Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

benthiavalicarb-isopropyl (ISO); isopropyl [(*S*)-1-{[(*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]carbamate

EC Number: -
CAS Number: 177406-68-7

CLH-O-0000007106-79-01/F

Adopted

18 March 2022

Corrigendum: new version (replacing the previous version published on 10 June) in which some editorial changes have been made on page 71.

18 March 2022

CLH-O-0000007106-79-01/F

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

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

Chemical name: **benthiavalicarb-isopropyl (ISO); isopropyl [(*S*)-1-{[(*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]carbamate**

EC Number: **-**

CAS Number: **177406-68-7**

The proposal was submitted by **Poland** and received by RAC on **20 January 2021.**

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

PROCESS FOR ADOPTION OF THE OPINION

Poland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **8 February 2021**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **9 April 2021**.

ADOPTION OF THE OPINION OF RAC

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

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

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2022** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	benthiavalicarb-isopropyl (ISO); isopropyl [(*S*)-1-{[(*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]carbamate	-	177406-68-7	Carc. 2 Skin Sens. 1 Aquatic Chronic 2	H351 H317 H411	GHS08 GHS07 GHS09 Wng	H351 H317 H411			
RAC opinion	TBD	benthiavalicarb-isopropyl (ISO); isopropyl [(*S*)-1-{[(*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]carbamate	-	177406-68-7	Carc. 1B Repr. 2 Skin Sens. 1 Aquatic Chronic 2	H350 H361fd H317 H411	GHS08 GHS07 GHS09 Dgr	H350 H361fd H317 H411			
Resulting Annex VI entry if agreed by COM	TBD	benthiavalicarb-isopropyl (ISO); isopropyl [(*S*)-1-{[(*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]carbamate	-	177406-68-7	Carc. 1B Repr. 2 Skin Sens. 1 Aquatic Chronic 2	H350 H361fd H317 H411	GHS08 GHS07 GHS09 Dgr	H350 H361fd H317 H411			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance is currently not listed in Annex VI of Regulation (EC) No 1272/2008.

Substance identity

The producer of benthiavalicarb-isopropyl developed the code number KIF-230R-L, which describes the ratio of the active optical isomers present (the exact ratio is marked as confidential information).

Vehicle Alembicol D (fractionated coconut oil:

In most of the available toxicological studies, Alembicol D was used as a vehicle. Given the lipophilicity of benthiavalicarb-isopropyl (log Kow of 2.6) the use of this vehicle is considered acceptable.

Relevant information on toxicokinetics

Benthiavalicarb-isopropyl's toxicokinetic properties were investigated in two rat studies according to EPA OCSPP Guideline No 870.7845, OECD TG 417, EC B.36 and EPA OCSPP Guideline No 870.7845, OECD TG 417, EC B.32 (presented in the CLH report). The results indicate that the substance is rapidly and efficiently absorbed at low doses (5 mg/kg bw/d: 89-97% within < 48 h), but less efficient at high dose (400 mg/kg bw/d: 41-54%). It is widely distributed and extensively metabolised at low dose, while at high dose it is less metabolised. While one study reports rapid excretion (within 48 h: 73-81% (low dose) and 80-86% (high dose); means (48 h): 12% urine/cage wash, 65% faeces; evidence of biliary excretion, enterohepatic circulation) the second study indicated some potential for accumulation (apparent accumulation after repeated administration, most probably due to recruitment of valine).

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosives

A study in accordance with EEC 440/2008 A.14 was performed. Mechanical sensitivity to shock (BAM fall hammer) and to friction (BAM friction test) as well as thermal sensitivity (Koenen steel tube test) was tested. All tests performed were negative. EEC method A.14 is not completely in line with the CLP criteria; however, the results are sufficient for classification. Moreover, the analysis of the chemical structure shows that there are no chemical groups present in the molecule, which are generally associated with explosive properties. As a conclusion, the dossier submitter (DS) proposed no classification of benthiavalicarb-isopropyl.

Flammable gases

The hazard class is not applicable (benthiavalicarb-isopropyl is a solid and not a gas according to the definitions laid down in Annex I, Part 1, 1.0, CLP Regulation).

Oxidising gases

The hazard class is not applicable (benthiavalicarb-isopropyl is a solid and not a gas according to the definitions laid down in Annex I, Part 1, 1.0, CLP Regulation).

Gases under pressure

The hazard class is not applicable (benthiavalicarb-isopropyl is a solid and not a gas according to the definitions laid down in Annex I, Part 1, 1.0, CLP Regulation).

Flammable liquids

The hazard class is not applicable (benthiavalicarb-isopropyl is a solid and not a liquid according to the definitions laid down in Annex I, Part 1, 1.0, CLP Regulation).

Flammable solids

A study in accordance with EEC 440/2008 A.10 was performed. Benthiavalicarb-isopropyl melted, ignited and extinguished after 15 seconds failing to propagate combustion. According to this test, the substance is not highly flammable. The CLP Regulation refers to UN RTDG test N.1 for the purposes of classification. However, the result "not highly flammable" complies with the ECHA 'Guidance on information requirements and chemical safety assessment' (R.7.1.10.3), wherein it is stated that data from an A.10 test method indicate that classification as a flammable solid does not apply (result: not highly flammable), no more testing is necessary. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Self-reactive substances

As a result of the screening procedure there were no chemical groups identified that are generally associated with explosive or self-reactive properties. This is in accordance with the CLP criteria set out in Annex I, 2.8.4.2, CLP Regulation. Additionally, the BAM fallhammer and friction test give some information on decomposition. Only after the friction test, a black mark on the porcelain plate was observed as a sign for decomposition. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Pyrophoric liquids

The hazard class is not applicable (benthiavalicarb-isopropyl is a solid and not a liquid according to the definitions laid down in Annex I, Part 1, 1.0, CLP Regulation).

Pyrophoric solids

Experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e., the substance is known to be stable at room temperature for prolonged periods of time (days)). This is in accordance with the CLP criteria set out in Annex I, 2.10.4.1, CLP Regulation. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Self-heating substances

A study in accordance with EEC 440/2008 A.16 was performed. No relative self-ignition up to a temperature of 179 °C was observed. This test is not in accordance with the CLP criteria as set out in Annex I, 2.11, CLP Regulation. Additional considerations on the melting point can be taken into account. There are two melting points (153.1 °C and 169.5 °C) due to polymorphism of benthiavalicarb-isopropyl. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Substances which in contact with water emit flammable gases

Benthiavalicarb-isopropyl came in contact with water in several studies conducted for this dossier. There was no report of violent reaction and emission of gas. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Oxidising liquids

The hazard class is not applicable (benthiavalicarb-isopropyl is a solid and not a liquid according to the definitions laid down in Annex I, Part 1, 1.0, CLP Regulation).

Oxidising solids

A study in accordance with EEC 440/2008 A.17 was performed. In this study barium nitrate is used as a reference oxidiser instead of potassium bromate or calcium peroxide as described in the UN RTDG tests O.1 or O.3, to which the CLP criteria refer as set out in Annex I, 2.1.4, CLP Regulation. Therefore, a conclusion on the need for classification under the CLP Regulation cannot be made. However, the results of the measured burning times indicate that no classification is warranted under the CLP Regulation. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Organic peroxides

The hazard class is not applicable (benthiavalicarb-isopropyl is not an organic peroxide with a bivalent O-O structure).

Corrosive to metals

The recommended test C.1 according to UN RTDG is only applicable to liquids and solids that may become liquids (solids with melting points up to 55 °C). As the melting points of benthiavalicarb-isopropyl are above 150 °C, testing is not feasible. This is in accordance with the ECHA 'Guidance on the Application of the CLP Criteria' (CLP guidance) in chapter 2.16.4.1. As a conclusion, the DS proposed no classification of benthiavalicarb-isopropyl.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Explosive

The first step is to assess whether chemical groups that are commonly associated with explosive properties are present in the molecule. No groups that are listed in table A6.1 of the UN RTDG are present.

The CLP Regulation refers to the UN RTDG (UN Recommendations on the Transport of Dangerous Goods; Manual of Tests and Criteria) test series 1 to 8 in part I for screening and classification purposes of explosives. Benthiavalicarb-isopropyl was tested according to EC method A.14, which is not totally in line with the procedure described in the UN RTDG. However, the results are negative regarding mechanical (impact and friction) and thermal (heating under confinement) sensitivity.

In conclusion, in line with the DS, RAC considered that there are sufficient data to conclude that **benthiavalicarb-isopropyl does not warrant classification for Explosive properties**.

Flammable solids

In line with the DS, RAC concluded that **benthiavalicarb-isopropyl does not warrant classification for Flammable solids**.

Self-reactive substances

In line with the DS, RAC concluded that **benthiavalicarb-isopropyl does not warrant classification for Self-reactive substances**.

Pyrophoric solids

In line with the DS, RAC concluded that **benthiavalicarb-isopropyl does not warrant classification for Pyrophoric solids**.

Self-heating substances

This hazard class is only applicable to solids (the surface of liquids is not large enough for reaction with air and the UN test method N.4 is not applicable to liquids). Due to the polymorphism, the substance is not completely molten below 160 °C, which would be an acceptable criterion for non-classification. The CLP guidance gives in chapter 2.11.7.1 some examples of substances, which are classified as self-heating. Therein, it is stated that many organic substances may self-heat and the tendency to self-heat increases with decreasing particle size. No information on the particle size distribution is available. Even though the reactivity profile appears low, the data are not sufficient to conclude on self-heating properties.

The DS' proposal of no classification is not supported. RAC considered that benthiavalicarb-isopropyl **does not warrant classification for self-heating properties due to lack of data**.

Substances which in contact with water emit flammable gases

Additional to the considerations of the DS, benthiavalicarb-isopropyl does not contain metals or metalloids as part of the chemical structure. This screening procedure is in accordance with the CLP criteria as set out in Annex I, 2.12.4.1, CLP Regulation. Therefore, in line with the DS, RAC concluded that benthiavalicarb-isopropyl **does not warrant classification for Substances which in contact with water emit flammable gases**.

Oxidising solids

Additional considerations on the chemical structure show that benthiavalicarb-isopropyl contains oxygen and fluorine only bonded to carbon or hydrogen atoms. This screening procedure is in accordance with the CLP criteria as set out in Annex I, 2.14.4.1, CLP Regulation. For this reason, RAC agreed with the DS that **benthiavalicarb-isopropyl does not warrant classification for Oxidising solids**.

Corrosive to metals

RAC agreed with the DS' reasoning but amends the overall conclusion to **conclusive but not sufficient for classification for Corrosive to metals**.

In conclusion, RAC agreed that **no classification for any of the physical hazards is warranted**. Furthermore, RAC agrees with the DS that only the hazard classes applicable to solid substances are relevant for benthiavalicarb-isopropyl.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity – oral route

Benthiavalicarb-isopropyl was tested in two oral acute toxicity studies, one in rat (Anonymous 3, 1998b) and one in mice (Anonymous 4, 1998a). Both were conducted according to OECD TG 401 and GLP.

***Table**: Acute oral toxicity studies*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute oral toxicity in rats Compliant with test method B.1 of directive 92/69/EEC GLP	Slc: Wistar, SPF rats 5/sex/dose	KIF-230, purity: 88.8% as KIF-230R-L	5000 mg a.s./kg bw, single exposure gavage	> 5000 mg/kg bw (male and female)	Anonymous 3, 1998b Exp. No 4062, DRAR Vol. 3 CA, B.6.2.1
Acute oral toxicity in mice Compliant with test method B.1 of directive 92/69/EEC OECD TG 401 GLP	Slc: ICR (SPF) mice 5/sex/dose	KIF-230, purity: 88.8% as KIF-230R-L	5000 mg a.s./kg bw, single gavage exposure	> 5000 mg/kg bw (male and female)	Anonymous 4, 1998a Report No.: 4061, DRAR Vol. 3 CA, B.6.2.1

a.s. = active substance

No mortality was observed in either study. There were no clinical signs, and no effects on body weight or at necropsy.

The resulting LD_{50} values were both above 5000 mg/kg bw, exceeding the upper limit value for classification for acute toxicity via the oral route, i.e., 2000 mg/kg bw.

The DS concluded that benthiavalicarb-isopropyl does not warrant classification for acute oral toxicity.

Acute toxicity – dermal route

Benthiavalicarb-isopropyl was tested in a single dermal acute toxicity study in rat (Anonymous 5, 1998c) conducted according to OECD TG 402 and GLP.

***Table**: Acute dermal toxicity study*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
Acute dermal toxicity in rats Guidelines No. 82-1, EPA OCSPP & No 870.1200 OECD TG 402 GLP	Slc: Wistar (SPF) rats 5/sex/dose	KIF-230, purity: 88.8% as KIF-230R-L	2000 mg a.s./kg bw 0.0145-0199 g/cm² Dermal occlusive application Duration: 24 h	> 2000 mg/kg bw (male and female)	Anonymous 5, 1998c Report No.: 4063, DRAR Vol.3 CA, B.6.2.2

No mortality was observed and there were no clinical signs, nor any findings at necropsy.

The resulting LD_{50} value was above 2000 mg/kg bw, exceeding the upper limit value for classification for acute toxicity via the dermal route, i.e., 2000 mg/kg bw.

The DS concluded that benthiavalicarb-isopropyl should not be classified for acute dermal toxicity.

Acute toxicity – inhalation route

Benthiavalicarb-isopropyl was tested in a single inhalation acute toxicity study in rat (Anonymous 6, 2000a), conducted according to OECD TG 403 and GLP.

***Table:** Acute inhalation toxicity study*

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LC_{50}**	**Reference**
Acute inhalation toxicity in rats Guidelines No. 81-3, EPA OCSPP & No 870.1300 OECD TG 403 GLP	Charles River Crl:CD® IGS BR rats 5/sex/dose	KIF-230, purity: 89.1% as KIF-230R-L Micronized dust aerosol MMAD= 3.9± 2.82 µm	Nominal: 19 mg/L Mean actual exposure concentration: 4.6±0.6 mg a.s./L Duration: 4.0 h Inhalation rate: 7 L/h Mean pre-exposure bw: 263g (males; M) and 227g (females; F)	> 4.6 mg/L (male and female)	Anonymous 6, 2000a Report No.: WIL-156011, DRAR Vol. 3CA, B.6.2.3

One female and one male died on day 1. The clinical signs in the deceased rats were laboured respiration, rales, gasping, hypoactivity and clear lacrimation. The clinical signs in the survivors were also laboured respiration and rales and in addition dried red material around nose/eyes/forelimbs, dried yellow material in urogenital area and decreased/mucoid faeces. Effects on body weight were seen between day 0 and 3 and day 0 and 7. Necropsy from the

deceased rats showed dark red adrenals (in both sexes), dark patchy lungs (female) and gas-filled stomach (male), while in the survivors there were no relevant findings, except dark red/mottled lungs in one female.

The resulting LC_{50} was indicated to be above 4.6 ± 0.6 mg/L, the only concentration tested, at which 1 out of 5 males and 1 out of 5 females died, i.e., equivalent to the LC_{20}. The lowest classification category for acute inhalation toxicity is between 1 mg/L (excluded) and 5 mg/L (included). This indicates that the tested concentration did not reach to the upper cut-off value for classification. The DS concluded, however, that as this value, where only 20% of the animals died, is very close to the upper limit for classification, it is likely that the LC_{50} lies above 5 mg/L.

The DS concluded that benthiavalicarb-isopropyl does not warrant classification for acute inhalation toxicity.

Comments received during consultation

One Member State Competent Authority (MSCA) supported no classification for acute toxicity.

Assessment and comparison with the classification criteria

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

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

Summary of the Dossier Submitter's proposal

Relevant studies with benthiavalicarb-isopropyl for this hazard class include two acute oral studies, one acute dermal study and one acute inhalation study, as well as an acute oral neurotoxicity study in rats (Anonymous 38, 2001; Anonymous 39, 2002).

The acute toxicity studies are presented in the section on acute toxicity. No effects relevant for classification as STOT SE were reported in these studies.

***Table**: Acute neurotoxicity study*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Acute oral neurotoxicity study in rats OPPTS Guideline 870.6200 OECD TG 424 Sprague Dawley Crl:CD® (SD)IGS BR rats 5/sex/dose	KIF-230, purity: 92.3% as KIF-230R-L 2000 mg/kg bw, single oral gavage	NOAEL = 2000 mg/kg bw LOAEL > 2000 mg/kg bw Functional Observational Battery (FOB): Statistically significant decreased motor activity in treated M compared to control animals on day 1 of treatment. Value (1184, -42%, $p < 0.05$) below the historical control data (HCD) range (2047-3902) of the performing laboratory (Springborn laboratories, Inc.), but study control value (2047) was also at the lower end. The decrease of motor activity in M on day 1 was not observed at later stages and was concluded as not toxicologically relevant. There was no statistically significant difference for F between control and treated group.	Anonymous 38 and 39, 2001 and 2002 - amended final report DRAR Report no. 3404.12, Vol. 3CA, B.6.7

In order to be classified as a substance targeting a specific organ after single exposure, the significant non-lethal toxic effect should be observable on a specific organ at a certain level. Depending on the level of toxic effect, a substance can either be in Category 1 (guidance value for classification: ≤ 300 mg/kg bw) or Category 2 (guidance value for classification: > 300 mg/kg bw and ≤ 2000 mg/kg bw).

The DS concluded that no such effects were seen in the acute toxicity studies via oral, dermal and inhalation route (see section on acute toxicity) and the slight decrease in motor activity observed in male rats in the acute neurotoxicity study after single exposure to 2000 mg/kg bw is not considered to fulfil these criteria.

Based on this, the DS concluded that benthiavalicarb-isopropyl does not have a significant toxic effect on any specific organ and should therefore not be classified as STOT SE 1 or 2.

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

Comments received during consultation

One MSCA supported no classification for STOT SE.

Assessment and comparison with the classification criteria

RAC discussed the relevance of the results from the acute toxicity studies. While no relevant toxicological findings were reported after acute oral (Anonymous 3, 1998b; Anonymous 4, 1998a) or acute dermal (Anonymous 5, 1998c) application of benthiavalicarb-isopropyl, toxicity was observed after acute inhalation exposure (Anonymous 6, 2000a). After 4 h exposure to 4.6 mg/L, 2 out of 10 animals (1 male and 1 female) died on day 1 (LC_{20}). Lethality is not covered under STOT SE, but under acute toxicity. However, the LC_{50} for benthiavalicarb-isopropyl is higher than 4.6 mg/L and most likely above the upper cut-off for classification for acute inhalation toxicity (5 mg/L). In the deceased rats, laboured respiration, rales, gasping, hypoactivity and clear lacrimation were reported, and upon necropsy, dark red adrenals, dark patchy lungs (females) and gas filled stomach (males) were seen. Laboured respiration, rales, dried red material around nose/eyes/forelimbs, dried yellow material in the urogenital area and decreased/mucoid faeces were also seen in surviving animals. Necropsy showed no relevant findings in the survivors. In conclusion, there were general signs of toxicity in all animals which were more severe in those animals that died later on, but no specific toxicity was observed. Overall, it was concluded that the observed effects did not support classification as STOT SE.

RAC agreed with the DS and concluded that **no classification for STOT SE is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Benthiavalicarb-isopropyl was tested in in a single study (Anonymous 7, 1999) according to Guideline No. 81-5, EPA OCSPP & No. 870.2500 and GLP.

***Table**: Skin irritation study*

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
Guidelines No. 81-5, EPA OCSPP & No 870.2500 GLP	Crl: NZW rabbits 6 M/ dose	KIF-230, purity: 87.9% as KIF-230R-L moistened with 500 µL water	0.5 g/ 10 cm^2 4 h	Average score for each animal (mean of 24, 48, 72 h observations): Score erythema: 0, 0, 0, 0, 0, 0 Score oedema: 0, 0, 0, 0, 0, 0	Anonymous 7, 1999 KCI Doc No. 198/993612/SE, DRAR Vol. 3 CA, B.6.2.4

None of the 6 rabbits tested showed any skin reaction at any time point. The DS concluded that benthiavalicarb-isopropyl does not fulfil the criteria for classification as skin irritant under the CLP Regulation and proposed no classification.

Comments received during consultation

One MSCA supported no classification for skin irritation.

Assessment and comparison with the classification criteria

RAC agreed with the DS and concluded that **no classification for skin irritation is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Benthiavalicarb-isopropyl was tested in a single study (Anonymous 8, 2000) according to FIFRA Guidelines No. 81-5, EPA OCSPP & No. 870.2400 and GLP.

***Table**: Eye irritation study*

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
Eye irritation study in the rabbit FIFRA Guidelines No. 81-5, EPA OCSPP & No 870.2400 GLP	Crl: Kbl©BR rabbits 6 M/ dose	KIF-230, purity: 96% as KIF-230R-L	solid form, 0.061 g, corresponding to 0.1 mL of undiluted a.s. one application	Average score for each animal (mean of 24, 48, 72 h observations): Corneal opacity: 0, 0, 0, 0, 0, 0 Iritis: 0, 0, 0, 0, 0, 0 Conjunctival redness: 0.3, 1.6, 1.3, 0.6, 0.3, 0.6 Conjunctival chemosis: 0, 0, 0, 0, 0, 0 All signs were reversible within 4 days	Anonymous 8, 2000 KCI Doc No. 199/993939/SE, DRAR Vol. 3 CA, B.6.2.5

Screening study – rinsed eye

One animal was treated in advance of the others. The treated eye was rinsed with distilled water 30 seconds after instillation lasting 30 seconds. Responses in rabbit were observed at 1, 24, 48 and 72 h after exposure. Only conjunctiva redness score 1 was observed 1 h and 24 h after instillation. Ocular reactions had resolved by two days after instillation.

Main study – unrinsed eye

All 6 treated rabbits showed positive response for conjunctival redness with a score of 1 to 2 from 1 to 72 h post instillation. Ocular reactions had resolved in all animals by either two or four days after instillation.

No cornea opacity, iridial inflammation or conjunctival chemosis was seen in any animal.

Twenty-four, 48 and 72 h after instillation, mean scores for corneal opacity or iris should be equal or above 1, or conjunctival redness or oedema should be equal or above 2, in 2 out of 3 (4/6) animals for the substance to be classified as eye irritant under the CLP Regulation. In this case, the highest score was 1.6 for conjunctival redness. On that basis the DS concluded that benthiavalicarb-isopropyl should not be classified for eye irritation.

Comments received during consultation

One MSCA supported no classification for eye irritation.

Assessment and comparison with the classification criteria

RAC agreed with the DS and concluded that **no classification for eye irritation is warranted**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Benthiavalicarb-isopropyl has been tested in a Guinea Pig Maximisation Test (GPMT; Anonymous 9, 2000a) and a Buehler Test (Anonymous 10, 2000b), according to OECD TG 406 and GLP.

***Table**: Skin sensitisation studies*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
GPMT EPA OCSPP Guideline No 870.2600 OECD TG 406 GLP	Guinea pigs (Dunkin-Hartley) Treated groups: 20 F/group Control groups: 20 F/group	KIF-230 (87.9% as KIF-230R-L; B.n.° G51-15-162)	Intradermal: 0.25% w/v KIF-230 in (FCA*) + Alembicol D, 3 pairs of injections Topical: 70% w/v KIF-230 in Alembicol D, 48 h Challenge:	Intradermal: slight irritations, comparable to effects in control* Topical: no effect Challenge: see table under RAC assessment	Anonymous 9, 2000a KCI Doc No. 201/993857/SS, DRAR Vol. 3 CA, B.6.2.6

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
			35 and 70% w/v in Alembicol D, 24 h		
Buehler test EPA OCSPP Guideline No 870.2600 GLP	Guinea pigs (Dunkin-Hartley) 20 F/group	KIF-230 (87.9% as KIF-230R-L; B.n.° G51-15-162)	Induction: 70% w/v in Alembicol D, 6 h, at day 1, 8 and 15 Challenge: 40% w/v in Alembicol D, 6 h	Induction: sporadic slight dermal reaction, 3/20 animals Challenge: No effect at 24 and 48 h.	Anonymous 10, 2000b KCI Doc No. 200/002387/SS, DRAR Vol. 3 CA, B.6.2.6

FCA = Freund's Complete Adjuvant

* All sites that were intradermally treated with FCA exerted necrosis in all animals of the control and treated groups, as well as in the positive control trials

Two skin sensitisation studies are available in Guinea pigs, a Magnusson-Kligman Maximisation Test (Anonymous 9, 2000a) and a Buehler test (Anonymous 10, 2000b).

For the **GPMT study** (Anonymous 9, 2000a) adequate exposure concentrations were determined in a preliminary study.

In a first pre-test (intradermal injection, range-finding), benthiavalicarb-isopropyl was injected at 0.1, 0.25, 0.5 and 1.0% w:v in Alembicol D at a volume of 100 µL each. Slight erythema and oedema occurred at 0.25% at 24 and 72 h, while necrosis was seen at higher doses.

In the second pre-test (topical induction, range-finding), benthiavalicarb-isopropyl was applied at 10, 20, 50 and 70% w:v in Alembicol D. No irritation was observed up to and including 70% mixture at 24 and 48 h.

The test facility concluded that a 70% w:v mixture was the maximum practical concentration that would not give rise to irritating effects during the challenge.

As topical treatment with a 70% w:v mixture did not induce any irritation, animals were pre-treated with 10% sodium dodecyl sulphate (SDS) 24 h prior to the topical induction in the main study.

In line with the requirements of the GPMT according to OECD TG 406, animals were treated in three phases:

- intradermal induction on day 1: 20 female Guinea pigs (Dunkin-Hartley) received three pairs of intradermal injections of respectively 100 µL of (i) 50% w:v Freund's Complete Adjuvant (FCA)/water emulsion, (ii) 0.25% w:v benthiavalicarb-isopropyl /Alembicol D, and (iii) 0.25% benthiavalicarb in 50% w:v FCA/Alembicol D. Twenty female control animals were given three pairs of intradermal injections of 100 µL with the concomitant blanks (FCA/water, Alembicol D, 50% w:v FCA/Alembicol D)

- topical induction on day 7: the same animals as above were topically treated with 400 µL of 70% w:v benthiavalicarb-isopropyl in Alembicol D (tests) or Alembicol D (controls) was administered by the occlusive application during 48 h

- challenge on day 22: 200 µL of 35% or 70% w:v benthiavalicarb-isopropyl in Alembicol D was applied on either an anterior or a posterior flank for 24 h

Additionally, 10 female Guinea pigs were treated with hexyl cinnamic aldehyde (HCA, 10% v:v intradermal and topical induction, 50% and 100% v:v challenge).

After intradermal injection, necrosis was observed with injections including FCA, while slight irritation was observed at the other injection sites (0.25% test article in Alembicol D), in both control and test animals. Necrosis was also seen at the sites treated with FCA in positive control animals (HCA).

One day after percutaneous induction (70% test article in Alembicol D), no irritation was observed.

The animals treated with HCA (positive controls) exhibited 100% response after both 24 h and 48 h challenge times as expected.

The challenge with a 70% w:v benthiavalicarb-isopropyl at 48 h elicited a skin response above the level observed in the controls, in more than 30% of the test animals.

The challenge with a 35% w:v benthiavalicarb-isopropyl at 24 and 48 h elicited a skin response in 45 and 50% of animals, respectively, while no effects in any control animals were observed.

In the **Buehler test** (Anonymous 10, 2000b), 20 female Guinea pigs (Dunkin-Hartley) were subject to a topical induction. On days 1, 8 and 15, 500 µL of 40% w:v benthiavalicarb-isopropyl in Alembicol D was applied topically for 6 h. On day 29 (challenge), 500 µL of 70% w:v benthiavalicarb-isopropyl in Alembicol D was applied for 6 h.

In the pre-test (topical induction, range-finding), benthiavalicarb-isopropyl was applied for 6 h at 40, 50, 60 and 70% w:v in Alembicol D. Slight erythema and oedema occurred in animals treated with 60% mixture and above at 24 and 72 h. It was concluded that a 40% w:v mixture was the maximum practical concentration that could be prepared which would not give rise to irritating effects during the challenge. Twenty-four hours after the 3 induction phases, sporadic incidences of slight to well-defined irritation were observed in the animals (induction 1: 1/20, induction 2: 3/20, induction 3: 1/20).

Twenty-four and 48 h after challenge phase, no positive reaction was elicited in either treated or control group.

The animals treated with HCA (positive controls) exhibited 90% and 100% response after 24 and 48 h challenge times, respectively, as expected.

Though the Buehler test (Anonymous 10, 2000b) was negative, the DS proposed to classify benthiavalicarb-isopropyl as skin sensitiser, based on the positive results from the GPMT (Anonymous 9, 2000a) in which more than 30% of the animals induced with benthiavalicarb-isopropyl elicited a skin response above the level observed in the controls. No concentrations ≤ 0.1% benthiavalicarb-isopropyl were applied for intradermal induction; therefore, the DS proposed no sub-categorisation and classification as Skin Sens. 1.

Comments received during consultation

One MSCA agreed with the proposed classification as Skin Sens. 1 without sub-categorisation based on the GPMT study by Anonymous 9 (2000a), based on limited reliability of this study.

The intradermal induction at a concentration of 0.25% benthiavalicarb-isopropyl resulted in 80% and 75% positive animals upon topical challenge with 70% benthiavalicarb-isopropyl after 24 and 48 h, respectively. In contrast, the challenge with 35% benthiavalicarb-isopropyl resulted in less than 60% positive animals, both after 24 and 48 h. According to the CLP Regulation, sub-

category 1A is indicated if ≥ 60% of the animals respond positive intradermal inductions doses of > 0.1% to ≤ 1%.

The MSCA considered the study to be of limited reliability as in several of the control animals a score of 1 was achieved for erythema and oedema. They criticised the approach taken by the study authors and the DS to disregard the animals with score 1 and referred to the CLP guidance (version 5.0, 2017, page 342 Table 3.5) which says that redness (i.e., erythema) with scores ≥ 1 demonstrate significant skin sensitising effects.

They noted the negative result obtained in the Buehler test (Anonymous 10, 2000b).

The DS responded that they considered it relevant that upon challenge exposure to benthiavalicarb-isopropyl, the response was more severe in induced animals compared to non-induced animals, suggesting that benthiavalicarb-isopropyl may cause allergic reactions.

The DS concluded that since more than 30% of the non-induced control animals seemed to be sensitised to the vehicle (Alembicol D), the data are not sufficient for sub-categorisation.

Assessment and comparison with the classification criteria

Two studies investigating the skin sensitising potential of benthiavalicarb-isopropyl, the GPMT (Anonymous 9, 2000a) and the Buehler Test (Anonymous 10, 2000b) are described in detail in the section "Summary of the DS's proposal".

Positive results were seen in the GPMT (Anonymous 9, 2000a). It was conducted according to OECD TG 406 and GLP; however, it has limitations.

First, it is unusual that a relatively high percentage of control animals, that were not induced with benthiavalicarb-isopropyl, had positive results in the topical challenge with 70% benthiavalicarb-isopropyl, which was not observed at a concentration of 35%. The following table lists the number of animals with positive results and the respective scores for the different treatment groups.

