Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide

EC Number: 256-625-6
CAS Number: 50563-36-5

CLH-O-0000007432-78-01/F

Adopted

14 March 2024

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIMETHACHLOR (ISO); 2-CHLORO-*N*-(2,6-DIMETHYLPHENYL)-*N*-(2-METHOXYETHYL)ACETAMIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

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

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

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

Substance name: dimethachlor (ISO); 2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(2-methoxyethyl)acetamide
EC number: 256-625-6
CAS number: 50563-36-5
Dossier submitter: Croatia

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
19.07.2023	United Kingdom	Health and Safety Executive	National Authority	1

Comment received

Carcinogenicity
We note comments from the RMS about histopathological findings in the nasal cavity for acetochlor, alachlor and butachlor. To further understand the carcinogenic potential of dimethachlor it would be useful to include any detailed histopathological findings for the nasal passage from the available repeated dose toxicity studies for dimethachlor.
We note there are some uncertainties about the nasalpharanygeal adenoma in rodents and their relevance to humans. We think it may be important to provide some comment on the known difference in enterohepatic circulation cut-offs in rodents and humans and the relative bioavailability's of the critical sulfur containing metabolites.

Dossier Submitter's Response

Thank you for your comments.
There is limited data on potential preneoplastic changes in the nasal cavity in repeated-dose toxicity studies for dimethachlor. Only for two short-term repeated-dose studies (25/26 day repeated dose dietary toxicity study in the rat, Anonymous 1992a; 28 day repeated dose dietary toxicity study in the mouse, Anonymous 1992b), it was specifically stated that the nasal cavity was histopathologically examined. In those two studies, preneoplastic or hyperplastic findings in the nasal turbinates were not observed.
In other studies, including 90-day studies, the nasal cavity was not on the list of organs/tissues that were routinely histopathologically examined. In these studies, the nasal cavity could have been examined only if gross macroscopical lesions were observed in the cavity. Therefore, in these studies, it is not expected that discrete changes (such as minimal hyperplasia of nasal mucosa), which do not produce a noticeable gross lesion, would be

discovered. Even nasopharyngeal adenomas found in two 2-year carcinogenicity study in rats were detected only by histopathological examination - these changes were not observed as gross macroscopic lesions at necropsy.

In the absence of actual data on bile excretion and enterohepatic circulation of dimethachlor in humans (or at least in primates) or physiologically based pharmacokinetic model for dimethachlor (at least to the Dossier Submitter's knowledge), potential toxicokinetic differences between species could only be speculated. Namely, it is considered that simple allometry and molecular weight threshold perform poorly for prediction of hepatic clearance and enterohepatic circulation (e.g., Fagerholm U. J Pharm Pharmacol 2008; 60(5):535-542). For example, although a 2-fold higher molecular weight threshold for biliary excretion of chemical substances has been suggested for humans compared to rats, very similar values for biliary excretion and enterohepatic circulation were found for certain compounds with molecular weight above the proposed threshold both in humans and in the rat (e.g., Kim TH et al. AAPS J. 2015; 17(5):1210-23).
Dossier Submitter would also like to point out that the critical sulfur containing metabolites could be enzymatically formed on site (i.e., in nasal mucosa), and that other potential mechanisms (which were not specifically investigated for dimethachlor) could be responsible for nasopharyngeal adenoma occurrence due to dimethachlor exposure. Namely, to the Dossier Submitter's opinion (as discussed in the CLH Report), if the "sulfoxide pathway" was responsible for nasopharyngeal adenomas observed in the present carcinogenicity study with dimethachlor, olfactory, and not respiratory, nasal tumours or hyperplasia could be expected in rats, since the type of enzymatic activity crucial in the creation of quinone metabolites is markedly lower in respiratory compared to olfactory nasal epithelium (in rats as well as in other species). Please also see the Dossier Submitter's response to Comment No. 2.

RAC's response

Thanks to the DS for the response on the questions asked.

Date	Country	Organisation	Type of Organisation	Comment number
21.07.2023	United Kingdom	Syngenta	Company-Manufacturer	2

Comment received

The Applicant considers that classification of dimethachlor as Carc. Cat. 2 is not justified. As explained in the detailed expert statement, "Dimethachlor Mechanism of Formation and Human Non-Relevance of Nasal Turbinate Tumours in Rat", provided separately, the weak carcinogenic effect observed with dimethachlor in the nasal tissue of the high dose male rats in the long-term rat toxicity study is not relevant for humans.
As mentioned in the expert statement, two in vitro metabolism studies have been conducted with dimethachlor, in order to support the mode of action for nasal tumour formation. As one of the previously submitted reports has been amended to correct information on the material used, and the second, GLP report has not been included in the submitted dossier, OECD summaries of both studies are provided. Study reports can be provided on request. These studies are considered key information with regard to dimethachlor metabolism and mode of action.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment dimethachlor.zip

Dossier Submitter's Response

Thank you for your comment.

