Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate

EC Number: -
CAS Number: 283159-90-0

CLH-O-0000006928-58-01/F

Adopted

10 December 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON METHYL *N*-(ISOPROPOXYCARBONYL)-L-VALYL-(3*RS*)-3-(4-CHLOROPHENYL)-B-ALANINATE; VALIFENALATE

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.

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Substance name: methyl *N*-(isopropoxycarbonyl)-L-valyl-(3*RS*)-3-(4-chlorophenyl)-β-alaninate; valifenalate
EC number: -
CAS number: 283159-90-0
Dossier submitter: Hungary

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	1
Comment received				
All comments are also submitted in a compiled table as attachment. General Comment – 10.6: Respiratory sensitisation: It is stated in the CLH Report (page 21): No formally recognised and validated animal or in vitro tests currently exist for respiratory sensitisation. Based on available data it is concluded that valifenalate does not indicate evidence for irritation of the respiratory tract. In the CLH Report (page 21) it is concluded that data are lacking. However, due to the lack of formally recognised and validated tests, tests for hazard class respiratory sensitisation cannot be provided. Therefore, the conclusion Data lacking is not correct. Please update the CLH report and remove that for Respiratory sensitisation data are lacking. General Comment - 10.13: Aspiration Hazard: Aspiration hazard is not relevant for solid substances. In chapter 10.13.3 (page 59 of CLH Report) it is concluded that data are lacking. However, on the same page it is mentioned that Aspiration hazard is Not relevant for solid substance. Please update the CLH report and remove that for Aspiration hazard data are lacking. ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				

Dossier Submitter's Response
Thank you for your comment. The points mentioned are not in the scope of this public consultation, however, in section 10.13, we agree that all statements are to be brought to consistency with the conclusion.
RAC's response
Thank you very much. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Germany		MemberState	2
Comment received				
The references in Table 7 don't have to be confidential.				
Dossier Submitter's Response				
Thank you for your comment and for bringing our attention to the issue.				
RAC's response				
Thank you very much. Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	3
Comment received				
Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified There is no evidence to support a classification in category 2 based on available in-vivo studies. In the available carcinogenicity study in rats, after 104 weeks of treatment there were no valifenalate-related changes in neoplastic findings up to and including the limit dose level of 1000 mg/kg/day (NOAEL for carcinogenicity = 1000 mg/kg in both sexes). In the available carcinogenicity study in mice, there was an increased incidence of hepatocellular adenomas and carcinomas in males receiving 850 or 5000 ppm, while the hepatocellular carcinomas at 850 ppm were within historical control incidences. In female mice an increase in adenomas only was reported. Although hepatocellular tumours are relatively common in mice, a full range of investigative toxicology studies including comparison of wildtype with knock-out mice has been performed to clarify the mode of action for formation of hepatocellular tumours induced by valifenalate and to confirm the non-relevance for human. These mechanistic studies identified that valifenalate induced hepatocellular tumours in mice by a phenobarbital/peroxisome proliferation-type mode of action via CAR/PXR and PPARα nuclear receptors. This mechanism of hepatocellular tumour formation is considered as not relevant for human. Alternative modes of action including activation of alternative nuclear receptors, genotoxicity or cytotoxicity could also be excluded. Therefore, Belchim Crop Protection agrees with the conclusion of the dossier submitter Hungary that valifenalate does not meet the classification criteria for carcinogenicity under CLP. ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				

Dossier Submitter's Response
Thank you for your contribution.
RAC's response
Thank you very much. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Germany		MemberState	4
Comment received				
In the long-term carcinogenicity study in mice (CD-1) statistically significant increased incidences of hepatocellular adenoma were observed in both sex, increased incidences of carcinomas were observed in males. Incidences of adenomas at the mid and high dose were outside the historical control background range (n= 14 and 16 in males, 2 and 5 in females, HCD range: 4-11 in males, 0-1 in females). Incidences of carcinomas at the highest dose (n=10) were also outside the historical control background range (n= 1-4) and equivalent to the maximum HCD range of carcinomas at the mid dose (n=4) in males. In mutagenic tests valifenalate showed no genotoxic potential (all the in vitro and in vivo tests are negative). No tumors were reported for rats. The DS considered, that the liver tumors in mice were secondary to adaptive metabolic changes and that the liver effects are mediated through activation of the nuclear receptors PPAR-alpha, CAR and PXR. It was concluded that the effects are not relevant to humans on a "quantitative basis" as "quantitative differences in kinetic and/or dynamic factors between experimental animals and humans" regarding the identified mechanism and involved receptors were identified by the DS. The DS proposed a MoA consisting out of 3 key events: 1) Nuclear receptor activation (CAR, PXR, PPAR-alpha), 2) Increased replicative DNA synthesis, 3) Formation of hepatocellular carcinoma. To demonstrate this hypothesis four mechanistic studies were reported, including analysis of gene expression, enzyme activity and cell proliferation in CD-1 mice, analysis in C57BL/6 PPAR-alpha-knockout mice and enzyme and DNA synthesis induction in CD-1 mouse hepatocytes. In summary, it was sufficiently shown that valifenalate is an inductor of CAR, PXR and PPAR-alpha in CD-1 mice as well as in C57BL/6 mice. Activation of AhR was not investigated. Replicative DNA synthesis in CD-1 mice after valifenalate treatment was demonstrated after 3 and 7 days, mean number of proliferating hepatocytes were similar for both time points (highest dose) and 4-fold higher than in the control. However, phenobarbital showed a more pronounced effect on cell proliferation, which was 9- fold higher after 3 days and 4 fold after 14 days of treatment in comparison to control animals. For the PPAR-alpha-knockout experiments for unknown reasons no CD-1 mice but C57BL/6 mice were used. A comparative study showed, that effects on liver weight were more pronounced in CD-1 mice as in C57BL/6 mice, while clinical chemistry parameters related to liver effects and enzyme activity related to activation of CAR were more pronounced in C57BL/6 mice than in CD-1 mice. We therefore wonder if experiments using CD-1 PPAR-alpha-knockout mice would have been more appropriate. The PPAR-alpha knockout experiments showed an implication of PPAR-alpha on the valifenalate mediated effects on liver weight, clinical chemistry alterations, replicative DNA-synthesis and liver histopathology as the values/incidences were lower in comparison to the WT mice. Nevertheless, PPAR-alpha-related enzyme activity was still induced in the PPAR-alpha-KO mice by valifenalate (i.e. LAH enzyme activity: 4-fold in comparison to control, WT: 8-fold in comparison to control) and the Cyp4a10 and Cyp4a14 mRNA levels were				