***Table**: Score from the GPMT (Anonymous 9, 2000a)*

		Challenge with 35%		**Challenge with 70%**	
		Control	**Test Group**	**Control**	**Test Group**
24 h	**Scores 1 & 2**	0/20 (0%)	9/20 (45%)	14/20 (70%)	16/20 (80%)
	Score 2		4/20 (20%)		8/20 (40%)
48 h	**Scores 1 & 2**	0/20 (0%)	10/20 (50%)	7/20 (35%)	15/20 (75%)
	Score 2		5/20 (25%)		10/20 (50%)

The challenge with 35% did not induce responses in the controls, and the responses in treated animals did not exceed 60% after 24 h nor after 48 h. After challenge with 70% a high percentage of animals of the controls had skin lesions, though not exceeding a score of 1. It should be noted, however, that according to the CLP guidance, erythema ≥ 1 should be regarded as significant skin sensitising effect. A high percentage of induction was also seen in the test group after challenge with 70%, exceeding 60% when score 1 and 2 lesions are considered together. In conclusion, compared to the control, in the test group more animals were affected at both challenge concentrations, the lesions reached a higher score (i.e., score 2) and after 48 h the animals of the treated groups also had a higher incidence of increased thickness, dryness and sloughing of the epidermis.

In their response to comments, the DS indicated that it could be possible that control animals got sensitised to the vehicle Alembicol D. This might be the case; however, this possibility was

not discussed by the study authors. In addition, there is no plausible explanation for the different response in the control animals when treated with either 35% or 70% benthiavalicarb-isopropyl, as the vehicle Alembicol D was present in both approaches.

The second unusual finding in this study was that upon intradermal treatment, all sites treated intradermally with FCA (either in a mixture with water or in a mixture with test material in Alembicol D or Alembicol D alone) exerted necrosis. The same was observed for the positive control animals, for the sites where FCA was applied. It seems that necrotic changes were regularly obtained upon FCA treatment in the conducting laboratory, which is unwanted from an animal welfare perspective and in addition the strong response observed in the animals may also have adversely influenced the test result. In the review of the GPMT procedure, Frankild *et al*. (1996) described that FCA-treated Guinea pigs have a lower skin irritation threshold and that Guinea pigs stressed by concomitant allergic or irritant inflammation remote from the site of skin testing can have a generalised state of hyperreactivity. They further described that an altered state of reactivity in the skin may sometimes lead to questionable challenge results in test animals and, occasionally, non-specific irritant responses in control animals. Though no signs of ill health or toxicity were reported in any of the animals on the study, the observed necrotic changes (i.e., 4 sites per animal) may have rendered the animals in a hyperreactive state. Although skin lesions were observed in control animals challenged with 70% benthiavalicarb-isopropyl, but not at the lower concentration of 35%, it might be that only the higher concentration exceeded the threshold for an unspecific, irritant response in the control animals.

According to the study report, a test animal was considered to show positive evidence of delayed contact hypersensitivity if the observed dermal reaction at challenge was definitely more marked and/or persistent than the maximum reaction seen in animals of the control group.

The study authors concluded that the study was positive and that benthiavalicarb-isopropyl had the potential to cause skin sensitisation.

RAC is of the opinion that the GPMT clearly indicates that benthiavalicarb-isopropyl has skin sensitisation potential.

In the available Buehler study (Anonymous 10, 2000b), which was carried out according to OECD TG 406 and GLP, no dermal reactions were observed after challenge with 40% w/v benthiavalicarb-isopropyl. The DS mentioned in their response to comments that they considered the test not fully reliable, as no skin reactions were seen in the induction phase. RAC was of the view that the Buehler test is less sensitive than the GPMT in general, as no intradermal injections or treatment with FCA is part of the protocol.

RAC further agreed with the DS that the absence of any irritation during the induction phase should be regarded as deficit of the study.

In addition, RAC noted that the information on the study available in the CLH report as well as in the DRAR is rather limited and includes conflicting information on the applied concentrations. In the text it is stated that the topical induction on days 1, 8 and 15 was conducted with 500 µL of 40% w:v benthiavalicarb-isopropyl in Alembicol D, while the challenge was done with 500µL 70% w:v benthiavalicarb-isopropyl in Alembicol D. In contrast, table 19 from the CLH report indicates that a concentration of 70% was used for induction and a concentration of 40% was used for the challenge exposure. The same information is included in the DRAR, and the discrepancy could not be clarified.

In conclusion, the negative result might be due to the lower sensitivity of this method compared to the GPMT and by the absence of irritation during the induction phase, and the provided information does not allow a full evaluation of the study. The result does not invalidate the observations from the GPMT.

In conclusion, RAC was of the opinion that the GPMT clearly indicates that benthiavalicarb-isopropyl induces skin sensitisation; however, based on the deficiencies of the study, mainly that effects were also seen in controls, the criteria for sub-categorisation cannot directly be applied. Therefore, and in line with the DS, RAC concluded that **benthiavalicarb-isopropyl warrants classification as Skin Sens. 1, without sub-categorisation**.

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

Summary of the Dossier Submitter's proposal

***Table**: Repeated dose toxicity studies*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
28-d oral toxicity in dog Range-finding study Not compliant with test method B.7. of Directive 96/54/EEC Beagle dogs 2/sex/dose	KIF-230, purity: 87.7% as KIF-230R-L Oral route 0, 100, 300, 1000 mg a.s./kg bw/d 28 days	NOAEL: 300 mg/kg bw/d LOAEL: 1000 mg/kg bw/d **Organ weight**: increased absolute and relative liver weight without dose-dependency: M: 100 mg/kg bw/d (absolute: 18%; relative: 17%); 1000 mg/kg bw/d (absolute: 16%; relative: 11%) F: 300 mg/kg bw/d (absolute: 14%; relative: 13%); 1000 mg/kg bw/d (absolute: 36%; relative: 45%). No statistical analysis was performed because of the low number of animals per group. **Gross pathology and histopathology**: Liver enlargement in both M and F. Panlobular hepatocyte hypertrophy at 1000 mg/kg bw/d. One F with unilateral kidney atrophy (fibrous scars) in the same dose group.	Anonymous 40, 1998 DRAR Report no. 3390, Vol. 3 CA. B.6.3.1/1
28-d dermal toxicity in rat OECD TG 410* Acceptable Rat, Crl:CD®(SD)IGS BR rats 10/sex/dose	KIF-230, purity: 87.9% as KIF-230R-L Dermal route 0, 100, 300, 1000 mg a.s./kg bw/d 28 days	NOAEL: 300 mg/kg bw/d LOAEL: 1000 mg/kg bw/d **Organ weight**: Dose-related decrease in thymus weight (22, 18 and 13% at 100, 300 and 1000 mg/kg bw/d, resp.) **Gross pathology and histopathology**: Marginal liver cell necrosis at 1000 mg/kg bw/d in M; marginal increased incidence of squamous hyperplasia in F at 1000 mg/kg bw/d (9/10 vs. 5/10 in control)	Anonymous 41, 2000b DRAR Report No.: WIL-156012, Vol. 3 CA 6.3.3

Sub-acute oral neurotoxicity OECD TG 424 Rat, Sprague-Dawley Crl:CD® (SD)IGS BR 10/sex/dose	KIF-230, purity: 92.6% as KIF-230R-L Oral route 0, 200, 2000, 20000 ppm M: 0, 17.7, 174.1, 1853.7 mg a.s./kg bw/d F: 0, 19.3, 185.7, 1845.8 mg a.s./kg bw/d 28 days	NOAEL: M: 174.1 mg/kg bw/d; F: 1845.8 mg/kg bw/d LOAEL: M: 1853.7 mg/kg bw/d; F: >1845.8 mg/kg bw/d **Body weight:** Decreased bw gain at top dose of 20000 ppm in M (18% at the end of the study); food efficiency also decreased (20%). **FOB:** Slight decrease of motor activity (low level) in M at 20000 ppm (25%)	Anonymous 42, 2002 Report no. KCI 207/022387, Vol. 3 CA, B.6.7
90-d oral toxicity in mice Test method B.26 of directive 2001/59/EC Acceptable B6C3F1, SPF mice 10/sex/dose	KIF-230, purity: 87.7% as KIF-230R-L Oral route 0; 50; 200; 7000 and 20000 ppm M: 0, 8.4, 33.0, 1293, 4031 mg a.s./kg bw/d F: 0, 11.3, 45.2, 1620, 4946 mg a.s./kg bw/d 90 days	NOAEL: M: 33.0 mg/kg bw/d; F: 45.2 mg/kg bw/d LOAEL: M: 1293 mg/kg bw/d; F: 1620 mg/kg bw/d **Body weight**: Bw gain, decrease in M: 7000 ppm, by 32%; 20000 ppm, by 43% over the entire treatment period of 13 weeks **Haematology**: Slight macrocytic anaemia (increase in reticulocyte ratio): M & F at 7000 and 20000 ppm; thrombocytosis in M ≥ 7000 ppm **Organ weight**: Liver weight, increase: 7000 ppm: M: absolute 63%, relative 87%, F: absolute 51%, relative 59%; 20000 ppm: M: absolute 97%, relative: 136%, F: absolute: 96%, relative: 109% Ovary weight, decrease: 20000: absolute: 29%, relative: 18% Kidney weight, decrease: 7000 ppm: M: 10%; 20000 ppm: M: 22%, F: 8% **Gross necropsy**: patches on stomach and liver, black and enlarged livers, brown thyroid glands at 7000 and 20000 ppm in M and F **Histopathology**: Liver: anisonucleosis: M ≥ 7000 ppm; fatty change: M at 20000 ppm; hepatocytic hypertrophy: M & F ≥ 7000 ppm; multinucleated giant cells: M at 20000 ppm; necrosis: M ≥ 7000 ppm, F at 20000 ppm; bile duct proliferation: M & F at 20000 ppm Ovaries: decrease in corpora lutea at 20000 ppm **Enzymatic induction** in the hepatocytes in both sexes ≥ 7000 ppm (increased and/or dilated rough endoplasmic reticulum)	Anonymous 43, 1998a, DRAR Report no. 3385, Vol. 3 CA 6.3.2/1

90-d oral toxicity in rats EPA OCSPP Guideline No 870.3100 Acceptable F344/DuCrj (Fisher) rats 10/sex/dose	KIF-230, purity: 87.7% as KIF-230R-L Oral route 0, 50, 200, 5000 and 20000 ppm M: 0, 3.5, 14.1, 353, 1444 mg a.s./kg bw/d F: 0, 3.9, 15.3, 379, 1552 mg a.s./kg bw/d 90 days	NOAEL: M: 14.1 mg/kg bw/d; F: 15.3 mg/kg bw/d LOAEL: M: 353 mg/kg bw/d; F: 379 mg/kg bw/d **Haematology**: Decrease of red blood cell (RBC) parameters ≥ 5000 ppm, both M & F **Blood chemistry**: Total cholesterol, increase: 5000 ppm: M (16%) & F (44%), 20000 ppm: M (30%) F (94%) Free cholesterol, increase: 5000 ppm (36%), 20000 ppm: M (27%) & F (100%) Phospholipids, increase: 5000 ppm: F (21%), 20000 ppm: M (14%) & F (51%) Total protein, increased: M of two top doses & F of top dose α2-globulin, increase: 5000 ppm: M & F (10%), 20000 ppm: M (17%) & F (25%) α1-globulin, increase: 20000 ppm: M (7%) & F (18%) β-globulin, increase: top dose F (10%) Albumin/globulin (A/G) ratio, decreased: 5000 & 20000 ppm: F (~10%) Gamma Glutamyl Transferase (γ-GT) activity, increased: 5000 ppm: M (167%) & F (307%), 20000 ppm: M (416%) & F (573%) **Organ weight**: Liver weight, increase: 5000 ppm: M: absolute 19%, relative 21%, F: absolute, 29%, relative 23%; 20000 ppm: M: absolute 27%, relative 34%, F: absolute 50%, relative 45% Adrenal weight, increase: 5000 ppm: M: absolute 14%, relative - , F: absolute 12%, relative - ; 20000 ppm: M: absolute 19%, relative 21%, F: absolute 14%, relative - Kidney weight, increase: F: 5000 ppm (8%), 20000 ppm (7%). **Gross necropsy**: livers were blackish and enlarged in top dose animals in both M & F **Histopathology**: Hepatocellular hypertrophy in M & F at 20000 ppm; mineralisation in F at 20000 ppm.	Anonymous 44, 1998b DRAR Report no. 3386, Vol. 3 CA 6.3.2/2
90-d oral toxicity in dogs EPA OCSPP Guideline No 870.3150;	KIF-230, purity: 88.8% as KIF-230R-L Oral route 0, 40, 200, 1000 mg a.s./kg bw/d	NOAEL: 40 mg/kg bw/d LOAEL: 200 mg/kg bw/d **Haematology**: Slight but significant decreases of RBC parameters (haematocrit (HCT) and	Anonymous 45, 1999 DRAR Report no. 3812, Vol. 3 CA

corresponding to OECD TG 409 Acceptable Beagle dogs 4/dose/sex	90 days	haemoglobin (Hb)) in F ≥ 200 mg/kg bw/d, in M at 1000 mg/kg bw/d. Platelets were higher in both sexes at top dose. For further details, see table 28 of the CLH report. **Blood chemistry**: Total bilirubin, increase: 1000 mg/kg bw/d: M (125%) & F (120%) ALP, increase: 1000 mg/kg bw/d: M (107%) & F (171%) γ-GT, increase: 1000 mg/kg bw/d: M (93%) & F (72%) Total protein, decrease: 200 mg/kg bw/d: F (10%), 1000 mg/kg bw/d: M (18%) & F (10%). A/G ratio, decrease: 200 mg/kg bw/d: F (27%), 1000 mg/kg bw/d: F (26%). Albumin level, decrease: in F at 40, 200 and 1000 mg/kg bw/d (10, 21 and 24%, resp.) & in M at 1000 mg/kg bw/d (26%). **Organ weight**: Liver weight, increase: 200 mg/kg bw/d: F: relative 43%, 1000 mg/kg bw/d: M: absolute 60%, relative 75%, F: absolute 58%, relative 70% **Gross necropsy and histopathology:** 1000 mg/kg bw/d: large liver, deposition of pigment & hepatocellular hypertrophy in M and F	6.3.2/3
Dog, 1 year US EPA FIFRA Pesticide Assessment Guidelines, Section 83-1, 1984 (OCSPP 870.4100) OECD TG 452 Beagle dogs 4/dose/sex	KIF-230, purity: 87.5 – 87.9% as KIF-230R-L Oral route 0, 4, 40, 400 mg a.s./kg bw a.s./d 1 year	NOAEL: 40 mg/kg bw/d LOAEL: 400 mg/kg bw/d **Blood chemistry:** Some parameters statistically significantly different from control during the test, but at termination, no significant difference at any doses seen **Organ weight**: Liver weight increase: 400 mg/kg bw/d: M: absolute 21%, relative - , F: absolute 28%, relative 18% **Gross pathology and histopathology**: No significant effect, only increase in the incidence of pituitary cysts (slight) in F without a clear dose-response relationship	Anonymous 46, 2001 DRAR Report no. 4551, CA 6.3.2/6
2-year chronic toxicity / oncogenicity study in rats EPA OCSPP Guideline No 870.4300; OECD	KIF-230, purity: 88.8-89.1% as KIF-230R-L 0, 50, 200, 5000, 10000 ppm M: 0, 2.5; 9.9, 249.6, 518.3 mg a.s./kg bw/d	NOAEL: M: 9.9 mg/kg bw/d; F: 12.5 mg/kg bw/d LOAEL: M: 250 mg/kg bw/d; F: 318 mg/kg bw/d **Body weight**: Slight but statistically significant decrease of bw in F at 10000 ppm (4%) and bw	Anonymous 18, 2001a DRAR Report no. 3822, Vol.3 CA, B.6.5/1

TG 453 adopted 8 September 2008 F344/DuCrj (Fischer, SPF) rats 80/sex/group	F: 0, 3.2, 12.5, 318.2, 649.4 mg a.s./kg bw/d 2 years	gain over the whole period of the study (7%) **Haematology**: Slight but statistically significant decrease in blood parameters, except platelet which increased (between 6 to 19%) at the two top doses in both sexes. For further details, see table 28 of the CLH report. **Blood chemistry:** At termination: In females, increase of: total cholesterol in females at 5,000 ppm (67%) and 10,000 ppm (72%), and free cholesterol at 5,000 ppm (70%) and 10,000 ppm (83%) phospholipid at 5,000 ppm (43%) and 10,000 ppm (47%) total protein at 5,000 ppm (6%) and 10,000 ppm (8%) γ-GT activity at 5,000 ppm (167%) and 10,000 ppm (217%) In females, decrease of: total bilirubin at the same doses (50%) AST (44% at 5,000 ppm and 39% at 10,000 ppm) ALT (41% at 5,000 ppm and 40% at 10,000 ppm) In males: only γ-GT activity was increased at termination at 5,000 ppm (31%) and 10,000ppm (164%) For details, see table 28 of the CLH report. **Organ weight**: Liver weight, increase: at all sacrifice times; 5000 ppm, day 104: M: absolute 22%, relative 21%, F: absolute 16%, relative 19%; 10000 ppm, day 104: M: absolute 29%, relative 33%, F: absolute 24%, relative 29% Kidney weight, increase: 5000 ppm, day 104: M: absolute 10%, relative: 7%, F: absolute 5%, relative 8% Adrenal weight, increase: 200 ppm: F: relative 7%; 5000 ppm, day 104: M: absolute & relative 20%, F: relative 14%; 10000 ppm, day 104: M: absolute 16%, relative 20%, F: absolute 10%, relative 14% Spleen weight, decrease: in F at 5000 ppm and 10000 ppm (~20-25%) while relative heart weight (6%) and relative brain weight (4%) were increased at 10000 ppm in F	

		Gross pathology: Liver enlargement in M & F at interim sacrifices (week 26: 10 M and 9 F at 10000 ppm, week 52: 5 M at 5000 ppm, 9 M and 9 F at 10000 ppm, week 78: 5 M and 4 F at 5000 ppm, 8 M and 9 F at 10000 ppm, no enlarged liver in control at any time point) but not at termination in the 5000 and 10000 ppm groups However, white and brown patches in M at 5000 ppm (brown: 36 at 5000 ppm vs. 12 in control; white: 16 at 5000 ppm vs. 5 in control) and 10000 ppm (brown: 34 at 10000 ppm; white: 24 at 10000 ppm) and red patches in F at 5000 (13 at 5000 ppm vs. 5 in control) and 10000 ppm (19 at 10000 ppm) at termination Granular kidney surface in both M & F at 10000 ppm at termination (9 in M and 3 in F at 10000 ppm vs. 0 in control) Black patches in the Harderian gland either in M (8 at 10000 ppm vs. 0 in control) or in F (28 at 5000 ppm and 41 at 10000 ppm vs. 0 in control) Tail nodules detected at the two top doses in M as well **Non-neoplastic histopathology**: Hepatocytic hypertrophy at 10000 ppm in M (24/44 vs. 1/38 in control) and at 5000 (7/46 vs. 1/40 in control) and 10000 ppm (25/45) in F. Fatty degeneration in F (39/42 at 5000 ppm, 36/45 at 10000 ppm vs. 21/40 in control) as well as focal changes, in association with spongiosis hepatis in M (39/46 at 5000 ppm, 42/44 at 10000 ppm vs. 19/38 in control) Glomerulosclerosis in F at 5000 (20/42 vs. 6/40 in control) and 10000 ppm (26/45). Calculus at 5000 and 10000 ppm in M (10/46 at 5000 ppm, 12/44 at 10000 ppm vs. 0/38 in control) and F (17/42 at 5000 ppm, 25/45 at 10000 ppm vs. 8/40 in control). Chronic nephropathy in M at 5000 (23/46 vs. 6/38 in control) and 10000 ppm (26/44). Brown pigment deposition in F at 5000 (19/42 vs. 2/40 in control) and 10000 ppm (19/45), dilated tubules in M at 5000 ppm (26/46 vs. 10/38 in control) and 10000 ppm (33/44). Hyaline droplets in M at 5000 (37/46 vs. 17/38 in control) and 10000 ppm (32/44) and at 10000 ppm in F (19/45 vs. 9/40 in control). Lymphocytic infiltration in F at 5000 and 10000 ppm (18/42 at 5000 ppm, 23/45	

<table>
<tr><td></td><td></td><td>at 10000 ppm vs. 9/40 in control). Fibrosis in M (5 at 10000 ppm vs. 0 in control) and transitional cell hyperplasia in M (10 at 10000 ppm vs. 1 in control) at 10000 ppm
Atrophy of the exocrine pancreas at 10000 ppm in M (22/44 vs. 11/38 in control) and F (15/45 vs. 3/40 in control)
Harderian gland dilatation in M at 10000 ppm (13/44 vs. 2/38 in control) and F at 5000 ppm (40/42 vs. 0/40 in control) and 10000 ppm (45/45)</td><td></td></tr>
<tr><td>2-year oncogenicity study in mice
EPA OCSPP Guideline No 870.4200, OECD TG 451
B6C3F1 mice
70/sex/group</td><td>KIF-230, purity: 88.8-89.1% as KIF-230R-L
0, 20, 100, 2500, 5000 ppm
Corresponding to ca.:
M: 0, 2.7, 13.7, 358, 731 mg a.s./kg bw/d
F: 0, 3.7, 18.6, 459, 928 mg a.s./kg bw/d
2 years</td><td>NOAEL: M: 13.7 mg/kg bw/d; F: 18.6 mg/kg bw/d
LOAEL: M: 358 mg/kg bw/d; F: 459 mg/kg bw/d
Body weight:
Bw gain calculated over the treatment period of 104 weeks statistically significantly decreased in M by 26% and 30% at 2500 and 5000 ppm, resp.
Haematology:
Increase of platelet counts in both M & F at 2500 ppm (week 52: increase of 13% in M; week 104: 19% increase in M and 17% in F) and 5000 ppm (week 52: increase of 25% in M and 16% in F; week 72: increase of 13% in M and 27% in F; week 104: 20% in M and F)
At termination, slight increases in M of haematocrit at 5000 ppm (9%), Hb content at 2500 ppm (5%) and 5000 ppm (8%), RBC count at 5000 ppm (12%)
Slight decrease in M of MCV at 2500 ppm (2%) and at 5000 ppm (3%) and of MCH at 5000 ppm (3%). However, changes in M not coherent with those from earlier sampling times
Statistically significant decreases (M) or increases (F) of WBC at all doses on week 104, not considered meaningful in absence of both a proper dose-related response and confirmative modifications in the differential leukocyte counts
<table>
<tr><th>Week 104</th><th colspan="2">WBC</th></tr>
<tr><th>Dose (ppm)</th><th>M</th><th>F</th></tr>
<tr><td>20</td><td>- 31%**</td><td>+131%*</td></tr>
<tr><td>100</td><td>- 45%**</td><td>- 23%*</td></tr>
</table></td><td>Anonymous 20, 2001b
DRAR Report no. 3823, Vol.3 CA, 6.5/2</td></tr>
</table>

2500	- 35%**	- 8%
5000	- 25%*	+ 54**

Statistically significant difference from control group; *: $p \leq 0.05$ **: $p \leq 0.01$

Organ weight:

Liver weights highly significantly increased at all sacrifice times at 2500 ppm (at termination, absolute: 113 and 67%; relative: 140 and 61%; in M and F, resp.) and 5000 ppm (at termination, absolute: 174 and 77%; relative: 218 and 73%; in M and F, resp.)

Absolute and relative adrenal weights increased in M at termination at 2500 ppm (absolute: 20%; relative: 31%) and 5000 ppm (absolute: 20%; relative: 38%)

Absolute and relative ovary weights decreased at termination (absolute: 11%; relative: 33%)

Gross pathology:

Similar effects at 2500 and 5000 ppm

Enlarged livers (week 52: 5 and 10 in M and 8 and 10 in F at 2500 and 5000 ppm, resp. vs. 0 in control; week: 78: 6 and 8 in M and 9 and 10 in F at 2500 and 5000 ppm, resp. vs. 0 in control; week 104: 13 and 27 in F at 2500 and 5000 ppm vs. 7 in control) and brown zones (week 104: in M, 26 at 2500 ppm and 20 at 5000 ppm vs. 6 in control and in F 25 at 2500 ppm and 24 at 5000 ppm vs. 0 in control) in M and F

White zones on the stomach of M statistically increased at 2500 ppm (31 vs. 19 in control) but not at 5000 ppm (18)

Liver nodules in M and F (week 78: 10 at 5000 ppm in M vs. 4 in control; week 104: in M, 34 at 2500 ppm and 28 at 5000 ppm vs. 22 in control) and in F, 27 at 2500 ppm and 30 at 5000 ppm vs. 7 in control) and white zones at top dose in M week 78 (8 at 5000 ppm vs. 3 in control) but not at termination while in F white zones was statistically significantly increased at 5000 ppm only at termination (23 vs. 6 in control)

Enlarged lymph node at top dose (12 at 5000 ppm vs. 4 in control) and Harderian

		gland nodules (6 at 5000 ppm vs. 0 in control) in F Red zones on the liver and thymus atrophy (not confirmed by histopathology) in M **Non neoplastic histopathology**: Statistically significant observations: Hepatocytic hypertrophy in M (30/35 at 2500 ppm and 20/28 at 5000 ppm vs. 0/41 in control) and F (40/41 at 2500 ppm and 42/43 at 5000 ppm vs. 0/41 in control), intermediate fatty change in M (30/35 at 2500 and 20/28 at 5000 ppm vs. 0/41 in control) and F (37/41 at 2500 ppm and 33/43 at 5000 ppm vs. 0/41 in control), foci of cellular alteration in M (35/35 at 2500 and 28/28 at 5000 ppm vs. 20/41 in control) and in F (36/41 at 2500 ppm and 34/43 at 5000 ppm vs. 9/41 in control), anisonucleosis in M (11/35 at 2500 and 13/28 at 5000 ppm vs. 1/41 in control) and in F (12/43 at 5000 ppm vs. 2/41 in control), necrosis in M (15/35 at 2500 and 22/28 at 5000 ppm vs. 2/41 in control) and at 5000 ppm in F (10/43 vs. 1/41 in control), single cell necrosis in M (33/35 at 2500 and 28/28 at 5000 ppm vs. 1/41 in control) and at 5000 ppm in F (6/43 vs. 0/41 in control), lymphocytic infiltration and in F (22/35 at 2500 ppm and 17/28 at 5000 ppm vs. 12/41 in control), multinucleated hepatocytes in M (8/35 at 2500 ppm and 4/28 at 5000 ppm vs. 0/41 in control), accumulation of macrophages in M (28/35 at 2500 ppm and 28/28 at 5000 ppm vs. 4/41 in control), bile duct proliferation in M (5/35 at 2500 ppm and 12/28 at 5000 ppm vs. 0/41 in control), extramedullary haematopoiesis in M (8/35 at 2500 and 14/28 at 5000 ppm vs. 2/41 in control) and fibrosis at 2500 ppm in M (5/35 vs. 0/41) Forestomach ulcers (13/35 at 2500 ppm and 16/28 at 5000 ppm vs. 6/41 in control), lymphocytic infiltration (22/35 at 2500 ppm and 17/28 at 5000 ppm vs. 12/41 in control) and squamous cell hyperplasia (29/35 at 2500 ppm and 19/28 at 5000 ppm vs. 18/41 in control) at 2500 and 5000 ppm in the M Ovary atrophy at 2500 (22/41 vs. 4/41 in control) and 5000 ppm (30/43). Uterus	

		angiectasis at 5000 ppm (6/43 vs. 0/41 in control) Thyroid follicular cell hyperplasia in M (12/35 at 2500 ppm and 27/28 at 5000 ppm vs. 2/41 in control) and F (20/41 at 2500 and 26/43 at 5000 ppm vs. 5/41 in control), as well as dilated follicles in M (12/35 at 2500 ppm and 17/28 at 5000 ppm vs. 2/41 in control) at the same doses and in F only at 5000 ppm (10/43 vs. 1/41 in control) Increase in megakaryocytes in bone marrow at 5000 ppm in the M (8/28 vs. 3/41 in control) Adrenal hypertrophy in M (13/35 at 2500 and 20/28 at 5000 ppm vs. 0/41 in control) and F (38/41 at 2500 ppm and 42/43 at 5000 ppm vs. 1/41 in control).	

* OECD TG 403 (acute inhalation toxicity) was indicated in table 28 of the CLH report but it was revised in the opinion to OECD TG 410 (repeated dose dermal toxicity: 21/28 days)

In order to be classified as a substance targeting a specific organ after repeated exposure, the significant non-lethal toxic effect should be observable on a specific organ (either Category 1 or 2 depending on the level of toxic effect and test) at a certain level. In the case of benthiavalicarb-isopropyl, while severe effects have been observed mainly in the liver, they were only seen at doses sufficiently above the guidance values given by the CLP Regulation.

Benthiavalicarb-isopropyl induced severe effects mainly in the liver of rat, mice and dogs, including in rat in a study via the dermal route. These effects interfered with the normal function of this organ in several instances; however, as these effects were consistently observed only above the relevant guidance value for classification as STOT RE 2, the criteria for classification are not fulfilled.

Comments received during consultation

One MSCA supported no classification for STOT RE.

Assessment and comparison with the classification criteria

RAC agreed with the DS that the major toxic effect induced by benthiavalicarb-isopropyl upon repeated exposure was liver toxicity.

Studies relevant for the assessment of STOT RE consisted of three 28-d studies, one dermal and one oral (neurotoxicity study, OECD TG 424) in rat and a 28-d range finding study in dog, three 90-d studies (rat, mouse and dog), a 1-year dog study as well as two carcinogenicity studies.

Liver

Based on the available studies, the DS concluded that liver toxicity was the major finding observed after repeated exposure. Effects started as early as after 28 days treatment (rat, Anonymous 41, 2000b), were seen in all species tested (rat, mouse, dog) and both sexes, increased in severity with dose and exposure duration, and were seen after oral as well as dermal exposure. RAC agrees with this conclusion.

To conclude on whether classification as STOT RE is necessary it has to be decided whether significant toxicity is observed below the guidance values indicated in the CLP Regulation. According to the CLP Regulation, an effect is considered severe enough for classification if it interferes with the normal function of the target organ. For benthiavalicarb-isopropyl such effects were seen in the liver only at dose levels above the guidance value for STOT RE 2 (after extrapolation to the exposure duration). The dose levels where severe liver toxicity occurred are summarised in the table below, which is taken from the CLH report and modified (addition of the relevant liver effects at the indicated doses and adaption of some values). Effects other than liver toxicity are discussed in the text below the table.

