Committee for Risk Assessment

RAC

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

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

30 November 2018

CLH-O-0000001412-86-252/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **-**

CAS Number: **78587-05-0**

The proposal was submitted by **Finland** and received by RAC on **19 October 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Finland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **5 December 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 February 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Andrew Smith**

[Co-Rapporteur, appointed by RAC: **João Carvalho**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-125-00-6	hexythiazox (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide	-	78587-05-0	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	613-125-00-6	hexythiazox (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide	-	78587-05-0	**Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H400 H410	**Retain** GHS09 Wng	**Retain** H410		**Add** M-factor (acute)=1 M-factor (chronic)=1	
RAC opinion	613-125-00-6	hexythiazox (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide	-	78587-05-0	**Retain** Aquatic Acute 1 Aquatic Chronic 1	**Retain** H400 H410	**Retain** GHS09 Wng	**Retain** H410		**Add** M-factor (acute)=1 M-factor (chronic)=1	
Resulting Annex VI entry if agreed by COM	613-125-00-6	hexythiazox (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide	-	78587-05-0	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Hexythiazox currently has an existing Annex VI entry. The current proposal seeks only to address the carcinogenicity and environmental endpoints. Assessments of the mutagenic potential of this substance and its general systemic toxicity following repeated dosing are included to the extent they relate to conclusions about the carcinogenicity endpoint.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of mutagenicity

NOTE: This assessment of mutagenicity is included only as supporting evidence for the carcinogenicity endpoint. No classification is discussed or proposed for the endpoint of germ cell mutagenicity.

Summary of the Dossier Submitter's proposal

Five *in vitro* and one *in vivo* mutagenicity studies were presented in the CLH report for hexythiazox.

The results of a bacterial reverse mutation test showed that hexythiazox did not induce gene mutations either in the presence or absence of metabolic activation. Hexythiazox did not induce point mutations (at the HPRT locus) in mammalian cells (Chinese Hamster V79) nor did it induce chromosomal aberrations *in vitro* in Chinese Hamster ovary cells. The results of both a bacterial recombination assay and an *in vitro* unscheduled DNA synthesis (UDS) test with primary rat hepatocytes were negative with hexythiazox. In an *in vivo* micronucleus assay in mice, the results were negative following single doses of hexythiazox of up to 2000 mg/kg bw.

Overall, the results of a series of adequately performed tests showed that hexythiazox is not mutagenic.

Comments received during public consultation

Germ cell mutagenicity was not open for commenting and no comments were received.

Hazard assessment

There is a wide range of both *in vitro* and *in vivo* mutagenicity studies are available. However, in the CLH report, the Dossier Submitter only included those studies considered as being of acceptable quality to the reporting Member State during the related Plant Protection Product review process. Accordingly, RAC considered only the relevance of these studies in the assessment of carcinogenicity.

In vitro studies

A bacterial mutation assay (Ames test) was carried out in accordance with OECD guidelines. *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA/pKM101 were treated with 0, 313, 625, 1250, 2500 and 5000 µg/plate of hexythiazox ±S9 mix. The results were negative with and without S9. Crystallisation of the test substance was noted at all concentrations tested. Positive and negative controls gave the expected results.

In a well-conducted mammalian cell gene mutation test carried out in Chinese Hamster V79 cells (target: HPRT gene locus), concentrations of 0, 9.38, 18.8, 37.5, 75.0 and 150 µg/ml hexythiazox were incubated with cells in the presence and absence of S9 mix. Appropriate positive and negative controls were used. The results showed that hexythiazox did not increase mutation frequency either in the presence or absence of metabolic activation when compared to negative controls.

In a guideline chromosome aberration test, Chinese hamster ovary (CHO) cells were treated with 0, 5, 20, 35 and 50 µg/ml hexythiazox in the absence of S9 and 0, 35, 50, 200, 350 and 500 µg/ml in the presence of S9. Harvest times were 10, 20 and 30 hours. The results showed there was no significant increase in the percentage of chromosomally aberrant cells in the presence or absence of S9. Positive and negative controls behaved accordingly.

An UDS test was carried out in compliance with OECD guidelines. Rat hepatocytes were incubated with 0, 2.5, 5, 10, 25, 50, 100 and 250 µg/ml of hexythiazox. The results of the test were negative. Cytotoxicity was observed at concentrations of 50 µg/ml and above. At the top concentration of 250 µg/ml, all cells died.