even more increased in the KO mice than in the WT. The presented argumentation, that the absolute levels of mRNAs were lower in the KO, demonstrated by slightly different CT values under control conditions (Cyp4a10 WT CT: 22.5 – 24.6, KO CT: 23-25.6) mice is not comprehensive and it seems questionable if the used KO mice is an appropriate model here. No information on responses using positive control were found. Overall, the reliability of the result obtained with the KO-strain appears questionable.

Activation of CAR and PXR by valifenalate treatment was demonstrated in the KO mice and overall liver weight and replicative DNA synthesis were still clearly increased in comparison to control (i.e. replicative DNA synthesis day 7: 5-fold, WT: 8-fold). No experiments using CAR/PXR knockout mice were conducted, which would have been helpful to show the role of these receptors.
In vitro experiments using CD-1 male hepatocytes revealed, that valifenalate under this condition is not able to induce clear effects on CAR or PPAR-alpha mediated gene expression or enzyme activity, while positive controls (PB, WY-14643, EGF) showed effects. The DS considered that this is due to insufficient metabolisation of valifenalate by the in vitro system and that therefore also experiments using human hepatocytes are expected to be not valuable. No comparative in vitro metabolism study is available to confirm this assumption.

All in all, receptor activation (CAR, PXR and PPAR-alpha and induction of replicative DNA synthesis by valifenalate in mice have been demonstrated (proposed key event 1 and 2). However, the non-relevance for humans was not sufficiently shown and alternative MoAs with known human relevance (AhR) were not addressed. Experiments using humanised mice and perhaps also human hepatocytes would have been valuable to conclude on the relevance of the observed liver tumor formation in mice and to demonstrate the proposed "quantitative" differences leading to non-relevance for humans. Besides this, it is questionable if differences of quantitative but not qualitative nature would warrant a non-relevance decision. We are of the opinion, that the mechanism for tumor formation is not sufficiently investigated to exclude its relevance for humans. Therefore, classification as Carc. 2, H351, is proposed.

Dossier Submitter's Response

Thank you for your comment. In a long-term carcinogenicity study with doses of 0, 150, 850 and 5000 ppm valifenalate in CD-1 mice, increased incidences of hepatocellular adenoma in males and females and of carcinomas in males were observed at 850 and 5000 ppm. The following table gives an overview of the liver tumour incidences and historical control data ranges (HCD). In addition to the HCD provided so far, additional HCD from 'Charles River Laboratories' (Spontaneous Neoplastic Lesions in the CrI:CD-1(ICR) Mouse in Control Groups from 18 Month to 2 year Studies, March, 2005, Mary L.A. Giknis Ph.D, Charles B. Clifford D.V.M, Ph.D) are tabulated.

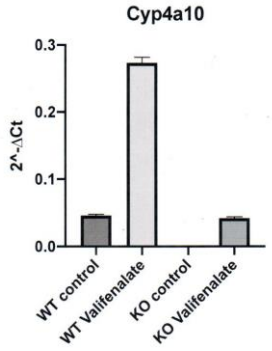
Parameter	Dose (ppm)				HCD[a] (%)	HCD[b] (%)
	0	150	850	5000		
Males, n=	50	50	50	50		
Liver						
Hepatocellular adenoma (%)	7 (14)	2 (4)	14 (28)	16* (32)	7.8-21.2	2.86-28.00
Hepatocellular carcinoma (%)	2 (4)	4 (8)	4 (8)	10* (20)	1.9-8.0	1.54-16.00
Females, n=	50	50	50	50		
Liver						
Hepatocellular adenoma (%)	0 (0)	0 (0)	2 (4)	5* (10)	0.0-1.9	0.85-7.84

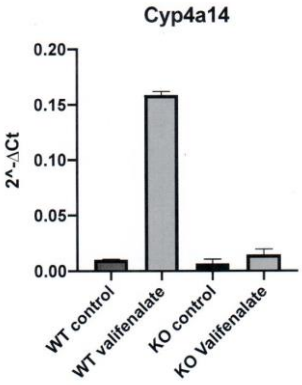
Hepatocellular carcinoma (%)	0 (0)	1 (2)	0 (0)	0 (0)	0.0-0.0	1.43-4.29

