Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

difenoconazole (ISO); 1-({2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl}methyl)-1H-1,2,4-triazole; 3-chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

EC Number: -
CAS Number: 119446-68-3

CLH-O-0000007004-85-01/F

Adopted

10 June 2021

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIFENOCONAZOLE (ISO); 1-({2-[2-CHLORO-4-(4-CHLOROPHENOXY)PHENYL]-4-METHYL-1,3-DIOXOLAN-2-YL}METHYL)-1H-1,2,4-TRIAZOLE; 3-CHLORO-4-[(2RS,4RS;2RS,4SR)-4-METHYL-2-(1H-1,2,4-TRIAZOL-1-YLMETHYL)-1,3-DIOXOLAN-2-YL]PHENYL 4-CHLOROPHENYL ETHER

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: difenoconazole (ISO); 1-({2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl}methyl)-1H-1,2,4-triazole; 3-chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether
EC number: 601-613-1
CAS number: 119446-68-3
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	1
Comment received				
FR: No comment.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	2
Comment received				
The CLH proposal by the Dossier submitter (DS) is supported.				
Dossier Submitter's Response				
Thanks for the support.				
RAC's response				
Thank you very much. Noted.				

Date		Country	Organisation	Type of Organisation	Comment number
28.05.2020		Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	3
	Comment received				
	1. Syngenta, on behalf of the Difenoconazole TF, supports the dossier submitter's conclusion of no classification for germ cell mutagenicity, carcinogenicity and reproductive toxicity. Additional information related to germ cell mutagenicity and carcinogenicity is herewith provided. 2. Syngenta, on behalf of the Difenoconazole TF, acknowledge the data gaps identified under point 8.1 (pg. 12 – Explosives) & point 8.7 (pg. 14 – Self-reactive substances) and confirms hereby that the missing studies according to the CLP regulation (UN RTDG methods) will be conducted to fulfil the data requirement. The studies will be available and can be submitted by October 2020. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Sanitised Difenoconazole - Mouse Micronucleus and hepatocyte studies.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Difenoconazole - Oral (Gavage) Mouse Micronucleus Test - Ame.pdf				
	Dossier Submitter's Response				
	Thanks for supporting dossier submitter conclusion for germ cell mutagenicity. With respect to point 2, studies could be considered by RAC if they are submitted according to the proposed calendar (October 2020).				
	RAC's response				
	The support for no classification of difenoconazole for germ cell mutagenicity, carcinogenicity and reproductive toxicity is noted. No additional studies for assessing the above stated physical hazards were finally submitted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	4
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	5

Comment received

DE-CA applauds the extensive work of the DS concerning the examination of the MoA and supports the MoA analysis and conclusions with regards to mice. However, we think the case for the MoA in rats is very weak and not supported by the data. We do not consider difenoconazole to be an activator of rat CAR3 with low potency. It is simply not an activator. A) The increase was not statistically significant and B) the increase was only 1.66-fold at the highest dose, compared to increases of 83 fold with the positive control and 17-fold with mouse CAR3. A "non-significant trend" (page 66) is not a trend. It is tempting to argue that there is low potency and hence "only" hepatocyte hypertrophy in the liver of rats was observed (page 70), but this is highly speculative based on the very limited data presented and there may well be other explanations, as mentioned in "6. Other modes of action".

Dossier Submitter's Response

Carcinogenicity – mice

Thanks for the support of the liver tumors MoA analysis and the final conclusion with respect to mice. The ES-CA concluded that these tumours are not relevant for humans and no classification is required for carcinogenicity.

Carcinogenicity - rats

By other hand, it has to be noted that evidences of key events in rats were included in the MoA for the comparison between species, but liver tumours were not observed in rats. In the 2-year available study (Anonymous 16, 1989a) no evidence of carcinogenicity was observed at tested dose levels. NOAEL for carcinogenicity in rats was considered greater than 2500 ppm, equivalent to 124 and 170 mg/kg bw/day for males and females respectively.

