Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON BENTHIAVALICARB-ISOPROPYL (ISO); ISOPROPYL [(S)-1-{[(R)-1-(6-FLUORO-1,3-BENZOTHIAZOL-2-YL)ETHYL]CARBAMOYL}-2-METHYLPROPYL]CARBAMATE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties. 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.

Substance 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
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.				
Dossier Submitter's Response				
Thank you for your comments. We agree with the proposed amendments. The SMILES notation for benthiavalicarb-isopropyl is available and should be reported in the CLH report as follows: CC(C)OC(=O)N[C@@H](C(C)C)C(=O)N[C@H](C)c1nc2ccc(F)cc2s1				
RAC's response				
RAC agrees with the DS's response.				

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

Dossier Submitter's Response

Thank you for your support, comments and additional data attached.

ECHA note – An attachment was submitted with the response from the dossier submitter. Refer to confidential attachment K-IIA 5.2.6_1_KCI201_993857_SS.pdf

RAC's response

The provided documents and new data have been thoroughly reviewed by RAC and considered for the opinion development.

RAC agrees that there is strong support for activation of the CAR/PXR pathway by benthiavalicarb-isopropyl, with relevance for the observed thyroid tumours as well as for the hepatocellular adenomas and carcinomas.

The key elements of this MoA have been demonstrated – these include morphological and histopathological evidence (increased liver weight, enlarged livers, hepatocellular hypertrophy), CAR/PXR activation (in vitro wild-type and knock-out studies), induction of total CYP 450 enzymes and specifically those downstream of CAR/PXR (i.e. CYP2B & CYP3A) as well as T4 UDPGT and the activities of PROD and BROD as indicators for CYP2b and CYP3A activities. It is, however, noted that in several of the assays also induction of CYP1A1/1A2 was detected, indicative of AhR activation, and though the induction was mostly not very strong, it sometimes was in the range or even exceeding that of the induction of enzymes downstream of CAR/PXR. In conclusion, a contribution of AhR activation cannot be excluded.

In addition, it is noted that no in vivo knock-out study is part of the data set, but would have been relevant, given the extensive metabolism of benthiavalicarb-isopropyl demonstrated in toxicokinetic studies. It can be assumed that, under in vitro conditions not all relevant metabolites were formed, which is equally relevant for the in vitro wild type and knock-out mouse hepatocytes, as well as for the in vitro studies investigating the response of hepatocytes from three human donors.

It is further noted in that in one of the studies investigating Wnt/ß-Catenin signalling in mouse liver (Anonymous, 2021), also activation of genes downstream of Wnt, including Myc, was observed. Though no activation was observed in two other mouse studies and in a study in rat liver, this finding cannot be completely dismissed.

RAC notes that the histopathological re-evaluation of the liver slides of the mouse carcinogenicity study confirmed most of the described hepatoblastomas and concluded that these tumours were almost always occurring within another liver tumour (either hepatocellular adenoma or carcinoma). This is noted; however, RAC is of the view that this does not explain why hepatoblastomas develop. No underlying mode of action (MoA) is identified that would explain why certain chemicals induce hepatocellular adenoma and carcinoma and other hepatocellular adenoma and carcinoma as well as hepatoblastoma. In addition, the development of hepatoblastoma has not been mentioned in any of the many review papers on the CAR/PXR MoA known to RAC. Turusov *et al*. (2002) also describe an

association between the majority of hepatoblastomas detected in four NTP carcinogenicity studies, but they also identified hepatoblastomas that occurred within the same liver that also had carcinomas, but separate from these lesions. The relevance of benthiavalicarb-isopropyl induced hepatoblastomas for humans cannot be excluded.

RAC agrees that there is currently no MoA identified that would explain the observed formation of uterine adenocarcinomas. These tumours are therefore considered relevant for humans. For further details and discussion, please see the RAC opinion.

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).

Dossier Submitter's Response

Thank you for your comment.