***Table**: Comparison of the relevant liver effects observed at the lowest dose in the respective study, extrapolated to 90-d exposure.*

Study reference	**Effective dose (mg/kg bw/d) & observed effects**	**Length of exposure**	**Effective dose when extrapolated to 90-d exposure**	**Classification supported by the study**
Rat, dermal Anonymous 41, 2000b Report no. WIL-156012, Vol.3 CA 6.3.3/1	1000 mg/kg bw/d: Marginal liver cell necrosis, accompanied by liver toxicity related changes in clinical chemistry parameters 300 mg/kg bw/d: No liver effects	28 days	311 mg/kg bw/d	No
Dog, oral Anonymous 40, 1998 Report no. 3390, Vol. 3 CA Range-finding study (only 2 animals/sex/dose)	1000 mg/kg bw/d: Liver enlargement in M & F, planlobular hepatocellular hypertrophy 100 & 300 mg/kg bw/d: Increase in absolute & relative liver weight in M & F between 13 and 18%, no clear dose-response	28 days	311 mg/kg bw/d	No
Rat, oral – neurotoxicity Anonymous 42, 2002 Report no. KCI 207/022387, Vol. 3 CA, B.6.7	1853.8 mg/kg bw/d: No liver effects described, despite decrease in bw by 18%	28 days	> 577 mg/kg bw/d	No
Mouse, oral Anonymous 43, 1998a Report no. 3385, Vol. 3 CA 6.3.2/1	Large gap between NOAEL and LOAEL: 33 and 1293 mg/kg bw/d, resp. Effects at LOAEL: Black enlarged liver, liver weight increases up to 63% (absolute, M) and 87% (relative, M), anisonucleosis, hepatocellular hypertrophy, necrosis (details on	90 days	Not applicable	No

Study reference	Effective dose (mg/kg bw/d) & observed effects	Length of exposure	Effective dose when extrapolated to 90-d exposure	Classification supported by the study
	histopathology are presented in the table below).			
Rat, oral Anonymous 44, 1998b Report no. 3386, Vol. 3 CA 6.3.2/2	Large gap between NOAEL and LOAEL: 14/15 (M/F) and 353/379 (M/F) mg/kg bw/d Effects at LOAEL: Liver toxicity related changes in clinical chemistry parameters, liver weight increases up to 29% (absolute, F) and 23% (relative, F)	90 days	Not applicable	No
Dog, oral Anonymous 45, 1999 Report no. 3812, Vol. 3 CA 6.3.2/3	200 mg/kg bw/d: Liver toxicity related changes in clinical chemistry parameters, 43% increase in relative liver weight in F (Further increase in liver weight in F and M, large liver, hepatocellular hypertrophy, and pigment deposition at 1000 mg/kg bw/d)	90 days	Not applicable	No GV for dog, but effect dose exceeds rat GV
Dog, oral Anonymous 46, 2001 Report no. 4551, CA 6.3.2/6	> 400 mg/kg bw/d (Only increase in absolute liver weight by 21% and 28% in M and F, resp. at this top dose of the study)	1 year	Not applicable	No GV for dog, but effect dose exceeds rat GV No relevant effects seen in the study
Rat, oral Anonymous 18, 2001a Report no. 3822, Vol.3 CA, B.6.5/1	Large gap between NOAEL: 9.9/12. (M/F) and LOAEL: 250/318 (M/F) mg/kg bw/d 250/318 (M/F) mg/kg bw/d: Liver toxicity related changes in clinical chemistry parameters, increased liver weight throughout the study (starting at week 26, around 20% in M & F, absolute & relative), liver enlargement in M & F (from week 26); some increase in hepatic hypertrophy in F, fatty degeneration in F, spongiosis hepatis in M	2 years	~ 2000 mg/kg bw/d	No
Mouse, oral Anonymous 20, 2001b Report no. 3823, Vol.3 CA, 6.5/2	Large gap between NOAEL: 13.7/18.6 (M/F) and LOAEL: 358/459 (M/F) mg/kg bw/d 358/459 (M/F) mg/kg bw/d:	2 years	~ 2900 mg/kg bw/d	No

Study reference	Effective dose (mg/kg bw/d) & observed effects	Length of exposure	Effective dose when extrapolated to 90-d exposure	Classification supported by the study
	Increased liver weight throughout the study up to 113% (absolute, M) and 140% (relative, M), enlarged livers in M & F, liver nodules in M & F (from week 78), hepatocellular hypertrophy in M & F, intermediate fatty change in M & F, foci of cellular alteration in M & F, anisonucleosis in M & F, necrosis in M, single cell necrosis in M & F, bile duct proliferation in M, fibrosis in M			

***Table**: Histopathological observations in the liver from male and female mice from the 90-d mouse study (Anonymous 43, 1998a).*

Dose	Males					Females				
mg/kg bw/d	0	8.4	33	1293	4031	0	11.3	45	1620	4946
Anisonucleosis	-	-	-	9/10	10/10 (8:1, 2:2)	-	-	-	-	2/10 (1:1, 1:2)
Fatty change	-	1/10	-	3/10	7/10	-	-	-	-	2/10
Hepatocellular hypertrophy	-	-	-	-	4/10	-	-	-	10/10	10/10 (4:1, 5:2, 1:3)
Multinucleated giant cell formation	-	-	-	-	4/10	-	-	-	-	1/10
Necrosis	-	-	-	8/10	9/10	-	-	-	5/10	8/10
Lymphocyte infiltration	-	-	-	-	1/10	-	-	-	-	-
Bile duct proliferation	-	-	-	3/10	10/10	-	-	-	-	5/10 (4:1, 1:3)

Degree of lesion was slight (1), except were indicated differently in brackets (Grading: 0: normal, 1: slight, 2: moderate, 3: severe)

The effects in the two top dose groups clearly indicate liver toxicity, but the doses exceed the cut-off values.

An oral 28-d mouse study (Anonymous, 1996) was made available to RAC on the 3rd of March 2022. Also in this study, liver was the main target organ and already after 28 days clear liver toxicity was observed, starting at a dose of 500 ppm (males: 105 mg/kg bw/d, females: 120 mg/kg bw/d). The effects seen at that dose included slight single cell necrosis in 3/5 males and 1/5 females, slight focal necrosis in 1/5 males, slight fatty change in 1/5 males and slight increase in mitosis in 1/5 males and 1/5 females. These observations demonstrate cytotoxicity after 28 days at doses ≥ 500 ppm. Centrilobular hypertrophy was only seen at the next higher dose (700 ppm: males: 1412 mg/kg bw/d, 5/5, slight; females: 1609 mg/kg bw/d, 2/5, slight). The dose of 500 ppm would be relevant for STOT RE 2; however, the effects are not considered severe enough to support a classification.

The two-generation study in rat, and 4 PNDT studies, two in rat and two in NZW rabbit, are also relevant. In addition, there are several mechanistic studies available in rat and mouse with exposure durations between 7 days and 16 weeks. In these studies, no liver effects relevant for classification were observed. These studies are described in detail in the section on reproductive toxicity and in the carcinogenicity section.

Other target organs of benthiavalicarb-isopropyl toxicity than liver

Thymus

In the dermal 28-d study in rat, a dose-related decrease in thymus weight was noted, starting from the lowest dose tested (100 mg/kg bw/d), however, without histopathological correlates. Reductions in thymus weight were also seen in top dose (> 1000 mg/kg bw/d) animals of the F0 (females), F1 (males & females) and F2 (males & females) generation of the two-generation study. Again, no histopathological findings were reported. In the 90-d dog study (Anonymous 45, 1999), thymus weight reduction was described, but only in mid dose males and females, not in the top dose (no histopathological findings were seen), as well as in the top dose of the mouse carcinogenicity study (thymus atrophy, not confirmed by histopathology).

These effects were not considered severe enough to support classification as STOT RE and occurred mostly at doses exceeding the guidance values.

Adrenals

Slight to moderate increases in adrenal weight were reported in the two-generation study in rat and the PNDT studies, the 90-d and the carcinogenicity studies in rat but were not corroborated by histological findings and exceeded guidance values for the 90-d and the carcinogenicity study.

In the mouse carcinogenicity study, adrenal cortical hypertrophy was observed at 2500 and 5000 ppm in males and females and adrenal weight was increased in males at 25000 ppm (absolute + 20%, relative + 31%). Though considered a relevant toxic effect, the doses clearly exceed the guidance values for STOT RE.

Kidney

A dose-dependent increase in kidney weight was seen in the two top doses (5000 and 10000 ppm) of the rat carcinogenicity study. In this study, at the same doses also glomerulosclerosis of the kidneys in females, calculus in males and females, chronic nephropathy in males, brown pigment deposition in females, dilated tubules in males, hyaline droplets in males (and at the top dose also in females), lymphocytic infiltration in females and in top dose males, fibrosis, and transitional cell hyperplasia were observed. The two top doses of this study clearly exceed the guidance values for STOT RE; therefore, these effects are not considered relevant for classification.

In addition, kidney weights were decreased in the top doses of the 90-d rat and mouse studies; no other effects were described.

Blood

Effects on blood parameters were described in the 90-d dog study, as well as in the rat and mouse carcinogenicity studies. The effect was seen in female dogs ≥ 200 mg/kg bw/d and in male dogs at 1000 mg/kg bw/d. In general, these effects were more pronounced after 13 weeks than after 6 weeks and were quite large, especially in females (e.g., -30% Hb in top dose females after 13 weeks). Pigment deposition was seen in the liver of top dose animals, which could be related to these effects. Platelet counts were reduced in top dose males and females. These observations were made at doses ≥ 200 mg/kg bw/d, exceeding the guidance values for STOT RE classification. Less pronounced effects were seen on blood parameters in the rat and mouse carcinogenicity studies, also at doses above the guidance values for classification. In both studies,

also white blood cell counts were affected; however, the findings were inconsistent in that in the rat study an increase was described, whereas in the mouse study the values were decreased at all dose groups, except for an increase in low dose females. The relevance of these findings is unclear.

Thyroid

Dose-dependent increases in thyroid follicular hyperplasia in male and female mice, and an increase in thyroid follicular adenomas in males were observed in the mouse carcinogenicity study. Detailed mechanistic investigations on the related mode of action (MoA), including thyroid hormone parameters and enzyme activities part of the hypothalamic–pituitary–thyroid (HPT) axis, were performed in mice as well as in rats. Thyroid hormone parameters were altered in mice and rats and can be characterised by a decrease in (thyroxine) T4 levels, but no effect on triiodothyronine (T3) levels. Increases in thyroid stimulating hormone (TSH) were seen only after longer exposure duration (detected after 16 weeks in mice, but not earlier, Anonymous 29, 2002a), though increases in thyroid stimulating hormone beta (TSHB) and thyrotropin-releasing hormone receptor (Trhr) mRNA transcripts in the pituitary were already seen after shorter exposure duration in mice (Anonymous 22, 2018a). The potential underlying MoAs of these effects are discussed under the section on carcinogenicity.

These findings resulted in an identification as endocrine disruptor (ED) of the T-modality under the EFSA regime (EFSA, 2021).

In conclusion, based on the absence of relevant effects at doses below the guidance values, RAC agreed with the DS that **no classification for STOT RE is warranted**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The database for benthiavalicarb-isopropyl covered *in vitro* tests in bacterial and mammalian cells as well as *in vivo* studies in somatic cells (For details on the studies see tables below).

***Table**: Summary table of the genotoxicity / mutagenicity tests in vitro, taken from the CLH report.*

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-15-162; 87.9% as KIF-230R-L	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2 uvrA) 8, 40, 200, 1000 and 5000 µg/plate 32, 80, 200, 500, 1000, 2000 and 5000 µg/plate	Positive (strain TA98) +S9 mixture Positive result due the impurity I-6 Sensitivity demonstrated by positive control	Dawkes, 1999 Report no. 535/44, Vol. 3 CA, B6.4.1/1
Bacterial assay for gene mutation	KIF-230 Lot: G51-35-184; 91.9% as	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2	Negative ± S9 mixture Sensitivity demonstrated by	Mizuhashi, 2001a Report no. 5839, Vol. 3

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OCSPP Test Guideline 870.5100 Acceptable	KIF-230R-L	uvrA) 39, 78, 156, 313, 625, 1250, 2500 and 5000 µg/plate	positive controls	CA, B.6.4.1/2
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-37-184; 95.8% as KIF-230R-L	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2 uvrA) 78, 156, 313, 625, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive controls	Mizuhashi, 2001b Report no. 5840, Vol. 3 CA, B6.4.1/3
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-36-184; 92.6% as KIF-230R-L	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2 uvrA) 39, 78, 156, 313, 626, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive controls	Mizuhashi, 2001c Report no. 5918, Vol.3 CA, B.6.4.1/4
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 TG - lot no. G51-08-158; 88.6% as KIF-230R-L	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2 uvrA) 28, 45, 72, 116, 186, 298, 477, 763, 1221, 1953, 3125 and 5000 µg/plate	Positive (strain TA98) + S9 mixture Positive result due to the impurity I-6 Sensitivity demonstrated by positive controls	Mizuhashi, 2001d Report no. 5919, Vol. 3 CA, B.6.4.1/5
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-47-190; 92.4% as KIF-230R-L	*S. typhimurium* (strain TA98) 313, 625, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive control	Mizuhashi, 2002a Report no. 6239, Vol.3 CA, B.6.4.1/6
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100	KIF-230 Lot: G51-48-190; 94.8% as KIF-230R-L	*S. typhimurium* (strain TA98) 313, 625, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive control	Mizuhashi, 2002b Report no. 6240 Vol. 3 CA, B.6.4.1/7

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Acceptable				
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-49-190; 92.7% as KIF-230R-L	*S. typhimurium* (strain TA98) 313, 625, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive control	Mizuhashi, 2002c Report no. 6241, Vol. 3 CA, B.6.4.1/8
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-50-190; 91.5% as KIF-230R-L	*S. typhimurium* (strain TA98) 313, 625, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive control	Mizuhashi, 2002d Report no. 6242, Vol. 3 CA, B.5.4.1/9
Bacterial assay for gene mutation OCSPP Test Guideline 870.5100 Acceptable	KIF-230 Lot: G51-51-190; 93.3% as KIF-230R-L	*S. typhimurium* (strain TA98) 313, 625, 1250, 2500 and 5000 µg/plate	Negative ± S9 mixture Sensitivity demonstrated by positive control	Mizuhashi, 2002e Report no. 6243, Vol. 3 CA, B.6.4.1/10
Bacterial assay for gene mutation OECD TG 471 Acceptable	KIF-230 TG - lot no. G51-56 ; 93.6% as KIF-230R-L	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2 uvrA) 16, 50, 158, 500, 1581 and 5000 µg/plate 8, 20, 51, 128, 320, 800, 2000 and 5000 µg/plate	Negative ± S9 mixture Negative result confirmed in a repeat study Sensitivity demonstrated by positive controls	Ballantyne, 2004a Report no. 535/114, Vol. 3 CA, B.6.4.1/11
Bacterial assay for gene mutation OECD TG 471 Acceptable	KIF-230 TG - lot no. G51-56, 93.6% as KIF-230R-L	*S. typhimurium* (strains TA98, TA100, TA1535, TA1537) *E. coli* (WP2 uvrA) 16, 50, 158, 500, 1581 and 5000 µg/plate 20, 51, 128, 320, 800, 2000 and 5000 µg/plate	Negative ± S9 mixture Negative result confirmed in a repeat study Sensitivity demonstrated by positive controls	Ballantyne, 2004b Report no. 535/115, Vol. 3 CA, B.6.4.1/12

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial gene mutation assay OECD TG 471 Acceptable	KIF-230 Lot: G51-58; 94.6% as KIF-230R-L	*S. typhimurium* (strain TA98, TA100, TA1535, TA1537) *E. coli* (strain WP2 uvrA) 16, 50, 158, 500, 1581 and 5000 µg/plate 8, 20, 51, 128, 320, 800, 2000 and 5000 µg/plate	Negative ± S9 mixture Negative result confirmed in a repeat study Sensitivity demonstrated by positive controls	Ballantyne, 2004c Report no. 535/116, Vol.3 CA, B.6.4.1/13
Bacterial reverse gene mutation OECD TG 471 Acceptable	KIF-230 Lot: G51-58; 94.6% as KIF-230R-L	*Salmonella typhimurium* (TA 1535, TA 1537, TA 98 and TA 100) *Escherichia coli* WP2 uvrA 16, 50, 158, 500, 1581 and 5000 µg/plate 20, 51, 128, 320, 800, 2000 and 5000 µg/plate	Negative ± S9 mixture Negative result confirmed in a repeat study Sensitivity demonstrated by positive controls	Ballantyne, 2004d Report no. 535/117, Vol. 3 CA, B.6.4.1/14
Bacterial reverse gene mutation OECD TG 471 Acceptable	KIF-230 Lot: G51-59; 92.7% as KIF-230R-L	*Salmonella typhimurium* (TA 1535, TA 1537, TA 98 and TA 100) *Escherichia coli* WP2 uvrA 16, 50, 158, 500, 1581 and 5000 µg/plate 8, 20, 51, 128, 320, 800, 2000 and 5000 µg/plate	Negative ± S9 mixture Negative result confirmed in a repeat study Sensitivity demonstrated by positive controls	Ballantyne, 2004e Report no. 535/118, Vol. 3 CA, B.6.4.1/15
Bacterial reverse gene mutation OECD TG 471 Acceptable	KIF-230 Lot: G51-59; 92.7% as KIF-230R-L	*Salmonella typhimurium* (TA 1535, TA 1537, TA 98 and TA 100) *Escherichia coli* WP2 uvrA 16, 50, 158, 500, 1581 and 5000 µg/plate 20, 51, 128, 320, 800, 2000 and 5000 µg/plate	Negative ± S9 mixture Negative result confirmed in a repeat study Sensitivity demonstrated by positive controls	Ballantyne, 2004f Report no. 535/119, Vol. 3 CA, B.6.4.1/16
Clastogenicity in	KIF-230 Lot: G51-	Chinese hamster lung (CHL) cells	Negative	Anonymous 11, 1998

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
mammalian cells OCSPP Test Guideline 870.5300 Acceptable	02-152; 96.9% as KIF-230R-L	955, 1910 and 3820 µg/mL	± S9 mixture Sensitivity demonstrated by positive control	Report no. 3391, Vol.3 CA, B.6.4.1/17
Gene mutation in mammalian cells OCSPP Test Guideline 870.5300 Supportive (presence of white powdery test substance)	KIF-230 Lot: G51-15-162; 87.9% as KIF-230R-L	Mouse lymphoma cells 3.75, 7.5, 15, 30, 60 and 120 µg/mL 7.5, 15, 30, 60 and 120 µg/mL	Negative ± S9 mixture Sensitivity demonstrated by positive control	Anonymous 12, 1999a Report no. 535/45, Vol. 3 CA, B.6.4.1/18
Unscheduled DNA synthesis Supportive	KIF-230 Lot: G51-15-162; 87.9% as KIF-230R-L	Rat hepatocytes 5, 16, 50, 158 and 500 µg/mL 16, 31, 63, 125, 250 and 500 µg/mL	Negative Sensitivity demonstrated by positive control	Anonymous 13, 1999b Report no. 535/46, Vol. 3 CA, B.6.4.1/
Comet assay Not stated/ No EC protocol is available Acceptable	KIF-230 Lot: FL-3003; 93.4% as KIF-230R-L	Human lymphocytes 62, 104, 173, 288 and 480 µg/mL	Negative Sensitivity demonstrated by positive control	Anonymous 14, 2003 Report no. 7445, Vol. 3 CA, B.6.4.1/20

***Table**: Summary table of mutagenicity/genotoxicity tests in mammalian somatic cells in vivo, taken from the CLH report.*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mouse bone marrow micro-nucleus test Test method B.12 of directive 2000/32/EC Deviations but acceptable	KIF-230 Lot: G51-15-162; 87.9% as KIF-230R-L	Mouse (CD-1) 2000 mg/kg dosed by oral gavage	Negative Sensitivity demonstrated by positive control	Anonymous 15, 2000 Report no. 535/47, Vol. 3. CA, B.6.4.2/1
Unscheduled DNA	KIF-230 Lot: G51-	Rat (Fischer CrJ:	Negative	Anonymous

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
synthesis Test method B.39 of directive 2000/32/EC Acceptable	24-176; 92.3% as KIF-230R-L	F344/Du) Hepatocytes 1000 and 2000 mg/kg bw dosed by oral gavage	Sensitivity demonstrated by positive control	16, 2001e Report no. 5336, Vol. 3 CA, B.6.4.2/2
Transgenic rodent mutation assay Methodology in line with open literature (Env. Mol. Mutagenesis 28: 363-375, 1996) Acceptable	KIF-230 Lot: G51-08-158; 88.6% as KIF-230R-L	Mouse (Muta™ mouse) Hepatocytes 1000 and 2000 mg/kg bw dosed by oral gavage for 5 days	Negative Sensitivity demonstrated by positive control	Anonymous 17, 2000a Report no. 4911., Vol.3 CA, B.6.4.2/3

The available data covered *in vitro* tests for gene mutation (base pair and frame shift mutations) in bacteria and mammalian cells, a clastogenicity test in mammalian cells *in vitro*, an unscheduled DNA synthesis test in rat hepatocytes and a Comet assay in human lymphocytes.

In vivo studies consisted of a mouse bone marrow micronucleus test, an unscheduled DNA synthesis tests in rat and a transgenic rodent mutation assay in mouse (Muta™ mouse). All *in vivo* studies were conducted via the oral route (gavage), representing a relevant route for *in vivo* mutagenicity studies.

All *in vitro* and *in vivo* studies were conducted according to accepted guidelines (where available) and GLP.

The results of these studies were consistently negative, with one exception. Among the first set of batches investigated in the bacterial reverse mutation assay, two of them gave positive results in *S. typhimurium* strain T98, suggesting a potential to induce frame shift mutations. In subsequent analytical studies, it was demonstrated that the mutagenic potential in both batches was caused by the presence of the impurity KIF.230-I-6 (for details, see CLH report). One of the two batches also contained the genotoxic relevant impurity KIF-I-12 (for details, see CLH report).

The applicant provided a new five batch analysis and claimed that it corresponded to the impurity profile of the commercially manufactured active substance. The new set of batches was tested in *S. typhimurium* strain T98 only. Negative results were obtained with and without metabolic activation.

A slight increase of polyploid cell incidence was detected in the *in vitro* chromosome-aberration assay (Anonymous 11, 1998) in the absence of exogenous metabolisation, but the DS concluded that its toxicological significance remains questionable as this may be a reflexion of cell toxicity, and no similar effects was observed when the test was performed in the presence of S9.

The CLH report further referred to mechanistic studies investigating the formation of 8-OH DNA-adducts in liver cells (2-week feeding studies in rat and mouse) that further supported the absence of mutagenic effects of benthiavalicarb-isopropyl. These studies are described in the carcinogenicity section (Anonymous 27, 2001a; Anonymous 28, 2001b). No increase in adduct formation was seen up to the highest dose tested in neither study.

Overall, the DS concluded that there was no evidence that would support classification for germ cell mutagenicity, despite a complete *in vitro* and *in vivo* data set and proposed no classification for germ cell mutagenicity.

Comments received during consultation

One MSCA supported no classification for germ cell mutagenicity.

Assessment and comparison with the classification criteria

RAC agreed with the DS's assessment and concluded that **no classification for germ cell mutagenicity is warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Benthiavalicarb-isopropyl's carcinogenic potential was investigated in two long-term toxicity and carcinogenicity studies in rats and mice (Anonymous 18, 2001a; Anonymous 20, 2001b). The results are summarised in the table below. The non-neoplastic findings of these studies are presented in the STOT RE section.

Seventeen mechanistic studies (Anonymous 22, 2018a; McMahon, 2018b-e; Anonymous 25, 26, 2001c-d; Anonymous 27-32, 2001a-b, 2002a-c, 2003; Anonymous 33, 2015) were also included in the CLH dossier. The studies investigated the Mode of Action (MoA) of the tumours observed in the long-term studies. These studies are summarised in the tables under 'CAR MoA', 'Thyroid follicular cell adenoma – potentially underlying modes of action', 'Uterine adenocarcinoma' and 'Other studies relevant for assessing the carcinogenic potential of benthiavalicarb-isopropyl' as well as in the section "Supplemental information – in depth analyses by RAC".

***Table**: Summary of animal studies on long-term toxicity and carcinogenicity, from CLH report (slightly modified)*

Method, guideline, deviations if any, species, strain, sex, no/group; Test substance, dose levels duration of exposure	**Results**	**Reference**
2-year chronic toxicity / oncogenicity study in rats OCSPP Guideline No 870.4300 OECD TG 453 Acceptable Dietary exposure F344/DuCrj (Fischer, SPF) rats 80/sex/group of which 10/sex/group were sacrificed at weeks 26, 52 & 78 Haematology, blood biochemistry, urine alysis, organ weight analysis & gross post-mortem histopathology evaluation at weeks 26, 52, 78 & 104 Conducting laboratory: BioSafety Research Centre for Foods, Drugs and Pesticides, "An-Pyo	*	Anonymous 18, 2001a DRAR Report no. 3822, Vol.3 CA, B.6.5/1

Method, guideline, deviations if any, species, strain, sex, no/group; Test substance, dose levels duration of exposure	Results	Reference
Centre" KIF-230, batch G51-08-158, purity: 88.8-89.1% as KIF-230R-L 0; 50; 200; 5000; 10000 ppm - M: 0, 2.5; 9.9, 249.6, 518.3 mg/kg bw/d - F: 0, 3.2, 12.5, 318.2, 649.4 mg/kg bw/d Duration: 104 weeks (5-6 weeks at the start of dosing)		
Historical control data from the testing laboratory (BioSafety Research Centre for Foods, Drugs and Pesticides, "An-Pyo Centre") for F344/DuCrj rats Long-term carcinogenicity studies performed between 1996 and 2005 The historical control database used covers 15 studies and 750 animals from control groups		Anonymous 19, 2017 DRAR Historical control Studies No.: 3822, Vol. 3 CA, B.6.5/5
2-years oncogenicity study in mice EPA OCSPP Guideline No 870.4200 OECD TG 451 Acceptable Dietary exposure B6C3F1 mice 70/sex/group of which 10/sex/group were sacrificed at weeks 52 & 78 Haematology, urinalysis, organ weight analysis & gross post-mortem histopathology evaluation at weeks 52, 78 & 104 Conducting laboratory: BioSafety Research Centre for Foods, Drugs and Pesticides, "An-Pyo Centre" KIF-230, batch G51-08-158, purity: 88.8-89.1% as KIF-230R-L 0; 20; 100; 2500; 5000 ppm - M: 0, 2.7, 13.7, 358, 731 mg/kg bw/d - F: 0, 3.7, 18.6, 459, 928 mg/kg bw/d Duration: 104 weeks (5-6 weeks at the start of dosing)	*	Anonymous 20, 2001b DRAR Report no. 3823, Vol.3 CA, 6.5/2
Historical control data from the testing laboratory: BioSafety Research Centre for Foods, Drugs and Pesticides, "An-Pyo Centre", for B6C3F1 mice: Long-term carcinogenicity studies performed between 1996 and 2005 The historical control database covers 14 long-term carcinogenicity studies with 705 animals from control groups		Anonymous 21, 2018 DRAR Historical control Studies No 3823 (001-209), Vol.3 CA, 6.5/4

* Information on mortality, general toxicity parameters and incidences of the most relevant non-neoplastic and neoplastic findings are presented in tables in the section "Assessment and comparison with the classification criteria"

Because of the adverse effects seen in the two top doses of both carcinogenicity studies in rats and mice, the DS concluded that the second highest dose should be considered the maximum tolerated dose (MTD), i.e., 2500 ppm and 5000 ppm in mouse and rat, respectively (for details on mortality and observed general toxicity, see section "Assessment and comparison with the classification criteria").

The CLH dossier contained several *in vitro* and *in vivo* mechanistic studies that aimed to clarify the underlying MoA for the different tumour types observed in rats and mice. These studies are presented in detail in the section "Assessment and comparison with the classification criteria".

Hepatocellular adenoma and carcinoma

The DS considered the increased incidence of hepatocellular adenoma in male rat and male and female mice, as well as the increased incidence of hepatocellular carcinoma in male mice, as treatment -related. The DS, however, considered the evidence from the mechanistic studies (see table under 'CAR MoA') sufficient to conclude that these tumours are the consequence of CAR activation and related molecular events and therefore rodent specific and not relevant for humans.

Hepatoblastoma

A statistically significant increase in the incidence of hepatoblastoma was seen in male mice of the two top doses, without dose-response, but exceeding HCD. The DS mentioned that hepatoblastomas differ fundamentally between B6C3F1 mice and humans in that they predominantly occur in early childhood in humans, whereas in mice they occur at the end of life. The DS further indicated that in humans, hepatoblastoma were associated with Familial Adenomatous Polyposis Coli and Beckwith-Wiedemann syndrome, and that there was a weaker association for occupational exposure to metals, petroleum products and paints or pigments. They further stated that hepatoblastoma in humans occurred in the absence of other non-neoplastic or neoplastic liver pathologies and was derived *de novo* from mutated hepatoblasts retained in the liver during development.

Regarding mouse hepatoblastomas, the DS referred to a hypothesis that says that based on similar mutational spectra among hepatocellular adenoma / carcinoma and hepatoblastoma appearing in the same mouse livers, it is unlikely that hepatoblastoma arise independently from these other lesions. They further stated that there was clear evidence that mouse hepatoblastoma can be chemically induced and that male B6C3F1 mice were considerably more sensitive than female mice of that strain.

Further, it was mentioned that there are also similarities between murine and human hepatoblastoma, i.e., morphological as well as on the molecular level (similar mutations and protein distributions, such as those affecting ß-catenin and other proteins related to the Wnt/ß-Catenin pathway).

The DS concluded that because of the divergent evolution, biology, and age of onset, differences between murine and human hepatoblastoma and the almost unique appearance of chemically induced hepatoblastomas in the B6C3F1 strain, chemically induced murine hepatoblastomas should not be considered relevant for human hazard characterisation. The DS finally stated that hepatoblastomas should be considered an artefact of the use of the B6C3F1 mice and that there was no evidence that other mouse strains, except Crj:BDF1 mice, can develop these neoplasms under the same conditions.

With regard to the above statements related to hepatoblastoma, the DS referred to Martens (2018b) and Foster & Provan (2018a-b), which are unpublished statements prepared on behalf of the applicant.

Thyroid follicular cell adenoma

Based on the standard toxicology and mechanistic data, the DS concluded that the observed increase in thyroid follicular cell adenoma in top dose male mice (9 vs. 0 in concurrent control; HCD range: 0 – 6%, mean 2.6%) was caused by increased catabolism of thyroid hormone by liver enzymes induced via CAR activation. The DS concluded that the key events of this MoA, i.e., increased activity of T4 UDP-GT resulting in increased TSH production by the pituitary, leading to thyroid follicular cell proliferation and thyroid follicular cell adenomas, were demonstrated for benthiavalicarb-isopropyl. The DS pointed out that concordance of dose-response relationship was demonstrated for the molecular events and the biological responses and was of the opinion that it was sufficiently demonstrated that other MoA were not active (i.e., thyroid peroxidase). The DS concluded that these tumours were not relevant for humans.

Uterine adenocarcinoma

The DS concluded that the relevance of the observed uterine tumours for humans cannot be excluded. Although it was demonstrated that benthiavalicarb-isopropyl is not mutagenic, no other MoA responsible for the tumour formation in female rat could be demonstrated.

Overall, the DS considered only the malignant uterine adenocarcinomas seen in female rat to be of human relevance, as no MoA could be identified causing these tumours and human relevance could therefore not be excluded.

For the remaining tumours, the DS considered the MoA evidence sufficient to conclude that they were not relevant for humans. The DS considered the malignant uterine adenocarcinomas, increased in the two top doses of one sex (females), in one species (rat), above HCD as supportive to classify benthiavalicarb-isopropyl as Carc. 2; H351.

Comments received during consultation

During public consultation comments were received from one Company-Manufacturer and one MSCA.

The Company-Manufacturer provided detailed opinion documents, review papers and study reports on the underlying MoA relevant for the assessment of carcinogenicity, and on the relevance of the different tumours observed in rats and mice. These documents also contain data from newly conducted mechanistic studies. The new studies cover investigations of the Wnt/ß-Catenin pathway in liver and uterine tissue, possible modes of action for uterine adenocarcinoma as well as a histopathological re-evaluation of the hepatoblastomas found in the mouse carcinogenicity study. The DS thanked the Company-Manufacturer for this information and considered it supportive for the conclusions drawn in the CLH report. A detailed discussion of the submitted documents with the related RAC views is provided in the section "Supplemental information – in depth analyses by RAC".

