day (F1)	1999, Doc. No. 553-001	7
Prenatal developmental toxicity study	Rat/ [redacted]CD (SD)[redacted]	Batch no.: 960108N purity: 99 %	0; 40; 200; 1000 mg/kg bw/day	1997, Doc. No. 551-002	8
Prenatal developmental toxicity study[b]	Rabbit/ NZW	Batch no.: 960108N purity: 99 %	0; 40; 200; 1000 mg/kg bw/day	1998, Doc. No. 551-001	N.a.
Prenatal developmental toxicity study (DRF)	Rabbit/ NZW	Batch no.: 1A0709	0; 111; 333; 1000 mg/kg bw/day	2021, Doc. No. 551-005	14

Study Type[a]	Spe-cies/Strain	Test substance	Dose levels or dietary concentrations	Reference (year and Doc. No.)	Study ID[a]
		purity: 99.2 %			
Prenatal developmental toxicity study	Rabbit/ NZW	Batch no.: 1A0709 purity: 99.2 %	0; 111; 333; 1000 mg/kg bw/day	2022, Doc. No. 551-006	15
Subacute oral toxicity study in rodent (DRF)	Rat/ [redacted]CD (SD)	Batch no.: 1L0108 purity: 99.4 %	0; 250; 500; 1000 mg/kg bw/day	2022, Doc. No. 545-006	9
Hershberger assay	Rat/ [redacted]CD (SD)	Batch no.: 1L0108 purity: 99.4 %	Phase 1: 0; 333; 1000 mg/kg bw/day Phase 2: 0; 111; 333; 1000 mg/kg bw/day	2022, Doc. No. 545-007	10
Uterotrophic assay	Rat/ [redacted]CD (SD)	Batch no.: 1L0108 purity: 99.4 %	0; 250; 500; 1000 mg/kg bw/day	2022, Doc. No. 545-008	11
Subacute oral toxicity study in rodent (DRF)	Rat/ [redacted]CD (SD) [redacted]	Batch no.: 1L0108 purity: 99.4 %	M: 0; 2.5; 25; 250 mg/kg bw/day F: 0; 63; 250; 1000 mg/kg bw/day	2021, Doc. No. 545-002	12
Peripubertal male and female assays	Rat/ [redacted]CD (SD) [redacted]	Batch no.: 1L0108 purity: 99.4 %	0; 250; 500 mg/kg bw/day	2022, Doc. No. 545-009	13

App.: appendix; DRF: dose range-finding studies; F: females; F_0: parental generation; F_1: first filial generation; M: males; N.a.: Not applicable; NZW: New Zealand White, n.a.: not applicable; SD: Sprague-Dawley.
[a:] In line with Appendix E (Annex 1).
[b]: This study did not show adverse findings. Based on the assessment of the RMS the study was repeated (see Study ID 14). Thus, the study from 1998 was not included in the ED assessment.

For more information, please refer to Appendix E and Vol.3 CA B6.
For the submission of the ED assessment in July 2019 a literature search for the active substance Benzobicyclon was performed in accordance with the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009".
The objective of the literature search was the assessment of scientific peer-reviewed open literature published within the year 2001 until 2019 and dealing with side-effects on health, the environment and non-target species and the ED properties of Benzobicyclon.

Literature was searched accessing the databases: TOXCENTER, BIOSIS, AGRICOLA, HCAPLUS, PQSCITECH, MEDLINE, ESBIOBASE, EMBASE and CABA via the service provider STN-International. The search strategy was based on a single concept search.
In total, 101 records were retrieved from bibliographic databases and were screened by expert reviewers for relevance. Based on the evaluation of the summary records (titles/abstracts) 93 publications were assessed as obviously not relevant with regard to the EU-data requirements and ED properties of Benzobicyclon.
Eight (8) full-text documents were assessed in detail. All of these publications did not provide relevant information.
For more information, please refer to the literature search report by [redacted] 2019 (Doc. No. 591-001).
For the submission of the present update of the ED assessment an update of literature search with regard to putative ED properties of Benzobicyclon was performed.

No record was retrieved from bibliographic databases since 2019 which were related to potential ED properties for Benzobicyclon and respective plant protection products.
Few putative EAS-mediated findings were observed in the available dataset of Benzobicyclon. However, none of these findings were considered to be indicative for a direct effect of Benzobicyclon on the endocrine system, but secondary to systemic toxicity *e.g.* associated with α2μ-globulin. For this reason, the RMS requested additional information on the MoA with regard to α2μ-globulin.
Benzobicyclon is likely to cause an increased binding to α2μ-globulin, an urinary protein of male rodents (Ghosh *et al*., 1991), and subsequently an increased accumulation of α2μ-globulin in the proximal tubular epithelium of male rats. As a consequence, kidney function is affected by this MoA. Features of the α2μ-globulin nephropathy include "*hyaline droplet accumulation in proximal tubules, tubular epithelial necrosis and regeneration, exacerbation of spontaneous renal disease, and induction of renal epithelial tumors*" (Dominick, M.A. *et al.,* 1991).

The induction of α2μ-globulin nephropathy and carcinogenesis is an "*unique male-rat-specific disease*" (Swenberg, J.A., 1993). It is known that many chemicals cause α2μ-globulin nephropathy in male rats, but no "*similar nephropathy in female rats or either sex of any other species*" (Swenberg, J.A., 1993). Especially the non-human relevance should be highlighted in this context. "*The contribution of α2μ-globulin to the species-specificity of the nephropathy has been shown in several ways. First, it has been determined that the NBR strain of rats that does not synthesize the hepatic form of α2μ-globulin, does not develop α2μ-globulin nephropathy and is not susceptible to renal tumour promotion by these agents. Additionally, it has been shown that, although mice are resistant to renal toxicity following exposure to agents that induce α2μ-globulin nephropathy, transgenic mice engineered to synthesize α2μ-globulin developed the nephropathy. Mechanistic studies have demonstrated that the requisite step in the development of the syndrome is the ability of a chemical (or metabolite(s)) to bind reversibly, and specifically, to α2μ-globulin. Binding of chemicals to α2μ-globulin appears to alter the lysosomal degradation of the protein, leading to its accumulation in phagolysosomes. Furthermore, a comprehensive survey of structurally-related proteins along with experimental analyses has provided evidence that, although other species, including humans, synthesize proteins that are similar to α2μ-globulin, differences in ligand-binding properties, physiological function and renal handling of these homologues preclude their involvement in this protein droplet nephropathy*" (IARC, 1999).

Following a commenting phase with the Co-RMS the applicant is requested to provide evidence on potential binding of benzobicyclone and it smajor metabolite(s) to α2μ-globulin.

Indications for an α2μ-globulin nephropathy were observed in several studies conducted with Benzobicyclon.
In order to comply with Malta's request for additional information on the non-endocrine MoA regarding α2μ-globulin a specific literature search was performed with regard to mechanistic information on the postulated MoA. This literature search was regardless of the active substance Benzobicyclon. The same databases were accessed as for the literature search in 2019 and the update of the literature search as described above.
In total, 204 records were retrieved from bibliographic databases in this literature search. After the review of title and abstracts, 8 publications were evaluated as potentially relevant and their full texts were ordered. Based on these full texts 6 publications were included in the MoA analysis of increased blood α2μ-globulin levels in rats and their secondary effect on hormone levels.
For more information on this literature search, please refer to Annex 4.
The publications considered relevant for the description of the relationship between the α2μ-globulin nephropathy and putative EAS-mediated findings observed in the available dataset of Benzobicyclon are shortly described in the following.

<u>Effect of α2μ-globulin on serum concentration of gonadotrophins and testicular activity in oestrogen-treated rats</u>

"Adult male rats were given injections of oestradiol-17β (50 μg/100 g body wt per day) for 7 days. When they were killed 14 days after the last injection, serum levels of gonadotrophins and testosterone and weights of accessory sex organs were less, testicular 17β-hydroxysteroid dehydrogenase (17β-HSD) activity was suppressed and spermatogenesis was inhibited. Administration of α2μ-globulin (1-5 mg/day) for 14 days to oestrogen-treated rats and for 10 days to control rats resulted in increased concentrations of gonadotrophins and testosterone in the serum. Accessory sex organ weight and spermatogenesis appeared to be normal while 17β-HSD activity increased in oestrogen-treated rats after treatment with α2μ-globulin. It was concluded that α2μ-globulin has an effect on testicular function in oestrogenized rats by inducing gonadotrophin and testosterone synthesis" (Biswas, N.M. et al., 1983).

<u>Effect of thyroidectomy, and thyroxine and α2μ-globulin replacement therapy on testicular steroidogenic and gametogenic activities in rats</u>

"*Adult male rats were thyroidectomized and killed after 22 days of treatment. Thyroidectomy lowered the weights of testes and accessory sex organs, decreased the activities of testicular Δ^5-3β- and 17β-hydroxysteroid dehydrogenases (HSD), and diminished spermatogenesis, serum levels of testosterone and α2μ-globulin. Supplementation with thyroxine at a dose of 5 μg/100 g body weight per day for 21 days or supplementation with α2μ-globulin at a dose of 1-5 mg/rat per day for 21 days in thyroidectomized animals partially reversed the decrease in HSD activities and serum concentrations of testosterone and α2μ-globulin, while spermatogenesis was restored to normal. The weights of testes and accessory sex organs were also reinstated after supplementation with thyroxine or α2μ-globulin in thyroidectomized rats in comparison with thyroidectomized animals. It was concluded that α2μ-globulin may be an intermediary in the thyroid hormone control of testicular function*" (Biswas, N.M. et al., 1994).

Effects of a major androgen-dependent urinary protein α2μ-globulin on the pituitary-gonadal axis and hypothalamic monoamines in adult male mice

"*The purpose of the present study was to evaluate the effects of alpha-2u-globulin, a sex-dependent male rat urinary protein on pituitary-gonadal functions and hypothalamic monoamine contents in male mice. Adult male mice, maintained under standardized laboratory conditions (L:D, 14:10) were injected subcutaneously with alpha-2u-globulin at a dose of 1 mg/animal/day or with vehicle daily for 14 days and killed 16 h after the last injection. Plasma levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T) and testicular levels of T were measured by radioimmunoassays. The concentrations of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in medial basal hypothalamus (MBH) and anterior hypothalamus (AH) were measured by high performance liquid chromatography. Administration of alpha-2u-globulin led to a significant increase in plasma FSH and LH levels ($P<0.05$) as well as in plasma and testicular T levels ($P<0.025$). In the MBH alpha-2u-globulin treated mice, there were significant elevations of NE ($P<0.025$), DA ($P<0.01$) and 5-HT ($P<0.025$) contents. In the AH, both DA ($P<0.025$) and 5-HT ($P<0.01$) contents were decreased while NE content remained unaltered. These results indicate that administration of alpha-2u-globulin can lead to a significant stimulation of pituitary-testicular axis and that this effect may be mediated through alteration of hypothalamic monoamines*" (Ghosh, P.K. et al., 1990).

Possible involvement of hypothalamic monoamines in mediating the action of alpha-2u-globulin on the pituitary-testicular axis in rats

"*A major androgen-dependent urinary protein of male rodents, α2μ-globulin, has been shown to influence adenohypophyseal hormone release. In an attempt to elucidate the mechanisms of its action, we have examined several parameters of hypothalamic and pituitary function in adult male rats treated for 2 weeks with two injections daily of 0.75 mg α2μ-globulin and sacrificed 16 h after the last injection. This treatment led to an increase in plasma luteinizing hormone levels, a decrease in plasma prolactin levels, an increase in testosterone concentrations in both plasma and testicular tissue, and increases in testicular weights. The norepinephrine turnover in median eminence and anterior hypothalamus was increased in α2μ-globulin-injected animals, while the norepinephrine turnover in the remaining medial basal hypothalamus was reduced. α2μ-Globulin-treated animals had a significantly decreased dopamine turnover in the anterior hypothalamus, while in the medial basal hypothalamus the dopamine metabolism was increased. These data suggest that α2μ-globulin-induced changes in gonadotropin and prolactin secretion are mediated by changes in catecholamine metabolism in several hypothalamic regions. Increased testosterone secretion appears to be due to increased secretion of gonadotropins from the pituitary*" (Ghosh, P.K. et al., 1991).

Effect of continual light deprivation and alpha-2u-globulin replacement therapy on serum concentration of gonadotropins and testicular activity in rats

"*Prolonged darkness caused a fall in testicular 17β-hydroxysteroid dehydrogenase (17β-HSD) activity and diminished spermatogenesis, serum levels of gonadotropins, testosterone and α2μ-globulin. Administration of α2μ-globulin at a dose of 1.5 mg rat^{-1} per day for 7 days after 68 days of light deprivation, reversed the 17β-HSD activity and serum levels of gonadotropins, testosterone and α2μ-globulin, while spermatogenesis was restored to normal. The animals kept in prolonged darkness for 68 days and then received saline (7 days in light-dark cycle, 14 L: 10 D), showed no significant changes of testicular activity, serum levels of gonadotropins, testosterone and α2μ-globulin, when compared with dark-exposed animals (68 days) receiving rabbit serum (7 days in light-dark cycle, 14 L: 10 D). These results suggest that α2μ-globulin plays an important role in testicular function in dark-exposed rats by inducing gonadotropins and testosterone secretion*" (Ghosh, P.K. et al., 1996).

Effect of dihydrotestosterone on serum concentrations of α2μ-globulin and on spermatogenesis in melatonin-treated rats

"*Adult male rats were given s.c. injections of melatonin (400 μg/100 g body weight per day) for 14 days. On day 15, the weights of the testis and accessory sex organs were less, testicular 17β-hydroxysteroid dehydrogenase (17β-HSD) activity was inhibited, spermatogenesis was suppressed and serum levels of gonadotrophins, testosterone and α2μ-globulin were decreased compared with control animals injected with vehicle. In a third group of rats given the same dose of melatonin for 14 days, administration of dihydrotestosterone (DHT) at a dose of 25 μg/100 g body weight per day on days 8-14 resulted in serum levels of α2μ-globulin, FSH, LH and testosterone and testicular 17β-HSD activity similar to those seen in vehicle-injected control animals. Weights of the testes and accessory sex organs and spermatogenesis were normal after administration of DHT in melatonin-treated rats. In another group of rats, the depressive effects of melatonin treatment on plasma gonadotrophins were reversed by the administration of α2μ-globulin on days 8-14. It was concluded that treatment with DHT*

prevents the depressive action of melatonin on testicular function by inducing the synthesis of α2μ-globulin" (Mandal, H. et al., 1990).

In addition to the literature search described above a non-formal search for potentially relevant publications was performed via an internet search engine using similar search terms as in the literature search with regard to the MoA literature search. The following publication was obtained and included in the MoA description.

α2μ-globulin is present in the rat anterior pituitary

"*The possibility that the pituitary gland may contain as yet undiscovered regulatory factors is intriguing. Recent reports have suggested the presence, in the anterior pituitary, of a number of proteins of extrapituitary origin. α2μ-Globulin, a rat serum and urinary protein, previously shown to be synthesized in the submaxillary gland and in the liver under anterior pituitary control, has now been localized by immunocytochemistry in the cytoplasm of some cells of the anterior pituitary. No α2μ-globulin could be detected in either the intermediate or posterior pituitary. The presence of α2μ-globulin was confirmed and quantitated by radioimmunoassay. Using RNA blot analysis and cloned α2μ-globulin cDNA probes, we could not detect α2μ-globulin mRNA sequences in pituitary RNA, indicating that α2μ-globulin is not synthesized therein. The presence of a2u-globulin, presumably of circulatory origin, in certain anterior pituitary cells suggests that it may play a role in anterior pituitary function*" (Antakly, T. et al., 1983).

2.10.1 Database search in accordance with Appendix D.1 of the ED GD (ECHA and EFSA, 2018)

In addition to the primary data sources (*i.e.* data generated using standardized test methods, systematic literature review), information from other sources including but not confined to databases of compiled data and *in vitro* data, which were not part of the dossier for Benzobicyclon, were gathered. This is in line with the ED GD (ECHA and EFSA, 2018) where databases to be searched are listed in Appendix D.1.

2.10.1.1 US EPA CompTox Chemistry Dashboard

To obtain *in silico* and *in vitro* mechanistic information for potential EATS-related endocrine activity of Benzobicyclon, the US EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard, accessed on 27th January 2022) was searched and the obtained information was evaluated.
For Benzobicyclon, ER and AR predictions for agonism, antagonism and binding from CERAPP and CoMPARA were available, respectively. Benzobicyclon was stated as inactive with regard to AR and ER agonism and ER antagonism and active regarding AR antagonism and AR and ER binding. However, ER binding was categorized as very weak. Table 113 presents an overview of the model results.

Table 113: Model Score Values for Benzobicyclon

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	-	-	-
ToxCast Pathway Model (AUC)	Estrogen	-	-	-
CoMPARA (Consensus)	Androgen	Inactive	Active	Active
CERAPP Potency Level (from literature)	Estrogen	-	-	-
CERAPP Potency Level (Consensus)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Active (Very Weak)

AUC: area under the curve; CERAPP: Collaborative Estrogen Receptor Activity Prediction Project; CoMPARA: Collaborative modelling project for androgen receptor activity; –: no data.

The CompTox Chemicals Dashboard was additionally searched for the individual EATS-related HTS *in vitro* assays which are listed in the EFSA instruction sheet (EFSA, 2019) provided with the Excel file in line with the Appendix E. However, no HTS *in vitro* assays were available for Benzobicyclon.

2.10.1.2 Danish (Q)SAR Database

With regard to meaningful data to be assessed within the WoE of OECD CF level 1 information, the Danish (Q)SAR Database (http://qsar.food.dtu.dk, accessed on 28th January 2022) revealed *in silico* mechanistic information for Benzobicyclon within the applicability domain (AD) on thyroperoxidase (TPO) inhibition,

arylhydrocarbon receptor (AhR) activation and constitutive androstane receptor (CAR) activation at max. 20 and 50 µM. The data did not raise a concern towards EATS-related endocrine activity. All other information were either not applicable or outside the AD and therefore considered to be of very limited reliability.
According to profiling with the OECD QSAR Toolbox v.4.2, Benzobicyclon was depicted as ER non-binder, whereas the simulated metabolites (*in vivo* rat metabolism simulator only as well as rat liver S9 metabolism simulator only) were stated as strong binder due to hydroxyl groups. Mechanistic profiling alerts and especially hydroxyl groups are not very specific. Furthermore, the obtained mechanistic profiling alert for ER binding was not confirmed by the endpoint-specific profiler 'rtER expert system - US EPA' where parent alone and simulated metabolites (*in vivo* rat as well as rat liver S9 metabolism simulator) caused no structural profiling alert.
CERAPP and CoMPARA predictions have already been included in CompTox Chemicals Dashboard search (see Table 113).
Table 114 and Table 115 present the output of the Danish (Q)SAR Database for Benzobicyclon.

Table 114: Endocrine and molecular endpoints (according to the Danish (Q)SAR Database)

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	POS_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		INC_OUT	INC_OUT	INC_OUT	NEG_OUT
Estrogen Receptor α Activation (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	INC_OUT
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	POS_OUT	N/A
Androgen Receptor Inhibition (Human *in vitro*)		INC_OUT	INC_OUT	NEG_OUT	INC_OUT
Androgen Receptor Binding, CoMPARA data (*in vitro*)		N/A	N/A	POS_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)		N/A	N/A	POS_IN	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)		N/A	N/A	NEG_IN	N/A
Thyroid Receptor α Binding (Human *in vitro*)					
- mg/L		-	71495.45	5413.752	62.68712
- µM		-	159955.8	12112.12	140.2491
- Positive for $IC_{50} \leq 10$ µM		-	-	-	-
- Positive for $IC_{50} \leq 100$ µM		-	-	-	-
- Domain		OUT	OUT	OUT	OUT
Thyroid Receptor β Binding (Human *in vitro*)					
- mg/L		-	14463.67	154.3435	14.54382
- µM		-	32359.38	345.3106	32.53869
- Positive for $IC_{50} \leq 10$ µM		-	-	-	-
- Positive for $IC_{50} \leq 100$ µM		-	-	-	-
- Domain		OUT	OUT	OUT	OUT
Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)		N/A	N/A	INC_OUT	N/A
Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)		N/A	N/A	NEG_IN	N/A
Pregnane × Receptor (PXR) Binding (Human *in vitro*)	N/A	INC_OUT	POS_OUT	POS_OUT	POS_OUT
Pregnane × Receptor (PXR) Binding (Human *in vitro*) NEW	-	N/A	N/A	INC_OUT	N/A
Pregnane × Receptor (PXR) Activation (Human *in vitro*)	-	N/A	N/A	INC_OUT	N/A
Pregnane × Receptor (PXR) Activation (Rat *in vitro*)	-	N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)	-	N/A	N/A	INC_OUT	N/A
Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (*in vitro*)	-	N/A	N/A	NEG_IN	N/A
Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (*in vitro*)	-	N/A	N/A	NEG_IN	N/A

	Exp	Battery	CASE Ultra	Leadscope	SciQSAR
Constitutive Androstane Receptor (CAR) Inhibition at max. 50 μM (*in vitro*)	-	N/A	N/A	INC_OUT	N/A
CYP3A4 Induction (Human *in vitro*)	-	N/A	N/A	INC_OUT	N/A

AhR: Arylhydrocarbon receptor. CERAPP: Collaborative Estrogen Receptor Activity Prediction Project. CoMPARA: Collaborative modeling project for androgen receptor activity. CYP: Cytochrome P450. Exp: Experimental values, from EpiSuite experimental databases or DK DTU QSAR models training sets. IC_{50}: half-maximal inhibitory concentration. IN: Within the applicability domain. INC: inconclusive. NA: Not applicable, because training set data cannot be released for commercial models. NEG: Negative. OECD: Organisation for economic co-operation and development. OUT: Outside the applicability domain. POS: Positive. PXR: Pregnane × receptor. (Q)SAR: (Quantitative) Structure Activity Relationship. -: no data.

Table 115: Estrogen receptor (ER) binding alerts (obtained by Danish (Q)SAR Database)

Estrogen Receptor Binding, alerts in:	
- parent only	Non binder, without OH or NH2 group
- metabolites from *in vivo* Rat metabolism simulator only	Strong binder, OH group
- metabolites from Rat liver S9 metabolism simulator only	Strong binder, OH group
rtER Expert System – US EPA, alerts in:	
- parent only	No alert found
- metabolites from *in vivo* Rat metabolism simulator only	No alert found
- metabolites from Rat liver S9 metabolism simulator only	No alert found
OECD QSAR Toolbox v.4.2 profilers *Profiler predictions are supporting information to be used together with the relevant QSAR predictions*	

NH_2: amino group. OECD: Organisation for economic co-operation and development. OH: hydroxyl group. (Q)SAR: (Quantitative) Structure Activity Relationship. rtER: Rainbow trout estrogen receptor. S9: Supernatant fraction obtained from liver homogenate by centrifuging at 9000 g. US EPA: United States Environmental Protection Agency.

2.10.2 Relevant human health data and epidemiological data generated according to data requirements for PPPs

Medical surveillance data on manufacturing plant personnel and monitoring studies did not reveal any data indicative of adverse effects and impact on the endocrine system (see Supplementary Dossier, M-CA 5: Toxicology and Metabolism (Gowan Crop Protection Ltd., 2022)).
There are no epidemiological studies involving Benzobicyclon available that could be considered for assessment of possible adverse effects on the endocrine system of humans.

2.10.3 ED assessment for humans

2.10.3.1 ED assessment for T-modality

Table 116: Available studies investigating T-mediated parameters

	Sufficiently investigated
T-mediated parameters	Yes based on the availability of the following studies: – Repeated dose 90-day oral toxicity study in rats (Study ID 1) – Repeated dose 90-day oral toxicity study in dogs (Study ID 2) – Repeated dose 1-year oral toxicity study in dogs (Study ID 3) – Repeated dose 21-day dermal toxicity study in rabbits (Study ID 4) – Combined chronic toxicity/carcinogenicity study in rats (Study ID 5) – Combined chronic toxicity/carcinogenicity study in mice (Study ID 6) – Peri-pubertal male and female assays (Study ID 13)

Thyroid hormone (TH; T4) and thyroid-stimulating hormone (TSH) levels have been assessed *in vivo* in rats (Study ID 13), thyroid weights were reported for rats and dogs and thyroid histopathology reports are available for rats, dogs, rabbits and mice for study durations from 21 days onwards up to 2 years. The dataset for the T-modality is considered complete, especially since "*the duration and doses selection allow a proper assessment of the thyroid histology*" (EFSA, 2020). Lines of evidence for adverse effects and endocrine activity related to T-modality and for general or target organ toxicity

According to the ED GD (ECHA and EFSA, 2018) "the assembling of lines of evidence should take into consideration all the available evidence (positive and negative) that have been evaluated as relevant and reliable" during the data gathering (Chapter 2). These parameters were assessed to determine "whether and how they contribute to the lines of evidence for adversity and/or endocrine activity".