In the Dossier Submitter's opinion, the mechanistic studies performed by the Applicant do not provide evidence that local cytotoxicity secondary to quinone imine formation is responsible for nasopharyngeal adenomas arising from nasal respiratory epithelium in male rats. Namely, cytochromes P450 enzymes are crucial in the creation of quinone metabolites (Hughes TB et al. Predict the Formation of Quinone Species in Drug Metabolism. Chem Res Toxicol. 2017 Feb 20;30(2):642-656), and their activity is lower in respiratory compared to olfactory nasal epithelium and liver tissue. For example, Green et al. found a 6-fold higher hydroxylation rate of acetochlor sulfoxide in olfactory compared to respiratory microsomes in rats (Green et al. Acetochlor-induced rat nasal tumors: further studies on the mode of action and relevance to humans. Regul Toxicol Pharmacol. 2000;32(1):127-33). Dossier Submitter, therefore, considers that in case the MoA postulated by the Applicant is responsible for nasal adenoma formation in the rat, adenomas would be of olfactory rather than of respiratory origin.

As pointed out by the Applicant, an increased incidence of polypoid adenomas in the nasal epithelium that affected both the olfactory and respiratory epithelium in both male and female rats was found in the rat carcinogenicity study with acetochlor (Broadmeadow, 1988). However, it is not stated in what proportion the olfactory and respiratory epithelium were affected (RAC Opinion and Background document to the Opinion proposing harmonised classification and labelling at Community level of Acetochlor, CLH-O-0000001412-86-29/F, 2014). Increased incidence of nasal respiratory epithelial hyperplasia was observed in rats exposed to acetochlor in the 2-generation reproductive toxicity study with acetochlor, but the increase was much less pronounced than for olfactory epithelial hyperplasia. It is unclear which MoA(s) are responsible for the hyperplastic reaction of nasal respiratory epithelia systemically exposed to acetochlor and dimethachlor. Alternative MoAs were not specifically investigated for these substances, to the knowledge of the Dossier Submitter.

On the other hand, dimethachlor seems to be of lower carcinogenic potency compared to carcinogenic chloroacetanilides, such as acetochlor. It should be discussed whether the low incidence of nasal adenomas (3/60, i.e., 5%), which are a benign type of tumour observed only in one species and in one sex, and taking into account other uncertainties (including those discussed in the Applicant's detailed expert statement submitted to ECHA), warrants a decision for no classification for carcinogenicity.

RAC's response

Thanks for the response of the DS.
Indeed, other mode-of-actions cannot totally be excluded. RAC considered the low incidence of nasal adenomas (not carcinomas) (3/16) sufficient for classification in Cat. 2.

Date	Country	Organisation	Type of Organisation	Comment number
12.07.2023	Germany		MemberState	3

Comment received

The classification as Carc. 2 is supported. However, the following aspects should be taken into consideration:

a) Despite the results of the newer study (K-CA 5.1.1/04), an N-dealkylated metabolite MET 11U was detected in the study by <confidential> (K-CA 5.1.1/02), albeit at low levels (U7 in Table B.6.1.1-14). N-dealkylation of the alpha-carbon atom is a key step resulting in protein adduct formation.

b) S-metolachlor was wholly excluded from the argument; however, the DE CA opines it should also be included because it is structurally more similar at a key position, namely the beta rather than the alpha carbon, which as mentioned above is the crux of the

argument. In the recent RAC Opinion for S-metolachlor (CLH-O-0000007145-77-01/F), the RAC stated that the "nasal olphactory tumours induced by acetochlor were determined to be secondary to local cytotoxicity due to the formation of quinone imine. These tumours were considered relevant to humans, although rats appeared to be more sensitive than humans." The increased incidences of nasal turbinate adenocarcinomas in male rats were deemed to be of concern as it is a rare tumour type. For S-metolachlor the incidence was 2/69, compared to 3/60 at 4000 ppm dimethachlor.

c) The DE CA notes that there is an increase in kidney lipoma: 0,1,1,2 in the males. The last of these values lie just outside/at the high end the HCD range given in Table B.6.5.1-15. According to one publication (10.1002/jat.2550130207), the incidence in SD rats is 0.37% and another study (10.1177/0192623310373777) puts the incidence in SD rats at 0.42%, both about 10-fold lower than the incidence seen in this study. Given that the kidney is also affected by dimethachlor, some more information and/or a discussion of the kidney lipoma would be appreciated.

Furthermore, we would appreciate if the RMS could answer the following questions:

1) In the mechanistic study by Knowles et al. (2020), nasal microsomes from rats and humans were used. Do you know from which epithelium these microsomes originated, from the respiratory epithelium or from the olfactory epithelium?

2) Nasopharyngal ademonas were detected in 3/60 high dose male Tif:RAIf rats. Do you know in which animals these tumours were detected: in animals surviving until the end of the study or/and in animals dying before study termination? In light of the considerably lower survival rate of male control animals (48% vs. 72% in the high dose group), information on the age of tumour-bearing animals could supply another valuable piece for the interpretation of nasopharyngeal adenomas in top dose males.