AhR activation
A high level of AhR activation is needed to promote hepatocellular carcinogenesis regardless of species. Specific downstream target genes can be used for AhR activation monitoring. The Cyp1a1 and Cyp1a2 genes are the most specific and quantitatively measurable biomarkers for AhR activation in rodent liver. In order to investigate AhR activation by benthiavalicarb-isopropyl, the applicant company performed three in vivo and three in vitro tests.
In two in vivo assays (Murata, 2001c; Murata, 2001d), AhR activation was examined in Fischer 344 rats and B6C3F1 mice by measuring the increase of CYP1A1 protein content after oral treatment at 1,000 mg/kg bw for 7 days. In rats a statistically significant 1.6-fold increase was noted in males; in mice a statistically significant 1.5-fold increase was found in males and a 0.6-fold increase was found in females. In the third in vivo study, McMahon (2018a) measured the induction of Cyp1a1/1a2 gene expression in male C57Bl/6 mice after treatment up to 5,000 ppm in the diet for 7 days. At 5,000 ppm, a statistically significant 2.4-fold control increase was noted in the expression of the Cyp1a1 gene and a statistically significant 2.2-fold control increase was noted in the expression of the Cyp1a2 gene.
AhR activation has also been investigated in vitro in hepatocytes that were incubated for 96 hours in the presence of benthiavalicarb-isopropyl at concentrations up to 100 µM. In primary wild-type mouse hepatocytes (McMahon, 2018b), no increase in Cyp1a1 gene expression was observed whereas Cyp1a2 gene expression was statistically significantly increased (2.5-fold control). In Car/Pxr knockout mouse hepatocytes (McMahon, 2018c), Cyp1a1 and Cyp1a2 gene expressions were statistically significantly increased (2.1-fold control and 3.8-fold control, respectively). In human hepatocytes from three volunteers (McMahon, 2018d), a statistically significant increase in the expression of the CYP1A1 gene was noted in the hepatocytes from all volunteers but remained below 2-fold control; CYP1A2 gene expression was only increased in the hepatocytes from one volunteer but was less than 2-fold control. No increase in EROD activity was observed in primary wild-type mouse hepatocytes whereas in Car/Pxr knockout mouse hepatocytes it was statistically significantly increased (2.9-fold control).
In order to determine the biological relevance of the observed increases in Cyp1a1/ Cyp1a2 gene expression as a possible initiating event for hepatocellular carcinogenicity, Qin *et al*. (2019) investigated AhR activation in Wistar Han rats that had been treated by gavage once daily for 4, 7 and 31 days with AhR-activating carcinogens (TCDD and PCB126) and AhR-activating non-carcinogens (omeprazole, mexiletine and canagliflozin). In all studies, animals were euthanized 24 hours after the last dosing and liver tissues were collected, which were flash frozen for necropsy. The expression levels of mRNAs in liver tissue were determined using the Reverse Transcriptase quantitative Polymerase Chain Reaction (RT-qPCR) assay. Based on the analyses of the rat liver samples collected at 24 hours after the last administration of four daily doses, both AhR-activating carcinogens consistently induced Cyp1a1 gene expression over 400 x at carcinogenic dose levels and below 400 x at noncarcinogenic dose levels, whereas the AhR-activating noncarcinogens generally induced Cyp1a1 gene expression less than 100 x at the top doses used in the reported carcinogenicity studies. From the results of this study, criteria were developed to express

the degree of concern for AhR activation in carcinogenesis using an AhR-score, which is calculated according to the below formula.
[AhR-score] = 1/3 x log10 (Cyp1a1 fold induction) + 2/3 x log10 (Cyp1a2 fold induction)
An AhR-score greater than 1.33 indicates a higher concern; an AhR-score greater than 1.0 indicates a lower concern. The statistical cut-off for control values was set at an AhR-score of 0.4.
The induction of Cyp1a1 gene expression reported in the studies performed by the applicant company did not exceed 4-fold control, which is much lower than the threshold for AhR-activating noncarcinogens (increase of 100-fold in Cyp1a1 gene expression). When calculating the AhR-score using the data from the in vivo mouse study (McMahon, 2018a), the score obtained (0.35) is even lower than the statistical cut-off set for control values (0.40). This clearly indicates that the weak increases in AhR-activation by benthiavalicarb-isopropyl observed in vivo and in vitro have no biological relevance and therefore AhR activation can be excluded as a mode of action for hepatocellular carcinogenesis.
One of the direct consequences of significant AhR activation would be the production of reactive oxygen species (ROS) causing oxidative DNA damage. Oxidative DNA damage was investigated in rats and mice and no change in the formation of 8-OHdG in liver DNA was found (Inagaki, 2001a; Inagaki 2001b).