After assembling and assessing the lines of evidence they were integrated for the assessment of adversity and endocrine activity in respect to the T-modality.

The integrated lines of evidence for T-related endocrine activity and T-mediated effects are reported in Table 117. The data comprise *in silico* and *in vivo* mechanistic data and data on organ weight and histopathological evaluations.

According to the ED GD "*adverse effects that are nonspecific secondary consequences of other toxic effects shall not be considered for the identification of the substance as endocrine disruptor*" (ECHA and EFSA, 2018). For this reason, all parameters with regards to general adversity and target organ toxicity are presented in the integrated lines of evidence table, Table 118, as applicable.

Table 117: Assessment of the integrated lines of evidence for the T-modality

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
N.a.	*In silico* prediction	(Q)SAR prediction: Danish (Q)SAR Database	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	No effect	Negative predictions within the applicability domain were available for thyroperoxidase (TPO) inhibition and constitutive androstane receptor (CAR) and arylhydrocarbon receptor (AhR) activation. The predictions concerning TRα and TRβ binding were all outside the AD and therefore considered not reliable.	Supporting negative evidence for T-related endocrine activity.	Overall, supporting negative evidence for T-related endocrine activity (*in silico*).	E, A, T, S
10	*In vivo* mechanistic	Liver weight (Hershberger, considered T-mediated only in combination with other thyroid endpoints)	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	Phase 1: Not affected by the test substance. Phase 2: Not affected by the test substance.	No adverse effect on liver weight in the Hershberger assay. Negative evidence for T-related endocrine activity.	Overall, negative evidence for T-related endocrine activity (*in vivo* mechanistic).	T
13	*In vivo* mechanistic	T3 and T4 level	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	Only T4 levels were analysed. Compared to the historical control data (HCD), M+F receiving the vehicle control showed slightly elevated T4 levels. The animals on this study received a feed with a higher protein content (20 % vs. 16 %) than the historical control animals and this likely resulted in initial body weights that were elevated leading to elevated T4 levels.	No adverse effect on T4 and TSH. Negative evidence for T-related endocrine activity.		T
13	*In vivo* mechanistic	Thyroid-stimulating hormone level (TSH)	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			T
2	EATS-mediated	Thyroid weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	including parathyroid	Isolated finding regarding decreased thyroid weight was observed in a pubertal development study. This finding was in the absence of	Overall, negative evidence for T-mediated adversity (*in vivo*).	T
3	EATS-mediated	Thyroid weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			T
5	EATS-mediated	Thyroid weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	and parathyroid			T
13	EATS-mediated	Thyroid weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	500	mg/kg bw/day	Decrease	post fixation M receiving 500 mg/kg bw/day showed a stat. significant decrease in abs. thyroid			T

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										weight. Histopathology did not reveal any effect contributing to this finding.	histopathological changes and no similar findings were present in other studies.		
13	EATS-mediated	Colloid area (thyroid histopathology)	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			T
13	EATS-mediated	Follicular cell height (thyroid histopathology)	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			T
1	EATS-mediated	Thyroid histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			T
2	EATS-mediated	Thyroid histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	Kuersteiner's cysts of the parathyroid glands on histopathological examination were higher than those in the control group. This finding was considered not to be attributable to administration of the test substance, because the differences in the incidences were small, they were not lesional, and the incidences were within the range of the laboratory historical control data collected from the 14 studies performed over the past 7 years (1991 to 1997): Kuersteiner's cysts of the parathyroid glands: 10.6 % (0.0 to 50.0 %) in F.			T
3	EATS-mediated	Thyroid histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	Histopathologically, Kuersteiner's cysts of the parathyroid glands were observed in F in the 1000 mg/kg bw/d group alone. This finding was, however, considered not to be attributable to administration of the test substance, because the difference in the incidence was small, it was not lesional, and the incidence was within the range of the laboratory historical control data for Beagle dogs of the same age [F: 16.7 % (0.0 to 66.7 %), 1991 to 1998, 12 studies, 48 dogs, 18 to 21 months old]. C-cell compleyes in the thyroid glands were observed in several M+F, including the control animals, however, these findings were considered not to be			T

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										attributable to administration of the test substance, because they were unrelated to the dose.			
4	EATS-mediated	Thyroid histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			T
5	EATS-mediated	Thyroid histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	At necropsy, M at 100 ppm (3.43 mg/kg bw/day) showed stat. significant increases in incidence of mass(es) of the thyroid at terminal kill after Week 104. Except one case which was hyperplasia of the parathyroid, other 12 cases were all diagnosed as C-cell tumors (adenoma or adenocarcinoma) in histopathology. In the absence of treatment related increases of preneoplastic lesions or neoplastic findings in any experimental groups (interim kill sacrifices, found gead or killed in extremis and in the overall incidence) the occurrence of these masses was considered unrelated to treamtment..			T
6	EATS-mediated	Thyroid histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			T
13	EATS-mediated	Thyroid histopathology	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			T
2	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-	An isolated finding regarding pituitary histopathology was reported. The lietarture evidence pro vided partially supopport the postulated MoA (related to the male rat specific α2μ-globulin nephropathy). Although the	Overall, supporting negative evidence for EATS-mediated adversity based on parameters considered sensitive to, but not diagnostic of, EATS-modalities	N
3	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not	Pituitary weight	Rat	18-19 (P adult to weaning	Weeks	Oral	-	mg/kg bw/day	No effect	-			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS			of F2 pups)							proposed MoA and related evidence for the effect on the pituitary are considered plausible. No other effects were observed. Supporting negative evidence for EATS-mediated adversity.	(pituitary; *in vivo*).	
7	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	Abs. pituitary weight for F1 M in the 20000 ppm (1324 mg/kg bw/day) group was stat. significantly decreased (↓ 8.8 %: 12.5 vs. 11.4 mg). This change was considered not to be due to test substance treatment because the rel. pituitary weight for F1 M at 20000 ppm was comparable to that in the control group.			N
13	Sensitive to, but not diagnostic of, EATS	Pituitary weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			N
1	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			N
2	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	The incidence of cysts in the pituitary glands was macroscopically and histopathologically higher. This finding was considered not to be attributable to administration of the test substance, because the differences in the incidences were small, they were not lesional, and the incidences were within the range of the laboratory historical control data collected from the 14 studies performed over the past 7 years (1991 to 1997): cysts in the pituitary glands: 25.5 % (0.0 to 100.0%) in M and 36.2 % (0.0 to 100.0 %) in F			N
3	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	Cysts in the pituitary glands were macroscopically and histopathologically observed in several M+F, including the control animals, however, these findings were considered not to be attributable to administration of the test substance, because they were unrelated to the dose.			N
4	Sensitive to, but not	Pituitary histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS												
5	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	Macroscopical finding: Although M at 100 ppm (3.43 mg/kg bw/day) showed a stat. significant increase in incidence of mass(es) of the pituitary in animals found dead or killed in extremis during the treatment, the change was considered to be incidental without relationship to the treatment, because the degree of difference from the control was very small and no stat. significant increases in incidence of pituitary tumors were observed in histopathology in the animals subjected to other phases of examination. Histopathological finding: M at 20 ppm (0.667 mg/kg bw/day) showed stat. significant increases in incidence of cysts, Rathke's cleft, of the pituitary at terminal kill after Week 104 and in overall incidence. However, these findings were considered to be incidental without any toxicological significance because of no dose dependency.			N
6	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Pituitary histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	1176	mg/kg bw/day	Change	Treatment related effects on the pituitary from M in F0 and F1: Increased hydropic degeneration cells (basophilic cells) were noted at incidences of 2/24 and 6/24 for F0 and F1 M, respectively, in the 20000 ppm (1176 and 1324 mg/kg bw/day, respectively) group; stat. significant increase. Since it has been known that an increase in the blood α2μ-globulin level in male rats causes increases in blood LH and FSH levels, and in the testicular weight, increased hydropic degeneration cells (basophilic			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										cells) in the pituitary and increased testicular weight and epididymal weights observed in parental M at 20000 ppm. The provided literarure evidence supports the postulated MoA for effects on testes and epididymides weights but it is considered to partially support the effect observed in the pituitary (hydropic degeneration basophilic cells), although the proposed MoA and related evidence for the effect on the pituitary are considered plausible.			

A: androgen; AD: applicability domain; abs.: absolute; AhR: arylhydrocarbon receptor; bw: body weight; C-cell: parafollicular cells; CAR: constitutive androstane receptor; E: estrogen; EATS: estrogen, androgen, thyroid, steroidogenesis; F: female(s); F0: parental generation; F1: first filial generation; F2: second filial generation; FSH: follicle stimulating hormone; HCD: historical control data; LH: luteinising hormone; M: male(s); N: endpoints potentially sensitive to, but not diagnostic of, EATS-modalities; N.a.: not applicable / not available; P: parental generation; ppm: parts per million; (Q)SAR: (quantitative) structure activity relationship; rel.: relative; S: steroidogenesis; stat.: statistical(ly); T: thyroid; T3: triiodothyronine; T4: thyroxine; TPO: thyroperoxidase; TR: thyroid hormone receptor; TSH: thyroid stimulating hormone; vs: versus; -: no data.

Table 118: Assessment of the integrated lines of evidence for general adversity and target organ toxicity

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
1	Target organ toxicity	Kidney weight	Rat	90	Days	Oral	630	mg/kg bw/day	Increase	F at 10000 ppm (630 mg/kg bw/day) showed stat. significant increases in both abs. and rel. weights of the kidneys. No effects observed in the recovery group.	Increases in kidney weight and changes in kidney histopathology were only present in rats, especially in M individuals. These findings were most likely due to the M rat specific α2µ-globulin nephropathy. Furthermore, dose-dependent	Signs of kidney impairment were observed in the available data, most of them associated with the M rat specific α2µ-globulin nephropathy, which is not relevant to humans. Effects on liver weight were minor and supported only in one case by	-
1	Target organ toxicity	Kidney weight	Rat	90	Days	Oral	22.74	mg/kg bw/day	Increase	M at 20 and 100 ppm (1.13 and 5.73 mg/kg bw/day, respectively) showed a stat. significant decrease in rel. weight of the kidneys (↓95 %, 93 %). No histopathological changes observed for male animals at 20 or 100 ppm. M at 400 ppm (22.74 mg/kg bw/day) showed a stat. significant increase in rel. kidney weight (↑ 105 %). Not stat. significant for abs. weight. F at 10000 ppm (630 mg/kg bw/day) showed stat. significant increases in			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										both abs. and rel. weights of the kidneys.	or high dose only effects on kidney weight and sporadical findings in kidney histopathology provide evidence for kidney impairment in F rats.	histopathological findings. Overall, the test substance was considered to possess a low target organ toxicity.	
2	Target organ toxicity	Kidney weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Target organ toxicity	Kidney weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Target organ toxicity	Kidney weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-
5	Target organ toxicity	Kidney weight	Rat	24	Months	Oral	427	mg/kg bw/day	Increase	F at 10000 ppm (427 mg/kg bw/day) showed stat. significantly increased abs. kidney weight at interim kill after 52 weeks and stat. significantly increased rel. kidney weight at interim kill after 26 weeks. Changes in kidney and liver weights were considered treatment related. No stat. significant changes observed in F at Week 78 and 104 or in M at any dose level at any kill.			-
6	Target organ toxicity	Kidney weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	No stat. significant changes in abs. weight in M and F.			-
6	Target organ toxicity	Kidney weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	Increase in rel. kidney weight in F at 30000 ppm (4807 mg/kg bw/day), not stat. significant for abs. kidney weight. No stat. significant change for F at 3000 ppm (473 mg/kg bw/day) or M up to 30000 ppm (3817 mg/kg bw/day). Not associated with any histopathological change. Considered not to be of toxicological significance.			-
7	Target organ toxicity	Kidney weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	M: 1176/F: 1741	mg/kg bw/day	Increase	F0 and F1 M and F at 20000 ppm (M: 1176 and 1324 mg/kg bw/day, respectively; F: 1741 and 1817 mg/kg bw/day, respectively) showed stat. significantly increased abs. and rel. (except F1 F) kidney weight. F0 M abs. ↑ 20.3 % (1639 vs. 1972) and F1 M abs. ↑ 28.6 % (1665 vs. 2141)			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										F0 F abs. ↑ 13.6 % (1069 vs. 1214) and F1 F abs. ↑ 13 % (1104 vs. 1247)			
7	Target organ toxicity	Kidney weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	56.1	mg/kg bw/day	Increase	F0 M and F at 20000 ppm (1176 and 1741 mg/kg bw/day, respectively) showed stat. significantly increased abs. and rel. kidney weight. F0 M at 1000 ppm (56.1 mg/kg bw/day) showed also stat. significantly increased rel. kidney weight (but not stat. significantly increased abs. kidney weight). No stat. significant effect in F0 F at 1000 ppm (85.4 mg/kg bw/day). F0 M rel. ↑ 8.2 % (1000 ppm) and ↑ 20.7 % (20000 ppm) F0 F rel. ↑ 9.5 % (20000 ppm)			-
7	Target organ toxicity	Kidney weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	62.8	mg/kg bw/day	Increase	F1 M at 20000 ppm (1324 mg/kg bw/day) showed increased abs. and rel. kidney weight. F1 M at 1000 ppm (62.8 mg/kg bw/day) showed increased relative kidney weight, not stat. significant for abs. kidney weight. F1 M rel. ↑ 9.8 % (1000 ppm) and ↑ 33.5 % (20000 ppm) No stat. significant effect on rel. kidney weight in F1 F at any dose level.			-
9	Target organ toxicity	Kidney weight	Rat	M: 10 F: 4	Days	Oral	250	mg/kg bw/day	Increase	M receiving 250, 500 or 1000 mg/kg bw/day showed a stat. significant increase in abs. kidney weight of approximately 20 %. This effect was likely due to the binding of the test substance to α2μ-globulin and was not apparent in F.			-
12	Target organ toxicity	Kidney weight	Rat	14	Days	Oral	250	mg/kg bw/day	Increase	M receiving 250 mg/kg bw/day showed a slight increase in abs. and rel. kidney weight. F receiving 250 or 1000 mg/kg bw/day also showed an increase in abs. and rel. kidney weight.			-
13	Target organ toxicity	Kidney weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	250	mg/kg bw/day	Increase	M receiving 250 mg/kg bw/day showed a stat. significant increase in rel. kidney weight and M receiving 500 mg/kg bw/day showed a stat. significant increase in abs. and rel. kidney weight. F receiving 500 mg/kg bw/day showed			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										a linear trend towards an increase in rel. kidney weight. Histopathology did not reveal any effect contributing to this finding. Furthermore, the abs. weight of the control group kidneys was elevated compared to the HCD which was most likely due to a change in the diet with a high protein content.			
1	Target organ toxicity	Kidney histopathology	Rat	90	Days	Oral	22.74	mg/kg bw/day	Change	Treatment related changes were observed in kidneys in M. 6/12 M at 400 ppm (22.74 mg/kg bw/day) (non-recovery group) showed deposition of hyaline droplets of tubular cells in the kidney which had been noted in M at 80 ppm or more in the 4-week dose finding study. The hyaline droplets of tubular cells were identified as α2μ-globulin. The hyaline droplets were not observed in the recovery group. Although tubular basophilic change seemed to be still magnified in M at 400 ppm, severity of the lesion was less than that examined before the recovery period.			-
2	Target organ toxicity	Kidney histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Target organ toxicity	Kidney histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Target organ toxicity	Kidney histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-
5	Target organ toxicity	Kidney histopathology	Rat	24	Months	Oral	3.43	mg/kg bw/day	Change	Stat. significant increases in incidence of deposition, hyaline droplet, proximal tubular cell in kidney were noted for M at 100 ppm (3.43 mg/kg bw/day) at interim kills after Weeks 52 and 78 and in overall incidence. In addition, M showed stat. significant increases in incidence of nephropathy, chronic with aggravated severity at terminal kill after Week 104 and in overall incidence. It			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										was assumed that the aggravated severity of nephropathy, chronic in these M was caused by M rat-specific α2μ-globulin nephropathy.			
6	Target organ toxicity	Kidney histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Target organ toxicity	Kidney histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	56.1	mg/kg bw/day	Change	Increased hyaline droplet degeneration in the proximal tubular cells of the kidney was noted for F0 and F1 M at respective incidences of 1/24 and 2/24 in the 100 ppm (5.65 and 6.46 mg/kg bw/day, respectively) group, 23/24 and 24/24 in the 1000 ppm (56.1 and 62.8 mg/kg bw/day, respectively) group and 24/24 and 24/24 in the 20000 ppm (1176 and 1324 mg/kg bw/day, respectively) group. Tubular basophilic change in the kidney was noted for F0 and F1 M at respective incidences of 9/24 and 12/24 in the 1000 ppm and 19/24 and 21/24 in the 20000 ppm group. Granular casts in the dilated tubules in the kidney were noted for F0 and F1 M at respective incidences of 2/24 and 3/24 in the 1000 ppm group and of 7/24 and 13/24 in the 20000 ppm group.			-
13	Target organ toxicity	Kidney histopathology	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	left and right			-
1	Target organ toxicity	Liver weight	Rat	90	Days	Oral	630	mg/kg bw/day	Increase	F at 10000 ppm (630 mg/kg bw/day) showed stat. significantly increased abs. liver weight in the non-recovery group at terminal kill after 13 weeks of treatment.	Increases in liver weight were supported only in one case by corresponding histopathological changes in mice (Study ID 6). Changes in liver weight were dose-		-
1	Target organ toxicity	Liver weight	Rat	90	Days	Oral	22.74	mg/kg bw/day	Increase	M at 400 ppm (22.74 mg/kg bw/day) (high dose group) showed stat. significant increases in rel. weight of the liver in the non-recovery group at terminal kill after 13 weeks of treatment; no effect in the 400 ppm recovery group			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										4 weeks after treatment. F at 2000 and 10000 ppm (125.9 and 630 mg/kg bw/day, respectively) (two highest dose groups) showed a stat. significant increase in rel. weight of the liver, no effect in the recovery groups 4 weeks after treatment. No changes in liver histopathology.	dependent or high dose only effects providing evidence for an adaptive response.		
2	Target organ toxicity	Liver weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Target organ toxicity	Liver weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Target organ toxicity	Liver weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-
5	Target organ toxicity	Liver weight	Rat	24	Months	Oral	427	mg/kg bw/day	Increase	F at 10000 ppm (427 mg/kg bw/day) showed stat. significant increases in the liver weight for absolute and relative weights at interim kill after Week 52, abs. weight at terminal kill after Week 104, and rel. weight at interim kill after Week 26 compared with the controls.			-
6	Target organ toxicity	Liver weight	Mouse	78	Weeks	Oral	4807	mg/kg bw/day	Increase	Increase in abs. and rel. liver weight in F at 30000 ppm (4807 mg/kg bw/day). No stat. significant change for F at 3000 ppm (473 mg/kg bw/day) or M up to 30000 ppm (3817 mg/kg bw/day).			-
7	Target organ toxicity	Liver weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	1741	mg/kg bw/day	Increase	F0 and F1 F at 20000 ppm (1741 and 1817 mg/kg bw/day, respectively) showed stat. significantly increased abs. (and rel.) liver weight: F0 abs. ↑ 14 % (12313 vs. 14042 mg) and F1 abs. ↑ 21.7 % (11436 vs. 13922 mg). No difference in abs. liver weight observed in F0 and F1 M.			-
7	Target organ toxicity	Liver weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	M: 1176/F: 1741	mg/kg bw/day	Increase	Rel. liver weight for F0 and F1 M in the 20000 ppm (1176 and 1324 mg/kg bw/day, respectively) group increased, no stat. significant effect on abs. weight. F0 and F1 F in the 20000 ppm (1741 and 1817 mg/kg bw/day,			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										respectively) group showed stat. significantly increased (abs. and) rel. liver weight. F0 M rel. ↑ 8.6 % (2.92 vs. 3.17) and F1 M rel. ↑ 7 % (3.02 vs. 3.23) F0 F rel. ↑ 10.3 % (3.98 vs. 4.39) and F1 F rel. ↑ 12.6 % (3.58 vs. 4.03)			
11	Target organ toxicity	Liver weight	Rat	4	Days	Oral	-	mg/kg bw/day	No effect	Liver weight was not affected.			-
12	Target organ toxicity	Liver weight	Rat	14	Days	Oral	62.5	mg/kg bw/day	Decrease	F receiving 62.5, 250 or 1000 mg/kg bw/day showed a decreased abs. and rel. liver weight compared to control. M were not affected.			-
13	Target organ toxicity	Liver weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	M receiving the vehicle control showed an elevated abs. liver weight compared to the HCD, which was due to the changes in feed containing a higher protein amount.			-
1	Target organ toxicity	Liver histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	Although there were stat. significant increases in organ weight of the liver in M at 400 ppm (22.74 mg/kg bw/day) and in F at 2000 ppm (125.9 mg/kg bw/day) or more, no lesions correspondent to the change were observed in histopathology.			-
2	Target organ toxicity	Liver histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Target organ toxicity	Liver histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Target organ toxicity	Liver histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-
5	Target organ toxicity	Liver histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			-
6	Target organ toxicity	Liver histopathology	Mouse	78	Weeks	Oral	M: 3817/F: 4807	mg/kg bw/day	Change	Increased incidences of centrilobular hepatocyte hypertrophy were reported in M and F at 30000 ppm (3817 and 4807 mg/kg bw/day, respectively). In M at 30000 ppm, there was also			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										evidence of an increased degree of this finding. This finding was considered to be associated with the slightly higher liver weights recorded in mice of this dosage group.			
7	Target organ toxicity	Liver histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
1	Systemic toxicity	Body weight	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	There were no stat. significant differences in body weight between the control and the treated groups of either sex in both non-recovery and recovery groups except a solitary significant decrease in F of the 2000 ppm (125.9 mg/kg bw/day) group at Week 10 in non-recovery group.	Effects on body weight and body weight gain were present only in two short-term studies. No effects were found in long-term studies. Therefore, these findings were considered to be of no toxicological significance.	All findings concerning systemic toxicity were minor in degree or considered to be of no toxicological significance.	-
2	Systemic toxicity	Body weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Systemic toxicity	Body weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Systemic toxicity	Body weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-
5	Systemic toxicity	Body weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			-
5	Systemic toxicity	Body weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			-
6	Systemic toxicity	Body weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Body weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Body weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	In the 100 ppm (8.76 mg/kg bw/day) group, bw of F1 F at Week 9 was stat. significantly increased; considered not to be treatment related. No differences for F1 F at 1000 ppm (89.0 mg/kg bw/day). In the 20000 ppm (1817 mg/kg bw/day) group, bw of F1 F were stat. significantly increased at Week 9, on GD 20, on LD 7 and 21 and on the day of necropsy (↑ 8.2 %; 319 vs. 345 g)			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										In the 20000 ppm group, body weight gains of F1 F were stat. significantly increased at Weeks 0-9 and 0-18. No significant effect on abs. body weight. In the 100 ppm group, bw gain of F1 F at Week 0-9 was stat. significantly increased.			
7	Systemic toxicity	Body weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
8	Systemic toxicity	Body weight	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			-
9	Systemic toxicity	Body weight	Rat	M: 10 F: 4	Days	Oral	-	mg/kg bw/day	No effect	-			-
10	Systemic toxicity	Body weight	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	In opposite to the positive control substances testosterone propionate (TP) and flutamide, the test substance did not lead to changes in body weight compared to vehicle control.			-
11	Systemic toxicity	Body weight	Rat	4	Days	Oral	-	mg/kg bw/day	No effect	In contrast to the positive control 17 alpha-ethinyl estradiol, the test substance did not affect the body weight.			-
12	Systemic toxicity	Body weight	Rat	14	Days	Oral	62.5	mg/kg bw/day	Decrease	F receiving 62.5, 250 or 1000 mg/kg bw/day showed a decrease in terminal body weight as well as in body weight gain. M were unaffected.			-
13	Systemic toxicity	Body weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	The animals on this study received a feed with a higher protein content (20 % vs. 16 %) than the historical control animals and this likely resulted in initial body weights that were elevated.			-
14	Systemic toxicity	Body weight	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			-
15	Systemic toxicity	Body weight	Rabbit	22 (GD6-GD27)	Days	Oral	1000	mg/kg bw/day	Decrease	F receiving 1000 mg/kg bw/day showed slightly but not stat. significantly lower body weight gains over GD6 to GD28 compared to the controls (-6.5 %). Lower dose groups were not affected. Terminal body weight adjusted for the gravid uterus weight was similar in all groups.			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
1	Systemic toxicity	Clinical chemistry and haematology	Rat	90	Days	Oral	22.74	mg/kg bw/day	Change	Haematology: M at 400 ppm (non-recovery) (22.74 mg/kg bw/day) showed mild but stat. significant changes in anemic parameters (less than 6 % compared with the control) including stat. significant decreases in haematocrit (Ht), haemoglobin (Hb), erythrocyte (RBC) together with stat. significant increases in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). The anemic condition observed in those M became ameliorated in association with recovery of renal lesions (alpha2μ-globulin nephropathy) after recovery period. Blood biochemistry: Although M at 400 ppm showed stat. significant increases in BUN and Creat. suggesting the toxic effects on the kidney by the treatment, these changes disappeared after the 4-week recovery period. No toxicological significance, however, was indicated in the significant decrease in AP observed in M at 400 ppm. Other significant changes were considered to be incidental due to no dose dependency or alterations observed in only recovery group.	Minor dose-dependent changes on clinical chemistry parameters predominantly at the high dose level indicative for liver and kidney impairments. In one case the effects were supposed to be associated with the M rat specific α2μ-globulin nephropathy.		-
2	Systemic toxicity	Clinical chemistry and haematology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	Clinical laboratory tests revealed rises in the white blood cell count, neutrophil ratio, and fibrinogen and phospholipid values in 1 F in the 2000 mg/kg bw/day group. These rises were, however, considered to be incidental and unrelated to the administration of the test substance, because there were no abnormalities in any of the other parameters examined, including in the pathological examination, and no such			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										rises were observed in any of the other animals in the same group.			
3	Systemic toxicity	Clinical chemistry and haematology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	An examination of blood chemistry after the initiation of dosing revealed declines in the K values in M at 100 and 1000 mg/kg bw/day. These findings were, however, considered not to be toxicologically significant because they were slight and there were no abnormalities in any of the other parameters examined. There were no effects attributable to administration of the test substance on the results of examinations of haematology.			-
4	Systemic toxicity	Clinical chemistry and haematology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	There were no test substance-related alterations in haematology, coagulation parameters and serum chemistry parameters.			-
5	Systemic toxicity	Clinical chemistry and haematology	Rat	24	Months	Oral	427	mg/kg bw/day	Change	Haematology: Although stat. significant alterations were observed in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and/or mean corpuscular haemoglobin concentration (MCHC) in F at 1000 and 10000 ppm (42,2 and 427 mg/kg bw/day, respectively) and M at 20 and 50 ppm (0.667 and 1.696 mg/kg bw/day, respectively), the changes were considered to be of no toxicological significance because there were no findings indicating apparent anemia at any intervals of examination. Blood biochemistry: F at 10000 ppm showed stat. significant increased total protein and globulin (both at interim kill after Week 52) and total cholesterol (at interim kill after Week 26). They were considered treatment related as these changes were			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										also observed in the 4-week dose finding study.			
6	Systemic toxicity	Clinical chemistry and haematology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
12	Systemic toxicity	Clinical chemistry and haematology	Rat	14	Days	Oral	1000	mg/kg bw/day	Change	F receiving 1000 mg/kg bw/day showed increased levels of tyrosine in the plasma. M were not affected. RBC, HB, MCV and creatinine levels were not affected in both sexes.			-
13	Systemic toxicity	Clinical chemistry and haematology	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	500	mg/kg bw/day	Change	Clinical chemistry only. M receiving 500 mg/kg bw/day showed a stat. significant increase in sodium, total protein and albumin levels. F receiving 500 mg/kg bw/day showed a dose-dependent trend towards increased gamma glutamyl transferase (GGT) levels lacking stat. significance. Levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, total bilirubin, creatinine, blood urea nitrogen, potassium, chloride, calcium and phosphorus were not affected			-
1	Systemic toxicity	Clinical signs	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	There were no stat. significant differences in incidence of clinical signs between control and treated groups of either sex in both non-recovery and recovery groups.	Test substance treatment had no effect on the expression of clinical signs, indicative of a low toxicity.		-
2	Systemic toxicity	Clinical signs	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	Observations of the general condition revealed a relatively high incidence of loose stools in 1 M in the 2000 mg/kg bw/day group. This sign was, however, considered not to be attributable to administration of the test substance, because loose stools were also observed at an equal frequency in naive beagle dogs and 1 M in the control group.			-
3	Systemic toxicity	Clinical signs	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	Loose and watery stools and vomiting were observed during the administration period, but there was no evidence to attribute their occurrence to			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										administration of the test substance.			
4	Systemic toxicity	Clinical signs	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	There were no test substance-related clinical observations.			-
5	Systemic toxicity	Clinical signs	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	Although stat. significance was noted for the incidences of clinical signs, no toxicological significance was conceived in these sporadic changes because of no dose dependency or decreasing wise of incidence.			-
6	Systemic toxicity	Clinical signs	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Clinical signs	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	No treatment related findings.			-
7	Systemic toxicity	Clinical signs	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
8	Systemic toxicity	Clinical signs	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	No treatment-related clinical signs were observed in any of the dose groups.			-
9	Systemic toxicity	Clinical signs	Rat	M: 10 F: 4	Days	Oral	-	mg/kg bw/day	No effect	-			-
10	Systemic toxicity	Clinical signs	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	-			-
11	Systemic toxicity	Clinical signs	Rat	4	Days	Oral	-	mg/kg bw/day	No effect	-			-
12	Systemic toxicity	Clinical signs	Rat	14	Days	Oral	-	mg/kg bw/day	No effect	-			-
13	Systemic toxicity	Clinical signs	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			-
14	Systemic toxicity	Clinical signs	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			-
15	Systemic toxicity	Clinical signs	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			-
1	Systemic toxicity	Food consumption	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-	Test substance treatment had no adverse effect on food consumption, indicative of a low toxicity.		-
2	Systemic toxicity	Food consumption	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Systemic toxicity	Food consumption	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Systemic toxicity	Food consumption	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
5	Systemic toxicity	Food consumption	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			-
6	Systemic toxicity	Food consumption	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Food consumption	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	In the 20000 ppm group, stat. significant increases in food consumption were observed for F0 parental F (1741 mg/kg bw/day) at Weeks 3, 4, and 6 and for F1 parental Fs (1817 mg/kg bw/day) at Weeks 2-10, on GD 14-20, and on LD 14-21			-
8	Systemic toxicity	Food consumption	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			-
14	Systemic toxicity	Food consumption	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			-
15	Systemic toxicity	Food consumption	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			-
1	Systemic toxicity	Mortality	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-	Test substance treatment had no effect on mortality, indicative of a low toxicity.		-
2	Systemic toxicity	Mortality	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
3	Systemic toxicity	Mortality	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
4	Systemic toxicity	Mortality	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			-
5	Systemic toxicity	Mortality	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			-
6	Systemic toxicity	Mortality	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Mortality	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Mortality	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
7	Systemic toxicity	Mortality	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			-
8	Systemic toxicity	Mortality	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			-