* P<0.05
a Laboratory in which study was conducted (Evreux)
b Giknis, 2005 (CRL)

These data show that hepatocellular adenoma incidences in males are almost within the HCD from CRL, whereas the hepatocellular carcinoma incidences are covered by them. The hepatocellular adenoma incidences in females are almost covered by the HCD from CRL.
Since a clear mode of action (MOA) mainly via PPARα induction and additional CAR and PXR induction was identified and other possible MOAs were excluded, based on solid experimental data, the classification of the substance for Carc 2, H351 is not deemed to be justified. Genotoxicity can be excluded based on the genotoxicity data package which was negative. Other MOAs, which might play a role, can also be excluded. Induction of Cyp1a2 and thus of AhR activation could be ruled out in a 14-day mechanistic study in male mice (Broich et al., 2015) in which neither gene expression nor enzyme activity of hepatic Cyp1a was induced in CD-1 mice exposed to valifenalate at any dose level. Other MOAs, like via steroidogenic effects or via oxidative stress could also be excluded experimentally.
Thus, based on the proposed MOA, the initiating event in liver tumour MOA of valifenalate is the co-activation of the nuclear receptors CAR/PXR and PPARα, which mediate the induction of replicative DNA synthesis in the liver of CD-1 mice. This increased cell replication is responsible for the subsequent induction of proliferative lesions of the liver including adenomas and carcinomas (Holsapple et al., 2006; Klaunig et al., 2003, Cohen 2010, Elcombe et al., 2014).
PPARα induction and additional CAR and PXR induction were demonstrated in a mechanistic study in male CD-1 mice (Broich et al., 2015), where the treatment with valifenalate for 3 and 14 days caused a dose dependent induction of the hepatic cytochrome P450 system, primarily a strong induction of P450 isoenzymes of Cyp4a1 and of peroxisomal β-oxidation, but also of Cyp2b and Cyp3a. Induction of replicative DNA synthesis based on BrdU incorporation measurement was seen at 1750 and 7000 ppm in this study and, thus, at doses clearly within and above the carcinogenic dose level range. This liver tumour MOA via activation of Cyp4a, Cyp2b and Cyp3a is not regarded as relevant to humans, according to a broad literature (Friedman, 2009; Corton, 2018; Peffer, 2018; Shizu, 2013; Tamura, 2013, 2015; Thatcher, 1994; Whysner, 1996).
This MOA was clearly demonstrated in the MOA studies conducted with valifenalate.
As part of the investigations of the mode of action, studies in PPARα- knockout mice were initiated, however, such an animal model was and is also today only available on a C57BL/6 strain background offered by Taconic. Since the oncogenicity study, in which the liver tumours were observed, was performed in CD-1 mice, a bridging study was performed to investigate the toxicological effects, including effects on PPARα in C57BL/6 wild-type mice in comparison to CD-1 mice to investigate any mouse strain differences (Vardy, 2015a).
In the main study the PPARα-mediated mode of action for valifenalate in PPARα knockout mice in comparison with the equivalent wild type (WT) mice was investigated. This study demonstrated a clear peroxisome proliferation response in the WT mice as compared to the PPARα KO mice. However, although PPARα was mainly responsible for the hepatic response to valifenalate, also CAR and PXR contributed to the liver effects. The results showed clear PPARα responses in the biochemical measurements and histopathological examinations, but also CAR and PXR activation in WT and KO animals. Responses were also seen in the PPARα KO mice, although to a lesser extent, and the absolute values were much lower in the PPARα KO mice, with the induction returning the Cyp4a levels to

the approximate levels measured in the WT mice under control conditions. If the data are not presented as a -fold of control view, but as the absolute values (2^-ΔCt), the data result in the following figures for Cyp4a10 and Cyp4a14:





The figures show that the absolute values for the induction in the KO animals are comparable to the Cyp4a levels in the WT control mice.
The relative response is explained by CAR and PXR induction, to which the PPARα KO are still responsive and, in addition, most likely by cross talk effects between nuclear receptors in general (Ueda, A. et al., 2002) and especially between the different PPAR subtypes (Deluca, J. et al., 2000; Faiola, B. et al., 2008).
These data suggest that the PPARα pathway and, as an additional mechanism, CAR and PXR activation are responsible for the liver effects of valifenalate. Neither the PPARα nor CAR/PXR MOA is regarded as relevant to humans.
Since the in vitro study showed that a metabolite, but not valifenalate, must be responsible for the liver effects, valifenalate would not cause any effect in in vitro studies in human hepatocytes, as proposed. Further studies in humanised or CAR/PXR KO mice would not add to the information which is already available, either, since the MOA via PPARα and CAR/PXR was sufficiently demonstrated, making additional vertebrate studies not justified.
To further support the MOA, a short conclusion based on the IPCS MOA framework is given below:

Key event 1:
It was demonstrated that as first key event nuclear receptors CAR, PXR and PPARα were activated in male CD-1 mice at higher concentrations of valifenalate, whereas the aryl hydrocarbon (AhR) nuclear receptor was unaffected by the substance.

Key event 2:
This was followed by the second key event which was induction of replicative DNA synthesis, as demonstrated in BrdU incorporation studies. Furthermore, they showed that the replicative DNA synthesis was dose-dependent and only present when mice were exposed to valifenalate at concentrations at or above carcinogenic dose-levels.

