COMPILED COMMENTS ON CLH CONSULTATION

Comments provided during consultation are made available in the table below as submitted through the web form. Please note that the comments displayed below may have been accompanied by attachments which are listed in this table and included in a zip file if non-confidential. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

ECHA accepts no responsibility or liability for the content of this table.

Last data extracted on 12.04.2021

Substance name: benthiavalicarb-isopropyl (ISO); isopropyl [(S)-1-{[(R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]carbamate
CAS number: 177406-68-7
EC number: -
Dossier submitter: Poland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	France		MemberState	1
Comment received				
FR: p1: The ISO common name: benthiavalicarb-isopropyl should be indicated. p1: The EC number (EINECS n°) 605-799-5 should be indicated. p1: The SMILES notation is available, please report it in the CLH report. p2: According to LOEP (September 2019), only toluene is identified as a relevant impurity. The other impurities KIF-230-I-6 and KIF-230-I-12 are confidential and should be removed.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
08.04.2021	Belgium	<confidential>	Company-Manufacturer	2
Comment received				
Prolonged exposure at high doses of benthiavalicarb-isopropyl during the carcinogenicity studies was linked to increased incidences of hepatocellular adenoma, carcinoma and blastoma. Benthiavalicarb-isopropyl was also linked to thyroid tumors in male mice and uterine tumors in female rats. 17 mechanistic studies were submitted in the context of the plant protection product review process in order to determine modes of action for each tumor type. The data and their analysis were included in four structured reports enabling risk assessors to determine the basis of the conclusions reached with respect to the key events for the underlying modes of action and their relevance to human health. These documents have been updated including the results and discussion of additional research in order to completely portray the current state of knowledge. Based on the weight of evidence of the available data, a CAR/PXR-mediated mitogenic mode of action has been established for liver tumors in mice and male rats (Attachment 1) and a liver-mediated altered thyroid hormone homeostasis mode of action has been established for thyroid tumors in male mice (Attachment 2). At this time, a mode of action for the uterine tumors in female rats has not been identified (see below). We therefore support the suggestion of the Polish Competent Authority to classify benthiavalicarb-isopropyl as a "suspected human carcinogen".				

However, the EFSA concluded that, in addition to CAR/PXR activation, benthiavalicarb-isopropyl may cause liver and uterine cancers by alterations in the Wnt/β-catenin signaling pathway. Because this mode of action is also operable in humans, the EFSA considered that the hepatocellular neoplasms and hepatoblastoma observed in B6C3F1 mice are relevant for human cancer hazard identification. We performed additional in vivo assays investigating the enhancement of β-catenin-mediated TCF/LEF transcriptional activity in the liver of benthiavalicarb-isopropyl treated mice and rats and abnormal accumulation of β-catenin in mouse liver. All studies were negative for β-catenin activation (Attachments 3, 4, 5, 6, 7 and 8).
Slides containing lesions diagnosed as hepatoblastoma were reviewed by an independent pathologist. The reviewing pathologist confirmed the diagnosis of the laboratory pathologist. The hepatoblastomas that were observed in the B6C3F1 mice used in the carcinogenicity study almost always occurred within an existing neoplastic lesion. We did not observe an upregulation of downstream oncogene targets of Wnt/β-catenin signaling (see above), which are associated with human hepatoblastoma. These findings suggest that hepatoblastomas are part of the spectrum of liver neoplasms that occur as a result of benthiavalicarb-isopropyl treatment. Pathologists experienced in rodent toxicology pathology consider the combination of hepatocellular adenoma, carcinoma and blastoma to be most adequate for human cancer hazard identification (Attachments 9 and 10).
Additional research that has not yet been presented in the CLH Report addresses EFSA's concern with regard to the observed uterine adenocarcinoma caused by benthiavalicarb-isopropyl as this adverse effect is likely related to a disruption of the estrogen hormone system. However, the increased incidence of uterine tumors was not accompanied by an increase in the incidence of uterine endometrial hyperplasia. Overall, in the available studies there is no evidence of an estrogen-mediated toxicity (Attachment 11). Subsequent mechanistic studies in rats show that benthiavalicarb-isopropyl does not act as a dopamine agonist as it did not cause a reduction in prolactin. Because benthiavalicarb-isopropyl does not reduce prolactin levels, the estrogen/progesterone-ratio was not changed (Attachments 12 and 13). Furthermore, we did not observe an activation of β-catenin target genes in the uterus of F334 rats (Attachment 14).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public Attachments.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential Attachment.zip