In the available CAR transactivation study (Omiecinski C., 2016) it was observed an increase in the activation of the receptor in rats compared to controls, but it was low (1.66-fold compared to controls; in mice it was 17.27-fold) and not significant. DS concluded that difenoconazole was a low activator of CAR (Table 25a). Since some associative events of the MoA such as hepatocellular hypertrophy and increased liver weight were observed in rats (Table 27), the ES-CA proposed that they could be explained by this low activation of the receptor by difenoconazole leading to these findings but not provoking tumours (adverse outcome). However, the ES-CA acknowledges that there are uncertainties in this CAR activation in rats and these findings could be due to another MoA. In any case, these consideration on the potential activation of CAR by difenoconazole in rats has no impact in carcinogenicity since liver tumours were observed in mice but not in rats.

RAC's response

The support for the work developed by the dossier submitted is noted. RAC concurs with the commenting member state about the uncertainties as regard the CAR activation in rats.

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	6

Comment received

Syngenta, on behalf of the Difenoconazole TF, agrees with the dossier submitter's conclusion of no classification for carcinogenicity.
The available data supports the conclusion that difenoconazole does not pose a carcinogenic hazard to humans. Administration of difenoconazole to mice resulted in statistically significant incidences of hepatocellular adenomas and carcinomas at dietary concentrations of 2500 and 4500 ppm for males and 2500 ppm for females; dose levels considered to exceed the MTD as demonstrated by mortality in the first few weeks of dosing. In addition, extensive and robust mode of action (MoA) studies have consistently demonstrated key events, either directly or via associative events, and have shown these tumours are initiated by activation of CAR. Due to qualitative differences in the activation of and response to CAR-activation between mice and humans, this MOA is not relevant for human hazard assessment.

Several other modes of action have been ruled out by using experimental data. Statin-like activity has not been ruled out experimentally as an alternative mode of action; however, no consistent effects on cholesterol have been observed in the repeat dose mouse studies. Therefore, it is unlikely difenoconazole is an HMG CoA reductase inhibitor.

Several uncertainties and inconsistencies have been raised during the review of the database. Double CAR/PXR knockout mice were utilised instead of single CAR knockout animals, as it is almost impossible to split the two nuclear receptors because of shared ligands, co-activators and response elements. A CAR MoA is likely to be a CAR/PXR MoA; therefore, it was considered appropriate to use double knockout mice. The humanised mice utilised in the 1- and 7-day in vivo (anonymous, 2017b) were humanised CAR/PXR mice were used

In addition to Vardy A, 2016b, a second in vitro investigative study using primary human hepatocytes isolated from two additional donors has been conducted (McGinnis and Chatham, 2019). The study assessed the effects of difenoconazole on the postulated key events in the liver tumour MOA, including the induction of CYP isoforms that are markers of CAR/PXR activation and hepatocellular proliferation. Briefly, hepatocytes were cultured for 96 h, exposed to 6 concentrations of difenoconazole (0.05, 0.1, 0.5, 1, 2 and 4 µM; donor 385: 0.1, 0.5, 1, 2, 4 and 8 µM - with the highest concentrations producing cytotoxicity as measured by intracellular ATP levels), and assessed for PROD and BROD enzyme activities and cell proliferation (measured as the change in replicative DNA synthesis, RDS). Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) were included as positive controls for CYP induction secondary to CAR activation and hepatocellular proliferation, respectively.

Treatment with difenoconazole did not affect PROD activity in either donor. A dose dependent increase in BROD activity was induced, with statistically significant induction observed at 1 µM in one donor. There were no increases in cell proliferation following treatment with difenoconazole at any concentration in hepatocytes of either donor. The

expected effects were observed for both positive control compounds indicating that the experimental system responded as expected. Only one donor showed significant increases in CYP2B/3A activity. This study shows difenoconazole does not cause an increase in cell proliferation in cultured human hepatocytes.