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
12	Systemic toxicity	Mortality	Rat	14	Days	Oral	-	mg/kg bw/day	No effect	1 F receiving 1000 mg/kg bw/day died due to a gavage error which was not related to the test substance.			-
13	Systemic toxicity	Mortality	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			-
14	Systemic toxicity	Mortality	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			-
15	Systemic toxicity	Mortality	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	1 F of the control group was sacrificed on Day 20 due to aborting. No abnormalities were observed in this animal. No other deaths or sacrifices before termination occurred.			-

↑: increase; ↓: decrease; abs.: absolute; AP: alkaline phosphatase; BUN: blood urea nitrogen; bw: body weight; Creat.: creatinine; F: female(s); F0: parental generation; F1: first filial generation; F2: second filial generation; GD: gestation day; GGT: Gamma-glutamyl transferase; Hb: haemoglobin; HCD: historical control data; Ht: haematocrit; K: potassium; LD: lactation day; M: male(s); MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; P: parental generation; ppm: parts per million; RBC: red blood cell count; rel.: relative; stat.: statistical(ly); TP: testosterone propionate; vs: versus; -: no data.

2.10.3.1.1 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Table 119: WoE for T-mediated adversity

• No treatment-related direct thyroid weight changes were observed in the following studies: o Repeated dose 90-day oral toxicity study in dogs (Study ID 2) o Repeated dose 1-year oral toxicity study in dogs (Study ID 3) o Combined chronic toxicity/carcinogenicity study in rats (Study ID 5) • In the peripubertal male and female assay (Study ID 13) males receiving 500 mg/kg bw/day, the highest dose level tested in this study, showed a statistically significant decrease in absolute thyroid weight. It is noted that all mean values were greater than the upper limit of the laboratory historical control data, including those of the concurrent control. Histopathology did not reveal any effect contributing to this finding. Females were unaffected.
• No thyroid histopathological changes were observed in the following studies: o Repeated dose 90-day oral toxicity study in rats (Study ID 1) o Repeated dose 90-day oral toxicity study in dogs (Study ID 2) o Repeated dose 1-year oral toxicity study in dogs (Study ID 3) o Repeated dose 21-day dermal toxicity study in rabbits (Study ID 4) o Combined chronic toxicity/carcinogenicity study in rats (Study ID 5) o Combined chronic toxicity/carcinogenicity study in mice (Study ID 6) o Peripubertal male and female assay (Study ID 13)

Stat.: statistical(ly); T: thyroid-modality; WoE: weight of evidence.

T-mediated adversity was evaluated examining thyroid histopathology and weight in the standard studies available for Benzobicyclon. T-mediated adversity with regard to mammals was not demonstrated for Benzobicyclon.
Pituitary weight and histopathology as parameters considered 'sensitive to, but not diagnostic of, EATS'-modalities did not show any direct adverse effects following treatment of rats, dogs, rabbits and mice with Benzobicyclon. An isolated histopathological finding in the pituitary of F0 and F1 males was considered to be a change plausibly associated with α2μ-globulin.

Table 120: WoE for T-related endocrine activity

• No treatment-related effects on T4 and TSH levels were observed *in vivo* in rats (Study ID 13). In this study, males and females receiving the vehicle control showed slightly elevated T4 levels compared to the historical control data (HCD). This effect was due to a change in feed containing a higher protein amount.
• No treatment-related effect on liver weight was observed in the Hershberger assay (Study ID 10).
• In the AMA with *Xenopus laevis* no treatment-related effects on survival, abnormalities or abnormal behaviour, developmental stage, wet weight, snout-to-vent length or normalised hind-limb length were observed. No treatment-related histological findings were associated with the exposure to Benzobicyclon (Study ID 24).
• In the Danish (Q)SAR Database TPO inhibition was predicted as negative (within the AD) by Leadscope.
• Predictions within the AD were obtained from the Danish (Q)SAR Database for AhR activation[a] and CAR inhibition[b]. All these predictions stated Benzobicyclon as negative.

AD. Applicability domain; AhR: Arylhydrocarbon receptor; AMA: amphibian metamorphosis assay; CAR: Constitutive androstane receptor; T4: thyroxine; TPO: thyroperoxidase; TSH: thyroid-stimulating hormone.
[a]: Effect addressed is in line with the ED GD (ECHA and EFSA, 2018) "other" in case of AhR.
[b]: Effect addressed is in line with Appendix E of the ED GD (ECHA and EFSA, 2018) "E, A, T, S" in case of CAR.

No concern was obtained for T-related endocrine activity based on specific *in vivo* hormone level measurements (T4 and TSH). Additionally, *in silico* prediction was negative with regard to potential T-related endocrine activity (TPO inhibition).

Overall, in a WoE approach the available *in vivo* and *in silico* information did not raise a concern for T-related endocrine activity.
This conclusion is supported by the results of an amphibian metamorphosis assay (AMA) according to OECD TG 231 (2009), *i.e.* a very sensitive assay which did not reveal T-related endocrine activity.

2.10.3.1.2 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

"*The initial analysis of the evidence comprises an assessment whether either EATS-mediated adversity or EATS endocrine activity has been sufficiently investigated*" (ECHA and EFSA, 2018) and assesses the observed effects in available toxicity studies.
A sufficiently large number of important T-mediated parameters were covered. The data requirements of the PPP Regulation were fulfilled and studies were generally carried out in accordance with current protocols.
T-mediated adversity was considered as sufficiently investigated (especially due to the availability of histopathological examinations and organ weight data of the thyroid) and revealed no indication for T-mediated adversity as a consequence of an endocrine MoA.
A reliable conclusion can be drawn for Benzobicyclon without generation of further information.
Overall, it can be concluded that based on sufficient investigation no T-mediated adversity was observed and therefore, "scenario 1a" applies to Benzobicyclon, the ED criteria are not met with regard to the T-modality (Table 121).

Table 121: Selection of relevant scenario

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "**T-mediated**" **adversity**	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no "**T-mediated endocrine activity**" observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.3.1.3 MoA analysis for T-modality

In accordance with the selected "scenario 1a" (Table 104), no MoA analysis is required for the T-modality.

2.10.3.1.4 Conclusion of the assessment of T-modality

In summary, T-mediated adversity was sufficiently investigated for Benzobicyclon and revealed overall no indication for T-mediated adversity as a consequence of an endocrine MoA. Thus, Appendix A does not need to be followed for further investigations according to the ED GD (ECHA and EFSA, 2018).

The preceding section of the present document indicates that the conclusion the "ED criteria for T-modality are not met" is applicable for Benzobicyclon ("scenario 1a"). No further investigations concerning the T-modality are required.

2.10.3.2 ED assessment for EAS-modalities

Table 122: Available studies investigating EAS-mediated parameters

	Sufficiently investigated
EAS-mediated parameters	Yes based on the availability of the following studies: – Repeated dose 90-day oral toxicity study in rats (Study ID 1) – Repeated dose 90-day oral toxicity study in dogs (Study ID 2) – Repeated dose 1-year oral toxicity study in dogs (Study ID 3) – Repeated dose 21-day dermal toxicity study in rabbits (Study ID 4) – Combined chronic toxicity/carcinogenicity study in rats (Study ID 5) – Combined chronic toxicity/carcinogenicity study in mice (Study ID 6) – Two-generation reproduction toxicity study in rats (Study ID 7) – Peripubertal male and female assay in rats (Study ID 13) – Prenatal developmental toxicity study in rabbits, DRF (Study ID 14) – Prenatal developmental toxicity study in rabbits (Study ID 15)

DRF: dose range-finding study.

It is noted that nearly all of the relevant EAS-mediated parameters have been assessed for Benzobicyclon. 'Coagulating gland weights' were investigated *e.g.* together with seminal vesicles (Study ID 13). Comparing the available dataset with Table 14 of the ED GD (ECHA and EFSA, 2018) 'anogenital distance', 'nipple development' and 'vaginal smear' are the only parameters which have not been explicitly mentioned in the available dataset. However, since 'estrus cyclicity' was evaluated, 'vaginal smear' is considered investigated. The analysis of the two remaining parameters, currently lacking in the available dataset, is considered not to modify the present assessment which relies on a comprehensive set of data and parameters with relevance for the EAS-modalities. In conclusion, the EAS-mediated adversity is considered to be sufficiently investigated for a reliable and scientifically sound assessment.
Furthermore, EAS-related endocrine activity is sufficiently investigated in line with the ED GD (ECHA and EFSA, 2018) based on the availability of OECD CF level 2 and 3 information such as a Hershberger assay (Study ID 10), an uterotrophic assay (Study ID 11), a steroidogenesis assay (Study ID 17) and an aromatase assay (Study ID 16). EAS-related endocrine activity is considered sufficiently investigated in line with the ED GD (ECHA and EFSA, 2018).

2.10.3.2.1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

The integrated lines of evidence for EAS-related endocrine activity and EAS-mediated adverse effects are reported in Table 123. The data comprise *in silico* predictions, *in vitro* and *in vivo* mechanistic data, data on organ weight, histopathological evaluations and reproduction and developmental parameters.
All parameters with regards to general or target organ toxicity are presented in the integrated lines of evidence table, as applicable.

Table 123: Assessment of the integrated lines of evidence for the EAS-modalities

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
N.a.	*In silico* prediction	(Q)SAR prediction: Danish (Q)SAR Database	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	No effect	Danish (Q)SAR database predicted the test substance negative within the applicability domain (AD) for constitutive androstane receptor (CAR) activation and arylhydrocarbon receptor (AhR) activation. Besides CERAPP and CoMPARA (see lines below), no predictions within the AD and therefore, no reliable information was available for AR, ER, pregnane × receptor (PXR) or CYP3A4.	Supporting negative evidence for EAS-related endocrine activity, due to the fact that CAR is considered to play a role in all four EATS-modalities (T-related endocrine activity was assessed in another table).	Supporting positive evidence for A-related endocrine activity and supporting negative evidence for E- and S-related endocrine activity (*in silico*). However, the reliability of this information is rather questionable since the underlying models are not validated and since no measurement data were generated.	E, A, T, S
N.a.	*In silico* prediction	(Q)SAR prediction: OECD QSAR Toolbox	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	No effect	The test substance was depicted as non-binder, but the proposed metabolites were stated as strong binder due to hydroxyl groups. This alert was not confirmed by the endpoint-specific profiler 'rtER expert system - US EPA' where parent alone and proposed metabolites caused no structural alert. Furthermore, mechanistic profiling alerts and especially hydroxyl groups are unspecific.	Supporting negative evidence for E-related endocrine activity.		E
N.a.	*In silico* prediction	(Q)SAR prediction: CERAPP	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	No effect	CERAPP Potency level (Consensus) predicted inactivity for ER agonism and antagonism and very weak activity for ER binding.	Although a very weak ER binding activity cannot be dismissed, supporting negative evidence for		E

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
											E-related endocrine activity (agonism and antagonism).		
N.a.	*In silico* prediction	(Q)SAR prediction: CoMPARA	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	Change	CoMPARA (Consensus) predicted inactivity for AR agonism and activity for AR antagonism and binding.	Supporting positive evidence for AR binding and antagonism. Supporting negative evidence for AR agonism.		A
16	*In vitro* mechanistic	CYP19	Human	15	Minutes	Uptake from the medium (*in vitro*)	-	µM	No effect	No notable inhibition of aromatase activity by the test substance was observed. Enzyme activity in the presence of the test substance ranged from 92.4 to 107 % of the control value.	Supporting negative evidence for S-related endocrine activity.	Overall, negative evidence for EAS-related endocrine activity *in vitro*	S
17	*In vitro* mechanistic	Estradiol synthesis	Human	48	Hours	Uptake from the medium (*in vitro*)	-	µM	No effect	In the first test, no stat. significant change in estradiol concentration was observed at any test substance concentration. In the second test, estradiol concentration was stat. significantly increased at a test substance concentration of 2 µM; however, the fold of estradiol increase did not reach the threshold of of ≥1.5 fold change for a positive result.	Supporting negative evidence for E-related endocrine activity.		E, A, S
17	*In vitro* mechanistic	Testosterone synthesis	Human	48	Hours	Uptake from the medium (*in vitro*)	0.633	µM	Decrease	In the first run statistically significant decrease (0.84 fold of control) of testosterone concentration was reportred at the highest (non-cytotoxic) dose tested of 2 µM. In a second experiment conducted with refined dose ranges a statististically sifgnificant dose related decrease of testosterone concentration was noted at 633 nM	Supporting negative evidence for T-related endocrine activity		E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										and 2 µM (and 0.91 fold and 0.81 fold of the solvent control, respectively). The results of both runs were considered negative as the statistically significant decreases of testosterone did not exceed the threshold for inhibition (≤0.5 fold vs control)			
10	*In vivo* mechanistic	Cowpers glands weight (Hershberger)	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	Phase 1: In contrast to the positive control TP, the test substance treatment did not show an androgenic effect. Phase 2: In contrast to the combination of TP and the positive control flutamide, the test substance treatment did not show an antiandrogenic effect.	No adverse effect on cowpers gland weight. Negative evidence for A-related endocrine activity.	Overall, negative evidence for EAS-related endocrine activity (*in vivo* mechanistic).	A
10	*In vivo* mechanistic	Glans penis weight (Hershberger)	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	Phase 1: In contrast to the positive control TP, the test substance treatment did not show an androgenic effect. Phase 2: In contrast to the combination of TP and the positive control flutamide, the test substance treatment did not show an antiandrogenic effect.	No adverse effect on glans penis weight. Negative evidence for A-related endocrine activity.		A
10	*In vivo* mechanistic	LABC weight (Hershberger)	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	Phase 1: In contrast to the positive control TP, the test substance treatment did not show an androgenic effect. Phase 2: In contrast to the combination of TP and the positive control flutamide, the test substance treatment did not show an antiandrogenic effect.	No adverse effect on LABC weight. Negative evidence for A-related endocrine activity.		A
10	*In vivo* mechanistic	Prostate weight (Hershberger)	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	Phase 1: In contrast to the positive control TP, the test substance treatment did not show an androgenic effect. Phase 2: In contrast to the combination of TP and the positive control flutamide, the test	No adverse effect on prostate weight. Negative evidence for A-related		A

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										substance treatment did not show an antiandrogenic effect.	endocrine activity.		
10	*In vivo* mechanistic	Seminal vesicles weight (Hershberger)	Rat	10	Days	Oral	-	mg/kg bw/day	No effect	Phase 1: In contrast to the positive control TP, the test substance treatment did not show an androgenic effect. Phase 2: In contrast to the combination of TP and the positive control flutamide, the test substance treatment did not show an antiandrogenic effect.	No adverse effect on seminal vesicles weight. Negative evidence for A-related endocrine activity.		A
13	*In vivo* mechanistic	Testosterone level	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on testosterone levels. Negative evidence for E-, A- and S-related endocrine activity.		E, A, S
11	*In vivo* mechanistic	Uterus weight (UT assay)	Rat	4	Days	Oral	-	mg/kg bw/day	No effect	In contrast to the positive control 17 alpha-ethinyl estradiol, the test substance did not affect the wet or the blotted uterus weight at any dose level tested and therefore it does not possess estrogenic activity.	No adverse effect on wet and blotted uterus weight. Negative evidence for E- and A-related endocrine activity.		E, A
6	EATS-mediated	Accessory sex organs histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on accessory sex organs was observed. Negative evidence for EAS-mediated adversity.	Besides some isolated findings from single studies as well as findings associated with male rat specific α2μ-globulin	E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	EATS-mediated	Age at balanopreputial separation	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the age at balanopreputial separation was observed. Negative evidence for EAS-mediated adversity.	nephropathy no consistent observations concerning EAS-mediated parameters were found. Additionally, it should be noted, that the most relevant and sensitive two-generation reproduction toxicity study (Study ID 7) revealed no effect on EAS-mediated parameters besides those in context of α2μ-globulin nephropathy. Overall, the results provide negative evidence for EAS-mediated adversity.	E, A, S
13	EATS-mediated	Age at balanopreputial separation	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	Age and body weight at preputial separation as well as age at partial preputial separation were assessed. M receiving 250 mg/kg bw/day showed a stat. significant increase in the age at partial preputial separation compared to the vehicle control. However, this value was within the historical control mean ± 2 SD range. Hence, and due to the lack of a dose dependency, this finding was considered not related to treatment.			E, A, S
7	EATS-mediated	Age at Vaginal opening	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the age at vaginal opening was observed. Negative evidence for EAS-mediated adversity.		E, A, S
13	EATS-mediated	Age at Vaginal opening	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	Age and body weight at vaginal opening and age at vaginal opening initiation were assessed. None of the parameters was affected.			E, A, S
1	EATS-mediated	Cervix histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on cervix was observed. Negative evidence for EAS-mediated adversity.		E, A, S
4	EATS-mediated	Cervix histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Cervix histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Cervix histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
1	EATS-mediated	Coagulating gland histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on coagulating gland was observed. Negative		E, A, S
5	EATS-mediated	Coagulating gland histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Coagulating gland histopathology	Rat	18-19 (P adult to	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S