The comment from the MSCA supported that classification for carcinogenicity was required but proposed to consider whether classification in Category 1B was more appropriate. They justified this proposal by the occurrence of different types of tumours (hepatocellular adenoma/carcinoma, thyroid follicular cell adenoma, hepatoblastoma and uterus adenocarcinoma) in two species and two sexes. They considered the uterine tumours as clearly relevant for humans and were also of the view that there were remaining uncertainties regarding the MoA analyses of the CAR/PXR MoA, relevant for the hepatocellular adenomas/carcinomas, including that there were also indications for AhR activation.

The MSCA was further of the view that evidence on hepatoblastoma provided by the applicant (Foster, 2018), did not allow to conclude on human relevance / non-relevance of these tumours, but rather described the difference between human und murine hepatoblastoma. The MSCA also

pointed out that the majority of experts at the Pesticide Peer Review Experts' Meeting 186 (2018) supported classification as Carc. 1B.

In their response, the DS stated that a prolonged and high level of AhR activation was needed to promote hepatocellular carcinogenesis and referring to the publication by Qin *et al*. (2019) they stated that the observed activation was far below the levels indicated in this publication. The DS further stated that a detailed re-evaluation of the histopathological samples from the mouse carcinogenicity study was performed in response to a request by EFSA (2 July 2018), demonstrating that all observed hepatoblastomas had developed within hepatocellular adenoma or carcinoma. The DS concluded that the hepatoblastoma were not a separate tumour entity and were considered not relevant for humans. They considered classification as Carc. 2 as adequate, based on the uterine tumours in rat, for which no MoA could be identified.

Assessment and comparison with the classification criteria

Chronic toxicity and carcinogenicity studies

Benthiavalicarb-isopropyl has been assessed in two long-term toxicity and carcinogenicity studies in rat and mouse. Historical control data from the conducting laboratory from a time period 5 years prior- and post-conduct of the studies were made available. Details on study design and general results are presented in the section "Summary of the Dossier Submitter's proposal". The tables below summarise the most relevant results of the two studies in relation to the observed carcinogenic effects.

***Table**: General toxicity in the chronic toxicity and carcinogenicity study in rat (Anonymous 18, 2001a), table 6.5.1/01-2 from the DRAR*

Dose (ppm)	**0**		**50**		**200**		**5000**		**10000**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Mortality (%)	23	20	15	26	12	15	10	15	12	10
Clinical signs	-	-	-	-	-	-	-	-	-	-
Tail nodule	1	-	1	-	1	-	3	-	6	-
Food consumption (% change)	-	-	-	-	-	-	↑ 5**	-	↑ 7**	-
Bw (% change)	-	-	-	-	-	-	-	-	↓ 4	↓ 4*
Bw gain (% change)	-	-	-	-	-	-	-	-	↓ 4	↓ 7*
Food efficiency# (% change)	-	-	-	-	-	-	-	-	↓ 4**	↓ 3**

Food efficiency only calculated up to week 52.
Statistically significant modification, Dunnett's t-test * $p < 0.05$, ** $p < 0.01$

***Table**: Relevant organ weights and neoplastic findings in the chronic toxicity and carcinogenicity study in rat at termination (week 104) (Anonymous 18, 2001a), information partly derived from DRAR. Non-neoplastic findings in the liver are also presented in order to help interpret the observed liver tumours (increase in hepatocellular adenomas in males)*

Doses (ppm)	**0**	**50**	**200**	**5000**	**10000**	**HCD#**
Males						
Doses (mg/kg bw/d)	**0**	**2.5**	**9.9**	**249.6**	**518.3**	
Liver weights:						
Absolute (g)	8.91	9.87	9.33	10.89**	11.51**	
Relative (%)	2.46	2.67	2.54	2.97**	3.28**	

Non-neoplastic findings: Number of findings (50 animals investigated)						
Liver:						
- Hepatocellular hypertrophy	1	0	0	5	24**	
- Hepatocellular fatty change	20	27	19	30	30	
- Spongiosis hepatis & focal alterations	19	23	19	39**	42**	
Neoplastic findings: Number of findings (50 animals investigated)						
- Hepatocellular adenoma	1	2	2	2	7* (14%)	0.0 - 18% (mean 6.1%)
- Hepatocellular carcinoma	0	2	0	0	2	
Females						
Doses (mg/kg bw/d)	**0**	**3.2**	**12.5**	**318.2**	**649.4**	
Liver weights:						
Absolute (g)	6.33	6.18	6.15	7.32**	7.87**	
Relative (%)	2.56	2.46	2.56	3.04**	3.30**	
Non-neoplastic findings: Number of findings for 50 animals investigated						
Liver:						
- Hepatocellular hypertrophy	1	0	0	7**	25**	
- Hepatocellular fatty change	21	16	15	39**	36**	
Uterus: no non-neoplastic findings	-	-	-	-	-	
Neoplastic findings: Number of findings for 50 animals investigated						
- Hepatocellular adenoma	4	0	2	1	2	0 - 10% (mean 1.5%)
Uterus adenocarcinoma	3	3	4	11* (22%)	10* (20%)	0 – 8% (mean 2.7%)

* Significantly different from the control: $p < 0.05$, ** Significantly different from the control: $p < 0.01$, # HCD: from the former BioSafety Research Center for Foods, Drugs and Pesticides ("An-Pyo Centre") for F344/DuCrj rats that were used in long-term carcinogenicity studies performed between 1996 and 2005.

Summary of the results of the chronic toxicity and carcinogenicity study in rat

There was no dose-related increase in mortality (survival was generally higher in treated groups than in controls) and only in top dose males and females slight effects on body weight, body weight gain and feed efficiency were observed (see table on general toxicity above), which did in no instance exceed 10%. RAC was of the opinion that the animals were not affected by severe general toxicity. RAC noted that although 4 doses were tested, the dose spacing was unusual with a large gap (more than 20-fold difference) between the two lower and the two higher dose groups. This is of special relevance when considering the oral absorption of benthiavalicarb-isopropyl in rat, which is quite high after exposure to low doses (89-97% at 5 mg/kg bw/d), but only ~50% at a dose of 400 mg/kg bw/d. The inappropriate dosing renders the interpretation of the results difficult.

Liver was a target organ in males and females and absolute and relative weight increase was seen in the two top doses in both sexes. In these two dose groups also the incidence of hepatocellular hypertrophy and hepatocellular fatty change was increased in males and females (statistically significant for both effects at both top doses in females, and for top dose males for hepatocellular hypertrophy). In males, spongiosis hepatis and focal alterations were also statistically significantly increased at the two top doses (for effects on other organs, see section on STOT RE).

In top dose males, a statistically significant increase above concurrent controls was seen for hepatocellular adenoma, reaching 14%, which is within the HCD, though in the upper range of these values (HCD: range 0.0 – 18%). No increase in hepatocellular tumours was seen in females.

In females, a statistically significant increase in uterine adenocarcinoma was seen in the two top doses, which was not dose-dependent but clearly exceeded the HCD in both groups (i.e., 22% and 20% in the two groups, respectively; HCD: range 0 – 8%, mean 1.1%). A dose-response might have become visible if more appropriate dose spacing had been applied (i.e., with a dose between the two lower and the two higher doses).

No increase in other neoplastic lesions was seen in males or females.

In the two top doses also haematological and blood biochemistry changes were observed (see table in the section on STOT RE), some of them are related to the observed liver toxicity, i.e., increase in cholesterol levels at all investigated time points; more pronounced in females than in males. Total bilirubin was decreased, but this effect was seen only in males throughout the whole study period. AST, ALT and ALP were decreased in the two top doses in males and females throughout the study, except for the final sampling (i.e., week 104). γ-GT was mostly increased in the two top doses in males and females.

A finding that may be relevant for the interpretation of the uterine adenocarcinomas is an increase in oestradiol levels in the two top doses in females, which was statistically significant except for the final sampling (i.e., week 104).

While the increase of benign hepatocellular adenomas in males was not exceeding HCD levels, the malign uterine adenocarcinomas in females were seen at the two top doses above HCD levels. Though no dose-response was observed, the plateau of the effect might have been reached and a slightly lower dose group (i.e., between the two higher and the two lower doses) might have given relevant information with regard to dose-response relation. The neoplastic findings were not invalidated by severe general toxicity at either of the two top doses. While the hepatocellular adenomas were within HCD levels, the increase in uterine adenocarcinomas clearly demonstrates a carcinogenic potential of benthiavalicarb-isopropyl above background levels in this study.

***Table**: Body weight (g) in the chronic toxicity and carcinogenicity study in mouse (Anonymous 20, 2001b), table 6.5.4/02-2 from DRAR.*

	Males					**Females**				
Dose (ppm)	**0**	**20**	**100**	**2500**	**5000**	**0**	**20**	**100**	**2500**	**5000**
Week 0	19.4	19.4	19.4	19.4	19.4	16.5	16.5	16.5	16.5	16.5
Week 52	40.6	41.5	40.1	39.1	37.3 **	30.2	30.5	30.1	30.9	30.6
Week 104	41.5	42.7	41.7	35.8 **	34.7 **	32.7	32.1	32.2	34.0 *	33.5 *
Body weight gain (weeks 0 – 104)	22.0	23.1	22.3	16.2 **	15.3 **	16.2	15.6	15.7	17.5 *	17.1 *

* … significantly different from control: $p < 0.05$; ** … significantly different from control: $p < 0.01$

***Table**: Relevant organ weights, non-neoplastic and neoplastic findings in the chronic toxicity and carcinogenicity study in male mouse at termination (week 104) (Anonymous 20, 2001b), information partly derived from DRAR.*

Doses (ppm)	**0**	**50**	**100**	**2500**	**5000**	**HCD[#]**
Males						
Doses (mg/kg bw/d)	**0**	**2.7**	**13.7**	**358.4**	**731.3**	
Liver weights:						
Absolute (g)	2.00	1.99	1.99	4.26 **	5.47 **	
Relative (%)	4.97	4.81	4.92	11.93 **	15.78 **	
Adrenal weights:						
Absolute (g)	5	23 ± 104 [#]	6 *	6 *	6 **	
Relative (%)	0.013	0.050[#]	0.015	0.017 *	0.018 *	
Non-neoplastic findings: Number of findings / animals examined						
Liver:						
Angiectasis	0/41	0/37	1/38	3/35	9/28**	
Anisonucleosis	0/41	2/37	1/38	11/35**	13/28**	
Multinucleated liver cell	0/41	0/37	0/38	8/35**	4/28**	
Intermediate fatty change	0/41	0/37	0/38	30/35**	20/28**	
Hepatocyte hypertrophy	0/41	0/37	0/38	34/35**	28/28**	
Focal alterations	20/41	19/37	24/38	35/35**	28/28**	
Bile duct proliferation	0/41	0/37	0/38	5/35*	12/28**	
Cellular infiltration	3/41	1/37	2/38	30/35**	25/28**	
Macrophage accumulation	4/41	1/37	0/38	28/35**	28/28**	
Necrosis	2/41	1/37	3/38	15/35**	22/28**	
Single cell necrosis	1/41	2/37	2/38	33/35**	28/28**	
Lymphocyte infiltration	6/41	6/37	5/38	21/35**	23/28**	
Stomach:						
Forestomach ulcer	6/41	7/37	6/38	13/35**	16/28**	
Lymphocyte infiltration	12/41	14/37	13/38	22/35**	17/28**	
Epithelial hyperplasia	18/41	14/37	14/38	29/35**	19/28**	
Pituitary:						
Cyst	0	3	2	4*	4*	
Hyperplasia	2	0	3	0	0	
Adrenal gland:						
Focal hypertrophy, cortex	11	7	10	1**	2*	
Hyperplasia, cortex	0	2	1	13**	20**	
Spindle cell hyperplasia	37	28	33	25*	24	

Bone marrow:						
Increase in megakaryocyte	3	2	2	6	8*	
Thyroid:						
Dilated follicle	2/41	1/37	1/38	12/35**	17/28**	
Follicular cell hyperplasia	1/41	3/37	4/38	17/35**	28/28**	
Neoplastic findings: Number of findings for 50 animals investigated [1]						
Liver:						
Hepatocellular adenoma	15 (30%)	6* (12%)	17 (34%)	43** (86%)	47** (94%)	16 – 56% (mean 39.3%)
Hepatocellular carcinoma	12 (24%)	12 (24%)	11 (22%)	35** (70%)	43** (86%)	10 – 40% (mean 18.9%)
Hepatoblastoma	0	0	0	12** (24%)	9** (18%)	0 – 2%
Thyroid:						
Thyroid follicular cell adenoma	0	1 (2%)	0	4 (8%)	9** (18%)	0 - 6% (mean 2.6%)
Bone marrow:						
Bone marrow, haemangioma	0	0	0	0	0	
Bone marrow, malignant mastocytosis	1	0	0	0	0	
Adrenal gland:						
Adrenal gland, adenoma	0	1	0	0	0	-
Adrenal gland, malignant phaeochromocytoma	0	1	0	0	0	-

* Significantly different from control: $p < 0.05$, ** Significantly different from control: $p < 0.01$, [1] This total number of animals (n = 50) includes animals sacrificed at termination and animals found dead or killed moribund, # this value is discussed in the DRAR, it might be caused by hyperplasia in a single animal, value not reliable, very large SD.

***Table**: Relevant organ weights, non-neoplastic and neoplastic findings in the chronic toxicity and carcinogenicity study in female mouse at termination (week 104) (Anonymous 20, 2001b), information partly derived from DRAR.*

Doses (ppm)	**0**	**50**	**100**	**2500**	**5000**	**HCD#**
Females						
Doses (mg/kg bw/d)	**0**	**3.7**	**18.6**	**459.3**	**927.8**	
Liver weights:						
Absolute (g)	1.45	1.51	1.45	2.42**	2.56**	
Relative (%)	4.44	4.76	4.52	7.14**	7.67**	
Adrenal weights:						
Absolute (g)	8	8	8	9*	9	
Relative (%)	0.025	0.025	0.026	0.028	0.026	

Ovary weights:						
Absolute (g)	9	9	11	8*	6*	
Relative (%)	0.027	0.028	0.037	0.023*	0.019**	
Non-neoplastic findings: Number of findings / animals examined						
Liver: §						
Intermediate fatty change	0/41	0/43	0/36	37/41**	33/43**	
Hepatocyte hypertrophy	0/41	1/43	1/36	40/41**	42/43**	
Focal alteration	9/41	11/43	8/36	36/41**	34/43**	
Bile duct proliferation	0/41	0/43	0/36	1/41	2/43	
Cellular infiltration	0/41	2/43	2/36	3/41	13/43**	
Macrophage accumulation	2/41	2/43	4/36	6/41	8/43	
Necrosis	1/41	0/43	1/36	3/41	10/43**	
Single cell necrosis	0/41	1/43	1/36	2/41	6/43*	
Thyroid:						
Dilated follicle	1/41	4/43	2/36	4/41	10/43**	
Follicular cell hyperplasia	5/41	1/43	1/36	20/41**	26/43**	
Ovary:						
Atrophy	9/41	9/43	14/36	22/41**	30/43**	
Reduced number of corpora lutea	10/41	11/43	10/36	9/41	13/43	
Bone marrow:						
Increase in megakaryocyte	1	2	0	3	4	
Uterus:						
Angiectasis	0	2	2	0	6*	
Adrenal gland:						
Hypertrophy, cortex	1	2	2	38**	42**	
Neoplastic findings: Number of findings for 50 animals investigated [1]						
Liver:						
Hepatocellular adenoma	4	2	4	20** (40%)	23** (46%)	6 – 26% (mean 13.6%)
Malignant lymphoma	2	6	8* (16%)	5	8* (16%)	4 – 26% (mean 15.6%)
Bone marrow:						
Haemangioma	0	2	0	1	0	
Malignant mastocytosis	0	0	0	0	0	
Uterus:						
Uterus, adenoma	0	0	1	1	1	

Uterus, endometrial stromal polyp	1	4	3	1	1	
Uterus, haemangioma	0	2	1	2	0	
Uterus, leiomyoma	1	0	1	1	1	
Uterus, adenocarcinoma	0	0	0	0	1	
Uterus, histiocytic sarcoma	3	0	1	3	2	
Uterus, leiomyosarcoma	1	0	0	0	0	
Uterus, stromal sarcoma	0	0	1	1	0	
Adrenal gland:						
Adrenal gland, adenoma	0	0	0	0	1	
Adrenal gland, malignant pheochromocytoma	0	0	0	0	0	

* Significantly different from control: $p < 0.05$, ** Significantly different from control: $p < 0.01$, [1]This total number of animals (n = 50) includes animals sacrificed at termination and animals found dead or killed moribund, § Regarding the non-neoplastic observations in the liver of female mice an inconsistency was noted between the DRAR and the CLH report. In the DRAR lymphocyte infiltration and epithelial hyperplasia is indicated, whereas this is not indicated in the CLH report. Based on the original study report it could be clarified that these effects were not seen in female mice, only in male mice lymphocyte infiltration was reported.

Summary of the results of the chronic toxicity and carcinogenicity study in mice

During the first 77 weeks of the study, there were no changes in the general condition of the animals attributable to treatment in either sex. From week 78 of the study, observations of the 2500 ppm and 5000 ppm males included wasting, piloerection, pallor, abdominal tissue mass, abdominal distension, and tachypnea. Viability was affected in males from week 78 onwards and survival declined to 56% in top dose males at termination. In females there were no treatment-related clinical signs.

At termination both body weight and body weight gain were decreased at the two top doses in males, while in females there was a slight increase at these doses. The decrease of male body weight gain in males was evident from week 90 at 2500 ppm, in the top dose it was evident already from week 9 onwards. Sporadic increases and decreases in food consumption and compound intake were observed, but overall it was comparable among the groups. Similar fluctuations were also noted in food efficiency, although in the two top doses food efficiency was marginally lower in males compared to controls during the first year of the study. Like for the other mouse studies (the 28-d and 90-d studies) it is noted that the original study reports (which were made available to RAC) did not contain any information on blood biochemistry parameters.

Some perturbations in haematology values mainly in males were observed for the two top dose groups.

Like in the rat carcinogenicity study also in the mouse study 4 dose groups were tested but, like in the rat study the dose spacing was suboptimal with a large gap between the two lower and the two higher doses (> than 20-fold). In males the two top dose groups were clearly affected by considerable general toxicity and lower doses would have been more informative.

Again, liver was a target organ of toxicity and males were more susceptible than females. Absolute and relative liver weights were increased (relative liver weight, males: 2500 ppm: +140%, 5000 ppm: +218%, females: 2500 ppm: +61%, 5000 ppm: +73%) and histopathological changes were seen in the majority of animals of the two top doses. These changes included hepatocellular hypertrophy, fatty change, focal alterations, cellular infiltration, necrosis, and single cell necrosis and more (see tables under 'Summary of the results of the

chronic toxicity and carcinogenicity study in rat') in males and females, again these effects were a bit more pronounced in males than in females.

Liver was already affected after 52 weeks and 78 weeks. These observations include lymphocyte infiltration, single cell and general necrosis, hepatocellular hypertrophy, focal alterations in the two top dose groups in males; not all of these effects were seen in females. In many cases these effects showed a dose-dependent increase in severity and in general they were more pronounced in males compared to females. A considerable part of the animals was affected, e.g., after 52 weeks 4/10 top dose males had general necrosis, and single cells necrosis was seen in 5/10 males at the second highest dose and 8/10 males of the top dose. These effects are indicative of cytotoxicity. A statistically significant increase in hepatocellular adenoma and carcinoma, exceeding HCD, was observed in males of the two top doses involving the majority of all animals. In addition, the incidence of hepatoblastoma was statistically significantly increased in males of the two top doses. No hepatoblastoma was seen in the concurrent control and the two lower doses, and the HCD were clearly exceeded. In females, a statistically significant increase in hepatocellular adenomas was seen in the two top doses, which exceeded HCD. While the neoplastic changes in the liver of males occurred in the presence of considerable general toxicity, the increase in hepatocellular adenoma in the two top doses in females were not accompanied by severe general toxicity, increasing the relevance of the finding. The fact that liver lesions were seen in both sexes also supports its relevance.

Details on tumours from the interim kills describe hepatocellular adenomas at week 52 (7/10 males of the top dose, 1/10 females of the second highest dose). At week 78, an increase in hepatocellular adenomas was seen in the two top doses in males and females and one incidence of hepatocellular carcinoma was seen in the two median doses in the males. In males, there were also 2 cases of hepatoblastoma in the top dose. In male mice, abdominal masses were described from week 77 onwards. As almost all male animals were affected by neoplastic liver lesions, the decreased survival in top dose males did not obscure the number of liver tumours.

Thyroid weights were not affected by benthiavalicarb-isopropyl treatment, but in both sexes dilated follicles and follicular cell hyperplasia in the thyroid were described (for males both effects were statistically significantly increased in both top doses, in females, comparable effects were described, except that no increase in dilated follicles was seen at 2500 ppm). Thyroid follicular cell adenoma was increased above HCD in males of the two top doses, in the top dose this was statistically significant. No increase was seen in females.

In females there was a statistically significant increase in malignant lymphoma at 100 ppm and at 5000 ppm. No dose-response was obvious, and the incidences were clearly within the historical control range, therefore this finding was not considered relevant.

It was further noted that single incidences of uterine adenomas were seen in the three highest doses and a single uterine adenocarcinoma in the top dose group. In their conclusion, EFSA noted that this observation could be relevant as the same tumours were seen in female rats and that uterine tumours is a rare finding in mouse (EFSA, 2021).

Mechanistic studies

In order to clarify modes of action that could be the underlying cause or be involved in the formation of the observed tumours, 17 mechanistic studies were presented in the CLH report. Six of these studies were conducted to investigate the involvement of CAR activation in benthiavalicarb-isopropyl-induced liver tumours, but also to inform on the relevance of this MoA for thyroid tumour development (Anonymous 22, 2018a; McMahon, 2018b-d; Anonymous 25, 2001c; Anonymous 26, 2001d). Four studies investigated whether liver enzyme induction or thyroid peroxidase (TPO) inhibition were the underlying cause of the observed thyroid tumours (McMahon, 2018e; Anonymous 29, 2002a; Anonymous 30, 2002b; Anonymous 31, 2003). Two

additional studies were conducted to assess benthiavalicarb-isopropyl's potential to induce oxidative DNA damage (Anonymous 27, 2001a; Anonymous 28, 2001b), three studies to investigate the initiation/promotion potential of benthiavalicarb-isopropyl (Anonymous 23, 2000a; Anonymous 24, 2000b; Nakajima, 2000b) and finally two studies to investigate possible mechanisms for the uterine tumours (Anonymous 32, 2002c; Anonymous 33, 2015)

As a consequence of the discussion under the EFSA regime, the applicant carried out further investigations on a possible influence of benthiavalicarb-isopropyl on the Wnt/ß-catenin signalling pathway and its potential role in the development of the observed liver tumours, as well as the uterine tumours. Summaries and study reports of these studies were made available during the consultation. Four studies investigated Wnt/ß-catenin signalling in liver, three studies in the mouse (Anonymous, 2019a; Anonymous, 2019b; Anonymous, 2021) and one rat study (Anonymous, 2020). A similar study was conducted to investigate Wnt/ß-catenin signalling in the rat uterus (Anonymous, 2020c). In order to further clarify the cause of the uterine tumours another two studies (Anonymous, 2020a; 2020b) were investigating the effect of benthiavalicarb-isopropyl on oestrus cyclicity, blood hormone levels (prolactin, progesterone, oestrogen) and the expression of mRNA of prolactin, follicle stimulating hormone (FSH) and luteinising hormone (LH) in the pituitary.

These studies were summarised in four opinion documents titled "Opinion Document on Mode of Action and Analysis of human Relevance of rodent Hepatocellular Tumours" (Anonymous, 2021a), "Opinion Document on Mode of Action and Analysis of human Relevance of rodent thyroid follicular cell tumours" Anonymous, 2021b), "Opinion document on the Wnt/ß-catenin pathway as a Mode of Action for the Mouse Hepatocellular Tumours and Hepatoblastoma" (Anonymous, 2021c), "Opinion Document on Hepatoblastoma in the Mouse" (Anonymous, 2021d) and a review report titled "Exploration of the Mode of Action of Uterine Adenocarcinoma in the Rat" (Anonymous, 2021e). In addition, study reports were provided (Anonymous, 2021; Anonymous 2019a, b; Anonymous, 2020; Anonymous, 2020a, b, c). The relevant information from the studies is summarised in the section "Supplemental information – in depth analyses by RAC".

In the review document by Anonymous (2021d), a re-evaluation of the histopathological slides of the hepatoblastomas from the mouse carcinogenicity study (Anonymous 20, 2001b) was provided. In the same review document (Anonymous, 2021d) also differences and similarities of mouse and human hepatoblastomas are discussed. These two reports were also cited in the CLH report (Foster, 2018a and 2018b).

Two further studies for the clarification of the thyroid effects (An *In vitro* Interaction Study with the human NIS (SLC5A5) Sodium Iodide Symporter (Anonymous 1, 2021a) and an *In vitro* mouse DIO activity assay (Anonymous 2, 2021b)) were submitted at a late stage in the process and are still included in the assessment. These studies were performed by the applicant in order to clarify open questions on the MoA underlying the thyroid tumours which were identified during the ED discussion at EFSA. A detailed description is also presented under section "Supplemental information – in depth analyses by RAC" in the Background Document.

Hepatocellular adenomas and carcinomas

CAR MoA

***Table**: Results of the mechanistic studies related to the CAR/PXR MoA postulated for the observed hepatocellular adenomas in male rat, male and female mice and the hepatocellular carcinomas in male mice. Parts of the table are taken from the CLH report, but were rearranged, complemented, and corrected (some values were not the same as in the DRAR)*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
In vivo study Evaluation of the KIF-230 TGAI hepatocellular and thyroid follicular cell toxicity No guideline Acceptable C57BL/6N mice 15 males/dose	KIF-230R-L, purity: 99.0% 0, 500 and 5000 ppm 7 days: 80 and 740 mg a.s./kg bw/d 28 days: 77 and 660 mg a.s./kg bw/d 7 and 28 days Positive control: phenobarbital (PB) sodium salt at 500 ppm 7 days: 87 mg PB/kg bw/d 28 days: 79 mg PB/kg bw/d	If not indicated differently these changes were statistically significantly different from the control. **Gene expression (only after 7 days):** - Cyp2b10 mRNA: 450- and 1900-fold increase at 500 and 5000 ppm, resp.; 1330-fold increase at 500 ppm PB - Cyp3a11 mRNA: 2.2- and 7.6-fold increase at 500 and 5000 ppm, resp.; 2.8-fold at 500 ppm PB - Cyp1a1 mRNA: 1.3- and 2.4-fold increase at 500 and 5000 ppm, resp.; 1.4-fold increase at 500 ppm PB - Cyp1a2 mRNA: 1.6- and 2.2-fold increase at 500 and 5000 ppm, resp.; 2.3-fold increase at 500 ppm PB **Enzyme activity (only after 7 days):** - PROD: 32- and 66-fold increase at 500 and 5000 ppm, resp.; 77-fold increase at 500 ppm PB - BROD: 29- and 153-fold increase at 500 and 5000 ppm, resp.; 149-fold increase at 500 ppm PB - EROD: 1.9- and 5.6-fold increase at 500 and 5000 ppm, resp.; 5.5-fold increase at 500 ppm PB - BQ: 1.5- and 4.2-fold increase at 500 and 5000 ppm, resp.; 3.1-fold increase at 500 ppm PB - T4-UDPGT: 1.3- and 2.3-fold increase at 500 and 5000 ppm, resp.; 1.8-fold increase at 500 ppm PB **Plasma hormone levels :** - Total T4: 1.3- and 1.3-fold increase at 500 ppm after 7 and 28 days, resp. 1.9- and 1.7-fold increase at 5000 ppm after 7 and 28 days, resp. - Total T3 & TSH: There were no significant treatment-related effects on T3 and TSH plasma levels either at 7 days or at 28 days. However, biological variation was very high for TSH in the present study. Comparable effects were seen for 500 ppm PB after 7 and 28 days (no further details presented). **Gene expression in the pituitary glands:** - TSHB mRNA: 4.8-fold increase at 5000 ppm after 28 days of treatment. - Trhr mRNA: 1.3-fold increase at 500 ppm and a 2.3-fold increase at 5000 ppm after 28 days of treatment. - With 500 ppm PB a 2.6-fold increase was seen for TSHB and a 1.8-fold increase for Trhr after 28 days treatment	Anonymous 22, 2018a DRAR Study No. CXR1882, Vol. 3 CA, B.6.5.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Histopathological investigation: - Hepatocellular proliferation (BrdU labelling): dose-dependent increase in proliferation, statistically significant 4.7-fold increase at 5000 ppm after 7 days of treatment. 500 ppm PB resulted in a 3.6-fold increase after 7 days (not included for 28 days) - At 500 ppm benthiavalicarb-isopropyl, hepatocellular hypertrophy was reported, at 5000 ppm high grade of hepatocellular hypertrophy and increased atypical hepatocellular vacuolation are reported, as well as an increase in mitotic figures. PB treated animals revealed hepatocellular hypertrophy and an increased incidence of mitotic figures in all animals. - Thyroid follicular cell proliferation (BrdU labelling): 1.3- and 1.5-fold increase at 500 and 5000 ppm, resp., after 28 days of treatment (no statistically significant increase after 7 days). 500 ppm PB resulted in a 1.5-fold increase after 28 days and a 1.4-fold increase after 7 days. - Microscopic examination of the thyroid did not reveal any effect on hypertrophy, probably because of aberrant histology in control animals.	
Induction of drug metabolic enzyme and proliferation of hepatocytes in mice No guideline Acceptable Slc:B6C3F1 (C57BL/6 x C3H®(SPF) mice 8/sex/dose	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L 0, 10 or 1000 mg/kg bw/d 7 days by oral gavage	**Mortality and clinical signs**: None **Body weight and bw gain**: No relevant findings. **Liver weight:** At top dose, absolute liver weights were increased with 25 – 30% in M and 13 – 15% in F; relative liver weights were increased with 20 – 31% in M and 13 – 24% in F. **Gross pathology and histopathology**: Hepatocyte hypertrophy in 3/3 M and 2/3 F at 1000 mg/kg bw/d. **Enzyme induction (protein content):** <u>Males - statistically significant increases at 1000 mg/kg bw/d were seen for:</u> - Total CYP450: 71% - CYP1A1/1A2: 149% - CYP2B1/2B2: 180% - CYP3A2: 104% <u>Females - statistically significant increases at 1000 mg/kg bw/d were seen for:</u> - Total CYP450: 92% - CYP1A1/1A2: 63% - CYP2B1/2B2: 553% - CYP3A2: 172% - CYP2E1 and CYP4A1: No relevant change in M or F. **Cell proliferation:** Hepatocellular proliferation: No increase.	Anonymous 26, 2001d DRAR Report n°4899 (001-258)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Induction of drug metabolic enzyme and proliferation of hepatocytes in rats No guideline Acceptable F344/DuCrj Fischer- SPF rats 8/sex/dose	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L 0, 10 or 1000 mg/kg bw/d 7 days by oral gavage	**Mortality and clinical signs**: None **Body weight and bw gain:** Body weight gain: 43% increase in M and 100% in F at 1000 mg/kg bw/d. **Gross pathology and histopathology**: Enlarged liver in M at 1000 mg/kg bw/d. 11 - 18% increase in M and 8 - 10% increase in F of relative liver weight at 1000 mg/kg bw. **Enzyme induction (protein content):** <u>Males - statistically significant increases at 1000 mg/kg bw/d were seen for:</u> - Total CYP450: 18% - CYP1A1/CYP1A2: 160% - CYP2B1/CYP2B2: 158% - CYP3A2: 101% - CYP2E1 and CYP4A1: No relevant change. <u>Females - statistically significant increases at 1000 mg/kg bw/d were seen for:</u> - CYP3A2: 131% - Inductions also for CYP1A1 (97%) and CYP2B1/2B2 (76%), but not statistically significant - CYP2E1 and CYP4A1: No relevant change **Cell proliferation**: Hepatocellular proliferation: increased by 62% and 58% at 10 and 1000 mg/kg bw/d in M, resp. The increase was not statistically significant. No effect in F.	Anonymous 25, 2001c DRAR Report n°4900 (001-259)
In vitro study KIF-230 mechanism of action in cultured wild-type mouse hepatocytes No guideline Acceptable Male C57BL/6 mouse hepatocytes	KIF-230R-L, purity: 99% 0, 3, 10, 30, & 100 µM Positive control: PB: 100 µM and 1 mM Epidermal growth factor (EGF): 25 ng/mL Cultured with KIF-230R-L and PB and Epidermal Growth Factor (EGF) 96 h + 72 h in the presence of BrdU for evaluation of cell proliferation	**Gene expression: fold change** See table below. **Enzyme activity:** See table below. **Cell proliferation:** Hepatocellular proliferation: 33, 51 and 54% at 10 µM, 30 µM and 100 µM, resp. **Cytotoxicity:** The tested concentrations were not cytotoxic, based on ATP content compared to control (ATP content was equal to control or slightly exceeded controls in all trials).	McMahon, 2018b DRAR Study No. 180071-1/45, Vol. 3 CA, B.6.5.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
In vitro study KIF-230 mechanism of action in cultured mouse (CAR-/-/PXR-/-) hepatocytes No guideline Acceptable Hepatocyte from CAR/PXR knock-out mice (KO-mice), male	Benthiavalicarb-isopropyl, purity: 99% 0, 3 µM, 10 µM, 30 µM, and 100 µM Positive control: PB:100 µM and 1 mM EGF: 25 ng/mL 96 h + 72 h in the presence of BrdU for evaluation of cell proliferation	**Gene expression:** See table below. **Enzyme activity:** See table below. **Cell proliferation:** Hepatocellular proliferation: No increase. **Cytotoxicity:** The tested concentrations were not cytotoxic, except for the highest dose of benthiavalicarb-isopropyl (100 µM), where the ATP content was reduced by 11% compared to the control.	McMahon, 2018c DRAR Study No. 180073-1/45, Vol. 3 CA, B.6.5.1
In vitro study KIF-230 mechanism of action in cryopreserved human hepatocytes No guideline Acceptable Human hepatocytes, 3 male donors	KIF-230R-L, purity: 99% 0, 3 µM, 10 µM, 30 µM, and 100 µM Positive control: PB: 100 µM and 1 mM EGF: 25 ng/mL 96 h + 72 h in the presence of BrdU for evaluation of cell proliferation	**Gene expression:** See table below. **Cell proliferation**: Hepatocellular proliferation: No increase. EGF treatment resulted in cell proliferation in cells of all three donors. **Cytotoxicity:** No decrease in ATP content up to the highest test concentration of benthiavalicarb-isopropyl of 100 µM was seen in hepatocytes of two out of three donors. In cells of the third donor a decrease in ATP content was seen at all concentrations of benthiavalicarb-isopropyl and PB. This decrease was dose-dependent for both substances reaching -21% at 100 µM benthiavalicarb-isopropyl and -14% at 1 mM PB.	McMahon, 2018d DRAR Study No. 180073 - 1/68, Vol. 3 CA, B.6.5.1