3) In the DRAR/CLH report, it is stated on page 97: "In contrast to top dose and control group, only limited number of tissue samples was examined in animals in the low and intermediate dose carcinogenicity sub-groups (lung, liver, kidney, testis, epididymidis, muzzle, and all gross lesions). This approach can create problems in the statistical analysis of dose-response trends and cannot be recommended if dose-response characterization is an objective of a study (OECD 2012). Namely, the actual number of tumours in low and mid-dose groups may be higher than the observed values. […]"

On page 98, by contrast, it is stated in footnote 7: "Trend test is considered justified, since the nasal tissues from all groups were analysed."

We think that there is a contradiction between these two statements. Even though nasal (muzzle) tissues were analysed from all groups, it seems that analyses were not conducted from nasal (muzzle) tissues from all animals. Consequently, a trend test should not be applied for nasopharyngal adenomas according to the first statement. Is this correct? If not, could you please clarify?

Dossier Submitter's Response

Thank you for your comments.

Ad a) Thank you for your comment.

Ad b) Thank you for your comment – Dossier Submitter agrees that nasal tumours observed in the study with S-metolachlor should be discussed as well, taking into account tumour type (for dimethachlor only nasopharyngeal adenomas were observed, while for S-metolachlor nasal adenocarcinomas were found in male rats).

Ad c) Thank you for your comment – Dossier Submitter agrees that an increase in the incidence of kidney lipomas should have been discussed. Kidney lipomas are expansile lesions, often well-circumscribed, which continue to grow over time and generally affect the architecture of surrounding cells (Frazier KS et al. Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol. 2012 Jun;40(4 Suppl):14S-86S). Histologically, they are monomorphic, consisting of interstitial aggregate of mature fat cells (lypocytes) (Hard GC et al. Proliferative Lesions of the Kidney in Rats. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, DC). They are rather rare renal tumours, which could arise as spontaneous lesions, especially in aging rats, but could be occasionally noted with increased frequency after xenobiotic treatment, especially that which affects lipid metabolism. Dossier Submitter, however, points out that the increase in the incidence over the dose levels applied in the study (0, 20, 300, and 4000 ppm) was small (0, 1, 1, 2).

Ad 1) In the mechanistic study by Knowles et al. (2020), mixed microsomes from olfactory and respiratory nasal epithelium were used, both from humans and rats. The study report submitted by the Applicant that was considered in the dRAR and CLH Report, contained a mistake about the origin of the microsomes used – it was stated that microsomes from rats were only from olfactory epithelium. The amended study report has been submitted by the Applicant during the Consultation. Also, the same study has been repeated as a GLP study (Report Number 20200208).

Ad 2) Nasopharyngeal adenomas were observed at 4000 ppm in one male at scheduled terminal sacrifice (732nd day of the study), and in two males that were found dead near the end of the study (study day 667 and 624, respectively).

Ad 3) Although only limited number of tissue samples was examined in animals in the low and intermediate dose carcinogenicity sub-groups, the muzzle was among the organs/tissues which were examined histopathologically (as stated in dRAR and CLH Report: "In contrast to top dose and control group, only limited number of tissue samples was examined in animals in the low and intermediate dose carcinogenicity sub-groups (lung, liver, kidney, testis, epididymis, muzzle, and all gross lesions)." Dossier Submitter, however, agrees that this sentence could be rephrased (in the Background Document) to be more understandable.

RAC's response

Thanks to the DS for answering the questions.
RAC in their opinion did consider S-metalachlor together with the other chloroacetanilides. Information provided by IND and DS are included in the opinion.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
12.07.2023	Germany		MemberState	4

Comment received

We notice that e.g. in Tables 91 and 95, multiple studies per taxonomic group are indicated as key studies. Generally, only studies yielding the lowest reliable toxicity endpoint per taxonomic group should be marked as key study. We recommend to change

the tables accordingly. However, as in Tables 98 and 99 only the actual key studies are given and used for classification, our remark does not influence the overall conclusion. As the tables in the aquatic section provide data on the active substance as well as formulated product, we recommend to add an indication clarifying whether the endpoints for the formulated product are given as mg a.s./L or mg product/L. We agree with the classification as aquatic acute 1 (M= 10) and aquatic chronic 1 (M=10).
Dossier Submitter's Response
Thank you for your comment. The tables 91 and 95 will be amended accordingly. Also, as recommended the clarification regarding the endpoints will be given (mg a.s./L or mg product/L).
RAC's response
RAC agrees with the suggested changes.

Date	Country	Organisation	Type of Organisation	Comment number
20.07.2023	France		MemberState	5
Comment received				
FR agrees the conclusion on classification and labelling for environmental hazards, i.e Dimethachlor is classified in acute aquatic hazard Cat 1 - H400 : Very toxic to aquatic life with M-factor = 10 based on L.gibba 7d-ErC50 = 0.0658 mg a.s/Lnom and long-term aquatic hazard Cat 1 - H410 : Very Toxic to aquatic life with long lasting effects with M-factor = 10 based on L. gibba 7d-NOErC = 0.005 mg a.s/Lnom and considering the substance as non-rapidly degradable.				
Dossier Submitter's Response				
Thank you for your comment. No further action needed.				
RAC's response				
Noted.				

PUBLIC ATTACHMENTS
1. dimethachlor.zip [Please refer to comment No. 2]