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				weaning of F2 pups)							evidence for EAS-mediated adversity.		
4	EATS-mediated	Epididymis weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-	A single effect on the epididymides weight related to the male rat specific α2μ-globulin nephropathy was observed, lacking supportive findings in epididymides histopathology. Furthermore, no similar findings in other studies with rats, dogs, mice or rabbits. All in all results on epidiymides were considered to provide negative evidence for EAS-mediated adversity.		E, A, S
6	EATS-mediated	Epididymis weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Epididymis weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Epididymis weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	1324	mg/kg bw/day	Increase	F1 M at 20000 ppm (1324 mg/kg bw/day) showed group increased abs. (↑6.1 %; 694 vs. 736 mg) and rel. (↑10.2 %; 0.1150 vs. 0.1267) epididymis weight. Since it has been known that an increase in the blood α2μ-globulin level in male rats causes increases in blood LH and FSH levels, in testicular and blood levels, and in the testicular and accessory sex organ weights, increased testicular weight and epididymal weights observed in parental M at 20000 ppm were considered to be changes associated with α2μ-globulin.			E, A, S
13	EATS-mediated	Epididymis weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	left and right separately			E, A, S
1	EATS-mediated	Epididymis histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			E, A, S
2	EATS-mediated	Epididymis histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Epididymis histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Epididymis histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Epididymis histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Epididymis histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	EATS-mediated	Epididymis histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
13	EATS-mediated	Epididymis histopathology	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	left and right			E, A, S
7	EATS-mediated	Estrus cyclicity	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the estrus cyclicity was observed. Negative evidence for EAS-mediated adversity.		E, A, S
13	EATS-mediated	Estrus cyclicity	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	Age at first cycle and cycle length were assessed and the percentage of cycling and regularly cycling F were calculated. The test substance had no effect on these parameters.			E, A, S
7	EATS-mediated	Genital abnormalities	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No genital abnormalities were observed. Negative evidence for EAS-mediated adversity.		E, A, S
13	EATS-mediated	LABC weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-	No effect on levator ani plus bulbocavernosus (LABC) muscles weight was observed. Negative evidence for EAS-mediated adversity.		E, A, S
4	EATS-mediated	Mammary gland histopathology (male)	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-	No adverse effect on mammary gland		E, A, S
1	EATS-mediated	Mammary gland histopathology (female)	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
2	EATS-mediated	Mammary gland histopathology (female)	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-	histopathology was observed. Negative evidence for EAS-mediated adversity.		E, A, S
3	EATS-mediated	Mammary gland histopathology (female)	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Mammary gland histopathology (female)	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Mammary gland histopathology (female)	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	F at 1000 ppm (42.2 mg/kg bw/day) showed stat. significant increases in incidence of fibroadenoma of the mammary gland at terminal kill after Week 104 and in overall incidence compared with the control. However, they were considered to be incidental without toxicological significance because of no dose dependency. Moreover, the incidences of neoplastic lesions observed in the present study (i.e. including mammary fibroadenoma) were all within the historical control limits of the test facility.			E, A, S
6	EATS-mediated	Mammary gland histopathology (female)	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	Although mammary adenocarcinomata were reported in 4 of 50 F at 30000 ppm (4807 mg/kg bw/day) compared with 0 of 50 control F, this incidence was not statistically significant in one-tailed pairwise comparison against control group, and they were considered unlikely to be related to treatment. Moreover, this incidence was only just outside the control background data.			E, A, S
1	EATS-mediated	Ovary weight	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	No effects in the non-recovery group at any dose level. In the 100 and 10000 ppm (6.29 and 630 mg/kg bw/day) recovery groups (low and high dose group), F showed stat. significant increases in abs. and rel. weights of the ovaries: 100 ppm; abs. weight ↑ 120 %, rel. weight ↑ 117 %; 10000 ppm: abs	A single effect in ovary histopathology was observed in rats. However, the findings were lacking stat.		E, A, S

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										weight ↑ 118 %, rel. weight ↑ 117 %. Considered incidental due to alterations observed in only recovery group. Addtionally, no corresponding changes observed in the histopathological examination.	significance and were within the historical control range or not dose dependent. All other examinations of ovary weight or histopathology in mice, rats, rabbits or dogs did not reveal adverse effects. Overall, negative evidence for EAS-mediated adversity.		
2	EATS-mediated	Ovary weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Ovary weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Ovary weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Ovary weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Ovary weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Ovary weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Ovary weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	F1 F at 20000 ppm (1817 mg/kg bw/day) showed increased abs. (↑ 14.9 %; 52.9 vs. 60.8 mg) ovary weight. This change, however, was considered not to be due to test substance treatment because the rel. ovary weight for F1 F in the 20000 ppm group was comparable to that in the control group.			E, A, S
7	EATS-mediated	Ovary weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
13	EATS-mediated	Ovary weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	-			E, A, S
1	EATS-mediated	Ovary histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	Animals from the non-reccovery and the recovery group examined.			E, A, S
2	EATS-mediated	Ovary histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S

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2	EATS-mediated	Oviduct histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Ovary histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Ovary histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Ovary histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Ovary histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Ovary histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	At gross pathological examination, cysts in the ovary were observed sporadically for F0 and F1 parental F without any relation to dose levels. This change, however, was considered not to be related to test substance treatment because of the occurrence at low incidence.			E, A, S
13	EATS-mediated	Ovary histopathology	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	250	mg/kg bw/day	Change	left and right F receiving 250 or 500 mg/kg bw/day showed a dose related decrease in small, medium, antral and atretic follicles in the left ovary. However, these findings were lacking stat. significance and were within the historical control range. Additionally, a decrease in corpora lutea was found, but this effect was not dose dependent.			E, A, S
2	EATS-mediated	Prostate weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on prostate weight or histopathology was observed. Negative evidence for EAS-mediated adversity.		E, A, S
3	EATS-mediated	Prostate weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Prostate weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	Decrease in rel. prostate weight in M at 30000 ppm (3817 mg/kg bw/day), not stat. significant for unadjusted abs. prostate weight, but stat. significant for adjusted to terminal bw prostate weight. No stat. significant change for M up to 3000 ppm (373 mg/kg bw/day). No corresponding histopathological			E, A, S

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										change. Therefore, considered to be of no toxicological importance.			
7	EATS-mediated	Prostate weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
13	EATS-mediated	Prostate weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	ventral and dorsolateral prostate weight			E, A, S
1	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			E, A, S
2	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Prostate histopathology (with seminal vesicles and coagulating glands)	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Seminal vesicles weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	A single effect on seminal vesicle weight was observed in rats. However, the effect was only apparent in the seminal vesicle weight containing the fluids but not		E, A, S
13	EATS-mediated	Seminal vesicles weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	500	mg/kg bw/day	Decrease	with coagulating gland, weighed with and without fluids M receiving 500 mg/kg bw/day showed a stat. significant decrease in abs. seminal vesicle weight including fluids. This effect was not apparent in seminal vesicle weight without fluids.			E, A, S

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1	EATS-mediated	Seminal vesicles histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-	in the weight without fluids. In this study no histopathology on the seminal vesicles was performed to support the findings. Additionally, the remaining studies did not provide supportive findings for adverse effects on seminal vesicle weight and histopathology. Overall, negative evidence for EAS-mediated adversity.		E, A, S
4	EATS-mediated	Seminal vesicles histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Seminal vesicles histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Seminal vesicles histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Seminal vesicles histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Sperm morphology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	Although percent normal morphology of epididymal sperm in F0 M in the 1000 (56.1 mg/kg bw/day) and 20000 ppm (1176 mg/kg bw/day) groups were stat. significantly increased, these changes were considered not to be toxicologically significant.	No adverse effect on sperm parameters was observed. Negative evidence for EAS-mediated adversity.		E, A, S
7	EATS-mediated	Sperm morphology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S

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7	EATS-mediated	Sperm motility	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Sperm numbers	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
1	EATS-mediated	Testis weight	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	In the 20 ppm (1.13 mg/kg bw/day) group of the recovery group, M showed stat. significant increased rel. testes weight. High dose group (400 ppm) showed no corresponding change.	A single effect on the testis weight related to the male rat specific α2µ-globulin nephropathy was observed, lacking corresponding findings in testis histopathology. Furthermore, no similar findings in other studies with rats, dogs, mice or rabbits. All in all results on testis were considered to provide negative evidence for EAS-mediated adversity.		E, A, S
2	EATS-mediated	Testis weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Testis weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Testis weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Testis weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Testis weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Testis weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Testis weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	1324	mg/kg bw/day	Increase	F1 M at 20000 ppm (1324 mg/kg bw/day) showed increased abs. (↑ 8.4 %; 1756 vs. 1903 mg) and rel. (↑ 12.3 %; 0.292 vs. 0.328) testes weight. Since it has been known that an increase in the blood α2µ-globulin level in male rats causes increases in blood LH and FSH levels, in testicular and blood levels, and in the testicular and accessory sex organ weights, increased testicular weight and epididymal weights observed in parental M at 20000			E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										ppm were considered to be changes associated with α2μ-globulin.			
13	EATS-mediated	Testis weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	left and right separately			E, A, S
1	EATS-mediated	Testis histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			E, A, S
2	EATS-mediated	Testis histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Testis histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Testis histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Testis histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Testis histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Testis histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	At gross pathological examination, softening and small in size of the testis were observed sporadically for F0 and F1 parental M without any relation to dose levels. This change, however, was considered not to be related to test substance treatment because of the occurrence at low incidence.			E, A, S
13	EATS-mediated	Testis histopathology	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	left and right			E, A, S
2	EATS-mediated	Uterus weight (with cervix)	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on uterus weight or uterus histopathology, both including the cervix, was observed. Negative evidence for EAS-		E, A, S
3	EATS-mediated	Uterus weight (with cervix)	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Uterus weight (with cervix)	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Uterus weight (with cervix)	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	Higher uterus weight (rel. to terminal bw), associated with thickening noted at post mortem, were recorded for F at 30000 ppm (4807 mg/kg bw/day), however, no statistical significance was attained.			E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	EATS-mediated	Uterus weight (with cervix)	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	mediated adversity.		E, A, S
13	EATS-mediated	Uterus weight (with cervix)	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	with and without fluid			E, A, S
14	EATS-mediated	Uterus weight (with cervix)	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	gravid uterus weights			E, A, S
15	EATS-mediated	Uterus weight (with cervix)	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	gravid uterus weights			E, A, S
1	EATS-mediated	Uterus histopathology (with cervix)	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			E, A, S
2	EATS-mediated	Uterus histopathology (with cervix)	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Uterus histopathology (with cervix)	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Uterus histopathology (with cervix)	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Uterus histopathology (with cervix)	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	In F at 1000 ppm (42.2 mg/kg bw/day) a decrease in endometrial stromal polyp in animals found dead or killed in extremis during the treatment was observed. Overall indicence showed no stat. significant change to control.			E, A, S
6	EATS-mediated	Uterus histopathology (with cervix)	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	There was no microscopic finding which could be associated with the greater number of F of all treated groups that showed thickening of the uterus, compared with terminal control F.			E, A, S
7	EATS-mediated	Uterus histopathology (with cervix)	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	At gross pathological examination, a mass in the uterus was observed sporadically for F0 and F1 parental F without any relation to dose levels. This change, however, was considered not to be related to test substance treatment because of the occurrence at low incidence.			E, A, S
13	EATS-mediated	Uterus histopathology (with cervix)	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	-	mg/kg bw/day	No effect	without fluid			E, A, S

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
1	EATS-mediated	Vagina histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on vagina histopathology was observed. Negative evidence for EAS-mediated adversity.		E, A, S
2	EATS-mediated	Vagina histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
3	EATS-mediated	Vagina histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
4	EATS-mediated	Vagina histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			E, A, S
5	EATS-mediated	Vagina histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	-			E, A, S
6	EATS-mediated	Vagina histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
7	EATS-mediated	Vagina histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			E, A, S
1	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	No stat. significant changes in rel. or abs. weight in the non-recovery and in the recovery group.	Isolated findings on adrenal weights without a clear consistent pattern and in the absence of corresponding histopathological findings. Overall, Benzobicyclon is considered not to have a direct effect on adrenals. Hence, supporting negative evidence for EATS-mediated adversity.	Overall, supporting negative evidence for EATS-mediated adversity.	N
2	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
3	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
4	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			N
5	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	Abs. adrenals weight was stat. significantly increased in M at 100 ppm (3.43 mg/kg bw/day) at interim kill after 78 weeks and rel. adrenals weight was increased in M at 10 ppm at interim kill after 78 weeks. Abs. and rel. adrenals weights in the 50 ppm (1.696 mg/kg bw/day) M group were increased at Week			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										104 (terminal kill, main group) but considered to be not related to treatment. No corresponding change was observed in the high dose group at terminal kill. Additionally, no particular abnormalities were detected in other parameters corresponding to this change.			
6	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	F0 F at 20000 ppm (1741 mg/kg bw/day) showed increased abs. (↑ 20.8 %; 33.6 vs. 40.6 mg) and rel. (↑ 17 %; 0.01091 vs. 0.01277) adrenals weight. No stat. significant effect in M. However, no corresponding change in the histopathology and thus, the changes in the weights were considered not to be treament related.			N
7	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	F1 F at 20000 ppm (1817 mg/kg bw/day) showed increased abs. (↑ 14.9 %; 35.6 vs. 40.9 mg) adrenals weight, not stat. significant in rel. weight. No stat. significant effect in M. However, no corresponding change in the histopathology and thus, the changes in the weights were considered not to be treament related.			N
7	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
13	Sensitive to, but not diagnostic of, EATS	Adrenals weight	Rat	M: 31 or 32 F: 21 or 22	Days	Oral	500	mg/kg bw/day	Increase	M receiving 500 mg/kg bw/day showed stat. significantly increased rel. adrenal glands weight,			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										exhibiting a corresponding stat. dose response.			
1	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	-			N
2	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
3	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
4	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			N
5	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	M at 10 ppm (0.334 mg/kg bw/day) showed a stat. significant increase in incidence of adrenal hyperplasia, medullary cell in animals found dead or killed in extremis and in overall incidence. However, these findings were considered to be incidental without any toxicological significance because of no dose dependency.			N
6	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	Adrenals from all F0 and F1 F were examined histopathologically due to treatment related increases in weight observed in this organ.			N
1	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	90	Days	Oral	-	mg/kg bw/day	No effect	No stat. significant changes in rel. or abs. weight in the non-recovery and in the recovery group.	No adverse effect on brain weight was observed.		N
2	Sensitive to, but not	Brain weight	Dog	13	Weeks	Oral	-	mg/kg bw/day	No effect	-	Supporting negative		N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	diagnostic of, EATS										evidence for EATS-mediated adversity.		
3	Sensitive to, but not diagnostic of, EATS	Brain weight	Dog	52	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
4	Sensitive to, but not diagnostic of, EATS	Brain weight	Rabbit	21 (6h per day)	Days	Dermal	-	mg/kg bw/day	No effect	-			N
5	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	24	Months	Oral	-	mg/kg bw/day	No effect	In the main group: stat. significantly decreased abs. brain weight in F at 100 ppm (4.19 mg/kg bw/day) after 104 weeks of treatment. In the satellite group: Stat. significantly increased abs. brain weight in M at 20 ppm (0.667 mg/kg bw/day) after 52 weeks and in M at 100 ppm (3.43 mg/kg bw/day) after 78 weeks. In F at 10000 (427 mg/kg bw/day) stat. significantly increased brain weight after 87 weeks of treatment. For this change, no relationship with the treatment was considered because no particular abnormalities were detected in other parameters corresponding to this change.			N
6	Sensitive to, but not diagnostic of, EATS	Brain weight	Mouse	78	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	Sensitive to, but not diagnostic of, EATS	Dystocia	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No dystocia was observed. Supporting negative evidence for EATS-mediated adversity.		N
7	Sensitive to, but not diagnostic of, EATS	Fertility (mammals)	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on fertility was observed. Supporting negative evidence for EATS-mediated adversity.		N
8	Sensitive to, but not diagnostic of, EATS	Fetal development	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on fetal development/ fetal weight was observed. Supporting negative evidence for EATS-mediated adversity.		N
14	Sensitive to, but not diagnostic of, EATS	Fetal development	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Fetal weights were unaffected.			N
15	Sensitive to, but not diagnostic of, EATS	Fetal development	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Litter or fetal weight were unaffected by the treatment.			N
7	Sensitive to, but not diagnostic of, EATS	Gestation length	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on gestation length was observed. Supporting negative evidence for EATS-mediated adversity.		N
8	Sensitive to, but not diagnostic of, EATS	Gestation length	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
7	Sensitive to, but not diagnostic of, EATS	Litter size	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on litter size was observed. Supporting negative evidence for EATS-mediated adversity.		N
8	Sensitive to, but not diagnostic of, EATS	Litter size	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Litter viability	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on litter viability was observed. Supporting negative evidence for EATS-mediated adversity.		N
7	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on litter/pup weight was observed. Supporting negative evidence for EATS-mediated adversity.		N
7	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-			N
8	Sensitive to, but not diagnostic of, EATS	Litter/pup weight	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the number of implantations and/or corpora lutea was observed. Supporting		N
8	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
14	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Numbers of corpora lutea and implantations were assessed.	negative evidence for EATS-mediated adversity.		N
15	Sensitive to, but not diagnostic of, EATS	Number of implantations, corpora lutea	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Number of live births	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the number of live births was observed. Supporting negative evidence for EATS-mediated adversity.		N
8	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-	No effect on the numbers of embryonic or fetal deaths and viable fetuses was observed. Supporting negative evidence for EATS-mediated adversity.		N
14	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Numbers of early and late deaths and of live and dead fetuses were assessed.			N
15	Sensitive to, but not diagnostic of, EATS	Numbers of embryonic or foetal deaths and viable foetuses	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Numbers of live fetuses were unaffected.			N
8	Sensitive to, but not diagnostic of, EATS	Post implantation loss	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-	No effect on post-implantation loss was observed. Supporting negative evidence for EATS-		N
14	Sensitive to, but not diagnostic of, EATS	Post implantation loss	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	F receiving 1000 mg/kg bw/day showed a slight increase in the number of early intra-uterine deaths resulting in a higher mean incidence of post-implantation loss. However, this effect was mainly			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										due to a single F having 4 intra-uterine deaths, and, due to the fact that the number was within historical control data range, considered to be a result of the small group size and not an adverse effect of the test substance.	mediated adversity.		
15	Sensitive to, but not diagnostic of, EATS	Post implantation loss	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			N
8	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-	No effect on pre implantation loss was observed. Supporting negative evidence for EATS-mediated adversity.		N
14	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			N
15	Sensitive to, but not diagnostic of, EATS	Pre implantation loss	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			N
7	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No effect on the presence of anomalies was observed. Supporting negative evidence for EATS-mediated adversity.		N
8	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-			N
14	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	external and skeletal The minor and variant skeletal fetal abnormalities seen were within historical control data ranges.			N
15	Sensitive to, but not diagnostic of, EATS	Presence of anomalies (external, visceral, skeletal	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	external visceral and skeletal anomalies were assessed The nature, incidence and intergroup distribution of the major fetal abnormalities did not indicate an adverse effect of the test substance. Furthermore, there was			N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
										no effect of the test substance on the incidence of minor or variant fetal abnormalities. The numbers of fetuses and litters affected were within known background data ranges.			
7	Sensitive to, but not diagnostic of, EATS	Pup development	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on pup development was observed. Supporting negative evidence for EATS-mediated adversity.		N
7	Sensitive to, but not diagnostic of, EATS	Pup survival index	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the pup survival index was observed. Supporting negative evidence for EATS-mediated adversity.		N
14	Sensitive to, but not diagnostic of, EATS	Reproduction	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Placental weights were unaffected.	No adverse effects on placental weight was observed. Supporting negative evidence for EATS-mediated adversity.		N
15	Sensitive to, but not diagnostic of, EATS	Reproduction	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Placenta weights were similar between the groups.			N
7	Sensitive to, but not diagnostic of, EATS	Sex ratio	Rat	18-19 (P adult to weaning of F2 pups)	Weeks	Oral	-	mg/kg bw/day	No effect	-	No adverse effect on the pup sex ratio was observed.		N

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
8	Sensitive to, but not diagnostic of, EATS	Sex ratio	Rat	10 (GD 6 to 15)	Days	Oral	-	mg/kg bw/day	No effect	-	Supporting negative evidence for EATS-mediated adversity.		N
14	Sensitive to, but not diagnostic of, EATS	Sex ratio	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	Due to the small group sizes, some apparent differences were present but mean fetal sex ratio was unaffected at all dose levels.			N
15	Sensitive to, but not diagnostic of, EATS	Sex ratio	Rabbit	22 (GD6-GD27)	Days	Oral	-	mg/kg bw/day	No effect	-			N

A: androgen; abs: absolute; AD: appicability domain; AhR: arylhydrocarbon receptor; AR: androgen receptor; bw: body weight; CAR: constitutive androstane receptor; CERAPP: Collaborative Estrogen Receptor Activity Prediction Project; CoMPARA: Collaborative Modeling Project for Androgen Receptor Activity; CYP: cytochrome P450 monooxygenase; E: estrogen; EATS: estrogen, androgen, thyroid and steroidogenesis; EPA: Environmental Protection Agency; ER: estrogen receptor; F: female(s); F0: parental generation; F1: first filial generation; F2: second filial generation; FSH: follicle stimulating hormone; GD: gestation day; LABC: levator ani plus bulbocavernosus muscles; LH: luteinising hormone; M: male(s); N: sensitive to, but not diagnostic of, EATS; N.a.: not applicable / not available; OECD: Organisation for Economic Co-operation and Development; P: parental generation; ppm: parts per million; PXR: pregnane × receptor; (Q)SAR: (quantitative) structure-activity relationship; rel.: relative; rtER: rainbow trout estrogen receptor; S: steroidogenesis; stat.: statistical(ly); T: thyroid; TP: testosterone propionate; vs: versus; -: no data.

2.10.3.2.2 Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

Table 124: WoE for EAS-mediated adversity

• No adversity towards the following EAS-mediated parameters was observed in any of the examined species (rat, dog, rabbit and/or mouse): Accessory sex organs histopathology, age at balanopreputial separation, age at vaginal opening, cervix histopathology, coagulating gland histopathology, epididymis histopathology, estrus cyclicity, genital abnormalities, LABC weight, mammary gland histopathology (male), mammary gland histopathology (female), ovary weight, prostate weight and histopathology, seminal vesicles histopathology, sperm morphology, sperm motility and sperm number, testis histopathology, (gravid) uterus weight and uterus histopathology, vagina histopathology.
• In the two-generation reproduction toxicity study (Study ID 7) F1 males treated with 1324 mg/kg bw/day, highest dose tested in this study, showed an increased absolute epididymis weight by 6.1 % and an increased relative to body weight testis weight by 10.2 %. Since it has been known that an increase in the blood α2μ-globulin level in male rats causes increases in blood LH and FSH levels, in testicular and blood levels, and in the testicular and accessory sex organ weights, increased epididymal weights observed in parental M at 20000 ppm were considered to be changes associated with α2μ-globulin. • Furthermore, no other study showed an effect on epididymis weight by Benzobicyclon and no change was observed in the histopathological examination of epididymis across the investigated species rat, mouse, rabbit and dog at dose levels up 3817 mg/kg bw/day or when treated up 24 months. • Overall, Benzobicyclon is considered not to have a direct effect on epididymis.
• In the peripubertal male and female assay (Study ID 13) females treated with 250 and 500 mg/kg bw/day showed a dose-related decrease in small, medium, antral and atretic follicles in the histopathological examination of the left ovary. However, these findings were lacking statistical significance and were within the historical control range. Additionally, a decrease in corpora lutea was found, but this effect was not dose-dependent. It is also noted that the relative proportion of the different follicle size were similar to that of the control. • Furthermore, no study showed an effect on ovary weight and no other study an effect in the histopathological examination of the ovary across the investigated species rat, mouse, rabbit and dog at dose levels up to 4807 mg/kg bw/day or when treated up to 24 months, or reproductive and fertility effects up to dietary the limit dose. • Overall, Benzobicyclon is considered not to have a direct effect on ovaries.
• In the peripubertal male and female assay (Study ID 13) males treated with 500 mg/kg bw/day, highest dose tested in this study, showed a statistically significant decrease (-2%) in absolute seminal vesicles weight, when including fluids. Without fluids no effect on the seminal vesicles weight was apparent. • Furthermore, no other study showed an effect on seminal vesicles weight by Benzobicyclon and no change was observed in the histopathological examination of seminal vesicles across the investigated species rat, mouse, and rabbit at dose levels up to 3817 mg/kg bw/day or when treated up to 24 months. • Overall, Benzobicyclon is considered not to have a direct effect on seminal vesicles.
• In the two-generation reproduction toxicity study (Study ID 7) F1 males treated with 1324 mg/kg bw/day, highest dose tested in this study, showed an increased absolute testis weight by 8.4 % and an increased relative to body weight testis weight by 12.3 %. Since it has been known that an increase in the blood α2μ-globulin level in male rats causes increases in blood LH and FSH levels, in testicular and blood levels, and in the testicular and accessory sex organ weights, increased testicular weight observed in parental M at 20000 ppm were considered to be changes associated with α2μ-globulin. • Furthermore, no other study showed an effect on testis weight by Benzobicyclon and no change was observed in the histopathological examination of testis across the investigated species rat, mouse, rabbit and dog at dose levels up to 3817 mg/kg bw/day or when treated up to 24 months. • Overall, Benzobicyclon is considered not to have a direct effect on testis.

EAS: estrogen, androgen, steroidogenesis; F1: first filial generation; FSH: follicle-stimulating hormone; LABC: levator ani-bulbocavernosus; LH: luteinising hormone; WoE: weight of evidence.