Histopathological review of mouse livers with hepatoblastomas by John R Foster
Of the 23 mice diagnosed with hepatoblastoma after 78 and 104 weeks of treatment, routine liver sections and liver sections with gross abnormalities were examined in order not to miss out any of the hepatoblastomas that were recorded by the original study pathologist in the mouse carcinogenicity study. The quality of the slides was excellent for all of those animals examined in the histopathological review. In contrast to the original study pathologist, John R Foster differentiated between benign and malignant neoplasms.
The hepatoblastomas diagnosed by the original study pathologist were largely confirmed. Two of the originally defined hepatoblastomas were found to be hyperplastic lesions while two other hepatoblastomas could not be confirmed in the slides. All confirmed hepatoblastomas (both benign and malignant) at 2,500 ppm and 8 out of 9 hepatoblastomas at 5,000 ppm were found to be present within an existing hepatocellular neoplasm. This provides evidence of a close association of these hepatoblastomas with existing hepatocellular adenomas and carcinomas from which they evolve and should therefore be included as part of the total liver tumours and not as a separate entity. These neoplasms do not arise separately. This mode of action is not relevant to humans because in humans hepatoblastomas arise de novo from mutated hepatoblasts. A detailed histopathological evaluation report was submitted as part of the Additional Information requested by the European Food Safety Authority (EFSA) on 2 July 2018.

We are of opinion that sufficient and adequate mechanistic data are available to demonstrate that CAR/PXR activation is the mode of action for hepatocellular tumour formation in the mouse. The key events (altered gene expression, hepatocellular proliferation, clonal expansion leading to altered foci and hepatocellular adenomas/carcinomas) and associative events (Cyp2b10 and Cyp3a11 enzyme induction, increase in relative liver weight and liver hypertrophy) that are typical for this mode of action have been demonstrated to be present in the mouse. The concordance of dose-response relationships, the temporal association, the strength, consistency and specificity of association of the tumour response with key events, the biological plausibility, the absence of alternative modes of action and the species specificity of these key events have been proven. These key events occur at dose levels at or above 2500 ppm. Cell proliferation subsequent to benthiavalicarb-isopropyl exposure in vitro was not observed in human hepatocytes. This indicates that the key event of cell proliferation does not occur in human

liver and therefore this mode of action is not relevant to humans. Since no mode of action could be identified for the uterine tumours in the rat, we propose that benthiavalicarb-isopropyl should be classified as a "suspected human carcinogen (Carc 2)". Reference Qin C, Aslamkhan AG, Pearson K, Tanis KQ, Podtelezhnikov A, Frank E, Pacchione S, Pippert T, Glaab WE, Sistare FD. (2019) AhR Activation in Pharmaceutical Development: Applying Liver Gene Expression Biomarker Thresholds to Identify Doses Associated with Tumorigenic Risks in Rats. Toxicological Sciences, 171(1), 2019, 46–55.
RAC's response
RAC concluded that classification as Carc. 1B is justified. Please see response to comment number 2.

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.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

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
Dossier Submitter's Response
Thank you for your comments and additional data attached.
RAC's response
RAC notes that thyroxine (T4) levels were decreased in mice and rats after exposure to doses between 80 and 855 mg/kg bw/day (mice, 7-14 days) and 661 mg/kg bw/day (rat, 14 days). Thyroid stimulating hormone (TSH) levels in mice were affected at a dose of 810 mg/kg bw/day after 16 weeks exposures. These doses are considered moderate. Induction of T4 UDPGT upon benthiavalicarb-isopropyl treatment is indicated. The arguments supporting this MoA are summarised in detail in the RAC opinion and it is concluded that 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. Three in vitro tests were submitted investigating TPO, NIS and liver DIO activities, to also cover other potential MoAs, and they were thoroughly assessed by RAC. All three tests were negative. While TPO was investigated in Yorkshire pig thyroid microsomes, but the effects were seen in mice and rats, there are still no standardised methods available to test for NIS and DIO. 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. Regarding the argument that despite a change in T4 concentrations, no adverse developmental or reproductive effects were produced, RAC concludes that there was developmental toxicity observed in rabbits (delayed ossification, nano-foetuses) which are considered supportive for classification as Repr 2; H361d, but they are not necessarily linked to effects on the thyroid axis (see also response to comment 6). However, RAC is of the view that the lack of thyroid-related effects is not necessarily contradictive, because current testing 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).

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 (Anonymous 37, 2000b); preliminary developmental study in rabbits not presented in the CLH dossier but in the DAR (Anonymous, 1999b)) 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.

Dossier Submitter's Response

Thank you for your comments.