B = benthiavalicarb; * p < 0.05; ** p < 0.01; ***p < 0.001; **** p < 0.0001

McMahon, 2018b

***Table**: Gene expression: fold change*

Treatment	mRNA expression			
	Cyp2b10	*Cyp3a11*	*Cyp1a1*	*Cyp1a2*
Control	1.00 ± 0.21	1.00 ± 0.23	1.00 ± 0.47	1.00 ± 0.27
Benthiavalicarb-iospropyl (3 µM)	1.01 ± 0.15	1.15 ± 0.30	**0.46 ± 0.21***	1.06 ± 0.24
Benthiavalicarb-iospropyl (10 µM)	1.02 ± 0.28	1.68 ± 0.49	**0.42 ± 0.21***	1.04 ± 0.29
Benthiavalicarb-iospropyl (30 µM)	0.82 ± 0.18	**2.5 ± 1.1*****	0.58 ± 0.29	1.20 ± 0.44
Benthiavalicarb-iospropyl (100 µM)	**0.56 ± 0.11***	1.76 ± 0.47	1.17 ± 0.31	**2.48 ± 0.34********
Phenobarbital (100 µM)	0.99 ± 0.26	1.05 ± 0.27	0.84 ± 0.36	0.99 ± 0.21
Phenobarbital (1 mM)	1.42 ± 0.54	**3.04 ± 0.66********	0.86 ± 0.35	**1.94 ± 0.47*****
EGF (25 ng/mL)	-	-	-	-

* p < 0.05; ** p < 0.01; ***p < 0.001; **** p < 0.0001

***Table**: Enzyme activity*

Treatment	Relative fold			
	PROD	BROD	BQ	EROD
Control	1.00 ± 0.07	1.00 ± 0.10	1.00 ± 0.17	1.00 ± 0.14
Benthiavalicarb-iospropyl (3 µM)	**2.42 ± 0.22****	1.88 ± 0.13*	1.396 ± 0.063	0.87 ± 0.38
Benthiavalicarb-iospropyl (10 µM)	**3.48 ± 0.10******	**3.02 ± 0.43******	**1.95 ± 0.24****	1.05 ± 0.22
Benthiavalicarb-iospropyl (30 µM)	**3.07 ± 0.12******	**2.57 ± 0.44******	**1.98 ± 0.24****	1.05 ± 0.26
Benthiavalicarb-iospropyl (100 µM)	1.52 ± 0.05	7.42 ± 0.17	1.05 ± 0.11	0.96 ± 0.06
Phenobarbital (100 µM)	**3.18 ± 0.35******	**3.06 ± 0.04******	1.79 ± 0.49*	1.54 ± 0.24
Phenobarbital (1 mM)	**7.32 ± 0.88******	**4.55 ± 0.57******	**8.46 ± 0.33******	**3.39 ± 0.49******

* p < 0.05; ** p < 0.01; ***p < 0.001; **** p < 0.0001

McMahon, 2018c

***Table**: Gene expression:*

Treatment	mRNA expression			
	Cyp2b10	***Cyp3a11***	***Cyp1a1***	***Cyp1a2***
Control	1.00 ± 0.20	1.00 ± 0.09	1.00 ± 0.15	1.00 ± 0.08
Benthiavalicarb-isopropyl (3 µM)	0.77 ± 0.33	0.83 ± 0.25	0.70 ± 0.24	**1.36 ± 0.19****
Benthiavalicarb-isopropyl (10 µM)	0.67 ± 0.23	0.77 ± 0.18	0.84 ± 0.24	**1.34 ± 0.10***
Benthiavalicarb-isopropyl (30 µM)	0.68 ± 0.26	0.78 ± 0.18	1.15 ± 0.33	**1.62 ± 0.25******
Benthiavalicarb-isopropyl (100 µM)	1.27 ± 0.39	0.99 ± 0.20	**2.14 ± 0.28******	**3.79 ± 0.18******
PB (100 µM)	0.83 ± 0.28	1.02 ± 0.14	1.05 ± 0.25	1.12 ± 0.09
PB (1 mM)	0.93 ± 0.42	**2.07 ± 0.54******	**1.49 ± 0.44***	**2.47 ± 0.28******
EGF (25 ng/mL)	-	-	-	-

* p < 0.05; ** p < 0.01; ***p < 0.001; **** p < 0.0001

***Table**: Enzyme activity*

Treatment	PROD	BROD	BQ	EROD
	relative fold	relative fold	relative fold	relative fold
Control	1.0 ± 1.7	1.00 ± 0.07	1.00 ± 0.18	1.00 ± 0.08
Benthiavalicarb-isopropyl (3 µM)	3.9 ± 4.3	1.06 ± 0.31	1.22 ± 0.33	0.68 ± 0.06
Benthiavalicarb-isopropyl (10 µM)	7.9 ± 8.1	1.43 ± 0.67	0.68 ± 0.15	**0.58 ± 0.08***
Benthiavalicarb-isopropyl (30 µM)	13.0 ± 4.2	0.91 ± 0.14	**0.52 ± 0.11***	0.69 ± 0.05
Benthiavalicarb-isopropyl (100 µM)	16.0 ± 7.1	1.35 ± 0.18	0.88 ± 0.12	**2.90 ± 0.31******
PB (100 µM)	14.6 ± 15.1	1.65 ± 0.48	1.09 ± 0.29	1.00 ± 0.08
PB (1 mM)	11.9 ± 10.4	1.74 ± 0.11	1.38 ± 0.06	**2.00 ± 0.23******

* p < 0.05; ** p < 0.01; ***p < 0.001; **** p < 0.0001

McMahon, 2018d

***Table**: Gene expression*

mRNA expression	Donor	Control	B. (3 µM)	B. (10 µM)	B. (30 µM)	B. (100 µM)	PB (100 µM)	PB (1 mM)
CYP2B6	8210	1.0 ± 0.2	1.2 ± 0.1	**2.0 ± 0.3*** **	**2.4 ± 0.3**** **	**2.0 ± 0.5*** **	**1.7 ± 0.4***	**3.7 ± 0.5**** **
	8219	1.0 ± 0.2	1.3 ± 0.1	**2.2 ± 0.4***	**2.7 ± 0.5*** **	**2.9 ± 1.2**** **	1.7 ± 0.4	**4.4 ± 0.8**** **
	385	1.0 ± 0.3	1.7 ± 0.4	2.5 ± 0.7	**3.4 ± 0.6****	**6.5 ± 1.5**** **	**3.3 ± 1.2****	**9.3 ± 2.1**** **
CYP3A4	8210	1.0 ± 0.2	**2.4 ± 0.4***	**4.4 ± 0.7**** **	**4.6 ± 0.7**** **	**4.8 ± 1.6**** **	**2.4 ± 0.7***	**7.2 ± 1.0**** **
	8219	1.0 ± 0.3	2.9 ± 0.5	**6.1 ± 1.3**** **	**7.7 ± 0.9**** **	**6.4 ± 2.2**** **	2.6 ± 0.5	**11.5 ± 2.1**** **
	385	1.0 ± 0.2	**4.6 ± 0.7**** **	**6.1 ± 1.0**** **	**7.3 ± 1.0**** **	**6.9 ± 0.7**** **	**4.3 ± 1.2**** **	**10.5 ± 1.5**** **
CYP1A1	8210	1.0 ± 0.2	1.1 ± 0.2	1.3 ± 0.3	**1.5 ± 0.3***	**1.7 ± 0.6****	1.3 ± 0.3	1.4 ± 0.3
	8219	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	**1.3 ± 0.2***	**1.6 ± 0.3**** **	1.1 ± 0.2	1.2 ± 0.2
	385	1.0 ± 0.2	0.9 ± 0.3	1.0 ± 0.2	1.2 ± 0.2	**1.8 ± 0.3**** **	1.0 ± 0.1	1.1 ± 0.2
CYP1A2	8210	1.0 ± 0.2	1.0 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	1.1 ± 0.2
	8219	1.0 ± 0.2	1.2 ± 0.3	1.3 ± 0.3	**1.6 ± 0.3***	**1.7 ± 0.5****	1.4 ± 0.3	**1.6 ± 0.3****
	385	1.0 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	1.3 ± 0.2	0.9 ± 0.3	1.0 ± 0.2

* p < 0.05; ** p < 0.01; ***p < 0.001; **** p < 0.0001

The available 6 studies investigating CAR/PXR activation in the liver covered the major key events relevant to assess this MoA, as e.g., described by Peffer *et al.* (2018); however, some drawbacks are pointed out in the following discussion of the studies.

In the 7-/28-d *in vivo* study in male mice by Anonymous 22 (2018a), liver and thyroid findings were in line with the results obtained in previous studies. Absolute and relative liver weights were statistically significantly increased in top dose animals and in the PB group, both after 7 and 28 days. Liver histopathology was only investigated after 7 days. The livers of PB-exposed mice showed centrilobular hepatocellular hypertrophy and increased mitotic figures in all mice in the group. The livers of the mice treated with 5000 ppm benthiavalicarb-isopropyl showed a higher grade of centrilobular hepatocellular hypertrophy than that seen with PB, with similar grades of mitotic figures. In addition, the majority of mice (12/15) showed increased atypical hepatocellular vacuolation. At 500 ppm, hypertrophy was less pronounced, but comparable to that seen with PB. At the low dose, the incidence of atypical vacuolation was lower (2/15).

There was no evidence of treatment-related histopathological effects on the thyroid glands after 7 neither after 28 days.

After 7 days, an increased incidence of hypertrophy of the basophilic cells was seen in the pituitary of all groups (including PB). After 28 days, the abundance of the cells was increased at 5000 ppm and in the PB group.

For most of the enzyme activity and gene expression parameters, an increase with dose was seen for the two doses of benthiavalicarb-isopropyl tested and in most cases the response at 5000 ppm benthiavalicarb-isopropyl was comparable to that at 500 ppm PB.

A considerable increase was seen for the gene expression of Cyp2b10 (450- and 1900-fold at 500 and 5000 ppm, respectively), which is also mirrored by strong increases in PROD and BROD activities, indicating CAR activation. A much lesser enzyme activity was seen for EROD (up to 5.6-fold in the top dose) and BQ, respectively, and Cyp1a1 and Cyp1a2 were only marginally induced.

Interestingly, a slight but dose-related increase in total T4 was seen after 7 as well as after 28 days and total T4 was also increased in PB treated mice, which is in contradiction with the increase in T4-UDPGT observed in the same groups. No effects were seen on the levels of total T3 and TSH. In contrast, in the pituitary, gene expression of TSHB was increased in the top dose and Trhr expression was increased dose-dependently, both after 28 days. Both transcripts were also increased in the PB group after 28 days.

A dose-dependent and statistically significant increase in cell proliferation was seen in the liver after 7 days, and in the thyroid after 28 days (not investigated in the liver after 28 days, not statistically significant in the thyroid after 7 days).

As thyroid tumours were only seen in male mice, and a much more pronounced tumour signal was seen in the liver of male mice, it would have been relevant to include both sexes in this mechanistic investigation, not only males. It would have been valuable to know if, or how strong, CAR signalling is induced in female mice, despite the much weaker (liver) and absent (thyroid) tumour response in females. For instance, the review paper on minimum data requirements to demonstrate that a CAR-mediated MoA is active (Peffer *et al.*, 2018), recommends investigating both sexes in case they differ with regard to tumour response.

Both sexes were investigated in the 7-d study by Anonymous 26 (2001d) in mice. While liver weight increase and hepatocellular hypertrophy were slightly more pronounced in males than in females of the top dose, total CYP450 content was more increased in females (92% in females vs. 71% in males, though absolute numbers were not presented). When looking at the content of specific CYP enzymes the levels were higher for CYP1A1/1A2 in males than in females, whereas for CYP2B1/2B2 it was the opposite.

In the DRAR it was concluded that in this study benthiavalicarb-isopropyl induced CYP450 related metabolism in mice liver. The finding included an increase of CYP1A1/1A2, CYP2B1/2B2 and CYP3A1 level. Except for the modification of CYP1A1/1A2, which is generally correlated with polycyclic aromatic hydrocarbon induction, the observed induction patterns (increase in CAP2B1/2B2 and CYP3A1) suggest PB-like activity. RAC agrees with this conclusion; however, it is noted that although in females the observed non-neoplastic and neoplastic liver lesions were much less pronounced in the available studies, including in this mechanistic study, the induction of total CYP450 and CYP2B1/2B2 as well as CYP3A2 in female mice of this study was clearly stronger than in males, which reduces the strength of the conclusion.

A comparable study was conducted in male and female rats (Anonymous 25, 2001c). Seven days exposure to 1000 mg/kg bw/d benthiavalicarb-isopropyl resulted in enlarged livers in males, and relative liver weight increase in males (11 – 18%) and females (8 – 10%). In males, a non-statistically significant increase in cell proliferation was seen without dose-response (10 mg/kg bw/d: 62% and 1000 mg/kg bw/d: 58%). CYP450 induction was seen in male rats and to a lesser extent in female rats. In top dose animals CYP1A1/1A2 as well as CYP2B1/CYP2B2 were increased more pronounced in males, while the increase in CYP3A2 was more pronounced in females. In the DRAR, it was concluded that except for the modification of CYP1A1, which is generally correlated with polycyclic aromatic hydrocarbon induction, the finding was suggesting a PB-like induction pattern. RAC agrees with this conclusion.

Two *in vitro* studies investigated the effects of benthiavalicarb-isopropyl in mouse hepatocytes, one study used cells from wild-type mice (McMahon, 2001b), the other study used cells from CAR/PXR knock-out mice (McMahon, 2018c).

In wild-type hepatocytes a clear increase in PROD and BROD activities was observed, indicating Cyp2b activity. No increase in Cyp2b10 mRNA compared to controls was detected, neither after treatment with benthiavalicarb-isopropyl nor after PB treatment. In the DRAR it was speculated that this could be caused by a gradual increase in expression of this gene in control cultures over the duration of the experiment (96 h). Overall, the observed changes indicate CAR-activation. The observed increase in BQ-activity indicates that also PXR was activated; however, as also in the trial with PB the activity of BQ was increased, the DRAR concluded that also this signal could be indicative for CAR activation (possibly via receptor cross talk). A dose-dependent increase in cell proliferation was noted. A slight increase in Cyp1a2 mRNA (2.5-fold) was seen in the top dose (comparable to the increase observed with 1 mM PB in this study), but no increase in EROD activity was observed, indicating that AhR was not activated.

As expected, the expression of the enzymes downstream of CAR/PXR was decreased in hepatocytes from CAR/PXR knock-out mice, as was the activity of PROD and BROD. Compared to the controls, a decrease was seen in EROD activities for benthiavalicarb-isopropyl trials and the lower PB dose, except for the highest doses of both substances (100 µM benthiavalicarb-isopropyl: 2.9-fold increase; 1 mM PB: 2-fold increase). Overall, this result supports that signal transduction is abolished when CAR and PXR receptors are knocked out. Slight increases in Cyp1a1 (2.1-fold) and Cap1a2 (3.8-fold) were seen in the top dose.

Despite a dose-dependent increase in the expression of CYP2B6, CYP3A4 and mRNA, no cell proliferation was induced in human hepatocytes derived from three male donors. It should be noted that the exclusion of female donors reduces the value of this result. It was further noted that in one donor, treatment with benthiavalicarb-isopropyl and PB induced a dose-dependent increase in cytotoxicity (i.e., decrease in ATP content), which was not seen in the other donors. Nevertheless, the cells of all donors reacted to EGF-treatment with a clear-cut increase in cell proliferation, demonstrating that cells from all three donors were responding to the strong proliferative signal of this growth factor.

Toxicokinetic studies have demonstrated that benthiavalicarb-isopropyl is extensively metabolised, but under *in vitro* conditions metabolism is much less active, especially if no measures are taken that could simulate normal metabolism like, e.g., the addition of metabolising enzymes (S9 mix). It could be that relevant metabolites were not formed in the *in vitro* tests carried out with benthiavalicarb-isopropyl. In this respect, it would have been highly relevant to also include an *in vivo* test in CAR knock-out animals.

Conclusion on CAR MoA

Overall, it can be concluded that in all six mechanistic studies investigating this MoA, increases in transcripts and enzyme activities downstream of CAR, as well as PXR, were observed. However, mainly in the *in vivo* studies, there was also an increase in the content of cyp1a1/1a2 mRNAs, CYP1A1/1A2 enzyme and increases in EROD activities indicating AhR activation, which was only slight in the study by Anonymous 22 (2018a) but more pronounced in the study by Anonymous 26 (2001d). It was further noted that there was a clear sex difference in liver effects and liver tumour incidence and severity between male and female mice upon benthiavalicarb-isopropyl treatment, which was not entirely mirrored by the results observed in the one *in vivo* mechanistic study investigating both sexes of mice (Anonymous 26, 2001d).

Another inconsistency was that no cell proliferation was induced in the study by Anonymous 26 (2001d) despite indications for CAR activation (plus 550% or 6.5-fold increase in CYP2B1/2B2 increase) and considerable effects on liver weight and histopathology, while in Anonymous 22 (2018b), with comparable study design (7 days exposure), dose-dependent cell proliferation was observed in males.

Furthermore, in the study by Anonymous 25 (2001c), considerable effects were seen on liver size and liver weight in males and females but increase in cell proliferation by ca. 60% at both tested doses was only seen in males (non-statistically significant) and only slight increases CYP expression were observed, mainly in males, where they were equal for CYP1A1/1A2 and CYP1B1/2B2 (+160% or 1.6-fold increase).

In contrast to the *in vivo* studies, the investigation of wild-type mouse hepatocytes *in vitro* did not indicate AhR activation (only at the top dose a slight increase in Cyp1a2 mRNA was noted). In hepatocytes from CAR/PXR knock-out mice, CAR and PXR signalling was abolished, and despite slight increases in AhR signalling, no cell proliferation was measured in the cells of the knock-out mice.

In human hepatocytes, no cell proliferation was induced, but the sample consisted of only 3 donors (males only). The reaction of cells from the different donors was not equal in that in one

of them an increase in cytotoxicity was observed with treatment. In addition, it is noted that EGF gives a rather strong proliferative signal to the cells. A stronger support could have been obtained from an *in vivo* knock-out study, which was also the view of the majority of member states in the EFSA discussion (EFSA, 2021). This is also supported by the fact that benthiavalicarb-isopropyl is extensively metabolised, which can hardly be mirrored under *in vitro* conditions.

The data support that benthiavalicarb-isopropyl is an activator of CAR and PXR, but the available data also indicate that under certain conditions activation of AhR may also occur.

The publication by Qin *et al.* (2019), cited by the DS in their response to comments, stated that a strong and prolonged increase of AhR activation was necessary to result in tumour formation. However, no respective investigation was available for benthiavalicarb-isopropyl (i.e., *in vivo* assessment of AhR activity was available for 7 days exposure, but no longer exposure duration was investigated).

Qin *et al.* (2019) also concluded that only increases in Cyp1a1/1a2 transcripts of more than 400-fold would be meaningful and indicative of AhR induced carcinogenesis. It was, however, noted that increases of such degree would then also be expected for the transcripts of the other Cyp-enzymes if considered to be the underlying cause for the development of tumours. Only in the study by Anonymous 22 (2018a), an increase in Cyp2b10 of more than 400-fold (i.e., 450- and 1900-fold for the two doses, respectively) was observed, while in the other studies the increases did not exceed 6.5-fold (CYP2B1/2B2, top dose females; Anonymous 26, 2001d). In addition, the relation between Cyps downstream of CAR and AhR, which was quite similar in some of the studies (i.e., Anonymous 26, 2001d: males, CYP1A1/1A2: 2.5-fold, CYP2B1/2B2: 2.8-fold or Anonymous 25, 2001c: males, CYP1A1/1A2: 2.6-fold, CYP2B1/2B2: 2.6-fold), should be considered.

It might be considered that a combined activation of these receptors could result in the observed tumour formation. Substances that activate all three receptors (CAR, PXR and AhR) have been described (e.g., Ayed-Boussema *et al.*, 2011). In addition, cytotoxicity is assumed to be another MoA contributing to benthiavalicarb-isopropyl-induced liver tumour formation.

Cytotoxicity

With regard to the observed liver tumours also the possible involvement of cytotoxicity needed to be considered as a relevant MoA.

In the mechanistic studies with 7 days duration, treatment with 5000 ppm benthiavalicarb-isopropyl also resulted in an increase in atypical hepatocellular vacuolation (not seen at 500 ppm or after treatment with PB). In addition, hepatocellular hypertrophy and liver weight increase was observed, but no cytotoxicity is reported. This observation does not exclude that cytotoxicity contributed to the observed tumour formation and it does not demonstrate that cytotoxicity was only a secondary consequence of CAR/PXR induced liver toxicity.

In all three long-term mouse studies there were clear signs of cytotoxicity (28-d study: Anonymous, 1996, 90-d: Anonymous 43, 1998a, Carcinogenicity study: Anonymous 20, 2001b). A detailed description and list of the observed histopathological changes can be found in the sections on STOT RE and for the mouse carcinogenicity study (Anonymous 20, 2001b) in the tables under 'Summary of the results of the chronic toxicity and carcinogenicity study in rat', the main findings are summarised below.

Signs of cytotoxicity were already seen after 28 days of exposure to benthiavalicarb-isopropyl. Only the 28-d study covered a dose of 500 ppm (equivalent to 105 mg/kg bw/d in males and 102 mg/kg bw/d in females). Already at this dose, slight single cell necrosis in 3/5 males and 1/5 females, slight focal necrosis in 1/5 males, slight fatty change in 1/5 males and slight increase in mitosis in 1/5 males and 1/5 females was reported. These observations clearly demonstrate

cytotoxicity after 28 days at doses ≥ 500 ppm. Centrilobular hypertrophy was only seen at the next higher dose (700 ppm: males: 1412 mg/kg bw/d, 5/5, slight; females: 1609 mg/kg bw/d, 2/5, slight), indicating that cytotoxicity was seen independent of CAR/PXR induced hepatocellular hypertrophy.

A summary of the histopathological observations from the 90-d mouse study can be found in the section on STOT RE (Anonymous 43, 1998a). Considerable liver toxicity was seen in the two top dose groups consisting of high numbers of animals with liver necrosis and single cells necrosis, bile duct proliferation, fatty change and anisonucleosis. Like for the carcinogenicity studies, also in the 90-d mouse study dose selection was inappropriate. The top two doses of the 90-d study even exceeded the doses used in the carcinogenicity study. It was therefore not possible to assess the occurrence of cytotoxicity after 90 days at doses that induced liver tumours after 2 years. However, given that cytotoxicity was seen after 28 days at clearly lower doses, it can be assumed that cytotoxicity was also induced after 90 days at doses relevant for tumour formation. This is supported by the respective findings in the mouse carcinogenicity study after 52 and 78 weeks (see description of the carcinogenicity study). There were clear signs of inflammation (lymphocyte infiltration, single cell necrosis, necrosis, bile duct proliferation) in a large number of animals, also at the final examination at the end of the carcinogenicity study (see tables under 'Summary of the results of the chronic toxicity and carcinogenicity study in rat'). It is unusual that the three mouse studies did not include blood biochemical parameters, which would have been useful to further assess the liver toxicity. Nevertheless, the described observations clearly support that benthiavalicarb-isopropyl induced cytotoxicity in the mouse liver.

Recurrent inflammation and regenerative growth is a well-known mechanism of tumour formation and the results described above indicate that the induction of cytotoxicity contributes to the formation of the observed liver tumours.

During the RAC 60 working group (January 2022) and plenary (March 2022) meetings, the Industry Stakeholder representatives argued that the only evidence of cytotoxicity was seen well above the doses used in the carcinogenicity studies, and that the incidence of various cytotoxic features (e.g.; inflammation and necrosis) were seen at lower incidences than the tumour incidence. In their view, the cytotoxicity was a consequence of the tumour formation and not vice versa. RAC did not agree with this conclusion (see detailed discussion above). For example, in the 90-d study, signs of cytotoxicity were seen already after 28 days, and in the 28-d study animals were affected by cytotoxicity at a not very high dose. As detailed above, RAC also noted that the dose at which the effects were seen in the 90-d study was not covered by the carcinogenicity studies. In addition, RAC noted that also in the carcinogenicity study animals were affected by cytotoxicity, see details above.

Thyroid follicular cell adenoma – potentially underlying modes of action

***Table**: Results of the mechanistic studies related to modes of action potentially relevant for the thyroid tumours observed in male mice, postulated for the observed hepatocellular adenomas in male rat and male and female mice, and the hepatocellular carcinomas in male mice. Parts of the table are taken from the CLH report but were rearranged and complemented*.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Effect on thyroid hormones in male rats No guideline	KIF-230 TGAI, purity: 88.6-89.1% as KIF-230R-L 0, 200 and	**Mortality and clinical signs:** No relevant findings. **Body weight and bw gain**: No relevant findings.	Anonymous 29, 2002a DRAR Report n°5903

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Acceptable, supplementary information F344/DuCjr-Fischer SPF rats 10 M/dose	10000 ppm 13.3 and 661.4 mg a.s./kg bw/d 14 days via the diet Levels of TSH, T3, T4 and T4 UDP-GT were measured.	**Gross pathology and histopathology:** Enlarged livers. No increase in thyroid size. 24% and 22% increase in absolute and relative liver weight at 10000 ppm, resp. **Enzyme activity**: - T4 UDP-GT: 16% increase at 10000 ppm after 14 days of treatment. **Serum hormone levels:** - Total T4: 15% and 18% decrease at 10000 ppm after 7 and 14 days of treatment, resp. - TSH and total T3: Unaffected.	(001-323),
Effect on thyroid hormones in male mice No guideline Acceptable, supplementary information Slc: B6C3F1, C57BL/6xC3H- SPF mice 6 M/sampling time	KIF-230 TGAI, purity: 88.6-89.1% as KIF-230R-L 0, 100 and 5000 ppm 17.0 and 855.0 mg a.s./kg bw/d 14 days via the diet Levels of TSH, T3, T4 and T4 UDP-GT were measured	**Mortality and clinical signs:** No relevant findings. **Body weight and bw gain:** No relevant findings. **Gross pathology and histopathology**: Dark and enlarged livers at 5000 ppm. No increase in thyroid size. 62% and 53% increase in absolute and relative liver weight after 14 days of treatment, resp. **Enzyme activity:** - T4 UDP-GT: 65% increase at 5000 ppm after 14 days of treatment. **Serum hormone levels:** - Total T4: 29% and 27% decrease at 5000 ppm after 7 and 14 days of treatment, resp. - TSH and T3: Unaffected.	Anonymous 30, 2002b DRAR Report n°5904 (001-324)
Effect on serum TSH of male mice No guideline Acceptable, supplementary information Slc: B6C3F1, C57BL/6xC3H- SPF mice 12 M/dose/sampling time	KIF-230 TGAI, purity: 88.6-89.1% as KIF-230R-L 0, 100 and 5000 ppm 15.7 and 809.8 mg a.s./kg bw/d 2, 4, 8 and 16 weeks via the diet Serum TSH concentration was measured via radio-immunoassay	**Mortality and clinical signs**: No relevant findings. **Body weight and bw gain:** 5000 ppm: 20% decrease in bw gain over the 16 weeks of treatment. **Liver:** 5000 ppm: livers of all animals were dark and enlarged, absolute and relative liver weights were increased by 31% and 29%, resp. **Serum hormone levels:** 5000 ppm: 14% increase in TSH after 16 weeks of treatment.	Anonymous 31, 2003 DRAR Report n°6655 (001-386)
In vitro study Investigation into the potential for KIF-230 to inhibit TPO activity *in vitro*	KIF-230R-L, purity: 99% Positive control: 6-propyl-2-thiouracil (PTU)	**TPO inhibition** (guaiacol oxidation): No effect up to 100 µM. The potential effect of benthiavalicarb-isopropyl on thyroid hormone synthesis was investigated at the level of the TPO enzyme.	McMahon, 2018e DRAR Study No. 180073 -

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No guideline Acceptable Female Yorkshire pig thyroid microsomes	9 conc.: from 0.01 to 100 µM	6-propyl-2-thiouracil (PTU) served as positive control substance, which is a well-described inhibitor of TPO. PTU strongly inhibited TPO-catalysed oxidation of guaiacol with an estimated IC_{50} value of 12 µM. In contrast, benthiavalicarb-isopropyl did not provoke any biologically significant inhibition of the TPO catalysed oxidation of guaiacol even at the highest tested dose of 100 µM. These findings suggest that benthiavalicarb-isopropyl does not affect thyroid hormone synthesis at the TPO level.	1/68, Vol. 3 CA, B.6.5.1

There are several indications from the available repeated dose studies that support liver enzyme induction and resulting catabolism of thyroid hormones as the underlying cause of the observed non-neoplastic and neoplastic thyroid effects. Liver weight increase and hepatocellular hypertrophy were seen in the majority of studies and CAR/PXR related liver enzyme induction is demonstrated in several *in vitro* and *in vivo* studies (see section above on *Hepatocellular adenomas and carcinomas – CAR MoA*).