Besides the putative EAS-mediated findings described in Table 107 there were some isolated findings which were - due to the lack of corresponding changes or since the increase ncidences observed in the study were within -the historical control limits of the test facility - considered not to be of toxicological significance and were not further considered in the current ED assessment. These findings include an increased absolute and relative ovary weights in the recovery group without corresponding changes in the histopathology and no ovary weight changes in the non-recovery group in a repeated dose 90-day oral toxicity study in rats (Study ID 1), increased

absolute ovary weight without corresponding histopathological change or change in the relative ovary weight in a two-generation reproduction toxicity study in rats (Study ID 7), increased incidence of fibroadenoma of the mammary gland without dose dependency in a combined chronic toxicity/carcinogenicity study in rats (Study ID 5) or decreased adjusted absolute and relative prostate weight without corresponding changes in the histopathology in a combined chronic toxicity/carcinogenicity study in mice (Study ID 6).
None of the few findings were considered to be indicative for a direct effect of the test substance Benzobicyclon on the endocrine system, but secondary to systemic toxicity *e.g.* associated with α2μ-globulin. The few and isolated observations revealed generally no consistent or coherent pattern within the organism or across studies and species as isolated cases occurred in one study and species but not in others. Furthermore, it has especially to be considered that the most relevant and sensitive OECD CF level 5 two-generation reproduction toxicity study (Study ID 7) showed no effect on EA(T)S-mediated parameters except increased epididymis and testis weight in males of the highest dose group, which were considered to be changes associated with α2μ-globulin. For more information on this topic see Chapter 2.10.3.2.1.4.
With regard to the isolated findings observed in the peripubertal male and female assay (Study ID 13) it should be noted that according to the ED GD (ECHA and EFSA, 2018) the "*the limitations of these assays, noticed during their validation, are that no chemical was shown to be completely negative in the assay, and that it does not detect specific aromatase inhibitors. The sensitivity of the assays for ER/AR agonists and antagonists is less than that of the uterotrophic and Hershberger assays."* In the Hershberger and uterotrophic assay (Study IDs 10 and 11) Benzobicyclon did not show estrogenic, androgenic or anti-androgenic activity.
According to the ED GD (ECHA and EFSA, 2018) adversity observed with regard to the parameters considered sensitive to, but not diagnostic of, EATS-modalities "*is not likely to be caused by alterations of the EATS modalities*". Since EAS-mediated adversity was considered sufficiently investigated for a reliable and scientifically sound assessment and no consistent and coherent pattern of EAS-mediated adversity was observed, findings on parameters sensitive to, but not diagnostic of, EATS-modalities cannot be considered diagnostic on their own of any one of the EATS-modalities and thus, such findings will not raise concern towards EATS-mediated adversity. However, in case of Benzobicyclon only two isolated putative EATS-sensitive findings such as adrenal weight changes (Study ID 13) and a histopathological finding in the pituitary (Study ID 7) were observed and thus, no concern was raised. In line with the ED GD (ECHA and EFSA, 2018), all available parameters are depicted in Appendix E and in the lines of evidence.
Overall, in a WoE assessment Benzobicyclon is considered to cause no EAS-mediated adversity.

Table 125: WoE for EAS-related endocrine activity

• In the Hershberger assay (Study ID 10) treatment with Benzobicyclon did not show an androgenic or antiandrogenic effect in castrated male rats. No weight change on androgen-dependent tissues (seminal vesicle, ventral prostate, LABC muscle, Cowper's glands and the glans penis) was observed.
• In the uterotrophic assay (Study ID 11) treatment with Benzobicyclon did not cause an estrogenic effect in ovariectomized female rats. No change was observed on wet and blotted uterus weight.
• In the peripubertal male and female assay (Study ID 13) no effect on testosterone levels was observed.
• In the aromatase inhibition assay (Study ID 16) no notable inhibition of aromatase activity was observed. Enzyme activity in the presence of the test substance ranged from 92.4 to 107 % of the control value.
• In the in vitro steroidogenesis assay conducted in human H295R cells (Study ID 17) no relevant estradiol and testosterone synthesis alterations were observed.
• CERAPP and CoMPARA predictions were available for Benzobicyclon and it was stated as inactive with regard to AR and ER agonism and ER antagonism and active regarding AR antagonism and AR and ER binding. However, ER binding was categorized as very weak.
• Besides CERAPP and CoMPARA, predictions within the AD were obtained from the Danish (Q)SAR Database for AhR activation[a] and CAR inhibition[b]. All these predictions stated Benzobicyclon as negative.
• Benzobicyclon was depicted as ER non-binder by the OECD QSAR Toolbox v4.2 profilers, but the proposed metabolites were stated as strong binder due to hydroxyl groups. This alert was not confirmed by the endpoint-specific profiler 'rtER expert system - US EPA' where parent alone and proposed metabolites caused no structural alert. Furthermore, mechanistic profiling alerts and especially hydroxyl groups are unspecific.

AD. Applicability domain; AhR: Arylhydrocarbon receptor; AR: androgen receptor; CAR: Constitutive androstane receptor; CERAPP: Collaborative Estrogen Receptor Activity Prediction Project; CoMPARA: Collaborative modelling project for androgen receptor activity; EAS: estrogen, androgen, steroidogenesis; ER: estrogen receptor; LABC: levator ani-bulbocavernosus; rtER: rainbow trout estrogen receptor; WoE: weight of evidence.
[a]: Effect addressed is in line with the ED GD (ECHA and EFSA, 2018) "other" in case of AhR.
[b]: Effect addressed is in line with Appendix E of the ED GD (ECHA and EFSA, 2018) "E, A, T, S" in case of CAR.

The available *in silico, in vitro* and *in vivo* mechanistic data provided negative as well as partial positive evidence for EAS-related endocrine activity. Positive evidence was obtained by OECD CF level 1 and 2 information such as the CoMPARA prediction (AR antagonism and binding). However, i*n vivo* no indication of positive evidence was obtained from the OECD CF level 3 information such as the Hershberger assay or testosterone level measurements, in the Uterotrophic assay and in an OECD CF level 4 study (peripubertal male and female assay, Study ID 13).
It should be noted that the *in silico* predictions were based on unvalidated models and thus, the reliability of this information is rather questionable.
Overall, in a WoE assessment no concern was raised towards EAS-related endocrine activity by Benzobicyclon.

2.10.3.2.3 Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

"*The initial analysis of the evidence comprises an assessment whether either EATS-mediated adversity or EATS endocrine activity has been sufficiently investigated*" (ECHA and EFSA, 2018) and assesses the observed effects in available toxicity studies.
A sufficiently large number of important EAS-mediated parameters were covered for a reliable assessment. The data requirements of the PPP Regulation were fulfilled and studies were generally carried out in accordance with current protocols.
EAS-mediated adversity was considered sufficiently investigated for a reliable and scientifically sound assessment and EAS-related endocrine activity was considered sufficiently investigated in line with the ED GD (ECHA and EFSA, 2018). Therefore, the conduct of further investigations is not triggered.
A reliable conclusion can be drawn for Benzobicyclon without generation of further information.
Since no EAS-mediated adversity as a consequence of an endocrine MoA was observed and since EAS-mediated adversity is considered sufficiently investigated for a reliable assessment, "scenario 1a" applies to Benzobicyclon, the ED criteria are not met with regard to the EAS-modalities (Table 126).

Table 126: Selection of relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated*)	Yes/No	1a	Conclude: ED criteria not met because there is no "**EAS-mediated**" adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no "**EAS-mediated endocrine activity**" observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

* Note by the Applicant: for a reliable and scientifically sound assessment

2.10.3.2.4 MoA analysis for EAS-modalities

In accordance with the selected "scenario 1a" (Table 126), no MoA analysis is required.
However, since putative EAS-mediated findings such as increased testis and epididymis weights were considered to be secondary to species-specific α2μ-globulin nephropathy, the relevant findings which were observed in the available dataset of Benzobicyclon are put into this context in the following.

The findings described below which are referring to general adversity and target organ toxicity indicate that Benzobicyclon is likely to cause an increased binding to α2μ-globulin, an urinary protein of male rodents (Ghosh, P.K. *et al.,* 1991), and subsequently an increased accumulation of α2μ-globulin in the proximal tubular epithelium of male rats. As a consequence, kidney function is affected by this MoA. Furthermore, although α2μ-globulin is not synthesized in the pituitary, presence of α2μ-globulin ("*presumably of circulatory origin*") in certain anterior pituitary cells was demonstrated and "*suggests that it may play a role in anterior pituitary function*" (Antakly, T. *et al*., 1983).
With regard to the available dataset of Benzobicyclon, relevant findings were observed especially in the two-generation reproduction toxicity study (Study ID 7), where the histopathological examination revealed increased hydropic degeneration cells (basophilic cells) in the pituitary of F_0 and F_1 male rats of the high dose group. Furthermore, increased hyaline droplet degeneration in the proximal tubular cells was observed in the kidney from parental males in all treated groups which was assessed as due to deposition of α2μ-globulin. This male rat specific effect is considered to be associated with pale in colour and enlargement of the kidney, increases in kidney weights, and tubular basophilic change and granular casts in the dilated tubules. The latter findings were observed in parental males in the mid and high dose groups in this two-generation reproduction toxicity study.
In addition to the two-generation reproduction toxicity study (Study ID 7), the α2μ-globulin nephropathy was also observed in males in the repeated dose 90-day oral toxicity study in rats (Study ID 1) and the combined chronic toxicity/carcinogenicity study in rats (Study ID 5).
The scientific literature was searched in order to provide a major description of the postulated MoA*, i.e.* effects in testes and epididymides of male rats resulting from test substance-related α2μ-globulin nephropathy. The relevant obtained information is presented in the following and Figure 4 illustrates the suggested relationship between the α2μ-globulin nephropathy and subsequent effects on testis and epididymis which were partly observed at high doses in the available dataset of Benzobicyclon (MoA).

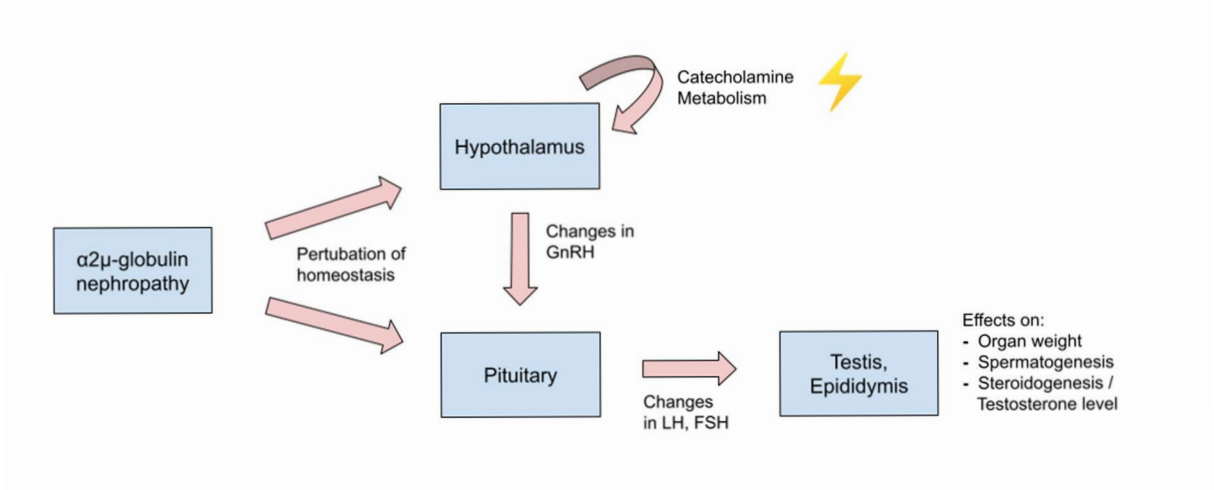


Figure 4: Illustration of the relationship between the α2μ-globulin nephropathy and effects on testis and epididymis based on Ghosh, P.K. *et al.*, 1991; Biswas, N.M. *et al.,* 1983; Mandal, H. *et al.*, 1990

FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; LH: luteinising hormone; ⚡: perturbation.

In order to investigate potential effects of α2μ-globulin adult male rats were treated for 2 weeks with 2 daily injections of 0.75 mg α2μ-globulin (Ghosh, P.K. *et al.*, 1991). This treatment led to "*an increase in plasma luteinizing hormone* [LH] *levels, a decrease in plasma prolactin levels and increase in testosterone concentrations in both plasma and testicular tissue, and increases in testicular weight*" (Ghosh, P.K. *et al.*, 1991). The data suggested that "*α2μ-globulin induced changes in gonadotropin and prolactin secretion are mediated by changes in catecholamine metabolism in several hypothalamic regions. Increased testosterone secretion appears to be due to increased secretion of gonadotropins from the pituitary*" (Ghosh, P.K. *et al.*, 1991). With regard to the catecholamine metabolism dopamine (DA) and norepinephrine (NE) turnover in different brain areas such as median eminence (ME), medial basal hypothalamus (MBH) and anterior hypothalamus (AH) were investigated. As a result of the α2μ-globulin treatment, NE turnover was statistically significantly elevated in ME and AH and statistically significantly reduced in the MBH, whereas the DA turnover was statistically significantly elevated in the MBH, statistically significantly reduced in the AH and not affected in the ME. Steady state levels of NE and

DA were not affected after α2μ-globulin treatment. It was suggested that "*the increase in plasma LH and FSH levels in α2μ-globulin-treated animals was due to NE-induced stimulation of the release of LH-RH from the hypothalamus*" (Ghosh, P.K. *et al.*, 1991). With regard to the influence of DA turnover, the authors indicated that "*the elevation of plasma FSH and LH following α2μ-globulin administration may be related to an increased DA turnover in the MBH"* (Ghosh, P.K. *et al.*, 1991). A study in mice which were treated with α2μ-globulin showed similar effects on the hypothalamo-pituitary-testicular axis (Ghosh, P.K. *et al.,* 1990).
In another study investigating the "effect of continual light deprivation and alpha-2u-globulin replacement therapy on serum concentration of gonadotropins and testicular activity in rats" (Ghosh, P.K. et al., 1996) treatment of rats with α2μ-globulin "after 68 days of light deprivation reversed the 17β-HSD activity and serum levels of gonadotropins, testosterone and α2μ-globulin, while spermatogenesis was restored to normal" (Ghosh, P.K. et al., 1996) after the enzyme activity, the respective hormone serum levels and the spermatogenesis was reduced due to prolonged darkness. The authors suggested that based on the observations described above "α2μ-globulin administration in rats increases DA turnover in medial basal hypothalamus (Ghosh et al., 1991) […] the significant rise of FSH and LH following α2μ-globulin replacement in dark-exposed rats may be due to increase DA turnover which stimulates hypothalamic LHRH release and thus gonadotropins secretion" (Ghosh, P.K. et al., 1996).
A further explanation of the increased FSH and LH secretion by the pituitary was discussed in the context of estrogen-treated rats (Biswas, N.M. *et al.,* 1983). At that time "*recent observations (Ghosh, Neuhaus & Biswas, 1981) show*[ed] *that α2μ-globulin prevents adrenal hypertrophy caused by excess adrenocorticotrophic hormone (ACTH) (Vogt, 1955) by stimulating the synthesis of corticosteroids in oestrogen-treated rats. Since the continued high levels of ACTH stimulate additional production of adrenocortical steroid hormones other than glucocorticoids (Gomes, 1970) and the steroid hormones by negative feedback action decrease gonadotrophin synthesis (Stevens & Goldzieher, 1968), α2μ-globulin possibly stimulates FSH and LH synthesis in oestrogen-treated rats by inhibiting excess synthesis of ACTH […] It was concluded that α2μ-globulin has an effect on testicular function in oestrogenized rats by inducing gonadotrophin and testosterone synthesis"* (Biswas, N.M. *et al.,* 1983).
A further study investigated the "*effect of dihydrotestosterone on serum concentrations of α2μ-globulin and on spermatogenesis in melatonin-treated rats*" (Mandal, H. *et al.*, 1990). 14-day melatonin treatment caused decreased weight of testis and accessory sex organs, inhibition of testicular 17β-HSD activity, suppression of spermatogenesis and decreased serum levels of gonadotropins, testosterone and α2μ-globulin in rats. Administration of α2μ-globulin on Day 8 to Day 14 reversed "*the depressive effect of melatonin treatment on plasma gonadotrophins […] testicular 17β-HSD activity and serum levels of FSH, LH and testosterone were increased in comparison with values seen in rats treated with melatonin alone* […]. *The weights of testis and accessory sex organs were also increased* […] *as were the number of type A spermatogonia, preleptotene spermatocytes and spermatids* […]" (Mandal, H. *et al.*, 1990).
In summary, it is suggested that 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. Thereupon, different peripheral findings such as effects on testosterone levels, testis weight and epididymis weight can be observed. Considering the available dataset of Benzobicyclon and the described research, this MoA is considered plausible for male rats treated at high doses. Since the α2μ-globulin nephropathy is a male rat specific effect without human relevance (ECHA, 2017; IARC, 1999), the findings in the context of this MoA are also without human relevance and therefore, the respective findings are not considered further in the current ED assessment.

2.10.3.2.5 Conclusion of the assessment of EAS-modalities

In summary, EAS-mediated adversity was sufficiently investigated for Benzobicyclon and the gathered information revealed no indication for EAS-mediated adversity as a consequence of an endocrine MoA.
The preceding section of the present document indicates that the conclusion the "ED criteria for EAS-modalities are not met" is applicable for Benzobicyclon ("scenario 1a"). No further investigations concerning the EAS-modalities are required.

2.10.3.3 Overall conclusion on the ED assessment for humans

For Benzobicyclon no reliable epidemiological or other human studies were available that could be considered for assessment of possible adverse effects on the endocrine system of humans.
Concerning mammalian toxicology, in vitro information (OECD level 2) was generated for the E, A, S-modalities based on a steroidogenesis assay (OECD 456) and an aromatase assay (US EPA 890.1200). No notable inhibition of aromatase or steroidogenesis alteration were observed. In vivo mechanistic Hershberger assay (OECD 441) and an Uterotrophic assay (OECD 440) were recently conducted and revealed no estrogenic, androgenic or anti-

androgenic effects caused by Benzobicyclon. Furthermore, TH and TSH level measurements in rats were available from an OECD CF level 4 study (peripubertal male and female assay) and no test substance-related effect was observed.
In vivo data (OECD CF level 4 and 5) revealed only isolated findings in EATS-mediated parameters. These findings (thyroid, epididymis, ovaries, seminal vesicles and testis) were seen without inter- and intra-species coherence or consistency and were considered not to be a direct effect of Benzobicyclon on the respective organ/tissue. The testis and epididymis findings were considered secondary to species-specific α2μ-globulin nephropathy.
Other parameters considered sensitive to, but not diagnostic of, EATS-modalities obtained from the toxicological studies did not provide any positive or negative diagnostic information and were not further discussed in this assessment, since T-mediated adversity is considered sufficiently investigated and as EAS-mediated adversity is considered sufficiently investigated for a reliable assessment and since no indication for EATS-mediated adversity was revealed. However, all available parameters are depicted in Appendix E and in the lines of evidence (Tables 100 and 106).
The evidence from all higher tier mammalian toxicity studies conducted in a range of animals and assessed according to current guidance allows to conclude that Benzobicyclon causes no biologically significant alterations to the endocrine system, i.e. no EATS-mediated adversity was observed. The few inconclusive indications for EATS related endocrine activity were not consistently observed, not supported by a mechanism of action and are of no toxicological significance in view of the lack of EATS-mediated adversity in the vast set of in vivo standard toxicity studies.
As T-mediated adversity is considered sufficiently investigated in line with the ED GD (ECHA and EFSA, 2018) and as EAS-mediated adversity is considered sufficiently investigated for a reliable assessment, a reliable conclusion in the course of a scientifically sound assessment can be drawn for Benzobicyclon. No generation of further information is considered neces-sary.
“Scenario 1a” of the ED GD (ECHA and EFSA, 2018) (i.e. sufficiently investigated and no potentially EATS-mediated adversity) applies to Benzobicyclon for the T- and EAS-modalities based on the toxicological dataset. No endocrine-specific MoA analysis was required.

2.10.4 ED assessment for non-target organisms

Ecotoxicological studies

Table 127 summarises the seven ecotoxicological toxicity studies included in the present assessment. Detailed information on the studies listed below were presented in Vol.3 CA B9.
A total of two avian reproduction studies with Bobwhite quail (Study ID 21) and mallard duck (Study ID 20), two fish early life stage toxicity studies with fathead minnow (Study ID 18) and sheepshead minnow (Study ID 19), a fish full life cycle study with fathead minnow (Study ID 22) and two OECD CF level 3 tests with Benzobicyclon (*i.e*. a fish short term reproduction assay (FSTRA with fathead minnow, Study ID 24) and an amphibian metamorphosis assay (AMA with *Xenopus laevis*, Study ID 23) are available and included in the present assessment.

Table 127: Summary of the relevant ecotoxicological in vivo studies included in the present assessment

Study principle (acc. to App. E)	Species/ strain	Test substance (purity and batch no.)	Nominal dose levels (dietary concentrations) / concentrations	Reference (year and Doc. No.)	Study ID (App. E)
Avian reproduction test	Northern Bobwhite Quail	> 99.9 %, batch no. 1A0110	0, 160, 400, 1000 ppm	2012a, 813-001	21
Avian reproduction test	Mallard duck	> 99.9 %, batch no. 1A0110	0, 160, 400, 1000 ppm	2012b, 813-002	20
Fish early life stage toxicity test	Fathead minnow	> 99.9 %, batch no. 1A0110	0, 0.0080, 0.020, 0.051, 0.13, 0.32, and 0.80 mg a.s./L	2012, 826-001	18
Fish early life stage toxicity test	Sheepshead minnow	> 99.9 %, batch no. 1A0110	0, 0.0041, 0.010, 0.026, 0.064, 0.16, and 0.40 mg a.s./L	2014a, 826-002	19
Fish life cycle toxicity test	Fathead minnow	> 99.9 %, batch no. 1A0110	0, 0.013, 0.025, 0.050, 0.100, and 0.200 mg a.s./L	2014b, 826-003	22
Fish short-term reproduction assay	Fathead minnow	99.4 % batch no. 1L0108	0, 2.00, 20 and 200 µg a.s./L	2020, 829-002	24
Amphibian metamorphosis assay	African clawed frog	99.4 % batch no. 1L0108	0, 1.0, 10 and 100 µg a.s./L	2022, 829-004	23

ED assessment for T-modality

Have T-mediated parameters been sufficiently investigated?

Table 128: Available studies investigating T-mediated parameters

	Sufficiently investigated
T-mediated parameters	Yes - based on the availability of the following study: - Amphibian metamorphosis assay (OECD 231)

Lines of evidence for adverse effects and endocrine activity related to T-modality

According to the ED GD (ECHA and EFSA, 2018) "*the assembling of lines of evidence should take into consideration all the available evidence (positive and negative) that have been evaluated as relevant and reliable*" during the data gathering (chapter 2). These parameters were assessed to determine "*whether and how they contribute to the lines of evidence for adversity and/or endocrine activity*".
After assembling and assessing the lines of evidence they were integrated for the assessment of adversity and endocrine activity in respect to the modalities. In the available ecotoxicology dataset, the amphibian metamorphosis assay investigated specific T-mediated parameters (*i.e.* development stage, hind-limb-length and thyroid histopathology) (Table 129).

Table 129: Assessment of the integrated lines of evidence for the T-modality and general toxicity

Grouping	Line(s) of evidence	Species	Duration of exposure	Route of administration	Lowest effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Amphibian metamorphosis assay – OECD 231 (Study ID 24)									
EATS-mediated (OECD 231)	Developmental stage	*Xenopus laevis*	60 d	Uptake from water	> 83.3 µg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	T
	Hind limb length				> 83.3 µg a.s./L	No effect			T
	Thyroid histopathology				> 83.3 µg a.s./L	No effect			T
Sensitive to, but not diagnostic of EATS (OECD 231)	Behaviour				> 83.3 µg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
	Body weight				> 83.3 µg a.s./L	No effect			-
	Malformations				> 83.3 µg a.s./L	No effect			-

Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

An amphibian metamorphosis study investigating T-mediated adversity is available for Benzobicyclon (Study ID 24). The test is evaluated and reported in CA B.9.2.3.
In the amphibian metamorphosis assay with *Xenopus laevis* no treatment-related effects on survival, abnormalities or abnormal behaviour, developmental stage, wet weight, snout-to-vent length or normalised hind-limb length were observed. No treatment-related histological findings were associated with the exposure to Benzobicyclon Thus, an overall negative evidence for endocrine activity and T-mediated adversity is concluded.
Based on the available results, no indications for T-mediated adversity are given (please also refer to Table 129).

Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

"*The initial analysis of the evidence comprises an assessment whether either EATS-mediated adversity or EATS endocrine activity has been sufficiently investigated*" (ECHA and EFSA, 2018) and assesses the observed effects in available ecotoxicology studies.
There was no indication for T-related endocrine activity or adversity in the available ecotoxicological dataset noting that T-related endocrine activity was investigated *in vivo* in the amphibian metamorphosis assay. Thus, in accordance with the ED GD (ECHA and EFSA, 2018) the "scenario 1a" was selected for T-mediated parameters of Benzobicyclon since a mechanistic OECD CF level 3 tests investigated no T-mediated adversity (please refer to Table 129 and Table 130).

Table 130: Selection of relevant scenario

Adversity based on T-mediated parameters	**Positive mechanistic OECD CF level 2/3 test**	**Scenario**	**Next step of the assessment**	**Scenario selected**
No (sufficiently investigated)	No	1a	Conclude: ED criteria not met because there is no "**T-mediated**" adversity	x
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no "**T-mediated endocrine activity**" observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

MoA analysis for T-modality

In accordance with the selected "scenario 1a", ED criteria are not met for the T-modality.