Adverse effects on development:

The study authors defined 'nano-foetuses' as foetuses weighing less than 60% of the mean foetal weight of the control group. Crown-to-rump lengths were not recorded.
In the main study (Itoh, 2000), 12 foetuses with a decreased body weight and 14 foetuses with delayed ossification of the talus bone were found at the highest dose tested (40 mg/kg bw/day). 10 out of the 12 nano-foetuses found at that dose level were from one dam (no. 2303) showing signs of severe toxicity, including (1) a decrease in body weight gain (75 g vs the group mean of 210 g from day 6 to day 29 of gestation), (2) a decrease in food consumption with no food intake on days 27 to 29 of gestation (cumulative food consumption was 1266 g vs the group mean of 1470 g from day 7 to 29 of gestation), (3) an increase in relative liver weight (3.389 g% vs the group mean of 2.647 g%), (4) an increase in relative kidney weight (0.636 g% vs the group mean of 0.437 g%) and (5) an increase in relative adrenal weight (11.189 mg% vs de group mean of 7.402 mg%). Necropsy revealed multiple organ lesions such as brown patches on the lungs, pale and enlarged liver and white patches on the kidneys. Therefore, the cluster of 10 nano-foetuses that were limited to the litter of dam no. 2303 should be considered as an indirect effect of the severe toxicity caused by benthiavalicarb-isopropyl in this dam. No such effects were seen in the other dams.

In the preliminary study (Itoh, 1999) suppression of body weight gain (−30%) due to reduced food consumption was also observed in the dams at 40 mg/kg bw/day as well as increases in relative and absolute liver weights. Decreased foetal weight (8 'nano-foetuses') and delayed ossification of the pubic and hyoid bones were recorded in the presence of maternal toxicity. Less weight should be attributed to the results of this preliminary study in the weight-of-evidence analysis because of the lower number of litters investigated (4 vs 19 litters at 40 mg/kg bw).
Overall, when taking into account these two studies, 40 mg/kg bw/day can be established as the LOAEL for maternal toxicity. At this dose there were in the main study no effects on the number of litters, live foetuses, pre-implantation losses, resorptions (early or late), sex ratio, foetal weights or on the incidence of external, visceral or skeletal malformations. No skeletal retardations other than a delayed ossification of the talus bone (14 out of 155 foetuses in 4 out of 19 litters) were noted.

The thyroid gland was not examined in the preliminary and main study. In other toxicity studies the doses at which thyroid follicular cell hyperplasia was observed in rats (249.6 – 318.2 mg/kg bw/day) and mice (358.4 – 459.3 mg/kg bw/day) are considerably higher than the highest dose tested in the rabbit embryo-foetal developmental studies (40 mg/kg bw/day). Given the overall weight of evidence, we consider that the decreased foetal weight and delayed ossification are not related to a thyroid-mediated developmental delay but secondary to toxicity in the dams.

RAC's response

Adverse effects on development:

RAC thanks the German CA for highlighting the results of the preliminary rabbit study. Upon request, the study reports for all rabbit studies, including the main and the preliminary study, as well as another preliminary study (with shorter exposure duration: i.e. from gestation day 6 to 18) were made available to RAC.

A detailed analysis of the included information was carried out by RAC. Based on the results for the individual animals, it could be derived that there is no correlation between the maternal toxicity and foetal effects (nano-foetuses, incomplete ossification). In the absence of severe maternal toxicity, the foetal weight reduction of more than 40% is regarded as adverse effect, even if the foetal length was not affected (no information on body length

contained in the study reports, low birth weight is known to adversely affect post-natal development in animals and humans).

There is some uncertainty related to the findings, i.e.; 10 of 12 nano-foetuses from the main study were seen in the litter of one dam; however, as in the preliminary study, at equal dose, all four litters were affected, the finding is considered relevant. Also the second effect (incomplete ossification) was seen in both studies in the top dose, though different parts of the skeleton were affected and incomplete ossification is regarded as variation only. In conclusion, these effects are considered supportive for a classification as Repr. 2; H361d.

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

In response to the DS's comment, RAC would like to highlight that upon detailed analysis of the data of the individual animals, no correlation between maternal and foetal effects could be found. For instance, it is mentioned that food consumption was reduced in the dam with 10 nano-foetuses; however, similar effects on food consumption were also seen in other dams, that had no nano-foetuses (including some from the control group). It is correct that this dam had an enlarged liver, but again, the same effect was seen in two other dams without nano-foetuses, including one from the low dose group. Body weight gain and corrected final body weight were comparable to the values from other dams of this group, which had no nano-foetuses.