One of these studies already presented in the previous section also investigated thyroid toxicity (Anonymous 22, 2018a). In this study thyroid hormone levels were measured in the blood. Interestingly total T4 was slightly increased at 500 ppm (1.3-fold after 7 days, 1.3-fold after 28 days) and at 5000 ppm (1.9-fold after 7 days, 1.7-fold after 28 days), despite increases in the expression of T4-UDPGT in the liver (after 7 days: 1.3-fold and 2.3-fold increase at 500 ppm and 5000 ppm, respectively). No effects were reported for blood T3 and TSH levels. This study also investigated the gene expression of TSHB and Trhr in the pituitary. Statistically significant increase was seen for both mRNAs at the top dose after 28 days (not after 7 days): TSHB: 4.8-fold, Trhr: 2.3-fold. Dose-dependent thyroid follicular cell proliferation was also induced after 28 days (not after 7 days): 1.3-fold at 500 ppm and 2.3-fold at 5000 ppm.

Two 14-d dietary studies were conducted in male rats (Anonymous 29, 2002a) and male mice (Anonymous 30, 2002b). Similar results were obtained for both species, including liver enlargement and liver weight increase and absence of histopathological effects in the thyroid. In male rats and mice of the top dose, considerable increase in T4-UDPGT activity was detected after 14 days and related decrease in total T4 was seen, both after 7 days and after 14 days.

Anonymous 31 (2003) investigated exposure to 100 ppm and 5000 ppm in the diet for two, four, six and 16 weeks. Serum TSH levels were increased by 14% after 16 weeks, before that no statistically significant changes were measured.

McMahon (2018e) studied the effect of benthiavalicarb-isopropyl on TPO activity. For this *in vitro* assay, thyroid microsomes were obtained from female Yorkshire pigs. While the well-known TPO inhibitor PTU (6-propyl-2-thiouracil) used as positive control gave expected results (i.e., clear cut inhibition of TPO activity), no effect was seen with benthiavalicarb-isopropyl concentrations up to 100 µM. Though this study gave clearly negative results, it can be discussed whether thyroid microsomes obtained from male as well as female mice would have been more appropriate to assess the thyroid response in male and female mice, than those obtained from Yorkshire pigs.

An *in vitro* Interaction Study with the human NIS (SLC5A5) Sodium Iodide Symporter (Anonymous 1, 2021a) and an *in vitro* mouse deiodinase (DIO) activity assay (Anonymous 2,

2021b) were submitted to ECHA in October 2021 and the study details can be found in the section "Supplemental information – in depth analyses by RAC". Both studies are well reported and conducted according to generally accepted standards, but the test protocol is not an accepted guideline yet. In contrast to the included positive control substances, benthiavalicarb-isopropyl was clearly negative in both studies, indicating that human NIS in cultured cells and microsomal mouse liver DIO were not inhibited or blocked by benthiavalicarb-isopropyl in these test systems.

Overall, it can be concluded that there are strong indications that the observed non-neoplastic thyroid changes in male and female mice as well as the increase in thyroid follicular cell adenomas in male mice are caused by liver enzyme induction and resulting thyroid hormone catabolism. This MoA has been investigated in several *in vitro* and *in vivo* studies, which found respective histopathological changes in liver and thyroid, identified CAR/PXR activation (initiating event), liver enzyme induction including T4 UDPGT induction, decreases in T4 levels (although in one study (Anonymous 22, 2018a) an increase in T4 was observed), and after prolonged exposure increase in TSH levels, increased transcription of TSHB and Trhr mRNA in the pituitary and cell proliferation in thyroid of male mice.

Although TPO inhibition was not investigated in mice (males and females), the species in which the thyroid changes were observed, the complete absence of an effect of benthiavalicarb-isopropyl on female Yorkshire pig TPO can be seen as an indication that this MoA is not relevant here. The positive control PTU induced a dose-dependent inhibition of TPO.

Moreover, the results of the two newly conducted studies were negative, giving no indication that benthiavalicarb-isopropyl inhibits NIS (*in vitro*) or liver DIO, although it has to be considered that the methods followed are currently still under development in the OECD programme and need further validation before approval. In addition, it is noted that the HPT axis is complex and not all potentially relevant modes of action can currently be covered by test methods.

Rodents are considered more sensitive towards liver enzyme induced thyroid toxicity than humans, but there is no qualitative difference regarding this MoA between rodents and humans. As there was only a slight increase in benign thyroid adenomas in males of the top dose only, RAC in line with the DS gives lower weight to this tumour type.

<u>Hepatoblastoma</u>

No separate MoA analysis was conducted for the investigation of hepatoblastomas; however, in the CLH dossier a possible involvement of Wnt/ß-catenin activation in the formation of these tumours is evaluated and an in-depth discussion of similarities and differences between murine and human hepatoblastoma was provided (Foster & Provan, 2018b). In this review, the occurrence of hepatoblastoma was proposed to arise from hepatocellular adenoma or carcinoma. In the DS's view the hepatocellular adenomas and carcinomas are not relevant for humans as they considered the CAR/PXR MoA as sufficiently demonstrated, and also the hepatoblastomas were considered not relevant for humans by the DS.

The relevance of the CAR/PXR MoA is discussed above and RAC concluded that it is very likely to be active and to play a role in the formation of liver adenomas and carcinomas, though there are some remaining uncertainties on the activity of alternative modes of action. However, RAC considered the evidence to exclude the human relevance of the observed hepatoblastomas as insufficient as outlined in detail in the section "Supplemental information - in depth analysis by RAC".

Uterine adenocarcinoma

***Table**: Studies investigating potential modes of action related to uterine adenocarcinomas and their relevance for benthiavalicarb-isopropyl.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Oncogenic mechanism of uterine cancer No guideline Acceptable F344/DuCjr-Fischer SPF rats 10 F/dose	KIF-230 TGAI, purity: 88.6-89.1% as KIF-230R-L 0; 200 and 10000 ppm 11.9 and 593.9 mg a.s./kg bw/d 56-d feeding study Aromatase activity was measured in liver, uterus, and ovaries. Serum concentrations of oestradiol, progesterone and LH were measured in serum at pre-dosing and at weeks 2, 4, 6 and 8 of dosing.	NOAEL= 11.6 mg/kg bw/d LOAEL= 576.2 mg/kg bw/d (increase in liver size/weight, increase in liver aromatase) **Mortality and clinical signs**: No relevant findings. **Body weight and bw gain:** No relevant findings. **Gross necropsy and organ weight**: Dark and enlarged livers. 31% and 29% increase in absolute and relative liver weight at 10000 ppm after 8 weeks of treatment, resp. No effect on uterus and ovary size and weight. **Enzyme activity:** Aromatase: 36% increase in liver, no increase in ovary and uterus at 10000 ppm after 8 weeks of treatment. **Serum hormone levels:** No significant change at any dose and sampling time.	Anonymous 32, 2002c DRAR Report n°5914(001-325)
Uterotrophic bioassay in the ovariectomised rat OECD TG 440, US EPA Test Guideline OPPTS 890.1600 Acceptable Sprague-Dawley Crl:CD®(SD) IGS BR rats 6 F/dose	KIF-230 TGAI, purity: 97% as KIF-230R-L 0, 10, 100 or 1000 mg/kg bw/d Positive control 17α-ethynyloestradiol 14 days	**Mortality and clinical signs**: No relevant findings. **Body weight and food consumption:** No relevant findings. **Organ weights:** No effect on uterine and vaginal weight. **Histopathology:** No effect on uterus and vaginal tissues. **Cell proliferation**: Uterine tissue: No increase. Vaginal tissue: No increase. There was no evidence of any oestrogenic effect.	Anonymous 33, 2015 DRAR Report n° 41401234

The CLH report presented two studies to investigate modes of action that could be relevant for the formation of the observed uterine adenocarcinomas.

A study titled "Oncogenetic mechanism of uterine cancer" (Anonymous 32, 2002c) in females Fischer rats did not detect any findings in uterus and ovaries after 8 weeks of treatment with up to 10000 ppm benthiavalicarb-isopropyl (no change in size or weight, no change in aromatase

activity). At 10000 ppm, livers were enlarged, and weights were increased and an increase in liver aromatase was detected (+35%). No changes in the serum levels of progesterone, oestradiol or LH were detected after 2, 4, 6 or 8 weeks.

Benthiavalicarb-isopropyl was also tested in an Uterotrophic assay according to OECD TG 440 and GLP, with 14 days exposure to 0, 10, 100 or 1000 mg/kg bw/d. A negative result was obtained.
Studies submitted during the consultation are presented in the section "Supplemental information – in depth analysis by RAC".

Anonymous (2020c) investigated the potential involvement of Wnt/ß-catenin signalling in uterine tumour formation. No change in investigated transcript was detected in uterine tissue after 14 days of exposure to 5000 ppm benthiavalicarb-isopropyl and the study is considered negative. No involvement of Wnt-ß-Catenin signalling was indicated.

Anonymous (2020a; b) assessed whether benthiavalicarb-isopropyl acts as a dopamine receptor agonist and induces the observed uterine tumours via changes in the oestradiol/progesterone ratio, comparable to decamethylcyclopentasiloxane (D5). For this purpose, 4 groups of 5 rats each were treated either with control diet, 5000 ppm benthiavalicarb-isopropyl, 500 ppm PB or 128 ppm D5. Parameters investigated consisted of oestrus cyclicity, pituitary gene expression of prolactin, follicle stimulating hormone beta (FSHB) and LH and plasma levels of prolactin, progesterone, and oestradiol.

Irregularities in oestrus cyclicity were seen in single animals of all groups but in 4 animals of the D5 group a decrease in cycle length was observed. For the remaining parameters, D5 reacted as expected (i.e., statistically significant effects on: prolactin mRNA (decrease), FSHB mRNA (increase), plasma prolactin and progesterone levels (decrease), oestradiol levels (increases)), but the other groups were not affected. It can be concluded that benthiavalicarb-isopropyl does not act as dopamine receptor agonist.

None of the investigated modes of action appeared to be activated upon treatment of rats with benthiavalicarb-isopropyl and could explain the formation of the observed uterine adenocarcinomas. In conclusion, RAC was of the view that human relevance of the uterine adenocarcinomas cannot be excluded.

Other studies relevant for assessing the carcinogenic potential of benthiavalicarb-isopropyl

***Table**: Studies investigating carcinogenic modes of action.*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
A two-stage hepato-carcinogenicity study in rats No guideline Acceptable F344/DuCrj Fischer-SPF rats 12 M/groups	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L Initiation study, 2 weeks: Saline: 0.9% Initiator: Diethylnitrosamine (DENA): 200 mg/kg bw i.p. (single dose) Promotion study, 6 weeks:	**Mortality and clinical signs**: None **Food consumption and bw**: 8% decrease in the group "DENA + a.s." compared to the group "saline + a.s." over the 8 weeks. Body weight gain was 10% lower in the group "DENA + a.s." compared to the group "saline + a.s." over the 8 weeks. **Liver weight and gross pathology**: Enlarged liver and increased liver weight (8%) in the group "DENA + a.s." compared to the group "saline + a.s.". **Cellular findings:**	Anonymous 23, 2000a DRAR Report n°4905 (001-260)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	KIF-230: 0; 10000 ppm (from week 2 to 8 via diet) 610.3 mg a.s./kg bw/d PB: 0.05% (from week 2 to 8 via diet) At week 3 after i.p. injection, a 2 / 3 - partial hepatectomy (PH) was performed on the animals of all groups, in order to induce mitosis	*Saline + a.s.:* - Increased incidence of hepatocellular hypertrophy - Marginal and not statistically significantly increased incidence of mitosis. *DENA + a.s.:* - Increased incidence of hepatocellular hypertrophy - Increased incidence of mitosis - Increased incidence of acidophilic cell foci - Increased incidence of clear-, mixed- and vacuolated cell foci	
A two-stage hepato-carcinogenicity initiator study in rat. No guideline Acceptable F344/DuCrj Fischer-SPF rats 12 M/groups	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L <u>Initiation study, 2 weeks:</u> DENA: 200 mg/kg bw i.p. KIF-230: 2000 mg/kg bw (single dose) <u>Promotion study, 6 weeks:</u> PB: 0.05% (from week 2 to 10 via diet) DGA3: 300 mg/kg bw i.p. (single dose at week 3) 2/3 hepatectomized (PH)	**Mortality and clinical signs**: None **Food consumption and bw**: No effect on food consumption in a.s. group over the 8 weeks. No effect on bw and bw gain in a.s. group. **Liver weight and gross pathology**: Absolute and relative liver weight statistically significantly increased when compared to DENA group (9%). **Cellular findings**: Lower incidence for mitosis, clear- and eosinophilic foci in the a.s. group when compared to DENA. Fatty change, hypertrophy, and necrosis at comparable level in a.s. group and DENA group.	Anonymous 24, 2000b DRAR Report n°4906 (001-261)
In vitro study Two-stage transformation assay on Balb/c 3T3 cells Test method B.21 of directive 88/302/EEC Pre-incubated Balb/c 3T3 cells	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L <u>Initiation assay:</u> KIF-230: 10.4, 17.3, 28.8, 48.0 and 80.0 µg/mL (72 h incubation) TPA: 0.1 µg/mL (promotor, 11 days incubation) <u>Promotion assay:</u> KIF-230: 0, 3, 6, 9, 12 and 15 µg/mL	**Main test:** No focal transformation when the a.s. was tested as an initiator. **Promotor test**: Increase in incidence of foci: 0.8, 0.5, and 0.3 mean number of foci/dish at 3, 6, and 9 µg/L, resp., vs. 0.1 mean number of foci/ dish in control.	Nakajima, 2000b DRAR Report n°4909 (001-262)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	(11 days incubation) 3-methylcholanthrene: 0.2 µg/mL (initiator, 72 h incubation)		
Oxidative DNA damage in the liver of rats No guideline Acceptable F344/DuCrj Fischer-SPF rats 5/sex/dose	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L 0, 200 and 10000 ppm M: 17.4 and 797.5 mg a.s./kg bw/d F: 17.1 and 914.5 mg a.s./kg bw/d 2 weeks via diet	Hepatic 8-OHdG remained unaffected after 2 weeks of treatment up to 10000 ppm.	Anonymous 27, 2001a DRAR Report n°5433 (001-284)
Oxidative DNA damage in liver of mice No guideline Acceptable Slc:B6C3F1(C57BL/6 x C3H®(SPF) mice 5/sex/dose	KIF-230 TGAI, purity: 88.8-89.1% as KIF-230R-L 0, 100 and 5000 ppm M: 19.4 and 1031.2 mg a.s./kg bw/d F: 26.1 and 1203.7 mg a.s./kg bw/d 2 weeks via diet	Hepatic 8-OHdG remained unaffected after 2 weeks of treatment up to 5000 ppm.	Anonymous 28, 2001b DRAR Report n°5434 (001-285)

An equivocal result was obtained in the *in vitro* two-stage transformation assay on Balb/c 3T3 cells for the promoting effect (colony formation at the lower dose range). However, benthiavalicarb-isopropyl did act as a tumour promoter in rat liver at high doses (increased GST-P positive foci) when previously exposed to a well-known initiator, in a comparable way to that of PB. In contrast, it did not have any tumour initiating activity in rat liver which was subsequently exposed to a well-known promotor. This is consistent with the absence of genotoxicity *in vivo*.

The capacity to induce oxidative DNA damage was assessed in two studies in rat and mouse (assessing the capacity to induce oxidative damage in the liver). Both were negative, indicating that oxidative damage is not relevant in benthiavalicarb-isopropyl induced tumour formation.

Overall conclusion on classification and comparison with CLP criteria

As there is no evidence of carcinogenicity in humans reported in the dossier, classification in Category 1A is not appropriate.

Based on the results of the chronic toxicity and carcinogenicity studies in rat (Anonymous 18, 2001a) and in mice (Anonymous 20, 2001b) there is clear evidence for carcinogenicity in animals.

According to the CLP Regulation (Annex I, 3.6.2.2.4), additional considerations like human relevance and background incidences as part of a weight of evidence approach have to be considered for a classification for carcinogenicity. These are assessed in the following table.

***Table**: Weight of evidence assessment of the available information for the tumours seen in mouse and rat upon treatment with benthiavalicarb-isopropyl*

Factor	Evidence with benthiavalicarb-isopropyl	Conclusion
Tumour type Considering background incidence and HCD	Uterine adenocarcinoma in female F344 rat. Statistically significant increase at the two top doses. Exceeding HCD. Rare tumour type. Malignant. Relevant for humans – no MoA identified that could reduce human relevance.	Supportive for classification
	Uterine adenoma and adenocarcinoma in female B6C3F1 mice – only single incidences of uterine adenoma in the mid dose and the two top doses. One adenocarcinoma in the top dose. No dose-response, not statistically significant but considered a rare tumour type in mice.	Supportive for classification
	Hepatocellular adenoma and carcinoma in male B6C3F1 mice, hepatocellular adenoma in female mice. Statistically significant increase at the two top doses – almost all mice affected in these two dose groups. Exceeding HCD. Several MoAs investigated, CAR/PXR MoA plausible, but remaining uncertainties (AhR activation, no CAR/PXR *in vivo* study, only 3 male human donors). In addition, it is assumed that cytotoxicity contributes to tumour formation in the liver.	Supportive for classification
	Hepatocellular adenoma in male F344 rat. Dose-dependent and statistically significant increase in the two top doses. In the upper range of the historical control values. Similar MoA considerations as in mice.	Supportive for classification
	Hepatoblastoma in male B6C3F1 mice. Statistically significant increase at the two top doses. Exceeding HCD. Highly malignant tumour. Rare tumour. Relevant for humans.	Supportive of classification
	Thyroid follicular cell adenoma in male B6C3F1 mice. Dose-dependent increase, statistically significant at the top dose. Exceeding historical control. Mode of action via CAR activation and resulting hepatocellular enzyme induction leading to increased thyroid hormone catabolism. Several other MoAs could be excluded; however, some remaining uncertainties regarding study design and lack of test methods covering all relevant MoAs. Only weak increase in benign tumours in one species and one sex, liver mediated thyroid toxicity – considered not relevant for tumour formation in humans (humans less sensitive than rodents)	Not supportive for classification
	In females there was a statistically significant increase in malignant lymphoma at the low dose and at the second highest dose.	Not supportive for classification

	No dose-response was obvious, and the incidence was clearly within the historical control range.	
Multi-site responses	Yes	Increased concern
Progression of lesions to malignancy	Yes, for uterus and liver (carcinoma and hepatoblastoma). No progression to malignancy for thyroid follicular cell adenoma.	Increased concern
Reduced tumour latency	Not indicated – data not presented	-
Whether responses are in single sex or both	Both sexes in rats and mice reported tumours.	Increased concern
Whether responses are in a single species or several	Tumour formation occurred in rats and mice.	Increased concern
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	No	-
Routes of exposure	Oral	-
Comparison of ADME between test animals and humans	No species-specific differences identified in the available toxicokinetic studies.	-
The possibility of a confounding effect of excessive toxicity at test doses	Rat carcinogenicity study: there was no increase in mortality and only in top dose males and females a slight effect on body weight, body weight gain and feed efficiency were observed, which did in no instance exceed 10%. Animals were not affected by severe general toxicity, despite the liver and kidney toxicity seen at the two top doses. Mouse carcinogenicity study: no severe general toxicity in females, except considerable liver toxicity in the top dose. No clinical signs. In males, general signs of toxicity were seen at the two top doses from week 78 onwards. Almost all animals were affected by liver toxicity and tumour formation. There was an increase in mortality in males after week 77, at the top dose survival declined to 56%.	-
Tumour latency	After 52 weeks 70% of top dose males were affected by hepatocellular adenoma. After 78 weeks 80% of the second highest dose and 100% of the top dose males had hepatocellular adenomas, 60% and 50% of females of the second highest dose group and the top dose group, respectively, also had hepatocellular adenomas. At week 78 there were also two cases of hepatoblastoma in top dose males. In males of the two top doses abdominal masses are described from week 78 onwards.	Supportive for classification
Mode of action and its relevance for humans	a) Genotoxic MoA An extensive data-base covering all relevant aspects of genotoxicity/mutagenicity was presented. All studies were negative (except one Ames test, which used a different batch with an uncommon mutagenic impurity), supporting the absence of genotoxicity. b) CAR/PXR MoA Liver adenomas and carcinomas: plausible and several studies support this MoA is active,	a) Not relevant b) Mode of action plausible – non-relevant

	however, some remaining uncertainties with regard to alternative MoAs (AhR, no *in vivo* knock-out study, Wnt/ß-Catenin signalling not fully excluded). In addition, cytotoxicity is assumed to contribute to tumour formation. c) Cytotoxicity: clear signs of cytotoxicity were seen in the livers of male and female mice in the carcinogenicity study, with higher severity in males compared to females; cytotoxicity was also seen in the 28-d and 90-d mouse studies as well as in the 90-d and 1-year dog studies. In the 28-d mouse study, it could be demonstrated that cytotoxicity was seen at a dose lower than that leading to hepatocellular hypertrophy, supporting the relevance of cytotoxicity in tumour formation. Recurrent inflammation and regenerative growth is a well-known MoA leading to tumour formation. d) Hepatoblastoma: no MoA identified e) Uterine adenocarcinoma: several MoAs excluded but no MoAs demonstrated. f) oxidative DNA damage – not supported by two *in vivo* studies in rat and mouse g) Initiation/promotion studies indicate that benthiavalicarb-isopropyl acts as a promotor, not as initiator – in line with absent genotoxicity. h) ED MoA - Interference with the HPT axis as demonstrated in rodents (rat, mouse) and dogs. For thyroid adenomas: CAR activation and resulting T4 UDPGT induction leads to increased thyroid hormone degradation and in turn compensatory cell hypertrophy and hyperplasia in follicular thyroid cells → adenoma observed in male mice (carcinoma not observed with benthiavalicarb-isopropyl). Well-investigated, also alternative MoAs covered (TPO, NIS, DIO) – remaining uncertainties (study design, some aspects of HPT relevant mechanisms not covered by testing methods), CAR MoA highly plausible and only weak increase in benign thyroid tumours (though statistically significant and above HCD), quantitative difference between humans and rodents. - Oestrogenicity: negative Uterotrophic assay, no increase in serum oestradiol, progesterone, or LH levels in rat upon 8 weeks treatment with benthiavalicarb-isopropyl, though an increase in oestradiol was seen in the rat carcinogenicity study in the two top doses. Not always dose-dependent, up to +71%, detected after 26, 78 and 104 weeks.	to humans, but remaining uncertainties c) Mode of action plausible, relevant to humans d) No known MoA e) No known MoA f) Not substantiated by data g) – h) - Interference with the HPT axis concluded by (EFSA, 2021) Relevant for the identification as ED, not relevant for carcinogenicity classification - No relevant finding in relation to carcinogenicity classification

- no influence on the concern (neither increase nor decrease)

Three different tumour types were observed in two different species (uterine adenocarcinoma in female rat, hepatocellular adenoma / carcinoma and hepatoblastoma in male mice, hepatocellular adenoma in female mice).

During the RAC 60 working group (January 2022) and plenary (March 2022) meetings, the Industry stakeholder representative agreed that no MoA for the uterine tumours has been determined. They, however, also stated that no precursor lesions, such as endometrial hyperplasia, were seen, which indicates that the tumours are not hormonally-related. They noted that in humans, carcinomas in the endometrium are always due to oestrogen-excess, and hence, if that can be eliminated, the human relevance could be largely eliminated. In their view, the liver and thyroid tumours are not relevant for humans, leaving the uterine tumours which could justify classification as Carc. 2; H351.

RAC was, however, of the view that since no underlying MoA could be identified for the observed malignant uterine tumours, they occurred at the two top doses of the rat study, which were not affected by severe general toxicity, the increase was statistically significant and exceeded historical control levels, they are considered relevant for humans and for the classification for carcinogenicity. See further details and discussion above.

Liver tumours were more pronounced in male than in female mice. Progress to malignancy was seen in males, where also a second type of liver tumours, hepatoblastoma, was observed, while in females there was an increase in hepatocellular adenoma. These tumours affected almost all animals of the two top doses in males and almost half of the females of the two top dose groups, the increase for each tumour type was statistically significant and above HCD. While no underlying MoA could be identified as cause for hepatoblastoma, CAR/PXR activation was discussed for hepatocellular adenoma and carcinoma. Though there is strong support that this MoA is active, the contribution of other modes of action, i.e., Wnt/ß-Catenin signalling and AhR activation could not be fully excluded. In addition, the observed histopathological changes in the mouse liver indicate that cytotoxicity also contributes to the observed liver tumour formation. The tumours are therefore considered relevant for humans.

Considerable increase in tumours was detected in two species and in the liver, tumours were seen in two sexes. These tumours are considered relevant for humans as the underlying mode of action was either not identified or the contribution of modes of action which are relevant to humans could not be fully excluded.

Based on this RAC concluded that **classification as Carc. 1B is warranted.**

Potency

When calculating T25-values for the relevant tumour types, low potency was indicated. However, the inappropriate dose spacing of the available studies (i.e., two rather high doses and two rather low doses, with a lack of information on the medium dose-range) impedes the derivation of a proper dose-response curve and no clear conclusion on potency was therefore possible. In conclusion, **the use of generic concentration limits (GCLs) was recommended**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

One two-generation study (OECD TG 416; Anonymous 34, 1999) in rats was available in the CLH report for evaluation of effects on sexual function and fertility.

Two-generation study in rats (Anonymous 34, 1999)

In a two-generation study, CD (Sprague-Dawley) rats were exposed to 0, 100, 1000 and 10000 ppm of KIF-230 (benthiavalicarb-isopropyl; purity: 88.8-89.1%) as KIF-230R-L (technical benthiavalicarb-isopropyl) (corresponding to a test substance intake of approx. 0, 6.9, 68.5 and 702.4 mg/kg bw/d in males and 0, 6.8-15.5, 67.3-167.7 and 708.3-1672.9 mg/kg bw/d in females in the F0 generation and 0, 10.0, 99.7 and 1057.8 mg/kg bw/d in males and 0, 6.5-14.3, 67.2-146.7 and 702.5-1456.1 mg/kg bw/d in females in the F1 generation). The number of animals included in the study was 25/sex/dose in the F0 generation and 22±1/sex/dose in the F1 generation. The exposure period was at least 10 weeks for both generations before they were mated to produce the F1 and F2 generations, respectively.

F0 generation

Only mild general toxicity was observed in F0 parental animals and was not considered treatment-related by the DS.

There were no adverse effects on mating, fertility, oestrus cyclicity, sperm number, sperm activity or sperm morphology in any dose group.

F1 generation

Similar to the F0 generation, only mild general toxicity was observed in F1 parental animals.

The number of sperm was reduced at 100 ppm (38%; $p < 0.01$) and 10000 ppm (22%; $p < 0.05$) but not at 1000 ppm. This effect was not considered treatment-related by the DS.

Apart from the effect on sperm number, there were no effects on sexual function and fertility, including mating, fertility, oestrus cyclicity, sperm activity or sperm morphology in any dose group.

No human data were available.

Based on the results of the two-generation study, the DS concluded that no classification for benthiavalicarb-isopropyl for adverse effects on sexual function and fertility is warranted.

Adverse effects on development

In addition to the two-generation study (Anonymous 34, 1999), one prenatal developmental toxicity study (Anonymous 36, 2004) and one teratogenicity study (Anonymous 35, 2000a) in rats, as well as one teratogenicity study in rabbits (Anonymous 37, 2000b), were available for evaluation of effects on development.

Two-generation study in rats (Anonymous 34, 1999)

The DS considered that the only treatment-related effects observed in F1 and F2 offspring were increased absolute and/or relative liver weights and decreased absolute and relative weights of thymus and spleen in the top dose group.

Prenatal developmental toxicity study (OECD TG 414) in rats (Anonmous 36, 2004)

SD rats, 22 pregnant dams/dose, were exposed to KIF-230 TGAI (purity: 93.6%) as KIF-230R-L at doses corresponding to 0, 10, 100, 1000 mg a.s./kg bw/d on gestation day (GD) 5-19. No deviations from test guideline.

Dams

Relative liver weight was increased at 1000 mg/kg bw/d (6%). Adrenal weight was increased at 100 mg/kg bw/d (absolute: 14%; relative: 16%) and 1000 mg/kg bw/d (absolute: 29%; relative: 16%).

Foetuses

Low incidence of ribs/costal cartilage variations at the top dose level in 9/144 foetuses from 5/21 litters.

Teratogenicity study in rats (Anonymous 35, 2000a)

CD (Sprague-Dawley) rats, 25 pregnant dams/dose, were exposed to KIF-230 TGAI (purity: 88.8-89.1%) as KIF-230R-L at doses corresponding to 0, 10, 100, 1000 mg a.s./kg bw/d, by oral gavage, on GD 7-19. All dams were euthanised on GD 20 and their foetuses removed by Caesarean section and examined. The study was conducted according to EPA OCSPP Guideline No 870.3700, corresponding to the OECD TG 414 (2001). Deviation: exposure started on GD 7 instead of GD 5. The study was considered acceptable by the DS.

Dams

There were no effects on survival, clinical signs, body weight, body weight gain or food consumption.

Increased liver weight (top dose; absolute: 12%; relative: 11%), increased adrenal weight (mid dose; absolute: 12%; relative: 8%) as well as enlarged liver (2 dams in mid dose and 6 dams in top dose) were seen.

At Caesarean section there were no effects on number of corpora lutea, implantations or placental weights.

Foetuses

At Caesarean section, there were no effects on number of live foetuses, sex ratios or foetal weights.