Key event 3:
Based on broad literature data, it is known that increased hepatocyte DNA synthesis can lead to development of hepatic changes and eventually benign and malignant hepatocellular neoplasms. In a 78-week oncogenicity study with valifenalate in male CD-1 mice, liver tumours were observed at dietary dose levels of 850 and 5000 ppm, but not at the level of 150 ppm, which was thus a NOEL for liver tumours.

Based on the 'Bradford Hill Considerations' of the IPCS MOA framework for a weight of evidence analysis of all available data for the MOA the following conclusions can be drawn:

Dose Response Relationship:
In the 78-week oncogenicity study with valifenalate in male CD-1 mice liver tumours were observed at dietary dose levels of 850 and 5000 ppm, but not at the level of 150 ppm, which was a clear threshold. In the MOA studies evidence for key event effects 1-2 were only observed at doses at or above the carcinogenic dose levels.

Temporal relationship:
The first two key events must precede the third key event of tumour development. This was demonstrated in the MOA studies, in which the first two key events occurred already after short-term treatment of up to 14 days. The third key event consists of the longer-term development of liver tumours, which is consistent with the observations in the 78-week carcinogenicity study in mice.
In summary, the MOA data for valifenalate are consistent with the temporal association of a mode of action with three key events.

Consistency, specificity:
The data of the MOA studies and the standard toxicology studies with valifenalate are consistent with the three key events for the mode of action of liver tumour formation in mice. The results of the MOA studies including the PPARα KO study, together with the results of the apical toxicology studies support a high confidence in the proposed MOA for the development of liver tumours after valifenalate exposure in mice.

Biological plausibility:
The proposed MOA is supported by broad literature. Valifenalate induces not only PPARα, but also CAR/PXR, so the resulting effect in mice is activation of one or more of these nuclear receptors. For many other substances, e.g. phenobarbital, this liver tumour MOA via CAR activation is well-known. Phenobarbital is also used in human medicine and no increase in liver tumour incidences in humans were observed. Consequently, this MOA is not regarded as relevant to humans. Also, the PPARα liver tumor MOA is not regarded as relevant to humans (Klaunig et al., 2003: PPAR alpha agonist-induced rodent tumours: Modes of action and human relevance. Crit. Rev. Toxicol. 33, 655–780).

Relevance to humans:
Based on the data of the MOA studies, valifenalate acts as a co-activator of CAR/PXR and PPARα and, since neither the CAR/PXR nor the PPARα MOA is regarded as relevant to humans, this MOA via activation of these nuclear receptors by valifenalate in mice is not expected to occur in humans.

Other MOAs:
Genotoxicity MOA can confidently be excluded since valifenalate was negative for this in a guideline genotoxicity test battery. An AhR-mediated mode of action for the formation of adenomas and carcinomas in CD-1 mice can be ruled out since in a MOA study neither gene expression nor enzyme activity of hepatic Cyp1a was induced in CD-1 mice exposed to valifenalate. A receptor-mediated mode of action via estrogen can be excluded since there is no structural similarity between valifenalate and estrogen that might suggest a similar mode of action of hepatocarcinogenesis; in addition, no evidence of estrogenic activity in the two-generation toxicity study in the rat or in other studies was seen. Cytotoxicity, another possible MOA for liver tumour development in mice is not supported by the data of any toxicology studies.

Uncertainties, inconsistencies, data gaps:
The liver tumour MOA of valifenalate consists of co-activation of CAR/PXR and PPARα. Since no KO mouse model for the three receptors exists, a study with such model could not be conducted. However, in the study in WT and PPARα KO mice, an effect via CAR and PXR was seen, furthermore, some of the effects were most likely due to receptor cross talk in general and between the PPAR subtypes, PPARγ and PPARδ. In any case the three MOAs are not regarded as relevant to humans.

Assessment of postulated mode of action and human relevance:
The MOA studies demonstrated a MOA via nuclear receptor activation by valifenalate of CAR/PXR and PPARα, which subsequently leads to an increased replicative synthesis and proliferation. As eventual consequence, hepatocellular hypertrophy, hyperplasia and tumours can occur. This MOA, which was demonstrated for valifenalate, is not regarded as relevant to humans. Therefore, classification of valifenalate as Carc. 2, H351 is not warranted.
RAC's response
Thank you very much. Noted. Your arguments will be considered in the RAC opinion and brought to the Plenary for discussion and consideration.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	5
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
03.04.2020	Sweden		MemberState	6
Comment received				
Overall, the Swedish CA agrees that the available data are not sufficient for classification for adverse effects on sexual function and fertility or adverse effects on the development of the offspring. However, we lack some discussion on relevant effects observed in studies available in the CLH-report. Adverse effects on sexual function and fertility In the STOT RE section of the CLH dossier, one 90-day repeated dose toxicity study (OECD TG 409, 2003) and one chronic toxicity study (OECD TG 452, 2005) both performed with Beagle dogs are reported. Effects such as increased incidence in the immaturity of the prostate gland at all dose levels (50, 250 and 750 mg/kg bw/day; OECD TG 409); stat. sign. decreased relative prostate weight (by 29% at 250 mg/kg bw/day) and ovary weight (by 57% and 48% at 250 and 50 mg/kg bw/day, respectively) in the absence of mortality, effects on body weight or body weight gain or adverse clinical signs of toxicity (OECD TG 452) were seen. As detailed in Annex I of the CLH report, the decrease in the relative ovary weight was seen in a dose-dependent manner (Table 3.12.1.9-06 of Annex I), reaching statistical significance at mid- and high-dose levels.				