Regarding the four dams of the top dose of the preliminary study, the DS mentioned that body weight gain was reduced by 30% in this group; however, the effect was equally high in the second highest group, in which no nano-foetuses were seen. In addition, body weight gain was prone to considerable variation in this study and animals were affected also before treatment even started.

It is further noted that in a study by Cappon *et al*. (2015), which investigated the effect of feed restriction on pregnancy outcome in NZW rabbits, a reduction of feed to 15g/d resulted in 40% abortions and net body weight loss of the dams, but mean foetal body weight was only reduced to 83%.

It can be concluded that animals were not affected by severe maternal toxicity at the rather low top dose of 40 mg/kg bw/day. In the first preliminary study a dose of 60 mg/kg bw/d was recommended to be used as top dose in the main study (though in this study exposure was only up until GD 18). The fact that the effect was seen in two studies at equal doses further increases the relevance of this finding.

RAC does not exclude the possibility that the observed developmental effects were related to interference with the HPT axis. Dwarfism was discussed in relation to such interference in the DRAR, and EFSA (2021) identified benthiavalicarb-isopropyl as an endocrine disrupter (ED) for the T-modality. The DS stated that interference of the HPT was only demonstrated at rather high doses in the rat studies. RAC is of the view that these effects were not only seen at high doses and the rat is a different species (nano-foetuses were seen in rabbits). The available data are, however, considered insufficient to conclude on whether the observed nano-foetuses were caused by interference with the HPT axis.

This is discussed in more detail in the RAC opinion.

<u>Adverse effects on sexual function and fertility:</u>

In line with the DS, RAC agrees that the rat two-generation studies does not indicate adverse effects on reproduction. However, in all three mouse studies (i.e. 28-d, 90-d and carcinogenicity study) effects on the ovaries were reported. RAC considers these effects as

sufficient to support classification as Repr. 2; H361f. For further details on the ovary effects in the mouse studies see RAC opinion.

Adverse effects on or via lactation:

RAC also included the results from a preliminary two-generation study in rats (Anonymous, 1998a) in its assessment. In the top dose of this study, effects on post-natal body weight development were reported in males and females. No relevant histopathological findings were seen. These effects were not considered supportive for a classification for adverse effects on lactation.

Cappon GD, Fleeman TL, Chapin RE, Hurtt ME (2005): Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit. Birth Defects Research (Part B) 74:424–430 (2005)

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.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

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.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

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.				
Dossier Submitter's Response				
Thank you for your support				

RAC's response
Noted.

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 (GPMT; 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).

Dossier Submitter's Response

Thank you for your support and comments.

The DS agreed that the results of the GPMT study should be discussed (study report of Anonymous 9, 2000a, KCI Doc No. 201/993857/SS will be provided for RAC consideration). In the Guinea pig maximisation test 7 out of 20 non-induced control animals (35%) showed a slight persistent erythema and oedema after the challenge application of 70%-w/v benthiavalicarb-isopropyl, which elicited in 15 out of the 20 induced test animals (75%) slight to well defined dermal responses at 24 and 48 hours after patch removal. The test animals were induced intradermally and topically with 0.25%-w/v and 70%-w/v benthiavalicarb-isopropyl. Slight skin irritation was observed in the test and control animals after intradermal injection of the test substance and vehicle, respectively. The responses

observed in the induced test animals during the challenge phase were however more severe as compared to the responses observed in the non-induced control animals suggesting that benthiavalicarb-isopropyl may cause allergic skin reactions.

In the Buehler test, three topical applications of 70%-w/v benthiavalicarb-isopropyl to 20 Guinea pigs elicited slight skin reactions in a few animals (3 out of 20 test animals) during the induction phase. No dermal reactions were observed in the induced test animals after the challenge application of 40%-w/v benthiavalicarb-isopropyl. The Buehler test is not considered fully reliable because of the lack of skin reactions during the induction phase.
Reliable data are only available from the Guinea pig maximisation test, which showed a high response (75% of the test animals) after intradermal injection of a low concentration of the test substance (0.25%-w/v). Since more than 30% of the non-induced control animals seemed sensitised to the vehicle, the data are not sufficient for sub-categorisation and therefore the classification of benthiavalicarb-isopropyl as a Category 1 Skin Sensitiser is warranted.

