COMPILED COMMENTS ON CLH CONSULTATION

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

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Last data extracted on 02.06.2020

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
CAS number: 119446-68-3
EC number: 601-613-1
Dossier submitter: Spain

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	1
Comment received				
FR: 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.				

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

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	4
Comment received				
This section was not reviewed.				

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".				

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

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				

Date	Country	Organisation	Type of Organisation	Comment number
28.05.2020	France		MemberState	8
Comment received				
This section was not reviewed.				

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				

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

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.

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.				

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.				

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.				

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.				

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.				

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.				

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.				

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.				

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.				

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)				

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.				

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.				

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]