A bacterial recombination assay was also included in the dossier as supplemental information. Concentrations of hexythiazox of up to 3200 µg were incubated with *Bacillus subtilis* strains H17 (Rec+) and M45 (Rec-). The results of this test were also negative.

In vivo study

Hexythiazox was tested in a micronucleus assay that was in compliance with OECD TG 474. A single intraperitoneal injection of 0, 500, 1000 or 2000 mg/kg bw of hexythiazox was administered to mice. The test result was negative and the Dossier Submitter indicated that the nature of the results indicated that there was bioavailability of the test substance to the bone marrow. This assessment of bioavailability is consistent with the available information from both toxicokinetic and repeat dose toxicity studies.

Conclusions

The *in vitro* and *in vivo* data indicate with high confidence that hexythiazox does not have a mutagenic mechanism of action.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

NOTE: This assessment of repeated dose toxicity is included only as supporting evidence for the carcinogenicity endpoint. No classification is discussed or proposed for the endpoint of specific target organ toxicity.

Summary of the Dossier Submitter's proposal

Four dietary repeated dose toxicity studies were available in mouse, rat and dog. Liver and adrenal were found to be the target tissues for hexythiazox. Increased liver weights together with an increased incidence of hepatocellular hypertrophy were observed at high hexythiazox doses in all three species. Adrenal weights were significantly increased in rat and dog at high doses. In a rat 90-day study, increased adrenal weights were associated with fatty degeneration of adrenal cortex at high (3500 ppm) and intermediate doses (500 ppm), the effect being more pronounced in males. In one-year dog study, adrenocortical hypertrophy of all three cortical zones in both sexes was observed at intermediate (500 ppm) and high (5000 ppm) hexythiazox

doses. Weights of thyroids/parathyroids were slightly, but not statistically significantly, increased in dog one-year study (only four dogs were examined). In the same study mild parafollicular hyperplasia was observed in both sexes in all groups including controls without a clear dose response. No remarkable histological findings were observed in parathyroid gland in rat or mouse or in mammary gland of rat and dog (only female dogs were examined). Mammary gland was not examined microscopically in the 28-day mouse study.

Comments received during public consultation

Specific Target Organ Toxicity was not open for commenting and no comments were received.

Hazard Assessment

Rats

Hexythiazox was administered in the diet to Fischer rats (20/sex/dose) at dose levels of 0, 10, 70, 500 or 3500 ppm (equivalent to 0, 1.2/0.8, 8.1/5.4, 58.6/38.1 and 397.5/257.6 mg/kg bw/day males/females respectively) for 13 weeks.

All animals survived to study termination without any clinical signs of toxicity. Small decreases in body weight and body weight gain were observed in males of the top dose group and females of the top two dose groups, but it was only a reduction in body weight gain in females of the top dose group that was > 10% of controls (specifically, 17.9% reduction compared to controls).

Small changes were noted to blood chemistry parameters in males and females of the top dose group only. Total cholesterol, total protein, albumin and calcium were all increased and alkaline phosphatase was decreased in females only of the top dose group.

Absolute and relative liver weights were increased statistically significantly in both sexes in the top two dose groups. However, the group mean increases in absolute weight were only above 10% in the top dose groups (approx. 40% increase compared to controls). Group mean relative weights were above 10% of controls in top dose group males and females, and in females only in the second highest dose group. In top dose males, adrenal weights were slightly increased (absolute: 115% and relative: 119% compared to controls) and the relative testis weight was also very slightly increased (106% compared to controls). In females, the relative ovary weight was increased compared to controls (126%).

No other significant treatment-related changes were noted.

Mice

Groups of B6C3F1 mice (10/sex/dose) were fed a diet containing concentrations of hexythiazox of 0, 50, 300, 1800 or 10800 ppm (equivalent to 0, 9.9/13.2, 55.1/62.9, 319.1/388.2 and 1908.4/2045.0 mg/kg bw/day males/females respectively) for 28 days.

There were no changes in mortality rates during this study and no clinical signs of toxicity. Body weight and/or body weight gain were decreased in males towards the end of the study, in all dose groups except those dosed with 55.1 mg/kg bw/day, however the toxicological significance of this is unclear, particularly as the magnitudes were not presented in the dossier.

