Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

hexythiazox (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide

EC Number: -

CAS Number: 78587-05-0

CLH-O-0000001412-86-252/F

Adopted

30 November 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Comments provided during public 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 public 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 public 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.

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Substance name: hexythiazox (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide
EC number: -
CAS number: 78587-05-0
Dossier submitter: Finland.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2018	Spain		MemberState	1
Comment received				
The Spanish CA agrees with the dossier submitter that findings in hexythiazox treated rats and mice are considered as weak and inconsistent evidence and not sufficient to warrant carcinogenicity classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted, thank you.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2018	France		MemberState	2
Comment received				
Rat study: - Testicular interstitial cell (Leydig cell) adenoma: While it is acknowledged that strain F344 is not appropriate to investigate this type of tumours in respect to the high spontaneous incidence (almost 100% in control and treated groups at terminal sacrifice), it should however be noted that hexythiazox treatment seems to impact the age at onset. Indeed, at interim sacrifice the interstitial cell tumour incidences were 0/10, 0/10, 2/10 and 3/11 at 0, 60, 430 and 3000 ppm respectively. - Mammary glands tumours In the absence of relevant HCD supporting that the incidences reflect biology variability, it cannot be excluded that the increased incidence mammary gland tumours in males are treatment-related (fibroadenomas: 0, 1, 2 and 6 at 0, 60, 430 and 3000 ppm respectively; 1 adenocarcinoma at 3000 ppm)				

- Para-follicular cell adenoma
In the absence of relevant HCD supporting that the incidences reflect biology variability, it cannot be excluded that the increased incidence C cell tumours in high dose males are treatment-related.

Mouse study:
- Liver tumours
Total number of hepatic tumours was statistically increased at the top dose in both sexes, while adenoma was statistically increased only in top dose females.
Furthermore, the incidence of hepatoblastoma (rare tumour) was increased in both sexes at the top dose level of 1500 ppm.

Based on these data and in the absence of appropriate HCD or mechanistic data investigating the potential underlying mechanisms of increased incidences of tumours and their human relevance, it is considered that classification for carcinogenicity cat.2 H351 Suspected of causing cancer is warranted.

While JMPR in 2008 concluded that the increased incidences of tumours in rodents exposed to hexythiazox were likely to be threshold phenomena and that hexythiazox was unlikely to present a carcinogenic risk to humans at exposure levels associated with residues in food, the HED Cancer Assessment Review Committee (USEPA) classified hexythiazox as a "possible human carcinogen" in 1988.

Dossier Submitter's Response

Thank you for your comments. We agree that RAC should carefully consider whether these tumour findings warrant classification for Category 2.

Testicular interstitial cell (Leydig cell) adenoma:
Slightly increased incidences of Leydig cell adenoma were observed in interim sacrifice at 12 months in F344 rats (0/10, 0/10, 2/10 and 3/11 at 0, 60, 430 and 3000 ppm respectively). However, only trace interstitial cell hyperplasia was reported at 12 months and there were no clear relation in its incidences to adenoma incidences of different groups (5/10, 3/10, 3/10 and 6/11, in 0, 60, 430 and 3000 ppm). Therefore, in our opinion it remains unclear whether this finding is related to hexythiazox treatment. Moreover, due to properties of their LH receptor, F344 rats are particularly sensitive to this tumour type. Although earlier onset of these tumours in mid and high dose F344 males could reflect hexythiazox induced slight hormonal imbalance we consider this finding of low relevance for humans and not relevant for carcinogenicity classification.

Mammary gland tumours:
We agree that it cannot be excluded that this finding is related to hexythiazox treatment. We also note that the database suggests that hexythiazox could cause slight hormonal imbalance (see CLH report page 55), which could explain slightly increased incidence of this tumour type. However, since there were no histological and morphological findings in mammary gland or in other hormone sensitive tissues, that would support hormonal mechanism for tumor formation in male rats only and this tumour type is relatively common in F344 rats, we consider the causality not credible.
Moreover, we consider it unlikely that a slight increase in the incidence of mammary gland fibroadenoma (benign tumours) in high dose males only would be toxicologically significant.