Moreover, in the latter study, at 250 mg/kg bw/day one female did not show oestrus during the treatment period and no corpora lutea were present in the ovaries of 3/4 females. We note that lack of corpora lutea and decreased absolute and ovary/brain ratios were also seen in the F1 parental generation from the high-dose group, in the OECD TG 416 (2004) study performed in rats . Thus, these studies should be included and discussed in the Reproductive toxicity section since the results are relevant for the assessment of sexual function and fertility. Adverse effects on the development of the offspring The Swedish CA agrees that the occurrence of total litter loss seen in the F1 parental generation in the mid- and high-dose groups of the OECD TG 416 study in rats can be considered as incidental, due to the lack of dose-response. If available, the historical control data for the total litter loss of this rat strain would provide a clearer view over this effect as non-treatment related. Adverse effects on or via lactation The Swedish CA supports the proposal for no classification for effects on or via lactation.
Dossier Submitter's Response
Thank you for your comment and support. As regards the lack of discussion of some effects on sexual function and fertility observed in the 90-day and chronic toxicity studies in beagle dogs, the effects mentioned were not regarded as direct effects on sexual function and fertility, since, as an important aspect, no potential to impair fertility or reproduction was evident in the reproduction toxicity study. The mentioned increased incidence in the immaturity of the prostate gland at all dose levels (50, 250 and 750 mg/kg bw/day by capsule) in the 90-day dog study according to OECD TG 409, is a common background finding in such short-term studies in dogs. In these studies young dogs have to be used according to the valid guidelines and in the early study phase they show high variability in their maturation process. High variability within the small group size of 4 animals can lead to biased incidences which may look dose-dependent, but are chance findings and occur also in control dogs. This was the case in this study in which the immaturity finding of the prostate gland occurred also in the control group. Furthermore, neither in epididymides nor in the testes were any signs of immaturity noted in this study. In a 28-day dog study with capsule doses of even up to 1000 mg/kg bw/day, no treatment-related prostate findings occurred, only signs of immaturity in all groups, including control (Brown, 2003, RCC study number 842174). The incidences of immature prostates in this study were 3, 3, 3 and 3 for 0, 250, 500 and 1000 mg/kg bw, which are clearly not dose- and thus not treatment-related. They support that in such short-term studies, the finding of immature prostates is related to the age of the animals and not to the treatment. Therefore, this finding of immaturity of the prostate gland is not an evidence of an effect on fertility or reproduction. The lower relative prostate weight at the highest dose in the 52-week study in dogs (OECD TG 452) with doses of 0, 1, 7, 50 and 250 mg/kg bw/day, is not regarded as adverse since there was no histopathological correlation. Therefore, this finding was not regarded as of toxicological relevance. The same is valid for the decreased relative ovary weight. No ovary weight effects occurred in the other dog studies, either, which also supports that this was a chance finding with no toxicological relevance. Since the finding of absence of corpora lutea in 3 females at 250 mg/kg bw/day was of low severity and was absent after the 8-week recovery, this finding is not considered to be of toxicological importance. The observation that one 250 mg/kg bw/day female did not show estrus during the treatment period was also regarded as chance finding and not as adverse or toxicologically relevant, since no such finding occurred in the other dog studies.

The lack of corpora lutea in the parental F1 generation of the 2-generation rat study (OECD 416) cannot be confirmed because no difference between the high dose and control group occurred, according to the report. The report says: "Counts of ovarian follicles and corpora lutea (Differential ovarian follicle count) were similar in control and high dose F1 parental animals indicating no test item-related effect". Likewise, the mentioned decreased absolute and ovary/brain ratios in the F1 parental generation from the high-dose group, in the OECD TG 416 study cannot be confirmed by the data from the report (p. 244), since the organ/body weight ratios of the ovaries were 0.021, 0.020, 0.022 and 0.020 (ovaries right) and the organ/brain weight ratios 3.177, 2.830, 3.039 and 2.854 (ovaries right) in the order of the ascending doses. They were clearly not affected by the treatment. In conclusion, no findings, which might indicate an effect on fertility or reproduction, could be confirmed. The given explanations can be included and discussed in the Reproductive toxicity section as recommended, since they demonstrate that there were no negative effects on sexual function and fertility. It is agreed that the occurrence of total litter loss seen in the F1 parental generation in the mid- and high-dose groups of the OECD TG 416 study in rats can be considered as incidental, due to the lack of dose-response. The question for historical control data for the total litter loss of this rat strain is answered under question 8 in which HCD are presented.
RAC's response
Thank you very much. Noted. RAC supports the DS's response but your arguments will be considered in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	7
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Germany		MemberState	8
Comment received				
In the two-year reproductive study a statistically significant increased neonatal mortality at the mid and high dose was observed. Total numbers of pup loss during day 0-4 was 18, 8, 35 and 39 in order of ascending dose levels and were above the maximum value of the historical control data (HCD) from 10 studies (mean: 6.7, median: 2, range: 0-23). Mean postnatal loss days 0-4 was 1.5 and 1.7 in the mid and high dose, respectively, and				