The available data for difenoconazole support a proposed MoA in male mice involving activation of the constitutive androstane receptor (CAR), altered gene expression specific to CAR activation, increased cell proliferation, clonal expansion leading to foci/areas of altered hepatocytes and liver tumours. Contrary to mice, treatment of primary human hepatocytes (n=3) with difenoconazole had no effect on hepatocellular proliferation when tested up to the limit of cell viability. This pattern of effects matches the known species differences that have been demonstrated for other CAR activators, and the weight of evidence indicates that it represents a qualitative difference in the established MoA for difenoconazole between mice and humans. Numerous CAR knockout mice studies have been conducted to demonstrate this MoA for model compounds, which has been successfully demonstrated via alternative in vitro methods. Consequently, no further data is considered ethically or scientifically justified to support the MoA for liver tumours. Thus, the available data demonstrates that this MoA is not relevant to humans and classification is not appropriate.

Additional information related to carcinogenicity is herewith provided.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Sanitised Difenoconazole - Mouse Micronucleus and hepatocyte studies.zip
ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Difenoconazole - Oral (Gavage) Mouse Micronucleus Test - Ame.pdf

Dossier Submitter's Response

Thanks for the support.

RAC's response

Thank you very much. Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	7

Comment received

Syngenta, on behalf of the Difenoconazole TF, agrees with the dossier submitter's conclusion of no classification for germ cell mutagenicity.
An additional in vivo micronucleus study has been conducted to the current OECD TG 474 (2016) to include proof of exposure to the bone marrow (anonymous, 2019). There was no evidence of clastogenicity or aneugenicity following oral (gavage) administration of difenoconazole, up to the MTD of 320 mg/kg/day in male mice. Difenoconazole is considered to be neither clastogenic nor aneugenic in the mouse micronucleus test.
Additional information related to germ cell mutagenicity is herewith provided.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Sanitised Difenoconazole - Mouse Micronucleus and hepatocyte studies.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Difenoconazole - Oral (Gavage) Mouse Micronucleus Test - Ame.pdf
Dossier Submitter's Response
This additional in vivo micronucleus study provided during the public consultation demonstrates bone marrow exposure and supports the validity of negative results from the previous micronucleus study and the ES-CA conclusion of no classification for germ cell mutagenicity.
RAC's response
Thank you very much. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	8
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	Switzerland	Syngenta Crop Protection AG	Company-Manufacturer	9
Comment received				
Syngenta, on behalf of the Difenoconazole TF, agrees with the dossier submitter's conclusion of no classification for reproductive toxicity. ECHA note – An attachment was submitted with the comment above. Refer to public attachment Sanitised Difenoconazole - Mouse Micronucleus and hepatocyte studies.zip ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Difenoconazole - Oral (Gavage) Mouse Micronucleus Test - Ame.pdf				
Dossier Submitter's Response				
Thanks for the support.				
RAC's response				
Thank you very much. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	10
Comment received				
This section was not reviewed.				

Dossier Submitter's Response
No comment.
RAC's response
No comment.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	11
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

Date	Country	Organisation	Type of Organisation	Comment number
01.06.2020	Sweden		MemberState	12
Comment received				
The Swedish CA supports classification of difenoconazole (CAS No. 119446-68-3) as Acute Tox. 4, H302 and the oral ATE of 1453 mg/kg bw.				
Dossier Submitter's Response				
Thanks for the support.				
RAC's response				
Thank you very much. Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	13
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	14
Comment received				
This section was not reviewed.				

Dossier Submitter's Response
No comment.
RAC's response
No comment.