Para-follicular cell adenoma

We agree that it cannot be excluded that this finding is related to hexythiazox treatment. However, we consider this unlikely due to following reasons. There were no significant differences in the incidences of parafollicular cell hyperplasia in male rats, and no differences in parafollicular cell tumor incidences in female rats. In females, parafollicular cell hyperplasia was slightly increased in all hexythiazox treated groups compared to concurrent controls (combined incidences of hyperplasia of all severity grades 5.7%, 11.6%, 10.1% and 10% at 0, 60 ppm, 430 ppm and 3000, respectively) but only hyperplasia graded as trace or mild was observed in hexythiazox treated groups. Parathyroid gland neoplasms are generally rare in F344 rats (HC ranges 0-4.2% and 0-2.2% in F344 males and females respectively in 1984-1994 in NTP database). However, the performing laboratory of this study has reported occasional spontaneous incidences of parafollicular adenoma in F344 males in a few studies. Accordingly, in the present study parafollicular cell adenomas were observed in all female (incidence 4.3% in all groups) and male groups including the controls (incidences 4.3%, 4.3%, 2.9% and 10.3% at 0, 60 ppm, 430 ppm and 3000 ppm, respectively).
Moreover, we consider it unlikely that a slight increase in the incidence of thyroid parafollicular cell adenomas (benign tumours) in high dose males only would be toxicologically significant.

Mouse study
The data shows that hexythiazox promotes formation of liver tumours in a mice strain (B6C3F1) exceptionally sensitive to promotion of liver tumors with high spontaneous incidences. We consider this finding to be of low relevance for humans (in accordance with CLP criteria guidance). Despite hepatotoxicity, there were no effect on hepatic tumour incidences in hexythiazox treated rats. In B6C3F1 mice the alterations of tumour incidences occurred only after 18 months treatment with high hexythiazox dose and after two years hexythiazox treatment there were only slight differences in the incidences of malignant liver tumours (carcinoma and hepatoblastoma) between hexythiazox treated groups and controls and no metastases were found. Hexythiazox treatment had no effect on survivability of mice. These factors considerable decrease the level of concern regarding the hexythiazox carcinogenicity concern for humans. However, we agree that RAC should particularly pay attention to increased incidences of hepatoblastoma (rare malign tumors) in both sexes in this study.

We are aware of previous assesments of hexythiazox by JMPR and US EPA. Our proposal not to classify hexythiazox for carcinogenicity is based on careful consideration of strength of evidence and additional considerations (weight of evidence) according to CLP criteria. We consider these tumour findings weak and inconsistent evidence and as such not sufficient for carcinogenicity classification for Category 2.

RAC's response

Thank you for your comments. RAC agrees with the Dossier Submitter's assessment of the data and considers that the findings in rats or mice do not provide reliable evidence of carcinogenicity.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2018	Germany		MemberState	3

Comment received

"DE-CA comment on carcinogenicity.pdf"

ECHA note – An attachment was submitted with the comment above. Refer to public attachment DE-CA comment on carcinogenicity.pdf

Dossier Submitter's Response

Thank you for your comments. We agree that RAC should carefully consider whether these tumour findings warrant classification for Category 2.

Mammary gland fibroadenoma
There is no fully acceptable historical control data available from the performing laboratory. We note that historical control data presented in the CLH report and in the litterature is of lower relevance and should be interpreted with caution. The incidence of mammary gland fibroadenoma was increased in a dose-dependent manner in hexythiazox treated F344 male rats compared to concurrent controls (total incidences 0%, 1.4%, 2.9% and 9.0% at 0, 60 ppm, 430 ppm and 3000 ppm, respectively). Positive trend tests revealed statistical significances in incidences of these tumours in different groups suggesting dose-response but pairwise comparison of control and high dose groups did not reach statistical significance. We have not found evidence that incidence in concurrent controls would be abnormally low. In females, there were no significant differences in incidences of mammary gland fibroadenoma between the treated groups and controls (8.6%, 4.4%, 1.4%, and 7.1%, at 0 ppm, 60 ppm, 430 ppm and 3000 ppm, respectively).
We agree with your considerations on possible hormonal MoA and note that the database suggests that hexythiazox could cause slight hormonal imbalance (see CLH report page 55), which could explain slightly increased incidence of this tumour type. However, since there were no histological and morphological findings in mammary gland or in other hormone sensitive tissues, that would support hormonal mechanism for tumor formation in male rats only and this tumour type is relatively common in F344 rats, we consider the causality of this finding not credible.
Moreover, we consider it unlikely that a slight increase in the incidence of mammary gland fibroadenoma (benign tumours) in high dose males only would be toxicologically significant.

Para-follicular cell adenoma
We appreciate your input on statistical analyses of the data. Please see our response to comment number 2 for discussion on para-follicular cell adenomas. We further note that statistical significance does not necessarily mean that tumour findings would be biologically meaningfull and toxicologically significant, and findings may be toxicologically significant even if there is no statistical significance.