outside the range of the HCD (0-1). The viability index of the F1 generation was reduced: 85.2 % (mid dose), 84.8 % (high dose) in comparison to 92.6 % in the control and was also outside the range of the HCD (range: 91.5-100, median: 99.25, mean: 97.41). Also pup mortality during day 5-21 was increased at the mid (9 pups dying) and high dose (10 pups dying) in comparison to control (4 pups dying) and was far above the mean (=4) and median (=1) of the HCD, but inside the range (max=26). Weaning indices were reduced in the mid (94.2 %) and high (93.9 %) dose in comparison to control (97.5 %) and were below the mean (=97.6) and median (=99.4) of the HCD but inside the range (84.5-100). The incidence of the finding 'no milk in stomach' was increased in the mid dose and high dose group and 4 and 5 litters, respectively, were affected. In the mid dose in three out of four 'total loss litters' no milk in stomach between day 1 and 5 was reported. In the fourth litter all pups were already dead (cannibalized) at first check. The finding of 'no milk in stomach', as reported in annex 1, should be included in the CLH-report. The DS concluded, that there was "no indication of impaired nursing behaviour during lactation". We wonder how this conclusion is consistent with the finding 'no milk in stomach'.

The DS stated that the reduced viability is due to total litter loss of three dams in the mid dose and one dam in the high dose. Due to the provided data the German CA suggests, that not three but four total litter losses occurred at the mid dose. The DS is of the opinion that the total litter losses were incidental and not treatment-related as the effect was not observed in the P litters, no dose-response was obvious, and no deaths in the surviving litters occurred. The DS therefore decided to exclude the affected litters from the analysis. We are of the opinion that it is questionable if the exclusion of the litters with total loss of pups is appropriate (the above summarized incidences therefore include all animals). Moreover, further information on the dams (maternal toxicity) and pups of the litters with total losses is lacking in the CLH-report making it difficult to conclude on this finding. For some parameters (e.g. body weight gain of the F1 generation and more detailed information on historical control data) more information is available in the annex-documents.

We do not agree with the DS, that no deaths in surviving litters occurred, as in the high dose group the mean number of living pups at day 21 was 6.7, while in the control group a value of 7.5 was reported. Overall, the German CA is of the opinion, that there are indications for a need for classification regarding developmental toxicity or effects on/via lactation because of reduced pup survival.

Dossier Submitter's Response

Thank you for your comment. As referred, in the two-generation reproductive study the total numbers of pup loss during days 0-4 were 18, 8, 35 and 39 in the order of ascending dose levels. The following table gives an overview of relevant parameters with regard to pup mortality and survival of the F1 generation:

Parameter	Dose (ppm)				HCD[1]
	0	1250/850	4300/2900	15000/10000	
All dams					
Pup loss days 0-4 p.p. (total number)	18	8	35	39	0-23
Pup loss days 0-4 p.p. (% of living pups)	7.4	3.2	14.8	15.2	0-8.5
Mean no. postnatal loss/litter days 0-4 p.p.	0.9	0.3	1.5	1.7	0-1.0
Mean living pups/litter day 4 p.p.	7.7	7.7	6.7	7.1	7.1-8.0
Mean living pups/litter day 21 p.p.	7.5	7.7	6.3	6.7	6.8-8.0
Mean pup loss/litter day 21	0.19	0.04	0.39	0.43	0-1.2

Without dams with total litter loss					
Pup loss days 0-4 p.p. (total number)	18	8	11	25	0-23
Pup loss days 0-4 p.p. (% of living pups)	7.4	3.2	5.5	9.6	0-8.5
Mean no. postnatal loss/litter days 0-4 p.p.	0.9	0.3	0.6	1.7	0-1.0
Mean living pups/litter day 4 p.p.	7.7	7.7	7.8	7.5	7.1-8.0
Mean living pups/litter day 21 p.p.	7.5	7.7	7.7	7.0	6.8-8.0
Mean pup loss/litter day 21	0.19	0.04	0.10	0.43	0-1.2
% Viability index	92.6	96.8*	95.5	90.4	91.5-100
% Weaning index	97.5	99.4	98.6	93.9	84.5-100

[1] Historical control data from 10 studies conducted from May 2002 to December 2007 (current study started November 2002)
p.p. = post-partum, * $p<0.05$