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	3

Comment received

We agree with the DS that classification for carcinogenicity is required for benthiavalicarb-isopropyl. However, classification with Carc. Cat. 1B, H350 may be considered more appropriate.
Justification: Criteria of Regulation (EC) No 1272/2008 for Carc. 1B are fulfilled: Different types of tumours (hepatocellular adenoma/carcinoma, thyroid follicular cell adenoma, hepatoblastoma and uterus adenocarcinoma) occurred in 2 species and 2 sexes. Relevance for humans could not be excluded with sufficient certainty based on the available data.
In the rat chronic toxicity/carcinogenicity study, hepatocellular adenoma and uterus adenocarcinoma are noted. Observed incidences of hepatocellular adenoma (14 % in males at the highest dose) were above of the mean incidence (6.1 %) but within the range of the HCD (0 - 18 %). However, incidences of uterine tumours at both upper dose levels (22% and 20 %) were outside the range of the HCD (0 – 8 %). The survival was in general higher in the treated groups than in the controls, thus the treatment was not affecting adversely

survival.
In the second carcinogenicity study conducted in mice, a carcinogenic effect in the liver was observed. The incidence of hepatocellular adenoma was increased in both sexes above the historical control with 94 % at the top dose in males (HCD: 16.0 - 56.0 %) and 46 % at the top does in females (HCD: 6.0 - 26.0 %). This was accompanied by a statistically significant and biologically relevant increase in carcinoma in males (86% at top dose, HCD: 10.0 - 40.0 %) but not in females (not stat. significant 12 %, HCD: 2.0 - 16.0 %). Hepatoblastoma were clearly increased in the two high dose groups (males, 18 % at top dose, HCD: from 0 - 2.0 %). The viability was affected from week 78 in males and survival declined to 56 % at the top dose at termination.
Several mechanistic studies were evaluated by the DS to address the relevance of hepatocellular adenoma and carcinoma, thyroid follicular cell adenoma, hepatoblastoma and uterine adenocarcinoma in rodents for human hazard characterisation:
Mechanistic studies in mice in vivo (a 7-day study with wild-type mice) and in vitro with wild-type mice hepatocytes demonstrate CAR induction, but also AhR induction, albeit to a lesser extent. A study with hepatocytes from CAR/PXR knock-out mice (in vitro assay) showed deficiencies, and no conclusion on the PROD activity is possible due to high variability of the three analysed samples. In hepatocytes of one of the three human donors (in vitro assay) also a slight induction of AhR-mediated gene expression (Cyp1A1) was observed.
It is noticeable, that regarding hepatoblastoma "Pathology Reports by the Prof John R Foster BSC, PhD" were presented. However, these reports present the differences in mice and human hepatoblastoma rather than a histopathological re-evaluation.
Overall, as there were relevant tumor responses in at least two organs and two species, classification with Carc. Cat. 1B, H350 should be considered. The available information does not allow to conclude on the MoA for liver tumour formation with sufficient certainty and a lack of relevance for humans has not been demonstrated convincingly. Carc. 1B was also supported by the majority of experts at the Pesticide Peer Review Experts' Meeting 186 (2018).