Specific comments
Yes, your interpretation is right. Abbreviation DOS states for died on study/decedents/unscheduled sacrifice and SAC states for terminal sacrifice. This has been stated in the footnote to Table 24.
There are different explanations in the study report for missing results/tissues, for example "not in the plane of section". In the case of microscopical findings in mammary gland of two decedent high dose males it is stated: "not examined".

The study report states that survival data and data on time to neoplastic lesions were analysed using software including the following statistical procedures: Cox´s test for linear trend in proportions and both Cox´s test and Gehan-Breslow´s generalized Kruskal-Wallis test for comparing survival distributions. These tests were used for both mammary fibroadenomas and parafollicular cell adenomas.

Please see our response to comment number 2 for discussion on the mouse study.

RAC's response
Thank you for your comments. RAC agrees with the Dossier Submitter's assessment of the data and considers that the findings in rats or mice do not provide reliable evidence of carcinogenicity.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
26.01.2018	United Kingdom		MemberState	4
Comment received				
Acute toxicity to Daphnia (Additional Report IIA, 8.2/34 Saito, 2003): Does the study report include observation data for animal inspections? This is important to rule out physical effects for immobilisation given that particles were observed in treatments with mean measured concentrations above the quoted water solubility. In addition, are there any 24/48 hour immobilisation endpoints from the chronic toxicity to Daphnia study which support the 48h EC50 being based on an ecotoxic response? Chronic toxicity to Daphnia (DAR IIA 8.2/21 Lui, 1996): While we note the reduction in oxygen levels over the study, we recognise that levels were above similar test guideline levels. On this basis, we feel additional statistical analysis is required to consider if the immobilisation 21 day NOEC invalid. In addition, can adult immobilisation data available from the other chronic toxicity to Daphnia studies aid interpretation of the Lui, 1996 21-day NOEC for immobilisation?				
Dossier Submitter's Response				
Thank you for your comments. Regarding study 8.2/34, according to the study report "All test solutions were clear during the observation period of 48 hours. But precipitation or undissolved particles were observed in 0.640 and 1.28 mg/L test solutions at the observation period of 48 hours." Assuming that this means that all test solutions were clear during the test and precipitation or undissolved particles were observed only at the end of the test in these two highest concentrations, any possible physical effects for immobilisation may be ruled out or at least considered to be minor. According to test report no items which might have affected to the results were observed during the test. However, no further information on any observation for animal inspection was provided in the report. The available chronic tests do not provide suitable 24/48 hour immobilisation endpoint for supporting the result. In the study 8.2/22 the surviving of parent animals was checked daily but no mortality was observed during the first two days in any test concentrations (the highest concentration tested was 0.0836 mg/l). In the study 8.2/24 a static acute test was carried out prior to the chronic test with unfed and fed daphnias. The 48-hour EC50 (immobility) values were 1.22 mg/l and 1.4 mg/l, respectively. The chemical and physical parameters measured were all within acceptable range, but no analytical methods were used to measure test concentrations so results are based only on nominal concentrations. Chronic test did not provide any immobilisation data for 24/48 hours. Regarding the study 8.2./21 we chose to use the mean of live young produced per adult reproduction day as the most sensitive endpoint for the classification proposal (NOEC 0.0277 mg/l). We recognise that mortality (immobility) was accepted during EU review according to Directive 91/414/EEC. However, we considered mean live young more				

reliable compared to mortality endpoint in this case, as clearer concentration-response pattern could be found unlike with mortality (see the Table 5. below, extracted from the study report and Table 51 in the CLH proposal). Also growth (length) followed the same trend (see below study report Table 7), and may be used in addition to reproduction measures as supportive information.

This study faced severe problems with the dissolved oxygen concentrations which varied strongly in all replicates. In the control concentrations stayed in acceptable range (67-76 %), but in all replicates, including solvent control, the dissolved oxygen concentrations fluctuated appr. 43 – 73 % during the study (at the initiation 63 - 73 %, at the end 43 - 55 %) even with the help of aeration which was started on the day 12. The test was carried out under flow-through conditions. The reason for this variation was not speculated in the study report, nevertheless, different measures were done in order to increase the concentrations (i.e. increasing the cycle rate, cleaning the test chambers, and aeration). Anyhow, the dissolved oxygen concentration stayed in all test chambers above 3 mg/l throughout test, which is the limit indicated in the OECD 211 test guideline, and therefore this study was considered valid also for classification purpose despite of this problem.