Total cholesterol was significantly decreased in both males and females of the top dose group and in males dosed with 319.1 mg/kg bw/day. Hepatocellular hypertrophy was observed in both males and females at the top two doses. Absolute and relative liver weights were also increased in males and females of these dose groups.

No other significant treatment-related changes were noted.

Dogs

Two studies are available in dogs, a 28-day range finding study and a 1-year study.

28-day study

Beagle dogs (2/sex/dose) were administered hexythiazox in the diet for 4 weeks at dose levels of 0, 125, 500, 2000 and 8000 ppm (equivalent to 0, 5.58/5.54, 23.1/21.6, 89.4/78.9 and 324/346 mg/kg bw/day, males/females respectively).

No deaths occurred during the study. Body weight gain was reported to have decreased in females of the top dose group and relative liver weights were increased in top dose males and females treated with 78.9 mg/kg bw/day. There were no relevant histopathological findings.

1-Year study

Hexythiazox was administered to Beagle dogs in the diet (4/sex/dose) at dose levels of 0, 100, 500 or 5000 ppm (equivalent to 0, 2.87/3.17, 13.1/13.9 and 153/148 mg/kg bw/day) for one year.

All dogs survived to study termination. Body weight gain was decreased in high dosed males and all treated females when compared to controls. This was associated with a reduction in food consumption observed in all treated groups.

Absolute and relative adrenal weights were statistically significantly increased in top dose males and females (absolute: 160/176% and relative: 179/169% males/females compared to controls). This finding was attributed to adrenocortical hypertrophy. Cortical cells were enlarged and the number of lipid vacuoles in cells were increased.

Relative liver weights were also statistically significantly increased in top dose males only (129% compared to controls). Trace – mild hepatocellular hypertrophy was apparent in animals of this dose group.

Absolute and relative thyroid/parathyroid weights were also increased in males and females of the top dose and in females of the mid dose (top dose: absolute: 130/150%, relative: 40/150% males/females and mid dose: absolute 130%, relative: 136% in females only compared to controls). However, these increases were not found to be statistically significant when compared to controls. There were no histopathological correlates.

Conclusions

The results of four dietary studies in rats, mice and dogs indicate that the target tissues for hexythiazox are the liver (rats, mice and dogs) and adrenals (rats and dogs). There were no specific toxicological findings seen that might be considered to indicate a potential carcinogenic mechanism of action for hexythiazox, however, the mechanism of action was not specifically studied

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenicity of hexythiazox has been investigated in two 24-month dietary carcinogenicity studies, one in rats and one in mice.

In F344 rats, statistically significantly increased incidences of mammary gland fibroadenoma were observed in hexythiazox-treated groups of males when compared to the control group. In females, the incidence of mammary gland fibroadenoma was actually decreased in low and mid

dose-treated groups. The Dossier Submitter noted that there were no morphological or histological findings in the mammary gland that would support a hormonal mechanism of action of mammary gland tumour formation in males only. The DS concluded that mammary gland fibroadenoma was a relatively common tumour in F344 rats but the findings of a slight increase in tumours in high dose males and a slight decrease in low and mid dosed females were unlikely to be related to treatment.

In addition, thyroid parafollicular cell adenoma was noted in all treated groups (including controls) in both males and females. However, the incidence of the adenoma was slightly increased at the end of the study period in high dose males, when compared to concurrent controls. The tumours appeared without any clear dose response and no fully acceptable historical control data from the performing laboratory was available according to the DS. No such tumours were observed in females, but an increase in incidence of parafollicular cell hyperplasia was observed in all hexythiazox-treated females when compared to concurrent controls. In the absence of a clear dose-response and any other thyroid tumour findings in either sex, the Dossier Submitter considered the small increase in incidence of parafollicular cell adenoma in top dosed males as toxicologically insignificant.

The incidence of testicular interstitial cell (Leydig cell) adenoma was slightly, but not statistically significantly increased in mid and high dose group male rats (23 and 163 mg/kg bw/day) at the mid study (12-month) sacrifice compared to concurrent controls and historical controls. However, by the end of the 24-month study, there were no differences in testicular interstitial cell adenoma incidences between treatment groups. This tumour type was widely regarded as a common spontaneous finding in this strain of rats (F344) and its occurrence was not considered informative. Therefore, this finding was not considered relevant for classification.