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	4
Comment received				
Based on the available data the proposal for non- classification for mutagenicity/germ cell mutagenicity of benthiavalicarb-isopropyl is supported.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
08.04.2021	Belgium	<confidential>	Company-Manufacturer	5
Comment received				
Administration of benthiavalicarb-isopropyl via the diet at high doses resulted in decreased serum thyroxine levels in male rats and mice. This was found to be consistent with liver enzyme induction and increased activity of UDP-glucuronosyltransferase on thyroxine. Benthiavalicarb-isopropyl did not affect the iodide oxidation step in thyroid hormone synthesis via inhibition of thyroid peroxidase. According to ECHA/EFSA's recommendations on how to investigate thyroid effects in rodents, we are carrying out supplemental in vitro assays verifying whether benthiavalicarb-isopropyl affects (1) thyroid hormone synthesis via iodide uptake (sodium/iodide-symporter) or (2) the peripheral metabolism of thyroid hormones via deiodinases. These data can be subsequently provided within four months.				

The change in thyroxine concentrations despite the fact that benthiavalicarb-isopropyl does not produce adverse developmental or reproductive effects deserves some additional discussion. It appears that thyroxine is not a specific measure of thyroid-mediated toxicity (Attachment 15). This lack of specificity should be discussed in the RAC opinion document. We think that thyroxine measurements in rodents can only be interpreted in the context of some adverse effects in order to be meaningful.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Public Attachments.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential Attachment.zip

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	6

Comment received

Adverse effects on sexual function and fertility:
We agree with the DS that based on the available data from a 2-generation study in rats (Anonymous, 1999), the effects are not sufficient to require classification of benthiavalicarb-isopropyl as toxic for sexual function and fertility.

Adverse effects on development:
From the available data, it is not clear whether the criteria for classification for Repr. 2, H361d are met or not. Higher incidences of dwarfism/nano-foetuses were observed. However, the study authors defined nano-foetuses by body weight, not clarifying if the lower body weight is based on decreased length growth. Without this information, it cannot be concluded whether a classification for developmental adverse effects is required or not. It should be noted that the DS did not present all available data.
Justification: In two studies in rabbits (developmental study in rabbits as presented in the CLH dossier (Itoh 2000); preliminary developmental study in rabbits not presented in the CLH dossier but in the DAR (Itoh 1999)) dwarfism/nano-foetuses (and incomplete ossification) was observed in the foetuses and should be considered treatment related.

However, clarification of the used definition of dwarfism/nano-foetuses is required to finally conclude on adversity of developmental effects of benthiavalicarb-isopropyl: The study author defined nano-foetuses as foetuses with less than 60% of the mean foetal weight in the control group. It is not clear, if length growth was also affected, which might be relevant for classification.
This was also concluded by the experts at the Pesticide Peer Review Experts' Meeting 186 (2018).

1. Developmental study in rabbits, as described in the CLH-dossier: Benthiavalicarb-isopropyl was administered by oral gavage to New Zealand White rabbits (22 per sex and dose) from day 6 to day 28 of gestation at dose levels of 0, 10, 20 and 40 mg/kg bw/day (Anonymous 37, 2000b). Increased incidence of dwarfism (nano-foetuses) was observed at the highest dose: 12/155 nano-foetuses, 3/19 litters, with one litter with high foetal incidence (10/1, 1/2). Average maternal feed consumption and maternal body weight/bw change were not altered by treatment. (One dam showed reduced feed uptake in the latter half of gestation and aborted, not relevant for nano-foetuses findings.) In addition, 2 nano-foetuses are observed in the control group (2/183 nano-foetuses, 2/1 litter) and 2 nano-foetuses are observed in the low dose group (2/168 nano-foetuses, 1/2 litter). The litter incidence at the top dose is above the litter incidence range of the presented historical

control data. There were maternal toxicity effects in one dam which aborted (lower body weight gain and food consumption; however, maternal toxicity is not clearly reported), thus, no maternal effects are reported for the others. Thus, there is an effect on foetus that should be considered related to treatment. Furthermore, an increase of delayed talus ossification was observed at the top dose (hindlimb talus, 14 foetuses).