In the top dose group, 32/176 foetuses from 11/24 litters were found with thymic remnant in the neck and 13/175 foetuses from 9/24 litters were found with splitting of the rib cartilage. The findings were considered as potentially treatment-related, but the DS noted that they were not statistically significant and that they were within HCD of the testing laboratory (see Table 26 of the CLH report).

Teratogenicity study in rabbits (Anonymous 37, 2000b)

New Zealand White (NZW) rabbits, 22 pregnant dams/dose, were exposed to KIF-230 TGAI (purity: 87.5-87.9%) as KIF-230R-L at doses corresponding to 0, 10, 20, 40 mg a.s./kg bw/d on GD 6-28. All dams were sacrificed on GD 29 and their foetuses were removed by Caesarean section and examined. The study was performed according to EPA OCSPP Guideline No 870.3700 corresponding to the OECD TG 414 (2001), no deviations.

Dams

Relative liver weight was increased in the top dose group (11%). There was one animal with hardly any food consumption in the second half of the gestation period and that animal became malnourished. Apart from that, no effect was observed on body weight, body weight gain or food consumption. Two animals aborted in the top dose group, on GD 25 and 28. One of them was the animal with reduced/no food consumption.

At Caesarean section there were no effects on the number of corpora lutea, implantation sites, or placental weight.

Foetuses

At Caesarean section there were no effects on the number of live or dead foetuses, resorptions, sex ratio or live foetus weight.

In the top dose group, 12/155 nano-foetuses (dwarfism) from 3[1]/19 litters were recorded. Ten of the 12 nano-foetuses were seen in one litter and the two remaining were seen in one litter each. For the dam with 10 nano-foetuses, severe maternal effects were observed regarding food consumption, which rapidly declined from GD 23 to the end of pregnancy. Between GD 25 and 29 nearly no food consumption was observed. The DS concluded that since severe maternal toxicity was seen in this dam, the effect was likely due to the condition of that dam, rather than treatment-related. Nano-foetuses were defined as foetuses with less than 60% of the mean foetal bw in the control group by the study author. In addition, 2 nano-foetuses were observed in the control group (2/183 nano-foetuses, 1 litter) and 2 in the low dose group (2/168 nano-foetuses, 2 litters). No nano-foetuses were seen in the mid dose group. In the top dose group, also incomplete ossification of the hindlimb talus in 14/155 foetuses (in 4 litters) was observed at a slightly higher incidence, but as the incidence was low, the DS considered it as an incidental effect rather than treatment-related. The same effect was seen in 2 foetuses in 2 litters in the low dose and 1 foetus in the mid dose group. It was not seen in the control group.

Based on the available data, the DS concluded that no classification for benthiavalicarb-isopropyl for adverse effects on development is warranted.

Adverse effects on or via lactation

No substance-related effects were observed in the two-generation study during lactation. No human data or other relevant information were available. The DS concluded that no classification for benthiavalicarb-isopropyl for adverse effects on or via lactation is warranted.

Comments received during consultation

One Company-Manufacturer and one MSCA provided comments on reproductive toxicity.

The Company-Manufacturer commented that decreased serum T4 levels in male rats and mice have been seen in studies with high exposure of benthiavalicarb-isopropyl and that the fact that, despite this, no adverse effects on reproduction was seen in the reproductive toxicity studies should be further discussed by RAC.

RAC was of the view that this is not necessarily a contradiction, because current test methods might not include the relevant parameters needed to detect thyroid-related developmental defects like e.g., spatial cognitive abilities (learning and memory) or specific brain histological examination (heterotopias).

The MSCA agreed with the DS that there were no effects on sexual function and fertility nor any effects on or via lactation warranting classification. For developmental toxicity, they commented that classification in Category 2 may be warranted based on the higher incidence of nano-foetuses seen in the teratogenicity study in NZW rabbits. They also referred to a preliminary teratogenicity study in NZW rabbit (not included in the CLH report) where the same effect was seen at the same dose as in the main study. In addition, they noted that there was an increase of delayed talus ossification seen in the top dose group in the main rabbit study. The MSCA, however, also noted

[1] Corrected from 2/19 reported in the CLH dossier, to 3/19 based on information in the DRAR (cf. Table 6.6.3/03-3, DRAR 8 Volume 3CA_B-6).

that the definition of nano-foetuses was not clear, and that it should be clarified if the decreased bw was due to the length growth of the foetuses being affected. If the length growth was affected, they considered that the increase in nano-foetuses seen would be relevant for classification.

The DS responded that since 10 out of 12 nano-foetuses were seen in one dam, with severe maternal toxicity, they did not consider the effect treatment-related. In the preliminary rabbit study, there was a decrease in bw gain (-30%) due to reduced food consumption in top dose dams, together with increase in relative and absolute liver weights. Since the nano-foetuses were seen together with maternal toxicity, the DS did not consider them treatment-related. They also noted that less weight should be given to the preliminary study due to a low number of litters (4 vs. 19 in the main study).

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

RAC noted that in addition to the main two-generation study, also a preliminary two-generation study in rats with 5 animals/sex/dose is available in the DRAR (Anonymous, 1998a), with dose levels of 0, 50, 200, 5000 and 20000 ppm (corresponding to approx. 3.3, 13.2, 329.6 and 1367.2 mg/kg bw/d in males, and 3.3-3.8, 13.9-15.2, 352.2-384.3 and 1465.7-1528.4 mg/kg bw/d in females). Exposure was performed from 8 weeks before mating, for males until the end of mating, for females throughout gestation and lactation until weaning. F1 animals were dosed immediately after weaning, for one week up until day 28 after birth. This study was carried out according to GLP and with the aim to establish appropriate doses for the main study. It was not included in the CLH report, but the study report is available to RAC.

F0 generation

There was no mortality, no clinical signs, no effect on body weight, food consumption or food efficiency in any treated animals. Top dose females showed a slight decrease of bw gain (-24%) during the pre-mating period. Livers were enlarged in females ≥ 5000 ppm and hepatocellular hypertrophy was seen in males ≥ 5000 ppm and in females at 20000 ppm. Liver weight was also increased in females ≥ 5000 ppm and in males at 20000 ppm.

There was no effect on copulation index, fertility index and oestrus cycle.

F1 generation

At the top dose, 8 dead new-borns were observed, leading to a slightly lower birth index (82%) compared to control group; no dead new-borns were reported in any other group. Other indices showed no dose-dependent and relevant change. In the top dose group, there was a trend towards lower body weight in male offspring on post-natal day (PND) 4 (-16.5%) and in females on PND 4-21 (-8% to -13.5%). During growth, female bw was decreased on PND 22 (-14%) and PND 28 (-12%). These changes were not statistically significant. No relevant histopathological findings were seen.

RAC concluded that in none of the two two-generation studies effects indicating adverse effects on sexual function and fertility were observed.

Though not highlighted in the CLH report, it was noted that in the mouse 28-d study (Anonymous, 1996), the mouse 90-d study (Anonymous 43, 1998a) and the mouse carcinogenicity study (Anonymous 20, 2001b), effects on ovaries were seen in the top dose groups of these studies. These effects were considered treatment-related despite the decrease in body weight and body weight gain in females of the top doses of the 90-d study. In the carcinogenicity study, body weights of top dose females were increased. In the 28-d study ovary weight was already affected at the medium dose of 120 mg/kg bw/d (though not statistically significant).

***Table**: Ovary effects from the mouse 28-d study (Anonymous, 1996)*

Dose (mg/kg bw/d)	0	12.7	120	1609	4378	10847
Body weight (g)	22	23	23	23	22	21
Body weight gain (g)	5	6	6	6	5	4
Ovary weight, absolute (g)	13	14 (+8%)	13	12 (-8%)	8** (-39%)	6** (-54%)
Ovary weight, relative (%)	60	62 (+4%)	57 (-6%)	54 (-11%)	38** (-37%)	27** (-56%)

** $p < 0.01$

***Table**: Ovary effects from the mouse 90-d study (Anonymous 43, 1998a)*

Dose (mg/kg bw/d)	0	11.3	45	1620	4946
Body weight (g)	25.9	26	26.6	24.8 (-4%)	24.4 (-6%)
Body weight gain (g)	8.5	8.8	9.2	7.4 (-13%)	7.0 (-18%)
Ovary weight, absolute (g)	14	15	15	12 (-14%)	10 (-28%) **
Ovary weight, relative (g)	0.05	0.06	0.06	0.046 (-8%)	0.041 (-18%) **
Corpora lutea #	0	0	0	2/10; s	10/10 **; s

** $p < 0.01$, s = slight change, # animals with decreased number of corpora lutea

***Table**: Ovary effects from the mouse carcinogenicity study (Anonymous 20, 2001b)*

Dose (mg/kg bw/d)	0	3.7	18.6	459	927
Body weight (g)	32.7	32.1	32.2	34.0 (+6%) *	33.5 (+2%) *
Body weight gain (g)	16.2	15.6	15.7	17.5 (+8%) *	17.1 (+6%) *
Ovary weight, absolute (g)	9	9	11	8 (-11%) *	6 (-33%) *
Ovary weight, relative (%)	0.027	0.028	0.037	0.023 (-15%) *	0.019 (-30%) **
Ovary atrophy	9/41	9/43	14/36	22/41**	30/43**
Corpora lutea #	10/41	11/43	10/36	9/41	13/43

* $p < 0.05$, ** $p < 0.01$%, # animals with decreased number of corpora lutea

In the 90-d mouse study, no mortalities were observed, and no relevant clinical signs were described. Nevertheless, the DRAR concluded that the decreased number of corpora lutea in this study might partly be explained by a diminished general condition of the animals. It was, however, noted that the observed body weight decrease was slight and as the finding was associated with a decreased ovary weight, it could not be excluded that more specific processes of hormonal nature could be involved.

Also in the mouse carcinogenicity study, no severe general toxicity was described up to the highest dose tested. In this study, decreased ovary weight was associated with atrophy in the two top doses. The number of corpora lutea was also slightly decreased, but not statistically significant and no clear dose-dependence was observed (numbers were also reduced in control females).

Mice were the only species in which effects on the ovaries were observed; dogs and rats were not affected, and the DRAR stated that this effect is frequently observed in B6C3F1 mice. They

also cited the NTP Atlas on Non-Neoplastic lesions[1] in which ovarian atrophy is described as a decrease in size and weight and a decreased number of oocytes, developing follicles and corpora lutea and that it occurs at reproductive senescence. However, as the effects were already seen after 28 and 90 days, they cannot be considered to be related to reproductive senescence and the effects showed dose-dependence.

Overall, it can be concluded that the effects on ovaries were substance related and not related to reproductive senescence. However, the effects were largely seen at rather high doses (though the second highest dose of 459 mg/kg bw/d in the carcinogenicity study was not considered unusually high and, though not statistically significant, a decrease in ovary weight was already seen at 120 mg/kg bw/d after only 28 days) and were accompanied by some degree of general toxicity. In addition, the effect on corpora lutea was not very severe, as in the 90-d study at 1620 mg/kg bw/d only 2/10 animals showed a slight decrease in numbers of corpora lutea and in the top dose of 4946 mg/kg bw/d a slight decrease was seen in 10/10 animals. In the carcinogenicity study, the numbers of corpora lutea were decreased as well, but this was not strictly dose-dependent and also the control animals were affected.

No adverse effects on sexual function and fertility were reported in the available rat two-generation studies. In the main two-generation study in rats (Anonymous 34, 1999), apart from a decrease in sperm number in the low and top dose, but not in the mid dose, no effects on sexual function or fertility were seen. Two-generation studies are only available in the rat, and no such study was available in the mouse. It was therefore not possible to exclude that the effects seen in ovaries after 90 days and 2 years (i.e., reduced absolute and relative weight, atrophy, reduced numbers of corpora lutea) could lead to impaired fertility and sexual function in mice.

In the absence of an explanation that would demonstrate that these observations are not relevant for humans, the effects have to be considered relevant, although only seen in mice, but not in rats or dogs.

The effects were only seen at relatively high doses, especially in the 90-d study; however, it was noted that in the 90-d and the carcinogenicity study there was a large gap between the two lower and the two higher doses. The second highest dose from the carcinogenicity study was not considered extraordinarily high and still ovaries were clearly affected.

In conclusion Category 1A is not appropriate as there is no human evidence establishing a causal relationship between exposure to benthiavalicarb-isopropyl and an adverse effect on fertility.

Category 1B is not appropriate, as there is no clear evidence of an adverse effect on fertility and reproductive performance in experimental animals.

However, there was an adverse effect observed in ovaries in female mice (reduced absolute and relative weight, atrophy, reduced numbers of corpora lutea), which was seen in three studies, a 28-d study, a 90-d study and a 2-year study. Based on the observation of the described effects seen already after 28 and 90 days it can be concluded that young adult animals are affected, which could clearly have an impact on their capacity to reproduce. The effects were seen at rather high doses, but the animals were not affected by severe general toxicity and the second highest dose in the carcinogenicity study was not considered extremely high. The effect was only seen

[1] https//ntp.niehs.nih.gov

in mouse (three studies) but not in rat or dog, but as no underlying mechanism for the observed effect was known, human relevance cannot be excluded. Therefore, RAC concluded that **classification for benthiavalicarb-isopropyl as Repr. 2; H361f, is warranted**.

Adverse effects on development

The relevant studies to assess a potential adverse effect on development consist of the main and the preliminary two-generation studies, two rat teratogenicity studies (Anonymous 35, 2000a; Anonymous 36, 2004), two prenatal developmental toxicity studies in rat (Anonymous 36, 2004; Anonymous 35, 2000a) and a preliminary rat teratogenicity study (Anonymous, 1998b), as well as in total three rabbit teratogenicity studies, the main study (Anonymous 37, 2000b) and two preliminary studies (Anonymous, 1999; Anonymous, 1998). The preliminary two-generation study, the preliminary rat teratogenicity study and the two preliminary teratogenicity studies in rabbit were not part of the CLH dossier but were mentioned in the consultation (Anonymous, 1999), the DRAR (Anonymous, 1998a) or were made available after the working groups discussion (Anonymous, 1998b; Anonymous, 1998c). All study reports were made available to RAC.

In the main two-generation study in rats (Anonymous 34, 1999), in top dose (10000 ppm) F1 male offspring, absolute and relative liver weights were increased (absolute 8%, relative 19%) and in top dose F1 female offspring relative liver weight was increased (16%). There was also a decrease in thymus weight in both male and female F1 offspring in the top dose group (absolute: 18% and 18%, in males and females, respectively; relative: 12% and 6%, respectively), as well as spleen weight (absolute: 22% and 20%, respectively; relative: 13 and 11%, respectively). Similar effects were seen in the F2 generation. In top dose F2 offspring liver weight was increased in both male and female offspring (absolute: 4% and 8%, respectively, 16% and 13%, respectively), while thymus weight (absolute: 16% and 15%; relative: 4% and 16%, respectively), and spleen weight (absolute: 23% and 16%, respectively; relative: 13% and 11%, respectively) were decreased.

This was seen together with general toxicity in the dams, consisting of an increased bw and bw gain in F0 animals but not in a dose-dependent manner. In F1 dams, there was a decrease in bw (9% on day 77) and bw gain (10% during premating and earlier part of gestation) in the top dose group. In F1 dams, the effects on bw were seen also during lactation, but not in a dose-dependent manner. There were also effects on liver weight (increased in males in mid dose and both males and females in top dose) and adrenal weights (increased in top dose males), and thymus weight (decreased in top dose females). In F1 males and females, liver weight was increased in the top dose.

In the preliminary two-generation study in rats (Anonymous, 1998a), 8 dead F1 pups in the top dose group were observed, leading to a slightly lower birth index (82%) compared to control group. No other findings were observed. More details on the study are described in detail in the section on "Adverse effects on Fertility and Reproductive Function". F1 animals were not mated to produce F2.

Overall, the effects seen in the two-generation studies were not considered supportive for classification.

Developmental toxicity studies in rats

The PNDT study in rats (Anonymous 36, 2004) was part of the CLH report and details on the study protocol can be found under "Summary of the Dossier Submitter's proposal". There was an increased incidence of ribs/costal cartilage variations seen in the top dose group (9/144 foetuses (6.25%); 5/21 litters). This was also seen in the control (2 foetuses; 1.3%) and low dose (1 foetus; 0.71%), but not in the mid dose. The effects were seen together with some

general toxicity in dams, consisting of increased liver weight (relative: 6%) in the top dose, increased adrenal weight in the mid dose (absolute: 14%; relative: 16%) and top dose (absolute: 29%; relative: 16%).

RAC concluded that there were no effects relevant for classification observed in this study.

The teratogenicity study in rats, (Anonymous 35, 2000a), was part of the CLH report and details of the study protocol as well as effects on dams are described under "Summary of the Dossier Submitter's proposal".

There was an increased incidence of thymic remnant in the neck in top dose foetuses (32/176 foetuses (18.2%); 11/24 litters (46%)). In the control, low and mid dose the incidence of this finding was 16 foetuses (9.2%) in 10/25 litters (40%), 15 foetuses (8.8%) in 10/25 litters (40%), and 18 foetuses (10.9%) in 13/23 litters (57%), respectively.

In addition, there was an increase in foetuses with splitting of the rib cartilage (13/175 foetuses (7%); 9/24 litters (38%)) in the top dose. In the control, low and mid dose, the incidence of this finding was 7 foetuses (3.7%) in 5/25 litters (20%), 10 foetuses (5.5%) in 7/25 litters (28%) and 12 foetuses (7%) in 10/23 litters (44%), respectively.

Single incidences of nano-foetuses (< 60% of the mean body weight of control foetuses), 1 foetus in the control and 1 foetus in the top dose, were not considered relevant findings.

***Table**: Relevant results from the teratogenicity study in rats (Anonymous 35, 2000a):*

Effect	**Dose (mg/kg bw/d)**				**HCD ***
	0	**10**	**100**	**1000**	
Thymic remnant in the neck					
Foetal basis	16/174 (9.2%)	15/171 (8.8%)	18/165 (10.9%)	32/176 (18.2%)	5.24-25.4% Mean: 12.8%
Litter basis	10/25 (40%)	10/25 (40%)	13/23 (57%)	11/24 (46%)	
Splitting of the rib cartilage					
Foetal basis	7/189 (3.7%)	10/181 (5.5%)	12/171 (7%)	13/175 (7%)	6.4-8.3% Mean: 7.4%
Litter basis	2/25 (20%)	7/25 (28%)	10/23 (44%)	9/24 (38%)	

* HCD from the testing laboratory: 5 studies; 799 foetuses, 112 litters; same strain of rats; no years indicated

In conclusion, both effects showed an increase with dose, but the historical control incidences were not exceeded. Therefore, these effects are not considered relevant for classification. An uncertainty was however related to the HCD as no information on the time period when they were generated was available.

The preliminary rat teratogenicity study (Anonymous, 1998b), which was made available to RAC, was carried out in order to establish dosing for the main study. Five pregnant dams per group were orally (gavage) dosed with 0, 100, 250, 500 or 1000 mg/kg bw/d. Dosing was shorter than recommended (GD 7-17).

There were no effects on food consumption, body weight or body weight gain in the dams, except some changes in liver weight and some cases of enlarged livers.

Two nano-foetuses (i.e., foetuses with a body weight < 60% of the mean of the control) were seen in the top dose group (2/73 foetuses, 2/5 litters). Overall, foetal weight was not affected by treatment, but placental weight for male foetuses was reduced to -7% in the second highest dose and -14% in the top dose and to -6% in the second highest dose and to -9% in female foetuses. No relevant visceral or skeletal alterations were reported.

The effects on placental weight appear to be treatment-related but did not affect foetal weight and it was not seen in two comparable studies testing the same dose. The study authors concluded that 1000 mg/kg bw/d was an appropriate top dose for the main study.

Overall, no findings relevant for classification were seen in the available rat studies.

Developmental toxicity studies in rabbits

The main teratogenicity study in rabbits (Anonymous 37, 2000b) was part of the CLH report and details of the study protocol are described under "Summary of the Dossier Submitter's proposal".

The table below gives an overview of effects seen in the dams. Effects on body weight and body weight gain were not severe and also the liver weight increase did not exceed 10% (absolute weight) and 11% (relative weight). No treatment-related differences were noted in numbers of corpora lutea, implantation sites, live or dead foetuses, or resorptions; nor in sex ratio, mean live foetus weight or mean placental weight in the study.

***Table**: Relevant results from the main teratogenicity study in rabbits, effects on dams (Anonymous 37, 2000b)*

Dose (mg/kg bw/d)	**0**	**10**	**20**	**40**
Food consumption GD 7–29 (g)	1402 ± 244	1457 ± 266 (+4%)	1505 ± 274 (+7%)	1470 ± 246 (+5%)
Bw GD 29 (g)	4035 ± 219	4042 ± 241	4067 ± 223	3976 ± 308 (-1.5%)
Bw – gravid uterus weight (g)	3532 ± 253	3573 ±216	3582 ± 209	3503 ± 246 (-0.8%)
Bw gain GD 0–6 (g) (prior dosing)	190 ± 57	191 ± 86	217 ± 70 (+14%)	179 ± 64 (-6%)
Bw gain GD 6–29 (g) (during dosing)	235 ± 150	240 ± 145	208 ± 149 (-12%)	210 ± 176 (-11%)
Liver weight (g)	83.98 ± 12.97	90.40 ± 8.55 (+8%)	87.84 ± 10.52 (+5%)	92.60 ± 10.51 (+10%)
Liver weight (relative to corrected bw)	2.381 ± 0.364	2.536 ± 0.252 (+7%)	2.452 ± 0.521 (+3%)	2.647 ± 0.274* (+11%)
# of dams examined	21[#]	20[#§]	19[#+]	19[#@]
# of dams with live foetuses	21	20	19	19

* Significant difference from control, $p \leq 0.05$, [#] 1 animal from each group was infertile (absence of implantation sites), [§] 1 animal died, not treatment-related, [+] 2 animals died due to gavage error, [@] 2 animals aborted

There were no severe treatment-related effects on body weight, body weight gain or feed consumption, but single animals were affected more than others.

Two of the dams in the top dose group aborted on days 25 and 28. The cause was not clear from the results of necropsy, but the animal that aborted on day 28 had reduced/hardly any food consumption in the second part of the gestation period (from GD 13 onwards) and became malnourished, which probably lead to the abortion.

One control and two top dose animals were reported to have enlarged liver at necropsy (not the animals that aborted). One of the two animals at the top dose with enlarged liver, was the dam that had 10/12 nano-foetuses observed at this dose group (see effects on foetuses below). In this dam the liver was also described as pale and there were white patches on the kidneys and brown patches on the lung. In this dam food consumption was reduced considerably on the last

few days of gestation, however, the same was observed in other animals without nano-foetuses, including some of the control group. Body weight gain from GD 6–29 in this dam was lower than the mean of the control, but still within the standard deviation of the control and it was comparable to other dams of the top dose group, which did not have nano-foetuses. No clinical signs were reported in this dam or most other dams of the top dose group.

The following table gives an overview on the relevant findings in foetuses seen in this study.

***Table**: Relevant results from the main teratogenicity study in rabbits, effects on foetuses (Anonymous 37, 2000b)*

Effect	**Dose (mg/kg bw/d)**				**HCD[a]**
	0	**10**	**20**	**40**	
Weight of live foetuses					
Males	40.00 ± 4.85	38.41 ± 5.15	42.45 ± 4.57	42.73 ± 7.56	
Females	40.30 ± 5.41	39.59 ± 7.00	41.33 ± 5.92	40.88 ± 8.22	
Nano-foetuses					
Foetal basis	2/183 (1.1%)	2/168 (1.2%)	-	12/155 (7.7%)	0.0-3.1% Mean: 0.4%
Litter basis	1/21 (4.8%)	2/20 (10%)	-	3/19 (15.8%)	0-13.6% Mean: 1.7%
Delayed ossification of the hindlimb talus[b]					
Foetal basis	0/183	2/168 (1.2%)	1/159 (0.6%)	14/155* (9%)	-
Litter basis	0/21	2/20 (10%)	1/19 (5.3%)	4/19 (21.1%)	-

[a] HCD from the testing laboratory: foetal and litter incidence of dwarfism (nano-foetuses: defined as a foetus weighing < 60% of the mean foetal weight in the control group); 16 studies from 1996 to 2003 (1526 foetuses, 187 litters), in NZW rabbits; [b] No HCD were available for this effect
* significantly different from control: $p < 0.05$

The incidence of nano-foetuses was clearly increased above HCD in the main study in rabbits, but the relevance was reduced by the fact that 10/12 nano-foetuses of the top dose occurred in one dam. The effects observed in this dam are described under maternal toxicity above. There was also uncertainty regarding the definition of nano-foetuses. Although it was explained that their weight was reduced to < 60% of the mean of the control foetuses, it was not clear whether they also were smaller (reduced length). However, a weight reduction by more than 40% is also considered a relevant finding if seen without severe maternal toxicity. It was interesting to note that not all nano-foetuses also had incomplete ossifications and not all foetuses that had incomplete ossification were nano-foetuses.

Five foetuses of the litter with 10 nano-foetuses also had dilated renal pelvis, 3 of them were also nano-foetuses. There was only one other foetus with dilated renal pelvis in this study (low dose, not a nano-foetus).

In top dose foetuses, a statistically significant increase in incompletely ossified hindlimb talus was observed. In addition, other parts of the skeleton were affected by incomplete ossification, including hyoid bone and os pubis, but no dose-dependent increase was noted in these parts. It was also noted that the incidence for hyoid bone was rather high (36% of all foetuses in the control).

Other observations were incidences of abnormal origin of the left common carotid artery in all groups including controls, with a dose-dependent increase: 20/183 (11%), 19/168 (11%), 27/159 (17%), 29/155 (19%) in control, low, mid, and top dose, respectively.

The preliminary teratogenicity study (Anonymous, 1999) in NZW rabbits was not part of the CLH dossier but results from it were mentioned during the consultation, and the study report was made available to RAC. In this study, benthiavalicarb-isopropyl was administered by oral gavage on GD 6-28 at dose levels of 0, 5, 10, 20 and 40 mg/kg bw/d; i.e., the same as in the main study, with an additional dose of 5 mg/kg bw/d.

As presented in the table below, it can be concluded that the dams were not affected by severe maternal toxicity. Final body weight was only reduced by 3% in the top dose, whereas reduction of body weight gain during the dosing period (GD 6–29) was 26% and 29% in the two top doses. Body weight gain showed a high variation and large differences between the groups were also seen during GD 0 to 6, where no test material was administered to the animals. In none of the dams of all groups, abnormal clinical observations were made, and there were no effects on number of corpora lutea, implantation sites, or placental weight. Effects in single dams that had foetuses with developmental effects are discussed in the text further down.

***Table**: Relevant results from the preliminary teratogenicity study in rabbits, effects on dams (Anonymous, 1999)*

Dose (mg/kg bw/d)	**0**	**5**	**10**	**20**	**40**
Food consumption GD 7–29 (g)	1346 ± 162	1409 ± 357 (+5%)	1422 ± 109 (+7%)	1296 ± 92 (-4%)	1282 ± 80 (-5%)
Bw GD 29 (g)	3688 ± 157	3758 ± 202	3678 ± 135	3663 ± 215	3576 ± 154 (-3%)
Bw – gravid uterus weight (g)	3215 ± 198	3247 ± 142	3252 ± 224	3216 ± 177	3107 ± 95 (-3%)
Bw gain GD 0-6 (g) (prior dosing)	98 ± 66	145 ± 103 (+50%)	81 ± 32 (-17%)	82 ± 98 (-16%)	68 ± 58 (-31%)
Bw gain GD 6–29 (g) (during dosing)	324 ± 115	332 ± 163 (+3%)	339 ± 81 (+ 5%)	241 ± 79 (-26%)	229 ± 203 (-29%)
Liver weight (g)	74.31 ± 8.84	91.94 ± 6.99 (+24%)	85.28 ± 12.74 (+15%)	79.24 ± 1.84 (+7%)	84.93 ± 7.22 (+14%)
Liver weight (relative to corrected bw)	2.309 ± 0.179	2.521 ± 0.136 (+9%)	2.617 ± 0.271 (+13%)	2.467 ± 0.077 (7%)	2.733 ± 0.21 (+18%)
# of dams examined	4[#]	5	3[##]	3[#§]	4[§]
# of dams with live foetuses	4	5	3	3	4

* Significant difference from control, p ≤ 0.05, [#] 1 dam was infertile, [##] 2 dams were infertile, [§] 1 dam died due to gavage error

Like in the main study, also in this study nano-foetuses were observed, and occurred in all litters of the top dose group (8 foetuses in 4 litters: 2/1, 1/2 and 4/1). Nano-foetuses are defined as foetuses with a weight < 60% of the mean of the controls here as well. No information on length was available in the report, but the study authors concluded that the nano-foetuses were a result of growth inhibition. Like in the main study, incomplete ossification was also observed, and here it achieved statistical significance for the hyoid bone (14 foetuses in the top dose) and os pubis (11 foetuses in the top dose). In contrast to the main study, there was no effect on ossification of the hindlimb talus (see table below).

***Table**: Relevant results from the preliminary teratogenicity study in rabbits, effects on foetuses (Anonymous, 1999)*

Dose (mg/kg bw/d)	0	5	10	20	40
# of litters	4	5	3	3	4
# of foetuses	31	43	21	24	38
Nano-foetuses	0	1	0	0	8 * (21%)
Nano-foetuses / litter	0	1/1	0	0	4/1, 2/1, 1/1, 1/1
Weight of live male foetuses	43.47 ± 5.99	44.55 ± 6.46 (+2.5%)	45.26 ± 2.40 (+4%)	40.36 ± 1.77 (-7%)	38.31 ± 10.40 (-12%)
Weight of live female foetuses	40.58 ± 7.48	41.31 ± 7.84 (+1.8%)	43.69 ± 3.63 (+8%)	39.99 ± 3.02 (-1.5%)	31.18 ± 5.21 (-23%)
Incomplete ossification:					
Skull: hyoid bone	0	10 (23.3%)	10 (47.6%)	4 (16.7%)	14 (36.8%)*
Os pubis	0	2 (4.7%)	0	0	11 (28.9%)*

* Significant difference from control, $p \leq 0.05$

The study report concluded that the delayed ossification was a consequence of the hampered growth. Foetal body weight was only presented as a mean value for each litter (not for the single foetuses) and as all litters of the top dose were affected it was not possible to derive whether the nano-foetuses alone were responsible for the observed weight reduction of -12% in males and -23% in females. But foetal body weight was also reduced in the next lower dose (-7% in male and -1.5% in female foetuses) at which no nano-foetuses were described. Irrespective of the categorisation as nano-foetus, lowered body weight may adversely influence development and ossification. In this respect it was noted that not only the nano-foetuses were affected by incomplete ossification, but there were foetuses with incomplete ossification of os pubis and hyoid bone that were not nano-foetuses and the other way round. Given that the dams were not severely affected by general toxicity it can be concluded that the observed foetal weight reduction was a relevant finding.

Furthermore, when looking at the individual animal data it was noted that the dams having nano-foetuses were not more affected by general or specific toxicity than the other dams. For instance, the dam from the low dose group having one nano-foetus had the highest body weight gain of this dose group and also the corrected final body weight (body weight minus gravid uterus weight) was the highest for this dam in this group. In the top dose such a comparison was not possible as all dams of this group had nano-foetuses. It was however, noted that effect on body weight gain was comparable between the two top dose groups (-26% and -29% of the control), but nano-foetuses were only seen in the top dose group, not in the second highest dose group. Regarding individual gross findings, it was noted that only one dam of the top dose group had an enlarged liver, brown patches or zones in the lungs were seen in almost all animals of all groups including controls. No other relevant gross findings were reported.