Conclusion on the assessment of T-modality

T-related endocrine activity was sufficiently investigated in the amphibian metamorphosis assay. Based on the available ecotoxicological dataset, T-mediated activity and adversity was not observed in the dataset considering the specific T-related endocrine activity survey (*i.e.* in the AMA study). In accordance with the ED GD (ECHA

and EFSA, 2018) the "scenario 1a" was selected for T-mediated parameters of Benzobicyclon since no effects were observed in mechanistic OECD CF level 3 tests for T-mediated adversity.

ED assessment for EAS-modalities

Have EAS-mediated parameters been sufficiently investigated?

Table 131: Available studies investigating EAS-mediated parameters

	Sufficiently investigated
EAS-mediated parameters	Yes - based on the availability of the following study: - Fish short term reproduction assay (OECD 229)

Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

According to the ED GD (ECHA and EFSA, 2018) "the assembling of lines of evidence should take into consideration all the available evidence (positive and negative) that have been evaluated as relevant and reliable" during the data gathering (chapter 2). These parameters were assessed to determine "whether and how they contribute to the lines of evidence for adversity and/or endocrine activity".
After assembling and assessing the lines of evidence they were integrated for the assessment of adversity and endocrine activity in respect to the modalities.
In the available fish short term assay, specific EAS-mediated parameters are measured (*i.e.* secondary sex characteristics, VTG levels). This study can give information on EAS-mediated parameters.
In other available ecotoxicology studies no specific EAS-mediated parameters (*e.g.* hormone levels) were measured. The studies give information on general toxicity and include parameters that might be 'sensitive to, but not diagnostic of, EAS'. Based on the available results, no indications for EAS-mediated adversity were given.
The integrated lines of evidence for EAS-mediated effects are reported in Table 132 and also comprise parameters sensitive to, but not diagnostic of, EATS.

Table 132: Assessment of the integrated lines of evidence for the EAS-modalities

Grouping	Line(s) of evidence	Species	Duration of exposure	Route of administration	Lowest effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Fish Early Life Stage Toxicity Tests – OECD 210 (Study ID 18 and 19)									
Sensitive to, but not diagnostic of EATS (OECD 210)	Body weight (fish)	*Pimephales promelas*	28 d	Uptake from water	0.247 mg a.s./L	11.5-40.3 % decrease compared to pooled control	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
	Embryo time-to-hatch				> 0.527 mg a.s./L	No effect			-
	Length (fish)				0.247 mg a.s./L	3.7-13.8 % decrease compared to pooled control			-
	Survival of embryos				> 0.527 mg a.s./L	No effect			-
Sensitive to, but not diagnostic of EATS (OECD 210)	Body weight (fish)	*Cyprinodon variegatus*	28 d	Uptake from water	> 0.323 mg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
	Embryo time-to-hatch				> 0.323 mg a.s./L	No effect			-
	Hatching success				> 0.323 mg a.s./L	No effect			-
	Length (fish)				> 0.323 mg a.s./L	No effect			-
	Survival of embryos				> 0.323 mg a.s./L	No effect			-
Fish Life Cycle Toxicity Test – OPPTS 850.1500 (Study ID 22)									
Sensitive to, but not diagnostic of EATS (OPPTS 850.1500)	Body weight (fish)	*Pimephales promelas*	171 d	Uptake from water	0.0753 mg a.s./L	29.4 – 40.1 % decrease of F0 male weight at day 146 compared to pooled control	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
	Embryo time-to-hatch				> 0.154 mg a.s./L	No effect			-
	Hatching success				> 0.154 mg a.s./L	No effect			-
	Length (fish)				0.0753 mg a.s./L	10.4-13.3 % decrease of male length at day 110 compared to control			-
	Reproduction (fecundity, fertility)				0.0753 mg a.s./L	26.9-41.0 % decrease in reproduction compared to pooled control			-
Fish Short-term Reproduction Assay – OECD 229 (Study ID 23)									
In vivo mechanistic (OECD 229)	Vitellogenin (VTG) in females	*Pimephales promelas*	21 d	Uptake from water	> 0.121 mg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	E, A, S
	Vitellogenin (VTG) in males				> 0.121 mg a.s./L	No effect			E, A, S

Grouping	Line(s) of evidence	Species	Duration of exposure	Route of administration	Lowest effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
EATS-mediated (OECD 229)	Male 2nd sex characteristics in females				> 0.121 mg a.s./L	No effect			A
	Male 2nd sex characteristics in males				> 0.121 mg a.s./L	No effect			E, A, S
	Specific gonad histopathology				> 0.121 mg a.s./L	No effect			E, A, S
Sensitive to, but not diagnostic of EATS (OECD 229)	Body weight (fish)				> 0.121 mg a.s./L	No effect			-
	Reproduction (fecundity, fertility)				> 0.121 mg a.s./L	No effect			-
Bird reproduction studies – OECD 206 (Study ID 20 and 21)									
Sensitive to, but not diagnostic of EATS (OECD 206)	Body weight (bird)	*Anas platyrhynchos*	21 weeks	Oral	> 124.9 mg a.s./kg bw/d	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
	Cracked eggs				> 124.9 mg a.s./kg bw/d	No effect			-
	Egg production				> 124.9 mg a.s./kg bw/d	No effect			-
									-
	Egg viability (% viable embryos of eggs set)				> 124.9 mg a.s./kg bw/d	No effect			-
	Eggshell thickness				> 124.9 mg a.s./kg bw/d	No effect			-
	Embryo viability (embryonic day 15)				> 124.9 mg a.s./kg bw/d	No effect			-
	Gross pathology (bird)				> 124.9 mg a.s./kg bw/d	No effect			-
	Hatchability				> 124.9 mg a.s./kg bw/d	No effect			-
	No. of 14 day old survivors				> 124.9 mg a.s./kg bw/d	No effect			-
	Viable embryos				> 124.9 mg a.s./kg bw/d	No effect			-
Sensitive to, but not diagnostic of EATS (OECD 206)	Body weight (bird)	*Colinus virginianus*	21 weeks	Oral	> 88.8 mg a.s./kg bw/d	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
	Cracked eggs				> 88.8 mg a.s./kg bw/d	No effect			-
	Egg production				> 88.8 mg a.s./kg bw/d	No effect			-
	Egg viability (% viable embryos of eggs set)				> 88.8 mg a.s./kg bw/d	No effect			-
	Eggshell thickness				> 88.8 mg a.s./kg bw/d	No effect			-

Grouping	Line(s) of evidence	Species	Duration of exposure	Route of administration	Lowest effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	Embryo viability (embryonic day 15)				> 88.8 mg a.s./kg bw/d	No effect			-
	Gross pathology (bird)				> 88.8 mg a.s./kg bw/d	No effect			-
	Hatchability				88.8 mg a.s./kg bw/d	5.81 % decrease compared to control			-
	No. of 14 day old survivors				88.8 mg a.s./kg bw/d	14.5 % decrease compared to control			-
	Viable embryos				> 88.8 mg a.s./kg bw/d	No effect			-

Table 133: Assessment of the integrated lines of evidence for general toxicity

Grouping	Line(s) of evidence	Species	Duration of exposure	Route of administration	Lowest effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Fish Early Life Stage Toxicity Test with fathead minnow – OECD 210 (Study ID 18 and 19)									
Systemic toxicity (OECD 210)	Survival (fish)	*Pimephales promelas*	28 d	Uptake from water	> 0.527 mg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
Systemic toxicity (OECD 210)	Survival (fish)	*Cyprinodon variegatus*	28 d	Uptake from water	> 0.323 mg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
Fish Life Cycle Toxicity Test – OPPTS 850.1500 (Study ID 22)									
Systemic toxicity (OPPTS 850.1500)	Survival (fish)	*Pimephales promelas*	171 d	Uptake from water	0.0753 mg a.s./L	41.7-58.3 % of male F0 mortality at day 171 compared to pooled control	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
Systemic toxicity (OPPTS 850.1500)	Survival (fish)	*Pimephales promelas*	171 d	Uptake from water	0.173 mg a.s./L	12.3 % decrease of F1 survival at day 57 compared to pooled control	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
Fish Short-term Reproduction Assay – OECD 229 (Study ID 23)									
Systemic toxicity (OECD 229)	Survival (fish)	*Pimephales promelas*	21 d	Uptake from water	> 0.121 mg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-

Grouping	Line(s) of evidence	Species	Duration of exposure	Route of administration	Lowest effect dose	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Bird reproduction studies – OECD 206 (Study ID 20 and 21)									
Systemic toxicity (OECD 206)	Mortality	*Colinus virginianus*	21 weeks	Oral	> 88.8 mg a.s./kg bw/d	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
Systemic toxicity (OECD 206)	Mortality	*Anas platyrhynchos*	21 weeks	Oral	> 124.9 mg a.s./kg bw/d	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-
Amphibian metamorphosis assay – OECD 231 (Study ID 24)									
Systemic toxicity (OECD 231)	Mortality	*Xenopus laevis*	21 d	Uptake from water	> 83.3 µg a.s./L	No effect	No evidence for endocrine activity and endocrine mediated adversity	Overall negative evidence for endocrine activity and endocrine mediated adversity	-

Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity

Two fish early life stage studies with fathead minnow (Study ID 18) and sheepshead minnow (Study ID 19), a fish full life stage study with fathead minnow Study ID 22), two bird reproduction studies with bobwhite quail (Study ID 21) and mallard duck (Study ID 20) and one fish short term reproduction assay (Study ID 23) are available to assess EAS-mediated adversity for Benzobicyclon. The tests are evaluated and reported in CA B.9.1.1.3, CA B.9.2.2 and CA B.9.2.3, respectively.

The available reproductive toxicity study with northern bobwhite quail did not show any effects on the reproductive parameters reported in the study up to and including a dose of 36.2 mg a.s./kg bw/d. At the maximum tested dose of 88.8 mg a.s./kg bw/day) there were treatment-related reductions in hatchability and surviving hatchlings per eggs set. The available reproductive toxicity study with mallard duck did not show any effects on the reproductive parameters reported in the study up to and including the highest dose tested of 124.9 mg a.s./kg bw/d. Therefore, both valid avian reproduction studies, although 'sensitive to, but not diagnostic of EATS', adds further weight of evidence for the lack of EATS-mediated adverse effects in birds.

In the available fish ELS study with the fathead minnow, adverse effects on growth (length and weight) were the only two effects noted. There were no effects on survival and reproductive parameters up to and including the highest concentration tested (0.527 mg a.s./L). In the available fish ELS study with the sheepshead minnow, there were no adverse effects on growth, survival and reproductive parameters up to and including the highest concentration tested (0.323 mg a.s./L). Therefore, the two fish ELS studies, although 'sensitive to, but not diagnostic of EATS', add weight of evidence for the lack of EATS-mediated adverse effects in fish.

In the available fish full life cycle study with the fathead minnow, adverse effects on survival (F0 males and F1), on growth (length and weight of male fish) and fecundity/fertility were noted. The effects on growth and reproduction were in the range of test concentrations when survival is affected: F_0 post-hatch survival, F_0 growth (wet weight and length) and reproduction (fecundity/fertility) were all affected at 0.0753 mg a.s./L. F_1 post-hatch survival and F1 growth (wet weight) were both affected at 0.173 mg a.s./L. The effects on growth are likely related to general toxicity and stress and are showing up at lower concentrations, followed by decreased survival at higher concentrations.

In the short-term reproduction assay with the fathead minnow, the evaluation of the endocrine related endpoints revealed no treatment-related negative impact of the test item. No effect on survival, body length and weight, fecundity, secondary sex characteristics or on the biomarker vitellogenin was observed. No treatment-related histopathological changes in the gonads or gonadal staging testis and ovary) have been observed after exposure to the test item. A number of cellular changes and lesions have been encountered in the gonads and abdominal cavity. These findings, however, could not be attributed to treatment with the test item and were considered as natural background variations. Due to the absence of any indication of effects from diagnostic endpoints (*i.e.* hormonal activity as VTG level, secondary sexual characteristic development) and apical endpoints (*i.e.* fecundity), an overall negative evidence for EATS-mediated activity is concluded.

Overall, no relevant indications for EAS-activity and EAS mediated adversity were observed in the two FELS studies, the fish full life cycle study, in the bird reproduction studies or in the FSTRA study.

Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

"*The initial analysis of the evidence comprises an assessment whether either EATS-mediated adversity or EATS endocrine activity has been sufficiently investigated*" (ECHA and EFSA, 2018) and assesses the observed effects in available ecotoxicology studies.

There was no indication for EAS-related endocrine activity or adversity in the available ecotoxicological dataset noting that EAS-related endocrine activity in the form of specific diagnostic and apical endpoints (*i.e.* hormonal activity as VTG level, secondary sexual characteristic development, fecundity) was investigated *in vivo* in the FSTRA study. Thus, in accordance with the ED GD (ECHA and EFSA, 2018) the "scenario 1a" was selected for EAS-mediated parameters of Benzobicyclon since mechanistic OECD CF level 3 tests for EAS-mediated adversity are part of the available dataset and did not reveal EAS-mediated adversity (please refer to Table 132 and Table 134).

Table 134: Selection of relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	No	1a	Conclude: ED criteria not met because there is no "**EAS-mediated**" adversity	x
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no "**EAS-mediated endocrine activity**" observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

MoA analysis for EAS-modalities

In accordance with the selected "scenario 1a", no MoA analysis is required for EAS-modalities.

Conclusion on the assessment of EAS-modalities

Based on the available ecotoxicological dataset, EAS-mediated activity and adversity was not observed in the dataset considering that a specific EAS-related endocrine activity survey (*e.g.* hormone measurements) was performed using the FSTRA study design. To finalise the assessment and in accordance with the ED GD (ECHA and EFSA, 2018) the "scenario 1a" was selected for EAS-mediated parameters of Benzobicyclon since mechanistic OECD CF level 3 tests for EAS-mediated adversity are part of the available dataset and did not reveal EAS-mediated adversity (please refer to Table 134).

Overall conclusion on the ED assessment for non-target organisms

Following the assessment steps proposed in the ED GD (ECHA and EFSA, 2018) Benzobicyclon is considered "sufficiently investigated" with regards to endocrine adversity for the T-modality and EAS-modalities based on the available ecotoxicological dataset. Specific surveys that measure EAS-related endocrine activity (*e.g.* hormone measurements) and T-related endocrine activity (*e.g.* development stage and hind-limb-length) were performed using the FSTRA and the AMA study design.
T-related endocrine activity was not observed in the dataset considering the specific T-related endocrine activity survey (*i.e.* in the AMA study). In accordance with the ED GD (ECHA and EFSA, 2018) the "scenario 1a" was selected for T-mediated parameters of Benzobicyclon since no effects were observed in mechanistic OECD CF level 3 tests for T-mediated adversity.
EAS-mediated activity was not observed in the dataset considering the specific EAS-related endocrine activity survey (*i.e.* the FSTRA study). In accordance with the ED GD (ECHA and EFSA, 2018) the "scenario 1a" was selected for EAS-mediated parameters of Benzobicyclon since no effects were observed in mechanistic OECD CF level 3 tests for EAS-mediated adversity.
Overall, based on the results of the specific EATS-related endocrine activity surveys, Benzobicyclon is considered "sufficiently investigated" and the ED criteria are not met for non-target organisms.

RMS overall conclusion on the ED assessment for non-target organisms other than mammals

T-modality

The T-related endocrine activity is considered sufficiently investigated in the available AMA study (CA 8.2.3/02; [redacted], 2020; Study ID 24). In this study, one replicate of solvent control group and one replicate

of the tested concentration 100 ug Benzobicyclon Technical/L were considered compromised due to a high mortality rate (5 dead tadpoles in both replicates). By excluding these two compromised replicates, the validity criteria are still met, according to OECD 231. As regards the performance of the assay, some deviations from OECD 231 are noted on temperature (differences between replicates and treatments not always < 0.5 °C) and the variability of tested concentrations, with CV exceeding 20% (please refer to CA B.9.2.3, study CA 8.2.3/02 for details). Overall, the RMS believes that the reported deviations did not significantly impact the integrity and the outcome of the study.

No treatment-related effects were detected for the T-mediated parameters investigated in the study (developmental stage, hind limb length normalized by SVL and thyroid gland histology). No statistically significant differences were detected for the parameters "sensitive to, but not diagnostic of, EATS modalities" (i.e. Snout-Vent Length (SVL) and mean wet weight). Therefore, due to the lack of advanced, asynchronous or delayed development, and thyroid histopathology effects, there is no indication to associate Benzobicyclon with thyroid activity.

Overall, based on the assessment of the available evidences, the RMS considers that the **ED criteria for T-modality are not met for non-target organisms other than mammals, according to the scenario 2a (ii)** (no endocrine activity observed, but sufficiently investigated).

EAS-modalities

The EAS-related endocrine activity is considered sufficiently investigated in the available FSTRA study (CA 8.2.3/01; [redacted], 2020; Study ID 23). The validity criteria set in OECD 229 are met with the exception of the temperature-related one (variation of 2.3°C and 1.7°C between vessels at day 0 and day 7). Considering that this is a vertebrate study and that fish are in healthy conditions both in control and solvent group, the slight exceedance of the temperature validity criterion is considered not to have affected the integrity of the study. Other minor deviations were recorded (please refer to CA B.9.2.3, study CA 8.2.3/01 for details). Overall, considering that no statistically significant difference was observed for any investigated parameters, the robustness of the test can be considered adequate.

No significant effects were observed for both EAS-mediated parameters (VTG in females and males, secondary sex characteristics in males and females, gonads histopathology) and for "sensitive to, but not diagnostic of, EAS" parameters (behaviour, fecundity, wet weight). As for gonads histopathology, a number of cellular changes and lesions have been encountered in the gonads both in controls and treatment groups. These findings, however, could not be attributed to treatment with the test item and were considered as natural background variations. Thus, there is no indication of EAS-related endocrine activity in fish.

The other relevant toxicity studies considered for the ED assessment of EAS modalities (two fish early life stage studies with fathead minnow (Study ID 18) and sheepshead minnow (Study ID 19), a fish full life stage study with fathead minnow (Study ID 22), two bird reproduction studies with bobwhite quail (Study ID 21) and mallard duck (Study ID 20)) include only parameters considered "sensitive to, but not diagnostic of EATS" and do not provide any additional evidence of EAS-related endocrine activity, in the lack of significant effects on EAS-mediated parameters in the available FSTRA study. The RMS has updated the Table 132 and Table 133 and the Appendix E, in accordance with the current outcome of the two abailable fish ELS studies and the FFLC study, although further clarifications or new statistical analysis should still be provided by the applicant. Please refer to CA B.9.2.2.1 and CA B.9.2.2.2 for details. In case a different outcome would be indicated for some of the parameters investigated in these studies, the ED assessment will be updated, accordingly.

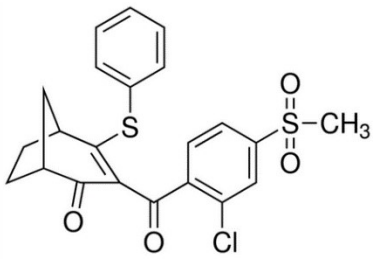
Overall, based on the assessment of available evidences, the RMS considers that the **ED criteria for EAS-modalities are not met for non-target organisms other than mammals, according to the scenario 2a (ii)** (no endocrine activity observed, but sufficiently investigated).

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 135 Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	benzobicyclon (ISO); 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one
Other names (usual name, trade name, abbreviation)	benzobicyclon
ISO common name (if available and appropriate)	benzobicyclon
EC number (if available and appropriate)	
EC name (if available and appropriate)	-
CAS number (if available)	156963-66-5
Other identity code (if available)	SB-500 and SAN 1315 H (producer's development code numbers)
Molecular formula	$C_{22}H_{19}ClO_4S_2$
Structural formula	
SMILES notation (if available)	CS(=O)(=O)c1ccc(C(=O)C2C(=O)C3CCC(C3)C=2Sc2ccccc2)c(Cl)c1
Molecular weight or molecular weight range	447.0 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Benzobicyclone is a racemate – please refer to Volume 4.
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	Min. 980 g/kg

2.11.1.2 Composition of the substance

Table 136: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Benzobicyclon	98	No Annex VI entry.	

Table 137: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
None	-	-	-	-

Table 138: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
None	-	-	-	-	-

Table 139: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
None	-	-	-	-

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 140: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	GHS 09 Wng	H410		M = 100 M = 100	-

2.11.2.2 Additional hazard statements / labelling

Not applicable.

Table 141: Reason for not proposing harmonised classification and status under CLH public consultation

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

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

2.11.3 History of the previous classification and labelling

Not applicable – No current harmonised classification.

2.11.4 Identified uses

Please refer to section 1.5.

2.11.5 Data sources

- DAR / data provided by the applicant.
- US EPA 2021a, 40 CFR Part 180 [EPA–HQ–OPP–2020–0391; FRL–8991–01– OCSPP] Benzobicyclon; Pesticide Tolerances Federal Register : Benzobicyclon; Pesticide Tolerances
- US EPA 2021b, Benzobicyclon: Section 3 Risk Assessment for Proposed New Formulation, Increase to the Established Tolerance, and National Use Expansion on Rice. EPA-HQ-OPP-2020-0391-0006_content.pdf

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

2.12.1 STEP 1: Exclusion of degradation products of no concern

According to SANCO 221/2000 rev.10-25/02/2003, CO_2 is considered as degradation product of no concern. The metabolites 1315P-070, 1315P-570, 1315P-960 and 1315P-966 were included in the groundwater exposure assessment.

2.12.2 STEP 2: Quantification of potential groundwater contamination

In the following, the results are presented which are based on the detailed groundwater risk assessment concerning the environmental fate of the formulation GWN-10235.

Table 142: PEC_{GW} values for benzobicyclon and its metabolites following application to rice (RMS re-calculation with MedRice_Modified version)

Substance	PEC_{GW} (µg/L)	
	Scenario 1	Scenario 2
Benzobicyclon	0.000	0.000
1315P-070	0.000	0.066
1315P-570	0.008	0.018
1315P-960	0.028	0.019
1315P-966	**0.524**	**0.278**

Table 143: PEC_{GW} values for benzobicyclon and its metabolites following a single application to flooded rice paddies at 300 g a.s./ha (RMS re-calculation with the revised approach)

Substance	PEC_{GW} (µg/L)
	Clayey scenario
Benzobicyclon	<0.0001
1315P-070	<0.0001
1315P-966	**0.524**
1315P-570	0.023
1315P-960	0.034

In conclusion, maximum PECgw values above the legal drinking water limit of 0.1 µg/L were obtained for the metabolite 1315P-966 with a maximum PECgw of 0.524 µg/L. Therefore, a drinking water risk assessment according to SANCO 221/2000 rev.10-25/02/2003 has been conducted for 1315P-966 as detailed in the following.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

In accordance with the PECgw calculations depicted above, 1315P-966 is the only relevant metabolite in groundwater exceeding the trigger value of 0.1 µg/L. 1315P-070 showed to have maximum PECgw value below 0.1 µg/L and is thus considered not to be relevant in the context of SANCO 221/2000 rev.10-25/02/2003.

2.12.3.1 STEP 3, Stage 1: screening for biological activity

The available data on the herbicidal activity of the metabolite 1315P-966 show that it has no biological activity (see page 28 of EFSA Scientific Report (2008) 150, Conclusion on the peer review of sulcotrione).

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

Based on available *in vitro* genotoxicity studies, the metabolite 1315P-966 is considered to be not genotoxic.

2.12.3.3 STEP 3, Stage 3: screening for toxicity

The available acute oral toxicity study in rats shows that 1315P-966 is not acute toxic (LD_{50} > 5000 mg/kg bw).

Overall considering that the metabolite is not genotoxic and does not derive from a parental compound having carcinogenic, reproductive or acute toxicity properties, it is considered to be not relevant.

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

1315P-966 has estimated concentrations in groundwater above 0.1 µg/L but below 0.75 µg/L for the intended use. Therefore, a refined risk assessment is not necessary.

2.12.5 STEP 5: Refined risk assessment

See 2.12.4, a refined risk assessment is not necessary.

2.12.6 Overall conclusion

1315P-966 is not of toxicological concern at the maximum predicted concentrations in groundwater and there is no hazard for the consumer.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

Benzobicyclon is a racemate comprising equimolar amounts of two enantiomers. Please refer to Volume 4.

2.13.1 Identity and physical chemical properties

The physical properties of Benzobicyclon were determined with the racemic mixture of Benzobicyclon comprising equimolar amounts of two enantiomers (purified were necessary).