2. Preliminary developmental toxicity study in rabbits, as described in the DAR: The previous Assessment Report (DAR) included a preliminary developmental toxicity study in rabbits (same strain, Itoh, 1999), but was neither reported in the CLH-Dossier nor in the RAR. Although it was agreed at the Pesticide Peer Review Experts' Meeting 186 (2018) that this study has to be included in the RAR. In this preliminary study, benthiavalicarb-isopropyl was administered by oral gavage from day 6 to day 28 of gestation at dose levels of 0, 10, 20 and 40 mg/kg bw/day. At highest dose of 40 mg/kg bw/d the same effect (nano-foetuses) was observed in all litters (8 foetuses out of 4 litters i.e. 2/1, 1/2 and 4/1). The maternal toxicity was not presented in detail, but at 40 mg/kg bw/d, liver weight was increased (14 to 18%) and both food consumption (10 %, day 2 - day 6 of gestation, but dosing started at day 6) and absolute body weight decreased (3 %). Additionally, this study also showed incomplete ossification (skull: hyoid bone up to 14 foetuses in the high dose, dose-relation questionable; os pubis 11 foetuses in the high dose).

Adverse effects on or via lactation:
We agree with the DS that based on the available data from a 2-generation study in rats (Anonymous, 1999), the effects are not sufficient to require classification of benthiavalicarb-isopropyl for effects on or via lactation.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	7
Comment received				
We agree with the proposal that classification for acute toxicity (oral, dermal and inhalation) is not required for benthiavalicarb-isopropyl.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	8
Comment received				
We agree with the proposal that classification for skin corrosion/irritation is not required for benthiavalicarb-isopropyl.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	9
Comment received				
We agree with the proposal that classification for serious eye damage/eye irritation is not required for benthiavalicarb-isopropyl.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	10
Comment received				
Based on the findings in the guinea-pig maximisation (Magnusson-Kligman) test, we agree with the DS that, benthiavalicarb-isopropyl meets the criteria to be classified as a skin sensitiser. While sub-categorisation (e.g. in Skin Sens. 1A) could be discussed, we consider that several uncertainties remain and, overall, agree with the DS to classify benthiavalicarb-isopropyl as Skin Sens. 1, H317 without further subclassification. Justification: According to Regulation (EC) No 12727/2008, results of a guinea pig maximisation test indicate the sub-category 1A if ≥ 60 % of the animals respond positive at > 0,1 % to ≤ 1 % intradermal induction dose. The available guinea pig maximisation test (Anonymous 9, 2000a, KCI Doc No. 201/993857/SS) was conducted using an intradermal induction dose of 0.25 %. As presented in Table 19 in the CLH-Dossier, the challenge with 70 % benthiavalicarb-isopropyl revealed 75 % positive animals (15/20 animals at 48h, taking into account scores ≥ 1). It should be noted, that the challenge with 35 % benthiavalicarb-isopropyl elicited a skin response in 50 % of the same animals (24 + 48 h, exposure with 70 % substance on anterior site, exposure with 35% substance on posterior site), i.e. in less than 60 % of the animals. Moreover, results of control animals appear somewhat arbitrary, as 14/20 control animals (24 h) and 7/20 (48 h) showed reactions (score 1, anterior site), but 0/20 showed reactions on posterior site (24, 48 h). Therefore, score 1 was disregarded in the study report. However, this approach is considered questionable and these findings could lead to a limitation of the reliability of the study. Furthermore, referring to the ECHA Guidance on the Application of CLP Criteria (version 5.0, 2017, page 342, Table 3.5), redness with scores ≥ 1 are used for the definition of significant skin sensitising effects. No positive reaction was elicited in the described Buehler test (Anonymous 10, 2000b, KCI Doc No. 200/002387/SS).				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	11
Comment received				
We agree with the DS that no significant neurotoxic effects were observed in available studies which should be considered for classification for specific target organ toxicity after single exposure.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	12
Comment received				
Based on the available data, we agree with the DS that, classification of benthiavalicarb-isopropyl for specific target organ toxicity after repeated exposure is not indicated.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
08.04.2021	Sweden		MemberState	13