Pup loss on days 0-4 p.p. was increased at mid- and high-dose if all dams were included in the evaluation, however, if dams with total litter loss were eliminated (3 at mid- and 1 at high-dose) the total numbers and values for % of living pups did not show a clear dose response and were within (control, low- and mid-dose) or almost within the HCD (high dose). Since the incidences of dams with total litter loss were not dose-related, a relationship with the treatment is very unlikely so that these litters can be excluded in order to have a meaningful evaluation and conclusion. At mid- and high dose, some degree of maternal toxicity was evident, like clinical signs (ruffled fur) and 2 of the 3 dams with litter loss had ruffled fur. Furthermore, at the highest dose, liver weight increase and centrilobular hepatocellular hypertrophy was observed and it cannot be excluded that these effects were more severe in the dams with total litter loss than in the others. An impairment of the dams with litter loss seems to be indicated also by the fact that the dams with total litter loss had a few pups which were already dead at first litter check. The exclusion of these litters from the calculated mean values would confirm the lack of an effect on the viability and survival of the offspring.
In addition, the survival data do not indicate a negative effect of the treatment. The mean number of living pups per litter on day 4 p.p. did not show a dose-dependent decrease if all dams are included and especially not if dams with total litter loss are excluded (see table). Overall, no negative effect on survival is evident. The difference in living pups at day 21 of 6.7 in the high-dose versus the control group value of 7.5 was almost within HCD and after elimination of dams with total litter loss, fully within HCD and with values of 7.5 at the high-dose versus 7.7 in the control group without any dose-related negative effect. Likewise, the pup loss and survival rate during days 5-21 was not negatively affected by the treatment. There was one dam with total litter loss in this period at the mid-dose. This was not indicative of a dose-related effect, furthermore, the survival data are within HCD. Pups normally start to eat from the dam's diet in this period and thus are increasingly exposed to the substance. Since there was no effect of valifenalate on mortality in the day 5-21 period, in which pups had access to the substance, this supports an absence of a treatment-related effect in this phase. In summary, the discussed results do not indicate a relationship of the day 0-4 mortality in the F1 generation with the treatment, especially because no similar changes occurred in the P litters.
The incidence of the finding 'no milk in stomach' was increased in the mid dose and high dose groups, but with regard to the litter incidences 1/21, 1/23, 6/23 and 4/23 in ascending order of doses, there is no clear relationship with doses could be established and this was most likely due to variability. Such findings, including cannibalism are background findings which often occur in reproductive toxicity studies as non-treatment-related phenomenon. It is consistent with the fact that this observation was also made in

the control group in this study and it occurred mainly in the litters with the mentioned losses, where the possibility of milk uptake by pups was apparently limited. There is no evidence of treatment-related impairment of the nursing behaviour of the dams. The discussed viability and weaning indices of the F1 generation would be within the HCD if the dams with total litter loss were taken out of the evaluation, as can be seen in the table above. Therefore, the treatment is unlikely to have had an effect on these parameters, which is further supported by the fact that no effects on these parameters occurred in the P generation. In conclusion, the reproductive toxicity study did not provide evidence of an effect of valifenalate on fertility, reproduction parameters or pup development, and no classification was deemed warranted.
RAC's response
Thank you very much. Noted. RAC supports the DS's response but your arguments will be considered in the RAC opinion.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	9
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	10
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	11
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	12
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	13
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	14
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
03.04.2020	France		MemberState	15
Comment received				
FR agrees with the proposed classification for valifenalate.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you for your comment. The support of DS proposal for classification of the substance as Aquatic Chronic 2 is noted by RAC. RAC agrees.				

Date	Country	Organisation	Type of Organisation	Comment number
03.04.2020	United Kingdom	UK Government	National Authority	16
Comment received				
Please see below comments on the OMS environmental classification proposal for valifenalate. We think Aquatic Chronic 2 is applicable but based on a different interpretation of the data. We consider the below points should be raised at public consultation to highlight inconsistent data interpretation and allow application of all data (i.e. most sensitive acute endpoint via the surrogate approach) for the chronic classification. This is important to consider the appropriate classification – for example if the algal data limitations were noted during the PC but the lack of surrogate approach was not, the harmonised classification could result in 'not classified' which we feel would be incorrect. Draft Comments:				

The proposed Aquatic Chronic 2 classification is based on the Skeletonema costatum 96 hour NOErC of 0.106 mg/L from the study by Hicks (2015). We agree that the study is valid and relevant to hazard classification although we note that statistical analyses should have involved comparison to the solvent control or pooled controls (if no difference between the solvent and procedural controls), rather than solely the procedural control. In this instance, given the similarity between the presented solvent control and procedural control mean data, we anticipate this would have little impact on endpoints.

We note growth inhibition after 96 hours was low, reaching a maximum of only 3%, and question whether this effect is statistically significant or a transcription error. The 96 hour ErC10 is greater than the highest test concentration and quoted as >9.48 mg/L reflecting the functional limit of solubility for valifenalate. As statistically based endpoints are preferred, we consider the 96 hour chronic endpoint from this study to be >1 mg/L.

While 72 hour endpoints appear more sensitive, these also indicate that the 72 hour ErC10 value is >1mg/L.

Overall, we consider that this study does not support the proposed Aquatic Chronic 2 classification for this NRD substance.

All other chronic toxicity endpoints are >1 mg/L.

There are no chronic toxicity data available for Americamysis bahia which was the most acutely sensitive species. Therefore, we note that the surrogate approach with the Americamysis bahia 96 hour EC50 of 2.8 mg/L (based on mm) should be used if the RAC decides not to base the chronic classification on the NOEC value for Skeletonema costatum growth rate. This surrogate approach results in an Aquatic Chronic 2 classification which is the same as the classification proposed by the DS of the CLH report.

Dossier Submitter's Response

Thank you for your comment and support. According to Regulation (EC) No 1272/2008 valifenalate is to be classified as Category Chronic 2 because the substance is not rapidly degradable and the lowest NOEC is ≤ 1 mg/L (Skeletonema costatum 96 hour NOErC = 0.106 mg/L; Hicks (2015)). It is agreed that the observed effects in this algae test are very low, but a significant effect on growth rate could be detected and a NOErC of 0.106 mg/L is reported.
As regards the application of the surrogate approach proposal, it does not seem to be warranted since a sufficient set of studies is available to classify valifenalate. Based on the available chronic Daphnia study, which resulted in a NOEC of 3.2 mg/L, no classification would be warranted and, in case classification would not be supported by the algae data, it would be more logical not to classify valifenalate than using a surrogate approach because a sufficient data set is available.