RAC's response

RAC agrees with the proposal to classify benthiavalicarb-isopropyl as Skin Sens. 1, without sub-categorisation.

Support for this classification mainly comes from the GPMT. Although this study has deficiencies (i.e. positive reactions were also seen in control animals, though to a lesser extent and less severe than in treated animals and only at the higher challenge dose; necrosis on the sites treated with FCA), it clearly indicates that benthivalicarb-isopropyl acts as a skin sensitiser.

The Buehler test is negative, but it also has deficiencies (no dermal reactions were observed after challenge with 40% w/v benthiavalicarb-isopropyl) and the protocol is in general less sensitive than the GPMT study. In addition, there were some inconsistencies in the study description (in the CLH report as well as in the DRAR), which could not be resolved based on the limited reporting. The negative result does not invalidate the positive result from the GPMT.

In conclusion, RAC proposes Skin Sens. 1, but the uncertainties related to the GPMT (described above) do not allow sub-categorisation.

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.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

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.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Noted.				

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.				
Dossier Submitter's Response				
Thank you for your comments. p. 75 Conclusion on classification and labelling for environmental hazards We agree with your comment regarding the precautionary statement P273. It should be used if release to the environment is not the intended use. This precautionary statement P273 should be removed.				
RAC's response				
Thank you for your comment.				

RAC notes the support for the proposed environmental classification.

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.

Dossier Submitter's Response

Thank you for your comments.

As was mentioned in CLH dossier point 11.6.1 page 77, the study (2001a, OECD TG 215) was already evaluated during Annex I inclusion of benthiavalicarb-isopropyl, and 28-d NOEC at 1.0 mg a.s./L was accepted and consider relevant for the risk assessment. According to the zRMS comments: " *the 95% confidence intervals could not be calculated and for this reason calculated EC10 value is not fully reliable and the NOEC is considered more relevant for the risk assessment purposes*." It should be also noted that this endpoint was considered acceptable by other Member States during the peer-review process. Detailed explanation of the RMS regarding choice of endpoint for the risk assessment may be found under study summary in Vol. 3CA, B.9.2.2.2/01.
Therefore we are of the opinion that 28-d NOEC at 1.0 mg a.s./L is considered relevant for classification purpose.

RAC's response

Thank you for your comment.

RAC notes the support for the proposed no Aquatic Acute classification of the substance.

RAC considers the endpoint derived from an OECD TG 215 study relevant for assessing chronic fish toxicity based on Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.7b: Endpoint specific guidance (version 4.0, June 2017) and therefore is appropriate to consider the endpoint relevant for chronic hazard classification of the substance.

RAC acknowledge 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 calculated EC_{10} value is not fully reliable and the NOEC is considered more relevant for the hazard assessment purpose. Therefore, RAC considers 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.

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.

Dossier Submitter's Response

The rate of degradation of benthiavalicarb-isopropyl was studied in four soils under standard laboratory conditions. The half-lives (DT_{50}) of benthiavalicarb-isopropyl under standard laboratory conditions varied from 10.8 to 18.8 days. The time for 70% degradation of benthiavalicarb-isopropyl would range from 18.8 to 32.7 days. On the basis of a geometric mean half-life of 13.8 days, benthiavalicarb-isopropyl can be classified as "readily degradable" according to the FAO classification scheme. Benthiavalicarb-isopropyl was not demonstrated to be readily biodegradable in a 28-day test for ready biodegradability. Benthiavalicarb-isopropyl was not demonstrated to be degraded by more than 70% within 28 days in an aerobic mineralisation study (OECD 309). Benthiavalicarb-isopropyl is demonstrated to be stable to abiotic hydrolysis in the aquatic environment. The water/sediment study provides evidence that benthiavalicarb-isopropyl dissipates rapidly from the water phase (DT_{50} = 3.69 – 7.71 days) through binding to sediment and through microbial degradation. The microbial degradation half-lives ($DegT_{50}$) calculated using the Level-PII kinetic fitting procedure were 17.2 and 25.7 days in the pond and lake system, respectively. Although microbial degradation may contribute to the dissipation of benthiavalicarb-isopropyl in viable water bodies, **the**

criteria to consider the substance to be rapidly degradable in the aquatic environment are not fulfilled.
RAC's response
Thank you for your comment. Noted.

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.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
Thank you for your comment. RAC notes the support for the proposed environmental classification.				

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]
2. K-IIA 5.2.6_1_KCI201_993857_SS.pdf [Please refer to response to comment No. 2, 5]