Table 5. (from the study report) Number of offspring produced by *Daphnia magna* per reproductive day during a 21-day chronic exposure to hexythiazox technical.

Mean measured concentrations (µg/L; ppb	Number of Offspring per reproductive day				
	Rep A	Rep B	Rep C	Rep D	Treatment
Control	6.15	9.54	10.2	8.89	8.81
Solvent control	8.95	9.15	8.79	7.02	8.52
6.07	8.03	9.24	8.06	8.42	8.44
12.7	8.23	9.22	8.63	8.10	8.57
27.7	8.59	7.91	6.88	8.06	7.87
53.8	7.64	4.31	4.42	3.48	4.78[a]
97.1	0.65	0.26	0.22	0.33	0.37[a]
228	0.00	0.00	0.00	0.00	0.00[b]

[a] Statistically significant (α = 0.05) reduction in the number of offspring produced per adult reproductive day from the pooled control.
[b] Concentration excluded from statistical evaluation on reproduction due to significant effect on survival.

Table 7. (from the study report) Mean lengths of *Daphnia magna*, after 21 days of exposure to hexythiazox technical under flow-through conditions.

Mean measured concentrations (µg/L)	Mean length (mm)					
	Rep A	Rep B	Rep C	Rep D	Mean[a]	(±SD)
Control	4.09	4.42	4.63	4.43	4.39	(0.40)
Solvent control	4.46	4.57	4.54	4.60	4.55	(0.36)
6.07	4.43	4.38	4.40	4.61	4.47	(0.26)
12.7	4.52	4.50	4.41	4.52	4.48	(0.14)
27.7	4.43	4.40	4.10	4.37	4.33	(0.22)
53.8	3.50	4.23	4.25	3.96	4.03[b]	(0.28)
97.1	3.38	0.00	0.00	3.84	3.74[b]	(0.22)

[a] Mean lengths based on individual lengths of Daphnia.
[b] Significantly (α = 0.05) reduced from the pooled control.

In other two chronic Daphnia test no statistically significant differences in mobilisation of parent daphnia in relation to the control were observed either at all or were found at an order of magnitude higher test concentrations;

In the chronic Daphnia study 8.2/22 no statistical significant effect on the parent mortality comparing to control was observed in the highest tested concentrations 0.0836 mg/l (LC0 ≥ 0.0836 mg/l). As for study 8.2/24, there were no statistically differences between test group mean and the solvent control group mean for survival, reproduction or length at the test concetrations of 0.05 mg/l and below. In the test design each test concentration was replicated three times with five *Daphnia magna* per beaker for survival measurement and seven times with one *Daphnia magna* per beaker for reproduction and growth measurement. At 0.17 mg/l test concentration there was no significant difference in mean survival, based on data from the beakers used to estimate survival. However, only three of seven daphnids in reproduction vessels survived for 21 days, and only one of them reproduced.

RAC's response
RAC agrees with the DS's response. Regarding the study 8.2./21, RAC agrees that the reproduction endpoint is more reliable than the immobilisation endpoint in this study as the latter did not follow a clear dose-response pattern. RAC considers it difficult to perform further statistical analysis to assess the validity of the endpoint by e.g. removing some of the samples from the analysis. This is because it seems difficult to justify which ones should be removed as the dissolved oxygen concentration dropped in all treatments. Furthermore, the results of the other available chronic studies with daphnia do not support the low NOEC for mortality determined in the study 8.2./21.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2018	France		MemberState	5
Comment received				
FR agrees with the classification and M factors (acute and chronic) proposals.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC takes note of the support.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2018	Germany		MemberState	6
Comment received				
We support the proposal for the classification of environmental hazards as Aquatic acute 1 (H400) and Aquatic chronic 1 (H410) and the acute/chronic M-factor of 1. ECHA note – An attachment was submitted with the comment above. Refer to public attachment DE-CA comment on carcinogenicity.pdf				
Dossier Submitter's Response				
Thank you for your support.				

RAC's response
RAC takes note of the support.

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Belgium		MemberState	7
Comment received				
BE CA agrees with FI's comparison of available data with the environmental CLP criteria and supports the proposed environmental classification of hexythiazox with Aquatic Acute 1, H400 (M=1) and Aquatic Chronic 1, H410 (M=1).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC takes note of the support.				

PUBLIC ATTACHMENTS
1. DE-CA comment on carcinogenicity.pdf [Please refer to comment No. 3, 6]