2.13.2 Methods of analysis

A stereo-specific method for analysis of the technical active substance is available

2.13.3 Mammalian toxicity

The toxicological studies have been performed using the substance as manufactured (comprising equimolar amounts of two enantiomers). Appropriate health based reference values for Benzobicyclon have been derived from critical dose descriptors (NOAELs) for Benzobicyclon as a racemate, representing the most sensitive biological/toxicological Benzobicyclon-related effect in mammals. However, considering the manufacturing process, benzobicyclon is obtained as a racemate. Moreover, the enantiomeric ratio of Benzobicyclon technical was investigated for six representative batches of TGAI and was found to be 1:1. Thus, it is concluded that Benzobicyclon technical is a racemate (see Vol. 4). However, no information is provided on the proportion of these enantiomers following oral and i.v. exposures. The metabolic pathways leading to 1315P-570, 1315P-076, 1315P-960 and 1315P-683 (…) do not specify if some steps involve enantiomeric selective reactions and therefore, the possibility of non equimolar amounts of the metabolites cannot be completely excluded.

2.13.4 Operator, Worker, Bystander and Resident exposure

See above.

2.13.5 Residues and Consumer risk assessment

See above.

2.13.6 Environmental fate

See above.

2.13.7 Ecotoxicology

Data point addressed:	N5 1.8/01
Author(s) (year):	[redacted] (2020)

Title:	BENZOBICYCLON: NON GLP MEASUREMENT OF BIOLOGICAL ACTIVITY - COMPARISON OF THE RACEMATE BENZOBICYCLON AND ITS TWO ENANTIOMERS GCP1_1 AND GCP1_2 IN A 7-DAY STATIC LEMNA GIBBA GROWTH INHIBITION TEST
Laboratory report / project Number (Doc. No.):	134393240 (347-001)
Testing facility:	Ibacon GmbH, Rossdorf, Germany
Published:	No
Test guideline used:	OECD No. 221 (2006)
Deviations:	None
Previous evaluation:	No, not previously submitted
GLP:	No
Acceptability/Reliability:	Yes

Executive Summary
A laboratory study was conducted to assess the efficacy of the racemate Benzobicyclon and its two enantiomers GCP1_1 and GCP1_2 on the test organism *Lemna gibba*, in terms of yield and growth rate for frond number and dry weight. The test has been conducted with an untreated control and two dose rates (5 µg/L and 20 µg/L) per test item in a 7-day static growth inhibition test.

In conclusion, Benzobicyclon technical and the two Benzobicyclon enantiomers GCP1_1 and GCP1_2 showed similar levels of efficacy in the 7-day static growth inhibition test, considering the parameters yield and growth rate based on frond number of *Lemna gibba* and yield and growth rate based on dry weight of this test organism.

I. MATERIALS AND METHODS
A. MATERIALS
1. Test material:

Test Material:	Benzobicyclon technical
Lot/Batch #:	1L0108
Test Material:	GCP1_1
Lot/Batch #:	1L0108
Test Material:	GCP1_2
Lot/Batch #:	1L0108

3. Test organisms

Species:	*Lemna gibba* G 3

B. STUDY DESIGN AND METHODS
1. Experimental Start / End: February 05, 2020 – February 12, 2020

2. Experimental treatment
Stock solutions were prepared in such a way that the rates of the two enantiomers GCP1_1 and GCP1_2 were equivalent to the rate of Benzobicyclon technical. The following dose rates were tested with all test items in comparison to a solvent control: 5 and 20 µg/L with Dimethylformamide (100 µl/L) being used as solvent control.

In order to be able to compare the toxicity of Benzobicyclon technical and its two enantiomers GCP1_1 and GCP1_2 at 5 and 20 µg/L, the parameters yield and growth rate for frond number and dry weight were assessed in a static test.

The experimental approach consisted of three replicates per treatment each containing 12 fronds at test start (corresponding to an approx. dry weight of 1.0 mg), using glass vessels of 250 mL volume with approx. 200 mL test medium, covered with evaporating plates. The test was conducted with a continuous illumination of 7010-7470 lux and a specific test medium (20x AAP-Growth Medium containing MOPS buffer).

For all treatments (including the control) the effects on yield based on the frond number of *Lemna gibba* and therefore the percentage inhibition were assessed at the following timings; 0-2 days, 0-5 day and 0-7 days after trial start. The effects on growth rate based on frond number were determined at the same timings. In addition the effects on yield and growth rate based on dry weight were assessed seven days after trial start.

3. Statistics

The static test design was chosen for the comparison of the biological activity of the racemate Benzobicyclon and its two enantiomers GCP1_1 and GCP1_2.

II. RESULTS AND DISCUSSION

The percentage inhibition of yield based on the frond number of *Lemna gibba* following the exposure to the different treatments in comparison to the untreated control are presented in the table below.

Table 144: Effects of Benzobicyclon treatments on yield based on frond number of *Lemna gibba* (percentage of yield inhibition)

Treatment	Inhibition of yield [%]		
	0 - 2 days	0 - 5 days	0 - 7 days
Control	/	/	/
Benzobicyclon technical (5 µg/L)	11.9	26.6	31.4
Benzobicyclon technical (20 µg/L)	33.3	55.7	71.0
GCP1_1 (5 µg/L)	7.1	26.6	27.2
GCP1_1 (20 µg/L)	45.2	48.7	66.6
GCP1_2 (5 µg/L)	0.0	11.1	14.3
GCP1_2 (20 µg/L)	33.3	51.3	64.3

For both tested dose rates (5 µg/L and 20 µg/L) the racemate Benzobicyclon and its two enantiomers GCP1_1 and GCP1_2 showed similar levels of efficacy in a 7-day static *Lemna gibba* growth inhibition test depending with respect to the yield based on the frond number *of Lemna gibba.*

The percentage inhibition of growth rate based on the frond number of *Lemna gibba* following the exposure to the different treatments in comparison to the untreated control are presented in the table below.

Table 145: Effects of Benzobicyclon treatments on growth rate based on frond number of *Lemna gibba* (percentage of growth rate inhibition)

Treatment	Inhibition of growth rate [%]		
	0 - 2 days	0 - 5 days	0 - 7 days
Control	/	/	/
Benzobicyclon technical (5 µg/L)	8.2	12.6	12.0
Benzobicyclon technical (20 µg/L)	25.3	31.6	37.9
GCP1_1 (5 µg/L)	4.9	12.5	10.2
GCP1_1 (20 µg/L)	36.5	26.2	33.9
GCP1_2 (5 µg/L)	-0.2	4.9	4.9
GCP1_2 (20 µg/L)	25.3	28.2	31.9

For both tested dose rates (5 µg/L and 20 µg/L) the racemate Benzobicyclon and its two enantiomers GCP1_1 and GCP1_2 showed similar levels of efficacy in a 7-day static *Lemna gibba* growth inhibition test depending with respect to the growth rate based on the frond of *Lemna gibba*.

The percentage inhibition of yield and growth rate based on the dry weight of *Lemna gibba* after seven days following the exposure to the different treatments in comparison to the untreated control are presented in the table below.

Table 146: Effects of Benzobicyclon treatments on yield and growth rate based on dry weight of *Lemna gibba* (percentage of yield and growth rate inhibition) after seven days

Treatment	Inhibition of yield [%]	Inhibition of growth rates [%]
Control	/	/
Benzobicyclon tech. (5 µg/L)	42.2	15.5
Benzobicyclon tech. (20 µg/L)	72.9	35.4
GCP1_1 (5 µg/L)	32.9	11.1
GCP1_1 (20 µg/L)	70.9	33.5
GCP1_2 (5 µg/L)	23.7	7.5
GCP1_2 (20 µg/L)	72.1	34.6

For both tested dose rates (5 µg/L and 20 µg/L) the racemate Benzobicyclon and its two enantiomers GCP1_1 and GCP1_2 showed similar levels of efficacy in a 7-day static *Lemna gibba* growth inhibition test depending with respect to yield and growth rate based on the dry weight of *Lemna gibba*.

III. CONCLUSIONS
In a 7-day static growth inhibition test with *Lemna gibba,* the Benzobicyclon racemate and its two enantiomers GCP1_1 and GCP1_2 show similar levels of efficacy. Thus, a comparable biological activity of the racemate and its two enantiomers GCP1_1 and GCP1_2 is assumed.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon

Food of animal origin: Not needed

Soil: Benzobicyclon, 1315P-070, 1315P-966, 1315P-570 and 1315P-960

Groundwater: Benzobicyclon, 1315P-070, 1315P-966, 1315P-570 and 1315P-960

Surface water: Benzobicyclon, 1315P-070, 1315P-570, 1315P-966, 1315P-960, 1315P-076, 1315P-683, 1315P-962

Sediment: Benzobicyclon, 1315P-070, 1315P-570, 1315P-966 and 1315P-960

Air: Benzobicyclon

2.14.2 Definition of residues for monitoring

Food of plant origin: sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon

Food of animal origin: Not needed

Soil: Benzobicyclon, 1315P-070

Groundwater: Benzobicyclon, 1315P-070

Surface water: Benzobicyclon, 1315P-070

Sediment: Benzobicyclon, 1315P-070

Air: Benzobicyclon

Level 3

BENZOBICYCLON

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

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

3.1.1.1 Article 4

		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		Active substance: Benzobicyclon; Formulation: Avanza (code: GWN-10235) Representative uses considered to comply with Article 4: pre-emergence and post-emergence systemic herbicide to control emerging and young monocotyledonous (grass and non-grass) weeds in rice. See table 1.5.1. Details of representative uses

3.1.1.2 Submission of further information

		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			Not applicable

3.1.1.3 Restrictions on approval

		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	

3.1.1.4 Criteria for the approval of an active substance

Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		Please refer to section 2.6.10 and detailed evaluation in Volume 3
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		Although rice is mostly considered a semipermanent crop, rice can be rotated with other crop. No data were submitted on rotational crop by the applicant. Based on the DT_{90} for Benzobicyclon and its relevant soil metabolites >100 days, RMS considers the studies on rotational crops necessary to predicts residues on food and feed.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		Please refer to section 2.8 and 2.9 and detailed evaluation in Volume 3
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Benzobicyclon containing products are envisaged to be used for control of various monocotyledonous grass and non-grass weed species in the representative crop paddy rice. In the last years an extensive efficacy program was conducted in countries of the Mediterranean EPPO zone to support this use. In this program Benzobicyclon has demonstrated a very high level of activity against monocotyledonous weed species, belonging to different families.

				Summary data concerning the level of achieved control (% control, number of trials), according to SANCO/10054/2013 – rev. 3 (Appendix I) has been submitted.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		The available data is sufficient to calculate PEC values for relevant metabolites to proceed with toxicological or ecotoxicological risk assessment. Ecotoxicological data are not available for all the relevant metabolites. The respective risk assessments are performed using surrogate toxicity endpoints from the parent compound.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	Y		Min purity 980 g/kg
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			No FAO specification
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not relevant
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		Adequate methods are available to determine benzobicyclon and significant impurities in technical active substance. Several unknown components could be seen in the analysis of the technical batches and their identification is required. Adequate method is available to determine benzobicyclon in the representative formulation.
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	In order to validate the primary method for post registration purposes, adequate ILV should be provided for benzobicyclon and metabolites 1315-070, 1315P-570 and 1315P-966 in all the commodity groups of the primary method except for benzobicyclone and 1315P-070 in dry commodities, covered by a specific adequate ILV.

				For monitoring metabolite 1315P-070 in water confirmatory validation data are required. Additional information on matrix effects is required for acceptability of the independent laboratory validation of the determination method for benzobicyclon and metabolite 1315P-070 in drinking water for enforcement purposes.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		The ADI of Benzobicyclon is 0.034 mg/kg bw/d based on the critical NOAEL with relevance to humans determined in a 2-year chronic toxicity/carcinogenicity study in rats (NOAEL: 3.4 mg/kg bw/d) and a safety factor of 100 (see section 2.6.10.1). The toxicological data set available for Benzobicyclon demonstrates that the setting of an ARfD is not needed (see section 2.6.10.2). The AOEL of Benzobicyclon is 0.060 mg/kg bw/d based on the relevant NOAEL determined in a 2-generation reproductive toxicity study in rats (NOAEL: 59.5 mg/kg bw/d), a safety factor of 100 and an oral absorption of 10% (see section 2.6.10.3). An ARfD was not derived for Benzobicyclon since no relevant effects/mortality were observed by acute exposure. Therefore, no AAOEL setting for Benzobicyclon is needed (see section 2.6.10.4).
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	The bacterial reverse mutation assay (Ames test) in different bacterial strains and a gene mutation assay in mammalian cells (mouse lymphoma assay) did not show any mutagenic potential of Benzobicyclon. In the chromosomal aberration assay, Benzobicyclon induced increases in structural and numerical aberration frequencies in mammalian cells. 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*. Overall, based on a weight of evidence approach the two in vivo MNT test in mice were considered adequate to conlude on the negative outcome for structural and numerical chromosomal aberrations potential of benxzobicyclone. Benzobicyclon is not genotoxic and not considered to trigger classification for genotoxicity according to Regulation (EC) No 1272/2008. Please refer to section 2.6.4 and Vol.3 CA B-6.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	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. Please refer to section 2.6.5 and Vol.3 CA B-6.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not applicable.*
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	The potential toxicity of Benzobicyclon on reproduction was investigated in a two-generation reproductive toxicity study in rats (dosed with 0, 100, 1000, 20000 ppm). The reproductive performance and fertility were not affected by the treatment with the test substance up to the highest dose tested of 20000 ppm, equivalent to 1250 mg/kg bw/d in males and 1779 mg/kg bw/d in females. Benzobicyclon is not considered to trigger classification for reproductive toxicity according to Regulation (EC) No 1272/2008. Please refer to section 2.6.6 and Vol.3 CA B-6.

ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not applicable.*
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009		X	ED assessment according to EFSA/ECHA GD (2018) reveals that Benzobicyclon causes no biologically significant alterations to the endocrine system, i.e. no EATS-mediated adversity was observed (please refer to section 2.10 for further details and Vol.3 CA B-6).
ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not applicable.*
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	Benzobicyclon **does not fulfill** the P criteria. DT_{50} in soil is less than 6 months (worst-case from lab studies of 38.2 days). DT_{50} in water/sediment system is less than 2 months (worst case of 1.06 days). Benzobicyclon **does not fulfill** the vB criteria, since the BCF is lower than 5000 (BCF_{ssl} = 126 L/kg and BCF_{kl} = 161 L/kg, 5% lipid-corrected)
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Benzobicyclon **does not fulfill** the P criteria. DT_{50} in soil is less than 120 days (worst-case from lab studies of 38.2 days). DT_{50} in water/sediment system is less than 40 days (worst case of 1.06 days).

				Benzobicyclon **does not fulfill** the B criteria, since the BCF is lower than 2000 (BCF_{ssl} = 126 L/kg and BCF_{kl} = 161 L/kg, 5% lipid-corrected) Benzobicyclon **does fulfil** the T criteria since the NOEC for *Lemna gibba* is <0.01 mg/L (i.e. 0.000167 mg a.s./L).
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Benzobicyclon **does not fulfill** the P criteria. DT_{50} in soil is less than 180 days (worst-case from lab studies of 38.2 days). DT_{50} in water/sediment system is less than 60 days (worst case of 1.06 days). Benzobicyclon **does not fulfill** the vB criteria, since the BCF is lower than 5000 (BCF_{ssl} = 126 L/kg and BCF_{kl} = 161 L/kg, 5% lipid-corrected)
Ecotoxicology				
		Yes	No	
i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		For birds and mammals, the dietary and drinking water risk assessment as well as the risk assessment for food chain behaviour and biomagnification indicated an acceptable risk from exposure to Benzobicyclon for the representative use of GWN-10235. Further discussions are deemed necessary for drawing a definitive conclusion on the long-term risk for herbivorous birds (mallard duck) The aquatic risk assessment for Benzobicyclon and the relevant metabolites performed according to standard and revised MED-Rice model indicates an acceptable acute and chronic risk for aquatic organisms for the representative use of GWN-10235. The acute oral and contact toxicity hazard quotients/ETR values for Benzobicyclon and the relevant metabolites indicate an acceptable acute risk to honey bees after application of GWN-10235. For Benzobicyclon, the chronic oral ETR values for adult honey bees do not exceed the trigger values, indicating an acceptable long-term risk after application of GWN-10235. The ETR values for honey bee larvae do not exceed the trigger values, except for "weeds" scenario. Considering the very low potential for exposure via flowering weeds after application of GWN-10235 in rice fields, an acceptable risk for honey bee larvae can be concluded. However, the outcome of the chronic risk assessment for honey bee larvae exposed to Benzobicyclon and the chronic risk assessment for honey bee exposed to the plant metabolites 1315P-570 and 1315P-966 should be further discussed.

				For non-target arthropods other than bees, the calculated field and drift rates do not exceed the relevant LR_{50}/ER_{50} values, indicating an acceptable risk for non-target arthropods based on the Tier II studies for the representative use of GWN-10235. For earthworms and other soil non-target macro- and meso-organisms, the calculated TER values for Benzobicyclon and the relevant metabolites exceed the trigger of 5, indicating an acceptable risk for the representative use of GWN-10235. The maximum PECsoil calculated for the representative use of GWN-10235 do not exceed the relevant NOEC values for nitrogen transformation, indicating an acceptable risk for soil micro-organisms. For non-target terrestrial plants the TER value based on a worst-case drift scenario and the lowest endpoint of the vegetative vigour study was above the trigger value of 5 for all representative uses of BWN-10235, indicating an acceptable risk. For further details please refer to Chapter 2.9.9.
ii	It is considered that, the substance **SHOULD BE identified as having endocrine disrupting properties** that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.		X	A Fish Short Term Reproduction Assay (FSTRA) and an Amphibian Metamorphosis Assay (AMA) were submitted by the applicant for the evaluation of the potential endocrine activity of benzobicyclon for non-target organisms other than mammals. Overall, based on the assessment of available evidences, the RMS considers that the **ED criteria for T-modality and EAS-modalities are not met for non-target organisms other than mammals, according to the scenario 2a (ii)** (no endocrine activity observed, but sufficiently investigated). For the conclusion on the potential endocrine disruption of benzobicyclon to wild mammals, please refer to the ED assessment for humans (Chapter 2.10).
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.	X		Applicable for all representative uses
iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist:	X		The acute oral and contact toxicity hazard quotients/ETR values for Benzobicyclon and the relevant metabolites indicate an acceptable acute risk to honey bees after application of GWN-10235. For Benzobicyclon, the chronic oral ETR values for adult honey bees do not exceed the trigger values, indicating an acceptable long-term risk after application of GWN-10235. The

	— will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			ETR values for honey bee larvae do not exceed the trigger values, except for "weeds" scenario. Considering the very low potential for exposure via flowering weeds after application of GWN-10235 in rice fields, an acceptable risk for honey bee larvae can be concluded. However, the outcome of the chronic risk assessment for honey bee larvae exposed to Benzobicyclon and the chronic risk assessment for honey bee exposed to the plant metabolites 1315P-570 and 1315P-966 should be further discussed.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		**Plant residue definition for monitoring and risk assessment** : sum of Benzobicyclon and 1315P-070 expressed as Benzobicyclon **Food of animal origin:** Not needed **Animal residue definition for monitoring and risk assessment**: Not required
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		For Benzobicyclon, the PECpgw values are < 0.1 µg/L in both scenarios calculated in accordance with the Guidance of the Working Group on MED-Rice (2003). The PECpgw for 1315P-070, 1315P-960 and 1315P-570 are < 0.1 µg/L in both scenarios. For the metabolite 1315P-966 the maximum PEC in groundwater amounts to 0.524 µg/L. A respective assessment of the relevance of 1315P-966 in groundwater was conducted. The available data on the herbicidal activity of the metabolite 1315P-966 shows, that it has no biological activity. Based on available *in vitro* genotoxicity studies the metabolite 1315P-966 is considered to be not genotoxic. The available acute oral toxicity study in rats shows that 1315P-966 is not acutely toxic. 1315P-966 is considered as a non-relevant metabolite. Based on these results, the risk to groundwater after application of Benzobicyclon according to GAP is acceptable.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	Considering its characteristics, benzobicyclon is not proposed as a candidate for substitution

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	Considering its characteristics, benzobicyclon is not considered a low risk substance

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

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

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
None	-	-	-	-
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
New study on the oxidising properties of the a.s. is required and, subsequently, the relative statement on the formulation should be amended.	All relevant uses	X	-	-
New study on the surface tension of the preparation is required.	All relevant uses	X	-	-
3.1.4.3 Data on uses and efficacy				
None	-	-	-	-
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None	-	-	-	-

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.5 Methods of analysis				
None	-	-	-	-
3.1.4.6 Toxicology and metabolism				
To provide evidence (e.g.: in silico) on potential binding of benzobicyclone and it smajor metabolite(s) to α2μ-globulin.	All relevant uses	X		
An up to date literture seacrh is needed to fully address human relevance of observed effects (decreased absolute pituitary weight, increased testicular and epididymal weight) in males in the two generation reproductive study.	All relevant uses	X		
3.1.4.7 Residue data				
Rotational crop studies.	Supporting for all representative uses	X	-	-
3.1.4.8 Environmental fate and behaviour				
A literature search for all relevant metabolites identified into the environmental fate section is required	All relevant uses	X	-	-

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.9 Ecotoxicology				
Study on an additional aquatic macrophyte species (i.e.. *Myriophyllum spicatum*) or further justifications for the lack of such study.	Yes			
Chronic toxicity studies on earthworms with the metabolites 1315P-966, 1315P-570 and 1315P-960 or a justification for the lack of such studies.	Yes			
Chronic toxicity studies on *Folsomia candida* and *Hypoaspis aculeifer* with the metabolites 1315P-070, 1315P-966, 1315P-570 and 1315P-960 or a justification for the lack of such studies.	Yes			
Nitrogen transformation studies with the metabolites 1315P-070, 1315P-966, 1315P-570 and 1315P-960 or a justification for the lack of such studies.	Yes			
Acute oral and contact toxicity study on bees with the formulation GWN-10235.	Yes			

3.1.5 Issues that could not be finalised

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

Area of the risk assessment that could not be finalised on the basis of the available data	Relevance in relation to representative use(s)

3.1.6 Critical areas of concern

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

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

Critical area of concern identified	**Relevance in relation to representative use(s)**
None	-

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

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

Representative use		Rice **1x 0.3 kg a.s./ha** (X[1])
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L[(a)] breached	
	Assessment not finalised	
Comments/Remarks		

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

3.1.8 Area(s) where expert consultation is considered necessary

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

Area(s) where expert consultation is considered necessary	Justification
None	-

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

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

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
Chronic risk for honey bee larvae potentially exposed to benzobyciclon via flowering weeds	*Results presented in DAR are insufficient to establish the absence of effects on honeybees from exposure to flowering weeds after application of the active substance in rice*	*There is sufficient evidence to assume a very low potential for exposure to bees via flowering weeds in rice fields after the application of benzobyciclon. Thus, an acceptable chronic risk for honeybee larvae can be expected for "weeds" scenario*