Date	Country	Organisation	Type of Organisation	Comment number
01.06.2020	Sweden		MemberState	15
Comment received				
The Swedish CA supports classification of difenoconazole (CAS No. 119446-68-3) as Eye Irrit. 2, H319.				
Dossier Submitter's Response				
Thanks for the support.				
RAC's response				
Thank you very much. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	16
Comment received				
Page 24, Table 18 There is an additional study by Mastrocco et al. (1987) examining eye damage and irritation. This (negative) study was included in Part B.6 of Volume 3 of the RAR and should also be taken into consideration when concluding on CLH.				
Dossier Submitter's Response				
This reference (Anonymous 9, 1987a) corresponds to an acute dermal toxicity study in rabbits included in Table 15. No data on eye is available in the study. With respect to the skin effects it was observed the following. At the treatment site, after 24-hour exposure under occlusion of 5 rabbits/sex at a dose level of 2010 mg/kg bw, it was observed slight erythema (Draize grade 1) in 3 animals (2 males and 1 female). Fissuring was noted in 1 male at 72 h. Desquamation of the skin at the treated site was observed in all animals on test day 7 and in the majority of males and two females on test day 14. The original report refers the observed erythema, fissuring and desquamation at treatment sites to be a result of exposure to the ethanol vehicle. However, according to the OECD TG 402 (1987) the influence of the vehicle should be considered, and the test chemical should be moistened with water if possible. Difenoconazole is soluble in water. This information observed in the acute dermal toxicity study can be regarded as additional to evaluate the skin irritation/corrosion potential of difenoconazole since there is an acceptable skin irritation study (Anonymous 1991a) following OECD TG 404 in which the average irritation scores at 24h, 48h and 72h were 0 for both erythema and edema after 4 hours exposure. Consequently, taking into account the absence on skin irritation in this guideline study, the RMS considers appropriate the proposal of no classification of difenoconazole due to				

skin irritation/corrosion.
RAC's response
Thank you very much. Noted.

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	17
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	18
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	19
Comment received				
This section was not reviewed.				
Dossier Submitter's Response				
No comment.				
RAC's response				
No comment.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	20
Comment received				
Page 97, B.6.3.2.1.2 The increase in relative liver weight in the females at 200 ppm is dose-dependent,				

statistically significant and well over 10 %. Given that the liver is the target organ, this increase should be considered adverse and not adaptive. Therefore, a lowering of the NOAEL to 20 ppm is strongly recommended. This remains, however, without impact on the CLH proposal.
Dossier Submitter's Response
Increase liver weights observed at 200 ppm (13 and 17 mg/kg bw/d for males and females respectively) and 750 ppm (51 and 66 mg/kg bw/d for males and females respectively) of approximately 20% are not regarded adverse by the RMS since this effect is the result of a normal adaptive response to increased workload. According to "A Review of Adaptive (Adverse and Non-adverse) Changes—Conclusions from the 3rd International ESTP Expert Workshop" (Hall et al., 2012) and adaptative response of liver is associated with the following: – The increase was less than 50%. – It had no associated histopathological changes. – There are no observed increases in the four liver enzymes i.e. ALT, ALP, AST and GGT which would indicate release of enzymes from damaged hepatocyte membranes and would be considered indicative of adverse hepatic injury and – No changes were observed in other clinical pathology markers that may indicate liver dysfunction (albumin, bilirubin cholesterol and total proteins). According to RMS it does not apply a lowering of the current NOAEL of 200 ppm to 20 ppm and this effect should not be taken into account for adversity for STOT RE.
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
28.05.2020	France		MemberState	21
Comment received				
FR: FR agrees with the proposal of classification for environmental hazards and with the proposed M factors (acute and chronic) : Aquatic acute 1 (M factor = 10) Aquatic chronic 1 (M factor = 10)				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment.				

Date	Country	Organisation	Type of Organisation	Comment number
20.05.2020	Germany		MemberState	22
Comment received				
We agree with the proposal of classification for environmental hazards as Aquatic Acute 1				

(H400) with an M-factor of 10 and Aquatic Chronic 1 (H410) with an M-factor of 10.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Thank you for your comment.

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	23
Comment received				
FR: No comment.				
Dossier Submitter's Response				
No comment.				
RAC's response				
Thank you for your comment.				

PUBLIC ATTACHMENTS
1. Sanitised Difenoconazole - Mouse Micronucleus and hepatocyte studies.zip [Please refer to comment No. 3, 6, 7, 9]

CONFIDENTIAL ATTACHMENTS
1. Difenoconazole - Oral (Gavage) Mouse Micronucleus Test - Ame.pdf [Please refer to comment No. 3, 6, 7, 9]