RAC's response

Thank you for your comment.

RAC notes that the commenting Member State agreed with the proposed chronic environmental hazard classification but suggested that the surrogate approach should be considered.

The comment regarding the use of control groups is noted by RAC.

RAC notes that the growth inhibition after 96 hours was low (3%) but the significant reduction in growth rate compared to negative control was reported.

RAC is aware that according to current CLP Guidance (Version 5.0, July 2017), if available, preference is given to the EC_{10} value over the NOEC value. However, in case of valifenalate the EC_{10} for growth rate in the 96 h growth inhibition test with *Skeletonema costatum* could not be determined. Therefore RAC is of the opinion that the NOEC of 0.106 mg/L over the EC_{10} value of > 9.48 mg/L for algae *Skeletonema costatum* shoul be selected as the lowest value for this species. This is also in line with the current CLP Guidance which indicate that the lowest of the available toxicity values will normally be used to define the hazard category.

In line with the current CLP Guidance (Version 5.0, July 2017), if available, the preference is given to the chronic toxicity data over acute toxicity data for defining the long-term hazard category. However, when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor.

The criteria for classification of a substance into the categories Chronic 1 to 3 follow a tiered approach where the first step is to see if available information on chronic toxicity merits long-term (chronic) hazard classification. In absence of adequate chronic toxicity data, the subsequent step is to combine two types of information, i.e. acute aquatic toxicity data and environmental fate data (degradability and bioaccumulation data) (see Figure 4.1.1).

RAC is of the opinion that adequate chronic toxicity data are available for all three trophic levels, although the data for the most acutely sensitive invertebrates species is not represented. RAC concludes that the lowest chronic toxicity value corresponds to a test with algae *Skeletonema costatum* with determined 96 h NOEC of 0.106 mg/L and the substance is not rapidly degradable. The substance therefore meets the criteria for classification with Aquatic Chronic 2. RAC notes that the chronic classification of Aquatic Chronic 2 would be reached also based on surrogate data for invertebrates (96 h EC_{50} of 2.8 mg/L for *Americamysis bahia*).

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	17
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the classification proposed by Dossier Submitter Hungary: Aquatic Category 2 ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				

Dossier Submitter's Response
Thank you for your comment and support.
RAC's response
Thank you for your comment. The support of DS proposal for classification of the substance as Aquatic Chronic 2 is noted by RAC. RAC agrees.

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Germany		MemberState	18
Comment received				
We agree with the proposal of classification for environmental hazards as Aquatic Chronic 2, H411.				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you for your comment. The support of DS proposal for classification of the substance as Aquatic Chronic 2 is noted by RAC. RAC agrees.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Ozone Layer

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	19
Comment received				
In line with the results of the available studies, Belchim Crop Protection agrees with the proposal by Dossier Submitter Hungary: Not classified ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf				
Dossier Submitter's Response				
Thank you for your comment and support.				
RAC's response				
Thank you for your comment. The support for no classification of the substance as hazardous to the ozone layer is noted by RAC. RAC agrees.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
02.04.2020	Belgium	Belchim Crop Protection	Company-Manufacturer	20
Comment received				
Some parameters are not evaluated and have been reported as data lacking in CLH Report for valifenalate (compare Table 6, page 4). These parameters are no data requirement under Regulation (EU) 1107/2009 and therefore no data are lacking. Further argumentation and the reason of non-relevance is provided for the respective hazard classes. Substances which in contact with water emit flammable gases				

• Valifenalate does not interact with water to become spontaneously flammable nor does it give off flammable gases in dangerous quantities. Therefore, no data lacking. Self-reactive substances • Valifenalate is not a thermally unstable substance and is not liable to undergo a strongly exothermic decomposition even without participation of oxygen (air). Valifenalate is not explosive, an organic peroxide nor is it oxidising. Therefore, no data lacking. Pyrophoric solids • Valifenalate, even in small quantities, is not liable to ignite within five minutes after coming into contact with air. Therefore, no data lacking. Organic peroxides • Not to evaluate and not applicable as valifenalate is not a peroxide. Therefore, no data lacking. Valifenalate has no physical hazards for classification. For some hazard classes lacking data is mentioned in CLH report. However, these data are no requirement for active substance regulated under Regulation (EU) 1107/2009. Therefore, no data are lacking. Additionally, the explanation provided above confirms that even without specific data no classification for these hazard classes is required. Belchim Crop Protection kindly ask to update the CLH report accordingly and kindly asks to replace for the above-mentioned points Data lacking by Not classified. ECHA note – An attachment was submitted with the comment above. Refer to public attachment VAL CLH comments_Belchim Crop Protection.pdf
Dossier Submitter's Response
Thank you for your comment. The requirements referred to are, however, set out in Regulation (EC) No 1272/2008, for the purposes of this CLH dossier.
RAC's response
RAC supports the Dossier Submitter's response.

PUBLIC ATTACHMENTS
1. VAL CLH comments_Belchim Crop Protection.pdf [Please refer to comment No. 1, 3, 5, 7, 9, 10, 11, 12, 13, 14, 17, 19, 20]