Comment received

p.73-74 Acute aquatic hazard
The Swedish CA agrees with the proposal that no acute aquatic environmental hazard classification is trigged for this fungicide. Acute aquatic toxicity data for all three trophic levels are available and indicate LC50/EC50/ErC50 >10 mg/L. Consequently, there is no need to determine an acute M-factor.

p. 74-75 Long-term aquatic hazard (including bioaccumulation potential and degradation)
The Swedish CA agrees with the proposed long-term aquatic environmental hazard classification; Aquatic chronic 2, H411. Benthiavalicarb-isopropyl, is not rapidly degradable and the lowest chronic endpoint was determined in the chronic fish study with rainbow trout and is equal to 1 mg a.s./L.

p. 75 Conclusion on classification and labelling for environmental hazards
This is out of the scope of the harmonized classification process, but since precautionary statements are mentioned in the dossier, we would like to add the following comment;
It is suggested to include the precautionary statement P273- Avoid release to the environment. However, according to Table 6.2 in CLP Regulation, Annex IV; List of precautionary statements, part 1, this precautionary statement should be used if release to the environment is not the intended use. The intended use of the fungicide benthiavalicarb-isopropyl is application in potato fields. The Swedish CA is therefore reluctant to the use of P273 in the labelling of benthiavalicarb-isopropyl.

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	United Kingdom	Health and Safety Executive	National Authority	14

Comment received

benthiavalicarb-isopropyl (CAS: 177406-68-7)
We agree that no Aquatic Acute classification is required because all aquatic acute toxicity endpoints are >1 mg/L.

The key endpoint for the proposed Aquatic Chronic classification is an OECD TG 215 (Fish, Juvenile Growth test) 28-d NOEC of 1.0 mg a.s./L for Oncorhynchus 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. While the study may not fully characterise long-term fish toxicity, it does appear to record a growth effect and all available data should be considered for hazard classification. On this basis, if the fish OECD TG 215 endpoint is considered relevant for classification, we note an EC10 of 3.5 mg a.s./L based on weight is available and should be used in preference to the NOEC value for classification. This EC10 would not lead to an Aquatic Chronic classification for this non-rapidly degradable substance given that it is >1mg/L.

Given the OECD TG 215 endpoint does not fully characterise long-term fish toxicity, we note an OECD TG 210 35-d NOEC of ≥5.0 mg a.s./L is available and suitable for to consider long-term toxicity to fish. This endpoint supports no classification.

All other aquatic chronic toxicity endpoints are also >1 mg/L resulting in no Aquatic Chronic classification.

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Germany		MemberState	15
Comment received				
We agree to the proposed classification of benthiavalicarb-isopropyl. However, from our point of view for a better understanding some more explanations why it is classified as not rapidly degradable are useful at point 11.1. The results of the different studies are given, but not directly discussed in regard to the evaluation of rapid degradation. In the rate degradation study (Purser D and Goodyear A, 2001) the decline of benthiavalicarb-isopropyl in soil is described as rapid without a reason why this is not meaningful for rapidly degradable (see table 30, p.58 and point 11.1.4.3, p. 63). At point 11.7.2 on page 74 only the result of water/sediment studies are given, but not the results of the ready biodegradation study.				

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	France		MemberState	16
Comment received				
FR agrees with the conclusion on classification and labelling for environmental hazards, i.e. Benthiavalicarb-isopropyl is classified as H411: Toxic to aquatic life with long lasting effects with the pictogram GSH09 but no signal word.				

PUBLIC ATTACHMENTS
1. Public Attachments.zip [Please refer to comment No. 2, 5]

CONFIDENTIAL ATTACHMENTS
1. Confidential Attachment.zip [Please refer to comment No. 2, 5]