3.2 PROPOSED DECISION

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

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

3.4 APPENDICES

APPENDIX 1: Proposed metabolic pathways

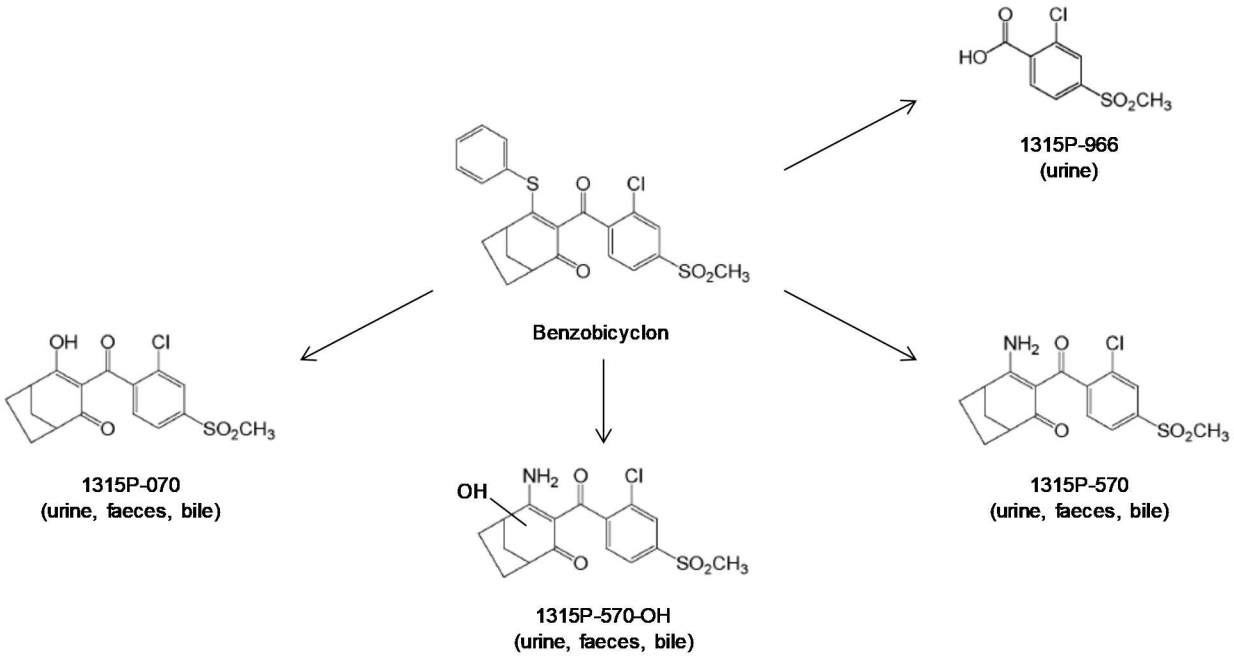


Figure 5: Proposed metabolic pathway of Benzobicyclon in rats

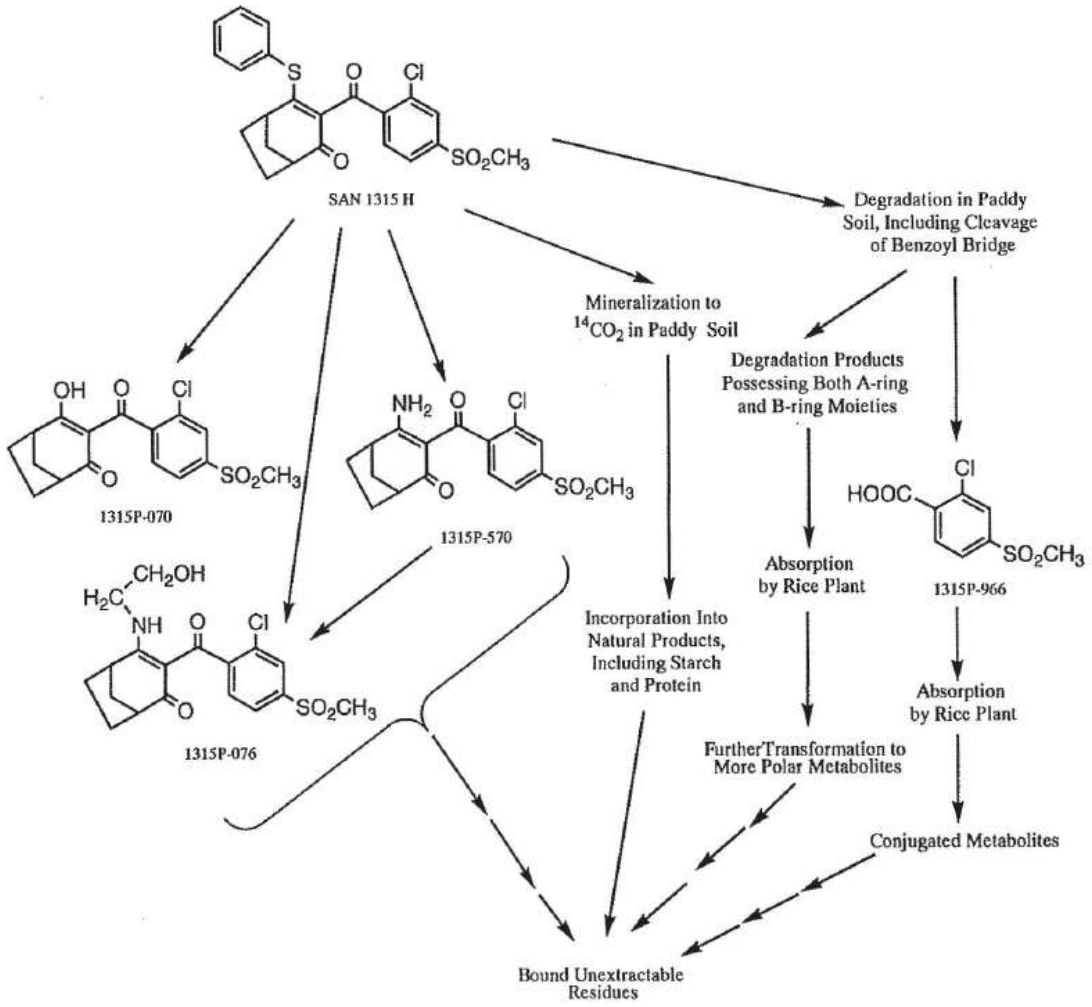


Figure 6: Proposed metabolic pathway of Benzobicyclon in rice

SAN 1315H: Benzobicyclon

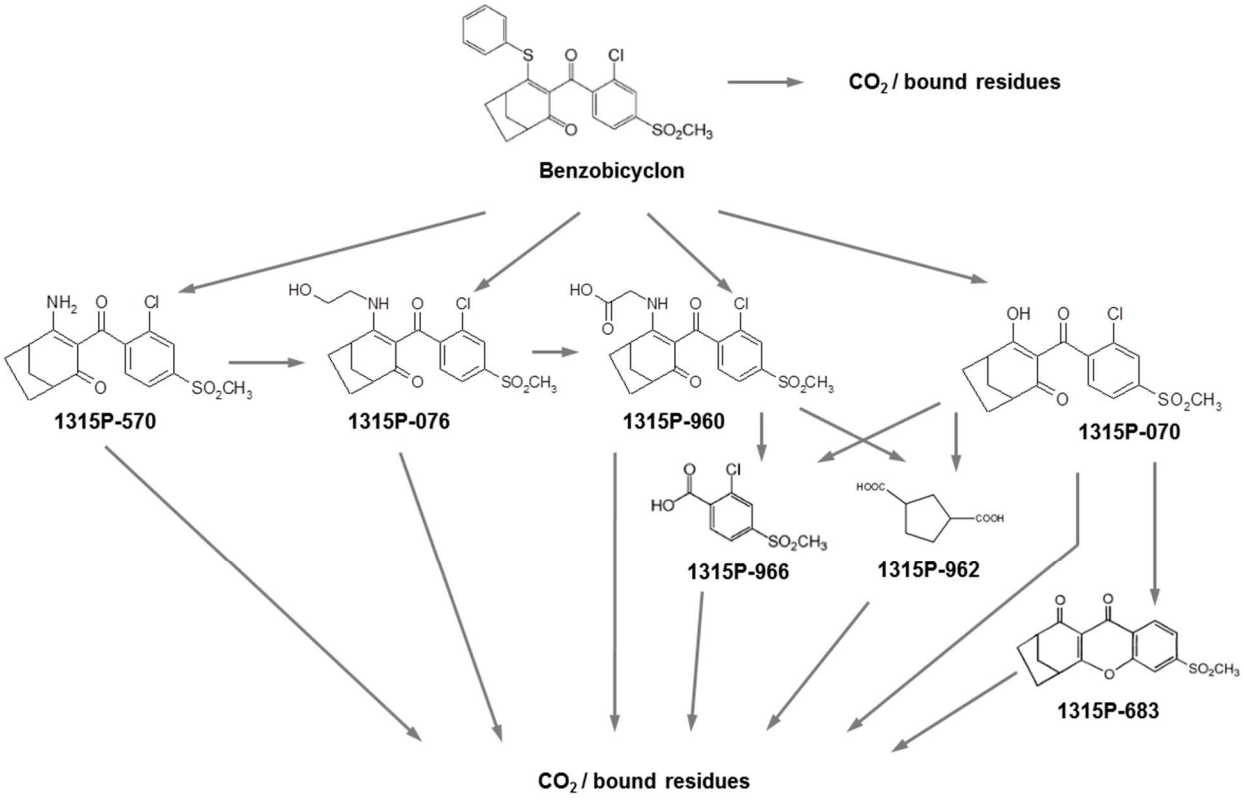


Figure 7 Proposed metabolic pathway for Benzobicyclon in soil

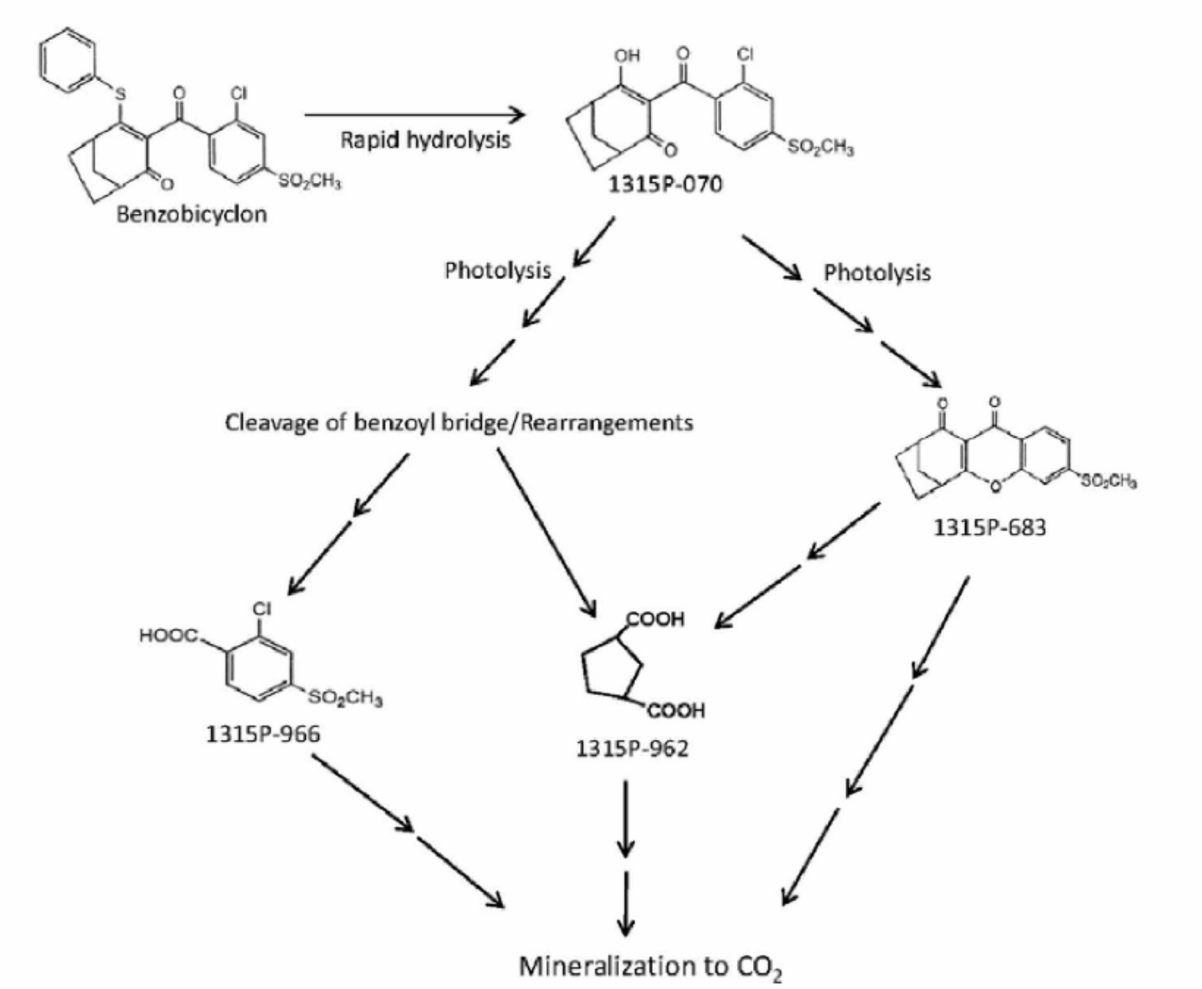
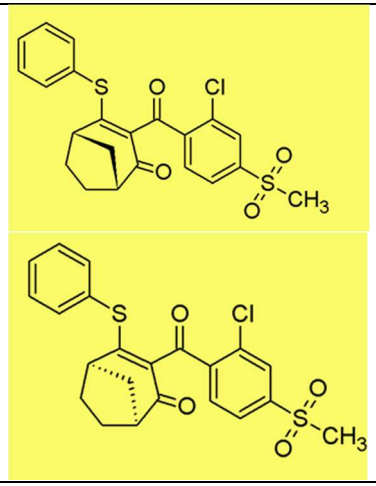
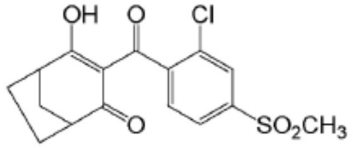
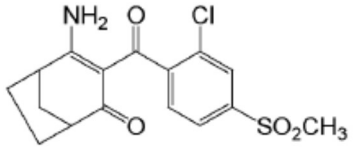
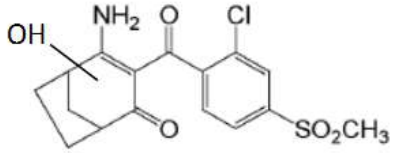


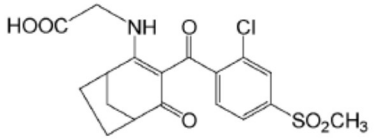
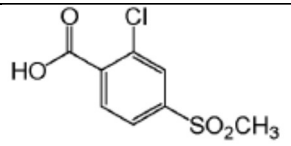
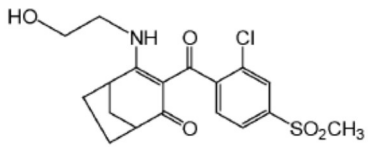
Figure 8: **Proposed metabolic pathway for Benzobicyclon and 1315P-070 in aqueous environments**

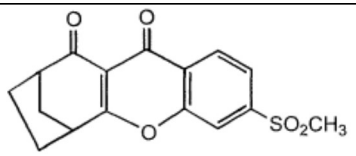
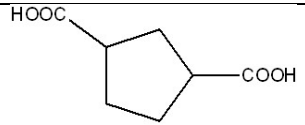
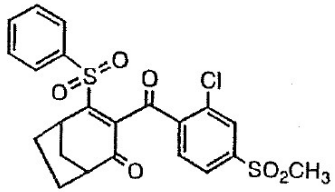
APPENDIX 2: Substances structures, codes, synonyms of the active components of Benzobicyclon and metabolites

Code Number (Synonyms) That indicated in bold was used in the summary dossier	**(IUPAC name /SMILES notation)**	**Structural formula**	**Compound found (level):**
Benzobicyclon	IUPAC name: (1*RS*,5*RS*)-3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one; CAS name: (1*RS*,5*RS*)-3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio)bicyclo[3.2.1]oct-3-en-2-one; CAS Reg, No: 156963-66-5 SMILES code: CS(=O)(=O)c1ccc(C(=O)C2=C(Sc3ccccc3)C3CCC(C3)C2=O)c(Cl)c1		**Rat:** – Faeces: 66.8 – 85.6 % of orally applied dose – Not present in urine or bile **Crop:** – Rice: immature plant/foliage (≤ 0.9%), rice grain (≤ 0.5% TRR)
1315P-070 Metabolite B U9 B16 F11	IUPAC name: 3-(2-chloro-4-methylsulfonylbenzoyl)bicyclo[3.2.1]octane-2,4-dione CAS Reg, No: 126656-88-0 SMILES code: CS(=O)(=O)c1ccc(C(=O)C2=C(O)C3CCC(C3)C2=O)c(Cl)c1		**Rat**: - Urine: 0.1 – 0.4 % and 1.5 - 3.9 % of the dose applied via oral and *i.v.* route, respectively - Bile: 0.1 – 0.8 % of oral dose applied - Faeces: 0.3 – 1.0 % of oral applie - d dose (combined with 1315P-570 and 1315P-570-OH) **Crop:** – Rice: immature plant/foliage (≤ 8.6%), straw (≤ 12.5% TRR), rice grain (≤ 0.6% TRR) **Soil:** - Max. total system: 16.9% (field study) after 0-1 h

Code Number (Synonyms) That indicated in bold was used in the summary dossier	(IUPAC name /SMILES notation)	Structural formula	Compound found (level):
			- Max.: 25.4% (lab. study non flooded, aerobic) at day 58 - Max. total system: 40.1% (lab. study flooded aerobic) at day 14 - Max. total system: 48.9% (lab. study flooded anaerobic) at day 78 **Photolysis in soil:** - Max. 4.3 % AR at day 12 **Aqueous photolysis:** - Max. 58.93 % at day 2 **Aerobic mineralization in surface water:** - Low dose (10 µl): 95.9% at day 14 - High dose (100 µl): 90.6 % at day 14 **Hydrolysis:** - pH 4: 104.05 % at day 10 (25°C) - pH 7: 104.85% at day 10 (25°C) - pH 9: 104.86 % at day 2 (25°C) **Water/sediment:** - Max. 88.7 % at day 6 (total system)

Code Number (Synonyms) That indicated in bold was used in the summary dossier	**(IUPAC name /SMILES notation)**	**Structural formula**	**Compound found (level):**
1315P-570 U11 B18 F13	IUPAC name: 4-amino-3-[2-chloro-4-(methylsulfonyl)benzoyl]bicyclo[3.2.1]oct-3-en-2-one (3-(2-chloro-4-methylsulfonylbenzoyl)- 4-amino)bicyclo [3.2.1]oct-3-en-2-one SMILES code: CS(=O)(=O)c1ccc(C(=O)C2=C(N)C3CCC(C3)C2=O)c(Cl)c1		**Rat:** - Urine: < 0.1 – 0.3 % and 0 - 0.8 % of the dose applied via oral and *i.v.* route, respectively - Bile: 0 – 0.5 % of oral dose applied - Faeces: 0.3 – 1.0 % of oral applied dose (combined with 1315P-570 and 1315P-570-OH) **Crop:** - Rice: immature plant/foliage (≤ 11.6%), straw (≤ 19.4% TRR), hulls (≤ 40.0% TRR), rice grain (≤ 1.1% TRR) **Soil:** - Max. total system: 0.7 % (field study) at day 7 - Max.: 10.1% (lab. study non-flooded, aerobic) at day 58 - Max. total system: 56.7% (lab. study flooded, aerobic) at day 120 - Max. total system: 21.7% (lab. study flooded, anaerobic) at day 120 **Photolysis in soil:** - Max. 0.8% AR at day 12 **Aerobic mineralization in surface water:** - Low dose (10 µl): 5.5% at day 35 - High dose (100 µl): 31.5 % at day 35 **Water/sediment**: - Max. 53.10 % at day 30 (total system)
1315P-570-OH U10 B17 F12	Hydroxylated form of 1315P-570		**Rat:** - Urine: 0.1 – 0.4 % and 0.7 - 0.9 % of the dose applied via oral and *i.v.* route, respectively - Bile: 0 – 0.6 % of oral dose applied - Faeces: 0.3 – 1.0 % of oral applied dose (combined with 1315P-570 and 1315P-570-OH)

Code Number (Synonyms) That indicated in bold was used in the summary dossier	(IUPAC name /SMILES notation)	Structural formula	Compound found (level):
1315P-960	IUPAC name: (4-(carboxymethyl)amino)-3-(2-chloro-4-methylsulfonylbenzoyl)-bicyclo [3.2.1]oct-3-en-2-one SMILES code: CS(=O)(=O)c1ccc(C(=O)C2=C(NCC(O)=O)C3CCC(C3)C2=O)c(Cl)c1		**Soil:** - Max. total system: 0.4% (field study) at day 3 - Max.: 5.0% (lab. study non-flooded, aerobic) at day 120 - Max. total system: 13.6% (lab. study flooded, aerobic) at day 78 **Water/sediment:** - Max.: 11.1 % at day 7 (total system)
1315P-966 CMSBA TTR CMSBA CMBA U6	IUPAC name: 2-chloro-4-methylsulfonyl benzoic acid SMILES code: CS(=O)(=O)c1ccc(C(O)=O)c(Cl)c1		**Rat:** - Urine: 0 – 0.4 % and 5.4 % of the dose applied via oral and *i.v.* route, respectively **Crop:** – Rice: immature plant/foliage (≤ 41.1%), straw (≤ 23.1% TRR), hulls (≤ 5.9% TRR) **Soil:** - Max. total system: 9.3% (field study) at day 0 - Max.: 20.8% (lab. study non-flooded, aerobic) at day 120 - Max. total system: 4.5% (lab. study flooded, aerobic) at day 120 **Photolysis in soil:** - Max.: 2.5% at day 16 **Aerobic mineralization in surface water:** 36.25 % at day 4 **Water/sediment:** - Max.: 2.3% at day 100 (total system)
1315P-076	IUPAC name: 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(2-hydroxyethylamino) bicyclo [3.2.1]oct-3-en-2-one		**Crop:** – Rice: immature plant/foliage (≤ 4.0%) **Soil:** - Max. total system: 1.1% (field study) after 6h

Code Number (Synonyms) That indicated in bold was used in the summary dossier	(IUPAC name /SMILES notation)	Structural formula	Compound found (level):
			- Max. total system: 7.4% (lab. study flooded anaerobic) at day 120 - Max. total system: 6.7 % (lab. study flooded aerobic) at day 30
1315P-683	IUPAC name: 3,4-dihydro-2,4-ethylene-6-methylsufonyl-1,9(2H)-xanthendione SMILES code: CS(=O)(=O)c1ccc2c(c1)OC1C3CCC(C3)C(=O)C=1C2=O		**Soil:** - Max. total system: 1.2 % (field study) at day 3 - Max. total system: 4.8% (lab. study non-flooded aerobic) at day 58 - Max. total system: 0.5 % (lab. study flooded anaerobic) at day 78) **Photolysis:** - Max. 5.6% (lab. study) at day 16 **Aqueous photolysis:** - Max: 13.63 % at day 2
1315P-962	IUPAC name: cyclopentane-1,3-dicarboxylic acid 1,3-cis-cyclopentanedicarboxylic acid SMILES code: COCC1CCC(C(O)=O)C1		**Soil:** - Max. total system: 0.2 % (field study) at day 1 **Aqueous photolysis:** - Max.: 39.25 % at day 11
1315P-168	IUPAC name: 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylsulfonyl)bicyclo[3,2,1]oct-3-en-2-one		**Soil:** - Max. total system: 5.8% (lab. study flooded aerobic) at day 30 - Max. total system: 0.6% (lab. study flooded anaerobic) at day 78

APPENDIX 3: Guidance documents used in this assessment

General

1. EFSA (2019): Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances. EFSA supporting publication 2019:EN-1612. 49 pp.
2. EFSA Guidance (2011): Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009. 49pp.

Section identity, physical chemical and analytical methods

Section physico chemical properties

1. Manual on development and use of FAO and WHO specifications for pesticides - third revision of the First Edition, WHO, Rome 2016
2. Chemicals Regulation Directorate, DATA REQUIREMENTS HANDBOOK, (Version 2.2, June 2012)
3. Technical monograph N°17, 2nd edition, Guidelines for Specifying the Shelf Life of Plant Protection Products, June 2009
4. Evaluation Manual for the Authorisation of plant protection products and biocides according to Regulation (EC) No 1107/2009, EU part, Plant Protection Products, Chapter 2 Physical and chemical properties, version 2.0; January 2014, Board
5. Guidance ST/SG/AC 10/11/Rev.5 for the safety properties
6. CLP regulation 1272/2008
7. Regulation (UE) N°283/2013 (1st March 2013) setting out data requirements for active substances, in accordance with regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market
8. Regulation (UE) N°284/2013 (1st March 2013) setting out data requirements for plant protection products, in accordance with regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

Section analytical methods

1. SANCO/3030/99 rev.4: Technical Material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414
2. SANCO/3029/99 rev .4: Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, section 4) and Annex III (part A, Section 5) of directive 91/414
3. SANCO/825/00 rev.8.1: Guidance document on pesticide residues analytical methods

Section Data on application and efficacy

1. SANCO/2012/11251 rev. 4 [Guidance Document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (the Renewal Regulation)], point 4.6 Substance efficacy

Section Toxicology

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2. EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874, 55 pp., doi:10.2903/j.efsa.2014.3874
3. EFSA (European Food Safety Authority), Buist H, Craig P, Dewhurst I, Hougaard Bennekou S, Kneuer C, Machera K, Pieper C, Court Marques D, Guillot G, Ruffo F and Chiusolo A, 2017. Guidance on dermal absorption. EFSA Journal 2017;15(6):4873, 60 pp. https://doi.org/10.2903/j.efsa.2017.4873

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5. EUROPEAN COMMISSION. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. COMMISSION GUIDANCE DOCUMENT SANTE-10832-2015 rev. 1.7. 24 January 2017.
6. Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003-rev. 10.1 (2012)
7. Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC. Sanco/221/2000-rev.10-final, 25 February 2003.
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10. OECD (2017), Test No. 402: Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070585-en
11. OECD (2009), Test No. 403: Acute Inhalation Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264070608-en
12. OECD (2015), Test No. 404: Acute Dermal Irritation/Corrosion, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264242678-en.
13. OECD (2017), Test No. 405: Acute Eye Irritation/Corrosion, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264185333-en.
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14. OECD guidance document No 73 on residue in livestock (Sep, 2013).
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16. Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue levels in honey (SANTE/11956/2016 rev. 9)
17. EU guidance document "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 10.3, June 2017).
18. EU guidance document "Calculation of Maximum Residue Levels and Safety Intervals e.g. Pre-harvest Intervals" (SANCO 7039/VI/95, 22/7/1997)
19. EFSA calculation model Pesticide Residue Intake Model "PRIMo" - revision 3.1.
20. OECD, 2015, OECD MRL calculator

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None

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None

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