Like in the main study, in the preliminary study there were incidences of abnormal origin of left common carotid artery, i.e., 5/37 (16%) in control and 10/38 (26%) in the top dose foetuses (animals of the other doses were not examined for visceral anomalies). The increase was not statistically significant.

The DS argued in his response to comments from the consultation that the relevance of this study was diminished by the low number of animals included (only 4 vs. 19 litters in the main study at the 40 mg/kg bw/d) and the limited information on maternal toxicity. However, during RAC assessment, the study report was made available to RAC and based on the information in the report, it can be concluded that dams were not affected by severe general toxicity. Despite the low number of animals assessed, there was a clear effect on the top dose group (statistically

significant increase in number of nano-foetuses and decrease in mean weight in male and female foetuses, incomplete ossification of hyoid bone and os pubis).

A pre-preliminary teratogenicity study in NZW rabbits (Anonymous, 1998c) was made available to RAC only after the first discussion in the working group (RAC working group meeting . This study was carried out in order to establish a dose level for the main teratogenicity study in NZW rabbits. Daily application with oral gavage was carried out in groups of 5 NZW rabbit dams per group at 1, 10, 30 & 60 mg/kg bw/d for the organogenesis period from GD 6 to 18, which was different from the two other rabbit teratogenicity studies in rabbit where dosing was from GD 6 to 28.

No death was observed throughout gestation period, but one dam of the 30 mg/kg bw/d group showed urogenital haemorrhage on GD 25 and abortion on GD 26. Trends towards decreased body weight gains were observed in the 30 & 60 mg/kg bw/d group after GD 6. Marked body weight decreases were noted in the female with abortion.

Necropsy was carried out in the surviving dams (5, 5, 5, 4 & 5 in control, low, mid, and top dose). Brown patch of the lungs was seen in one dam of each group, but in the control group in two animals indicating no relation to treatment. Other effects were single observations and therefore not considered relevant (e.g., black patch of the lungs, black patch of ovary, cysts of oviduct and deformation of uterus). Relative and absolute liver weight were increased in the two top dose groups, reaching statistical significance for relative liver weight in the top dose (30 mg/kg bw/d: absolute: + 7%, relative: +10%; 60 mg/kg bw/d: absolute: +15%, relative: +16%).

Though not treatment-related, it was noted that there was a trend towards lower implantation rate and higher pre-implantation loss rate of embryos in the 60 mg/kg bw/d group, compared with the control group. In the same dose group, there was a lower live foetus rate, however, this was caused by an increase in late resorption (10/12 implants) in only one dam.

No effects were seen on corpus luteum graviditatis, sex ratios, live foetal weights or placental weights and there was no indication for placental abnormalities.

Under external observations it was mentioned that one nano-foetus (< 60% of the mean weight of the controls group) each was seen in the 1 & 10 mg/kg bw/d groups. The incidence of these single cases was within the historical control range presented for the main study for foetal incidence, but not for litter incidence (20% (1/5), HCD range: 0.0 – 13.6%). However, as there was no dose-dependence, RAC considers it not treatment-related.

Upon visceral examination incidences of abnormal origin of the left common carotid artery (control: 29 (64.4%), 60 mg/kg bw/d: 14 (60.9%)) and thymic remnants in the neck (control: 9 (20.0%), 60 mg/kg bw/d: 7 (30.4%) were described. However, as these findings were not increased in the top dose, animals from the other doses were not examined for visceral abnormalities.

Skeletal examination of the control and 60 mg/kg bw/d group did reveal some incidences of incomplete ossification, but there was no difference in the incidence rate between the two groups. No increase in incidence rate of other skeletal variations (e.g., skeletal variation of the 13th rib) was observed or only single findings (e.g., sacralisation of lumbar vertebrae, asymmetry and connection of sternebrae) were reported. Animals from the other dose levels were therefore not examined and it was concluded that no relevant findings were induced by benthiavalicarb-isopropyl treatment in this study.

Despite slight effects on body weight gain and food consumption in the two top doses, no relevant findings were noted in the foetuses. The study concluded that 60 mg/kg bw/d would be an appropriate dose for the main teratogenicity study.

Overall analysis of the results of the rabbit studies:

The main effects observed in the rabbit foetuses were an increased incidence of nano-foetuses, reduced foetal weight, incomplete ossification and increases in the incidence of abnormal origin of the left common carotid artery. These findings were observed in the main (Anonymous 37, 2000b) and the preliminary study (Anonymous, 1999), but not in the pre-preliminary study (Anonymous, 1998c). Although the top dose used in the pre-preliminary study was higher than for the other two studies (60 instead of 40 mg/kg bw/d), dosing was considerably shorter (i.e., GD 6-18 instead of GD 6-28) which might explain why foetuses were not affected in this study.

Overall, these observations in foetuses were not accompanied by severe maternal toxicity as outlined in detail for each of the studies (see above), including upon comparison with the effects observed in the individual dams that had affected foetuses.

The most prominent effect was the increase in incidences of nano-foetuses in the top dose of the main and the preliminary study. Incidences were statistically significantly increased in the top doses of the main and preliminary study above HCD. Single incidences were also seen in the control group of the main study and low doses of all three studies, but within historical control values.

The following table brings foetal effects for top dose litters from the main and the preliminary rabbit study in relation to the individual findings in the respective dams. It also compares this information with the mean values of the top dose and the control group.

***Table**: Data from individual dams from the top dose group, in comparison with mean values of top dose and control group animals from the main study (Anonymous 37, 2000b) and the preliminary study (Anonymous, 1999).*

	Animal #	# of nano-foetuses	Bw, GD 29 (g)	Bw gain, GD 6 – 29 (g)	Food cons., GD 6 – 29 (g)	Bw – gravid uterus weight
Anon. 37, 2000b	#2301	1	3433	32 *	1204	3076 (-13%)
	#2303 §	10	3663	75 *	1266	3244 (-8%)
	#2306	1	3703	40 *	1595	3094 (-12%)
	Mean top dose @	12	3976 ± 308	210 ± 176	1470 ± 246	3503 (-0.8%)
	Mean control	2	4035 ± 219	235 ± 150	1402 ± 244	3532
Anon., 1999	#2402	2	3377	49	1201	2984 (-7%)
	#2403	1	3642	350	1361	3080 (-4%)
	#2404	1	3738	452	1285	3182 (-1%)
	#2405	4	3547	65	665 **	3184 (-1%)
	Mean top dose	8	3576 ± 154	229 ± 203	1282 ± 80	3107 (-4%)
	Mean control	0	3688 ± 157	324 ± 115	1346 ± 162	3215

***** In the same group some dams, that had no nano-foetuses (and that did not abort) had even stronger reductions in bw gain / final bw – gravid uterus weight: **#2309: 15g / 3280 (-7%)**, 11 foetuses, 3 with single incidences of incomplete ossification; **#2311: - 62g / 3228 (-9%)**, 6 foetuses, no foetus with incomplete ossification; **#2318: 10 g / 3366 (-5%)**, 8 foetuses, 3 with single incidences of incomplete ossification
@ Two dams aborted and are not included in these numbers: **#2315:** no reduction in food consumption, only on the day of abortion (GD 25); **#2318:** no food consumption from GD 13 onwards, abortion on GD 28
§ #2303: reduction in food consumption over the last 4-5 days, no food consumption on the last 3 days (GD 27–29); this was one of the two animals in the top dose with enlarged liver (2nd dam of this group with enlarged liver (#2302) had no nano-foetuses; enlarged liver was also seen in one control dam (#2005) that had 2 nano-foetuses; two dams of the low dose group that had nano-foetuses had no enlarged liver (#2107 & #2114))

Overall, this comparison indicates that there was no clear correlation between reduced food consumption/body weight gain and the occurrence of nano-foetuses or foetuses with incomplete ossification. Furthermore, enlarged liver was not correlated with the occurrence of nano-foetuses.

One exception was the dam which had 10 nano-foetuses (top dose, main study). When excluding this dam, there would be no relevant effect with respect to nano-foetuses in the main study. In this dam there was a slight reduction in final body weight compared to control and body weight gain (GD 6–29) was considerably lower (though there were dams for which this value was even lower, and no nano-foetuses were observed). In addition, food consumption was reduced on the last 4-5 days, and there was no food consumption on the last 3 days. It is, however, questionable whether the effect "nano-foetus" could be caused by this reduction in food consumption. It was also noted that food consumption was low in other dams that had no nano-foetuses (in the same but also in other groups, including controls) and food consumption generally goes down towards the end of gestation (e.g., Garcia-Garcia *et al.*, 2021).

Although there were some uncertainties related to that specific dam, this was not the case for the observed nano-foetuses in all of the 4 top dose dams in the preliminary study. There were no (relevant) changes in body weight, body weight gain or food consumption in the respective dams that might explain the occurrence of nano-foetuses.

Nano-foetuses were defined as foetuses with weight < than 60% of the mean control weight. The studies did not indicate whether these foetuses also had a decreased length. However, in relation to the nano-foetuses, the DRAR also mentioned "dwarfism" and some potential causes of this effect, including interference with the HPT axis, maternal toxicity or hereditary causes. Maternal toxicity was, however, not severe. The pre-preliminary study even concluded that a dose of 60 mg/kg bw/d could have been used in the main study. It was therefore unlikely that the observed nano-foetuses as well as the general reductions in foetal weights were caused by maternal toxicity. In this respect, it was also relevant to note that in a study investigating feed restriction in pregnant NZW rabbits (Cappon *et al.*, 2015) a restriction to 15 g feed/d resulted in net maternal body weight loss and 40% abortions, but mean foetal weight was only reduced to 83% of controls.

Regarding interference with the HPT axis as potential cause of the nano-foetuses, it was noted that in several repeated dose toxicity studies with benthiavalicarb-isopropyl in rats and mice, interference with the HPT axis was observed and these observations lead to the identification as ED of the T-modality under the EFSA regime (EFSA, 2021). Though a clear link between this MoA and the observed nano-foetuses was not demonstrated.

Regarding the possibility that the observed nano-foetuses could have hereditary causes, it was noted that they were seen in historical and concurrent controls, indicating that they can occur spontaneously. In addition, it was noted that 10/12 nano-foetuses in the top dose of the main study were from one litter and might therefore be the result of a genetic pre-disposition that might lead to the development of nano-foetuses. However, as the same effect was also seen in the preliminary rabbit study at the same dose, but in 4 different litters (i.e., all litters of this group) this explanation becomes less likely. In addition, no underlying MoA of such a hereditary cause was described, neither in the CLH report nor in the DRAR. In this respect it was noted that five foetuses of the litter with 10 nano-foetuses also had dilated renal pelvis, three of them were also nano-foetuses (dilated renal pelvis was only seen in one other foetus, low dose, no nano-foetus). This effect might be related to delayed development as indicated by low weight and incomplete ossification in the foetuses of this litter.

It was not known whether the foetuses also had a reduced body length, but RAC considered a reduction of foetal body weight by more than 40% alone, in the absence of severe maternal toxicity, as adverse. Reduced body weight is known to adversely affect post-natal development in animals and humans.

In this respect it should be noted that the dose of 40 mg/kg bw/d was rather low and higher doses could have been tolerated (as also indicated by the pre-preliminary rabbit developmental toxicity study, Anonymous (1998c) in which a dose of 60 mg/kg bw/d was recommended as top dose for the main study).

Incomplete ossification was statistically significantly increased in the top doses of main and preliminary study, though different parts of the skeleton were affected (see study description above). It is likely that this effect was related to the lower body weight and developmental delay. The individual body weights of foetuses are not known, but it can be derived that the delay in ossification was not only seen in nano-foetuses but also in other foetuses of the top dose groups of the two studies.

In the preliminary and the main study, increases in the incidence of abnormal origin of the left common carotid artery could be observed. However, these increases were not statistically significant. No increase was seen in the pre-preliminary study, but the background incidence was rather high (64% in the controls). The high background incidence and the fact that the increase with dose was not statistically significantly reduces the relevance of this finding.

In conclusion, it is striking that the same effects (nano-foetuses, delayed ossification) were observed in two different studies at the same dose. In the absence of severe maternal toxicity, the observed reduction in foetal body weight was considered to demonstrate an adverse effect, as it might adversely influence post-natal development.

Next to maternal toxicity, two other potential causes for the occurrence of nano-foetuses were mentioned. While interference with the HPT-axis was supported by related observations in several repeated dose toxicity studies in rats and mice (see section on carcinogenicity and STOT RE), a clear link was not demonstrated between the developmental effects and this MoA. The other cause that was proposed in the DRAR was a genetic pre-disposition that might lead to the occurrence of nano-foetuses. However, no detailed description of a possible MoA was available and the fact that a statistically significant increase in nano-foetuses was seen in two studies, with several litters affected in the top dose only, did not support this explanation.

Overall, it can be concluded that the relevance of the findings from the rabbit studies cannot be dismissed.

It seems that rabbits are more sensitive than rats towards developmental toxicity of benthiavalicarb-isopropyl, but without further information on species differences the relevance of these findings for humans cannot be excluded.

The criteria for Category 1A are not fulfilled as there was no human data available.

The criteria for Category 1B are not considered fulfilled, as uncertainties with respect to the observed effects in the available experimental animal studies make the evidence less clear.

The uncertainties include the clustered occurrence of 10/12 nano-foetuses in one litter of the top dose group of the main study which could indicate an undetected pre-disposition of the respective dam. However, in the preliminary study at the same dose, all 4 litters were affected. Incomplete ossification was seen in the top dose of the main and the preliminary rabbit study, but different parts of the skeleton were affected. In addition, it was noted that delayed ossification is only a variation, not a malformation, but it might indicate a general developmental delay of the nano-foetuses as well as the other foetuses of the relatively low top dose groups of both studies, where no severe maternal toxicity was observed. Reduced foetal weight and developmental delay may adversely influence post-natal development in animals as well as humans. These developmental effects were only seen in rabbits not in rats, but as there was no information on an underlying MoA not that it would not be relevant to humans, the findings in rabbits cannot be excluded. Taking this into account RAC considered that there was some evidence from experimental animals of an adverse effect on development and, thus, that the criteria for Category 2 are fulfilled.

In summary, RAC concluded that benthiavalicarb-isopropyl **warrants classification as Repr. 2; H360d**.

Adverse effects on or via lactation

In the preliminary two-generation study (Anonymous, 1998a) in the top dose group, there was a trend towards lower bw in male offspring on PND 4 (-16.5%) and in females on PND 4-21 (-8% to -13.5%). During growth, female pup bw was decreased on PND 22 (-14%) and PND 28 (-12%). No relevant histopathological findings were seen.

No findings indicating effects on or via lactation were seen in the main two-generation study in rats. No other relevant data were available.

Based on the available data, considering that the effects on pup bw seen in the preliminary study were not seen in the main study and the low number of animals used, RAC agreed with the DS that **no classification for benthiavalicarb-isopropyl for adverse effects on or via lactation is warranted.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Benthiavalicarb-isopropyl is an active substance used in plant protection products as a fungicide against *Peronosporales* fungi, except *Pythium spp.* and *Phytophthora infestans* in potato crops. The DS proposed to classify the substance as **Aquatic Chronic 2** based on lack of rapid degradation and a 28d-NOEC value of 1 mg/L for the rainbow trout *Oncorhynchus mykiss.*

Degradation

A hydrolysis study (Yeomans P and Swales S, 2000) according to Testing Method C.7 (Directive 92/69/EEC), EPA Subdivision N, Section 161-1 (October 1982); MAFF: 59 NohSan No. 4200 (January 1985) and OECD TG 111 (May 1981) was run at pH 4, 5, 7 and 9 and at 25 °C and 50 °C in sterile aqueous buffered solutions. Benthiavalicarb-isopropyl was considered hydrolytically stable. The half-live (DT_{50}) is assumed to be > 1 year at 25 °C. No major hydrolysis products were detected.

The study on direct aqueous photolysis of benthiavalicarb-isopropyl in sterile aqueous buffered solutions at pH 5, 7 and 9 under intermittent artificial light for 12 hours per day for 30 days at 25 °C was conducted according to SETAC Pesticides, Section 10 (March 1995); EPA Pesticide Guidelines, Subdivision N, Section 161-2 (October 1982). The photolytic half-life for benthiavalicarb-isopropyl was calculated to be 16.2 days at pH 5, 543 days at pH 7 and 191 days at pH 9. Many minor photolytic degradation products were formed at pH 5 in amounts of ≤ 5% of the applied radioactivity and were not identified. Evolution of CO_2 accounted for 28% of the applied radioactivity at pH 5, 2.2% at pH 9 and 0.5% at pH 7. Other volatile organic compounds accounted for < 0.5% of the applied radioactivity.

In an indirect photolysis screening study (Habeeb SB, 2016) according to EPA OPPTS 835.5270 (January 1998) using synthetic humic water and under natural sunlight for 16 days the calculated photolytic half-life (DT_{50}) was 795 days. No photolytic degradation products were formed. Indirect photolysis is unlikely to be a significant mechanism for dissipation of benthiavalicarb-isopropyl in aquatic environments.

In a ready biodegradation test (Bealing DJ, 1998) according to EC Method C4, OECD TG 301B (CO_2 Evolution (Modified Sturm Test)) using sewage plant effluent CO_2 evolution was found to be 2 - 3% of the theoretical maximum value after 28 days. The substance is therefore not readily biodegradable under test conditions.

In an aerobic mineralisation in surface water study (Feldmann S, 2015) performed according to OECD TG 309 the degradation and metabolism of benthiavalicarb-isopropyl were studied in a pelagic pond system (pH 8.28) under aerobic conditions for 62 days at 23 °C. The test material was applied at a low (11 µg/L) and high (108 µg/L) dose. In the low dose system, degradation of benthiavalicarb-isopropyl was rapid after ended lag phase (36 days) with a t½ value of 13.9 days (Hockey Stick), while in the high dose system, degradation after ended lag phase (37 days) was much slower with a t½ value of 65.7 days (Hockey Stick, HS). The DT_{50} on the whole study period for the low and high dose were 49.9 days (HS) and 103 days (HS), respectively. CO_2 and other volatile organic compounds did not exceed 1% of the applied radioactivity at any dose. In both test systems one major metabolite was detected (KIF-230-M-5 (~19 - 25% AR)). In addition, in the low dose test system the following major metabolites were detected: KIF-230-M-4 (24% AR), KIF-230-M-8 (~10% AR) and a minor metabolite (KIF-230-M-3 (~5% AR)).

The degradation and metabolism of benthiavalicarb-isopropyl was studied in a pond water/sediment system (pH 8.2) and a lake water/sediment system (pH 6.8) under aerobic conditions for 100 days at 20 °C. The study (Goodyear A, 2000) was conducted according to BBA Guidelines for PPP (Part IV, 5-1) (December 1990); SETAC Pesticides, Section 8.2 (March 1995) and EPA, subdivision N, Section 162-4 (October 1982). In the Millstream Pond water, benthiavalicarb-isopropyl degraded rapidly reaching 10.2% after 30 days and 5.1% at last sampling time (100 days). In the Emperor Lake water, benthiavalicarb-isopropyl also degraded rapidly reaching 3.3% after 59 days. The DT_{50} dissipation of benthiavalicarb-isopropyl from water, sediment and system were respectively: 3.69 days (Double First-Order in Parallel degradation kinetics equation, DFOP), 25.7 days (Single First-Order Rate Model, SFO) and 18.2 days (SFO) in pond and 7.71 days, 16.5 days (SFO) and 15.1 days (SFO) in lake. $DegT_{50}$ in water and sediment were respectively: 17.2 and 21.7 days (SFO) in pond and 25.7 and 9.63 days (SFO) in lake. The decline of benthiavalicarb-isopropyl from the aqueous phase was accompanied by a corresponding increase in residues in the sediment which represented 82 and 94% of the applied radioactivity after 100 days incubation. The main metabolites detected in the sediments were KIF-230-M-5 (~ 12% Applied Radioactivity, AR), KIF-230-M-4 (~23% AR) and KIF-230-M-3 (~26% AR). 6-fluoro-2-hydroxy-1,3-benzothiazole was a minor degradation product (5% AR). The degradation profile of benthiavalicarb-isopropyl was similar for aerobic soil and aerobic water/sediment systems, indicating that degradation in sediment was the result of microbial degradation. Volatile substances (CO_2 and other organic volatile compounds) did not exceed 4% of the applied radioactivity. In the water layer of both test systems three minor metabolites were formed: 1-(6-fluoro-1,3-benzothiazol-2-yl)ethanamine (< 3% AR), 1-(6-fluoro-1,3-benzothiazol-2-yl) ethanone (< 0.5% AR) and 1-(6-fluoro-1,3-benzothiazol-2-yl) ethanol (≤ 6% AR).

Aerobic degradation of benthiavalicarb-isopropyl in soil under aerobic dark conditions at 20 ºC and 30ºC was investigated in five different soils in three studies. The half-lives (DT_{50s}) in soils were in the range of 2.8 to 72.7 days. At studies termination, evolved CO_2 were from 4 to 54 % of applied radioactivity. Several degradation products were formed.

The DS concluded that benthiavalicarb-isopropyl is not considered rapidly degradable.

Bioaccumulation

For benthiavalicarb-isopropyl, measured octanol-water partition coefficient (log P_{ow}) determined according to OECD TG 107 (HPLC method) and at 20-25 °C is 2.37-2.93 at pH 5, 2.36-2.90 at pH 9, and 2.28-2.86 at pH unadjusted (distilled H_2O). In the CLH report also partition coefficient

test data for relevant metabolites (KIF-230-M-1, KIF-230-M-3, KIF-230-M-4, KIF-230-M-5 and KIF-230-M-8) were available. The measured log P_{ow} were 2.26 – 2.58 at 20 °C and 1.7 – 1.84 at 25 °C.

Considering that measured octanol-water partition coefficient (log P_{ow}) is < 4 no experimental bioaccumulation studies were performed for benthiavalicarb-isopropyl and its relevant metabolites.

Based on the data presented, the DS concluded that benthiavalicarb-isopropyl has a low potential for bioaccumulation as log P_{ow} of benthiavalicarb-isopropyl is below the cut-off value of 4 given in the CLP Regulation.

Aquatic Toxicity

The CLH report presents data from aquatic toxicity tests with benthiavalicarb-isopropyl and relevant metabolites (KIF-230-M-1, KIF-230-M-3, KIF-230-M-4, KIF-230-M-5 and KIF-230-M-8). For benthiavalicarb-isopropyl, there are reliable aquatic acute and chronic toxicity data for all three trophic levels. In case of relevant metabolites reliable aquatic acute toxicity studies for all three trophic levels are provided, while for chronic aquatic toxicity only the data for algae are available. The summary of the relevant information on acute and chronic toxicity for benthiavalicarb-isopropyl and relevant metabolites are provided in Table 32 (acute) and Table 33 (chronic) of the CLH report.

Acute toxicity

For fish, three limit tests with three different species (*Oncorhynchus mykiss, Cyprinus carpio* and *Lepomis macrochirus*) and performed according to OECD TG 203 were available for benthiavalicarb-isopropyl. In all three studies, the 96h LC_{50} was > 10 mg/L. For relevant metabolites, the studies using rainbow trout (*Oncorhynchus mykiss*) and performed according to OECD TG 203 reported the 96h LC_{50} from > 3.36 mg/L to > 100 mg/L.

For aquatic invertebrates, one acute toxicity study (limit test) using benthiavalicarb-isopropyl and five acute toxicity studies with relevant metabolites are available. All the studies were performed with *Daphnia magna* and according to OECD TG 202. The 48h EC_{50} of > 10 mg/L for benthiavalicarb-isopropyl and 48h EC_{50} of 6.28 - > 100 mg/L for relevant metabolites were reported.

For algae, one acute toxicity study with green algae *Selenastrum capricornutum* using benthiavalicarb-isopropyl and five acute toxicity studies with green algae *Pseudokirchneriella subcapitata* using relevant metabolites are available. All the studies followed OECD TG 201. The 72h E_rC_{50} and 72h E_yC_{50} for benthiavalicarb-isopropyl were > 10 mg /L while for the different metabolites the 72h E_rC_{50} were between > 10 and 100 mg/L and 72h E_yC_{50} between 7.42 and 100 mg/L.

The DS proposed **not to classify** the benthiavalicarb-isopropyl as acutely hazardous to the aquatic environment. The basis for this proposal is that acute aquatic toxicity test results showed no toxicity effects to aquatic organisms (algae, daphnia and fish) at concentrations ≤ 1 mg/L.

Chronic toxicity

Two chronic toxicity studies with two different fish species were available for benthiavalicarb-isopropyl. First study using zebrafish (*Danio rerio*) following OECD TG 210 (Anonymous 55, 2015) resulted in 35d NOEC of ≥ 5 mg/L. The second study using rainbow trout *Oncorhynchus mykiss* was carried out according to OECD TG 215 (Anonymous 56, 2001a). The 28d NOEC was 1 mg/L.

There was only one study carried out according to OECD TG 211 available for aquatic invertebrates for benthiavalicarb-isopropyl with 21d NOEC value of 3 mg/L (reproduction and length) and 21d EC_{10} value of 4.3 mg/L (weight) for *Daphnia magna*.

One toxicity study with benthiavalicarb-isopropyl performed according to OECD TG 201 was available for algae *Selenastrum capricornutum*. The 72h NOE_rC was 2.50 mg/L. In addition, five studies with green algae *Pseudokirchneriella subcapitata* using relevant metabolites are available. The studies followed OECD TG 201 and resulted in 72h NOE_rC of 5 – 33.3 mg/L.

Based on the results from chronic aquatic toxicity studies, the DS concluded that fish is the most sensitive taxonomic group. The lowest chronic toxicity value is 28d NOEC of 1 mg/L for rainbow trout *Oncorhynchus mykiss* using benthiavalicarb-isopropyl. This value fulfils the criteria for **Aquatic Chronic 2** (H411), i.e.; toxicity ≤ 1 mg/L for non-rapidly degradable substance.

Comments received during consultation

Three MSCAs and one National Authority provided comments. All MSCAs agreed with proposed classification for environmental hazards. The National Authority only agreed with no classification for acute aquatic hazard.

One MSCA commented that precautionary statement P273 - Avoid release to the environment should be removed from the CLH report as intended use of the fungicide benthiavalicarb-isopropyl is application in potato fields. The DS agreed.

In the view of the second MSCA, additional explanation why the benthiavalicarb-isopropyl is considered not rapidly degradable is needed.

The National Authority commented that the key endpoint for the proposed chronic classification is an OECD TG 215 (Fish, Juvenile Growth test) 28d NOEC of 1.0 mg/L for *O. mykiss* based on weight. The OECD TG 215 test endpoint is fish growth, and the method does not consider sensitive life-stages (e.g.; juveniles, eggs, larvae) relevant to long-term fish toxicity and therefore may not fully characterise long-term fish toxicity. However, the study recorded effect on growth and all available data should be considered for hazard classification. In the view of the National Authority, if the fish OECD TG 215 endpoint is considered relevant, the preference to EC_{10} value based on weight over NOEC value should be used. The use of EC_{10} value would lead to no classification for chronic aquatic hazard. In addition, given that OECD TG 215 endpoint does not fully characterise long-term fish toxicity, the NOEC value from OECD TG 210 is available and suitable to consider it as long-term toxicity to fish. The use of this NOEC value would lead to no classification for chronic aquatic hazard. All other aquatic chronic toxicity endpoints are also > 1 mg/L resulting in no aquatic chronic classification. DS considered the NOEC based on growth relevant for classification purposed.

Assessment and comparison with the classification criteria

Degradation

RAC agreed with the DS's proposal to consider benthiavalicarb-isopropyl as **not rapidly degradable**:

- The substance is hydrolytically stable at environmentally relevant pHs (pH 4-9) and has a long hydrolytic half-life (DT_{50} > 1 year at 25 °C).
- No significant degradation was observed in the ready biodegradability test (OECD TG 301B), indicating that the substance is not readily biodegradable.

- In the surface water simulation test the mineralisation was low (< 1%) and DT_{50} on the whole study period were 49.9 days (low dose) and 103 days (high dose). Three major metabolites were formed, namely KIF-230-M5, KIF-230-M4 and KIF-230-M8.
- The $DegT_{50}$ in the water and sediment in a water/sediment system study were respectively 17.2 and 21.7 days (SFO) in pond system and 25.7 and 9.63 days (SFO) in lake system. Low mineralization was observed (< 4%).
- It was also not demonstrated that benthiavalicarb-isopropyl is ultimately degraded in a soil simulation tests with a half-life of < 16 days (DT_{50}: 2.8 to 72.7 days; mineralisation from 4% to 54%).

Bioaccumulation

RAC agreed with the DS that benthiavalicarb-isopropyl has a low potential for bioaccumulation. The basis for this conclusion is the measured log P_{ow} values of 2.56 – 2.63 are below the CLP Regulation threshold value of 4.

Acute toxicity

RAC is of the opinion that reliable aquatic acute toxicity data for all three trophic levels are available for benthiavalicarb-isopropyl and relevant metabolites. RAC noted that all $L(E)C_{50s}$ for fish, invertebrates and algae (see Table 32 in CLH report) are above the threshold value of 1 mg/L. Therefore, RAC supported the DS´s proposal that **benthiavalicarb-isopropyl does not warrant classification for acute aquatic hazard**.

Chronic toxicity

RAC considered the endpoint derived from an OECD TG 215 study relevant for assessing chronic fish toxicity based on CLP guidance (version 5.0, July 2017) and therefore is appropriate to consider the endpoint relevant for chronic hazard classification of the substance.

RAC acknowledged that the CLP guidance (version 5.0, July 2017) indicates that when the EC_{10} value is available, this value is preferred over NOEC. Furthermore, this applies in cases where EC_{10} and NOEC values are available for the same endpoint and the same study. In case of benthiavalicarb-isopropyl, the NOEC of 1 mg/L and EC_{10} of 3.5 mg/L based on weight derived from OECD TG 2015 study are available for *O. mykiss*. However, as indicated by DS the 95% confidence intervals could not be calculated and for this reason the calculated EC_{10} value is not fully reliable, and the NOEC is considered more relevant for the hazard assessment purpose. Therefore, RAC considered that in case of benthiavalicarb-isopropyl it is more appropriate to use the NOEC based on weight for *O. mykiss* as the most critical chronic endpoint for fish.

RAC was of the opinion that reliable aquatic chronic toxicity data for benthiavalicarb-isopropyl are available. Furthermore, reliable studies with relevant metabolites are available for algae. RAC noted that for algae, in the toxicity studies with relevant metabolites the derived 72h NOE_rC is higher (72h NOE_rC = 5 – 33.3 mg/L) than for benthiavalicarb-isopropyl (72h NOE_rC = 2.5 mg/L). Based on available data, fish are the most sensitive trophic group, and the lowest chronic effect value corresponds to a test with fish *O. mykiss* with 28d NOEC value of 1 mg/L based on weight for benthiavalicarb-isopropyl. Benthiavalicarb-isopropyl was not rapidly degradable and had a low potential for bioaccumulation. Consequently, RAC concluded that benthiavalicarb-isopropyl warrants classification as **Aquatic Chronic 2 (H411)** for chronic aquatic hazards. This is consistent with the conclusion of the Dossier Submitter.

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ANNEXES:

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

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