The Dossier Submitter did not consider the findings of slightly increased incidences of benign mammary gland fibroadenoma and parafollicular cell adenoma in high-dosed F344 male rats related to treatment with hexythiazox. On the basis that hexythiazox was not genotoxic and there was no clear indication of a hormonal mode of action for tumour formation in males only, the Dossier Submitter concluded they were not relevant for classification.

In B6C3F1 mice, dietary administration of hexythiazox for two years resulted in a statistically significant increase in the total incidence of hepatic tumours.

In females the incidence of hepatocellular adenoma was statistically significantly increased at the top dose only. Three hepatocellular carcinoma were observed in the low, mid and high dose groups and one hepatoblastoma was observed in the high-dose group compared to no carcinoma in the control and no hepatoblastoma in the control, low and mid dose groups.

In the top-dose group of males, the incidences of hepatocellular adenoma and carcinoma were slightly and not statistically significantly increased at terminal sacrifice. Three hepatoblastoma were noted compared to zero in the control, low and mid dose groups.

Mice treated with the high dose of hexythiazox showed signs of liver toxicity. This was indicated by increased liver weights, hepatic nodules, hepatic necrosis and cytological alterations of liver cells. A proliferative effect was suggested by an increased incidence of hepatic nodules at the end of the study. Thus, increased incidence of liver tumours in high dose mice seemed most likely to have been be caused by hepatoxicity and a proliferative stimulus on the liver.

The Dossier Submitter noted that B6C3F1 mice had a very high spontaneous incidence of liver tumours and that the Guidance to the Application of CLP stated that "where the only available tumour data are liver tumours in certain strains of mice, without other supplementary evidence, the substance may not be classified in any of the categories". It was noted that liver tumours in B6C3F1 mice had been given as an example for tumours for high spontaneous incidence in the guidance.

The Dossier Submitted did not consider the evidence in mice as sufficient for classification.

Overall, the conclusion was that the findings observed in rats and mice after two years of treatment with hexythiazox were considered weak and inconsistent evidence, and thus, not sufficient for classification for carcinogenicity.

Comments received during public consultation

Three comments were received from MSCA during the public consultation. One was in agreement with the DS' proposal for no classification and two required at least a discussion as to whether classification in Category 2 for carcinogenicity was more appropriate due to the findings in both rats and mice. In particular it was noted by both these MSCAs that the hepatoblastoma observed in the livers of both male and female top-dosed mice was a rare finding. One MSCA also noted that the increase in testicular interstitial cell adenoma in F344 rats at the interim 12-month sacrifice might have been due to hexythiazox treatment.

Assessment and comparison with the classification criteria

There are two carcinogenicity studies presented in the CLH dossier for hexythiazox, one in rats and one in mice.

Rats

Hexythiazox was administered to F344 rats (80/sex/group) via the diet at dose levels of 0, 60, 430 and 3000 ppm for 24 months (equivalent to 0, 3.2/4.02, 23.1/29.3 and 163/207 mg/kg bw/day in males/females). At 12 months, an interim sacrifice and necropsy was performed on 10 rats/sex/group.

There was no effect on mortality rate in treated animals and no overt clinical signs of toxicity. Survival rates at study termination were 50/70, 57/70, 59/70 and 53/70 in control, low, mid and high dose males and 57/70, 49/70, 61/70 and 56/70 in females.

Tumour findings

Testis

Table: *Tumour findings in the testes of F344 rats:*

	Dose (mg/kg bw/day)			
Interstitial cell tumour, benign	0	3.2	23.1	163
After 12 months:	0/10	0/10	**2/10 (20%)**	**3/11 (27%)**
After 24 months:	67/70 (96%)	66/70 (94%)	66/70 (94%)	68/69 (99%)

At the end of the study there was a high incidence of testicular interstitial cell (Leydig cell) adenoma in all treated groups of male rats. This is not considered to have been related to treatment with hexythiazox given that the incidence rates were similar in all the treated groups and in the untreated controls. The findings are consistent with CLP Guidance on the Application of the CLP criteria indicating that this strain of rat is very sensitive to formation of benign Leydig cell tumours. Further, there was no obvious progression of tumours from hyperplasia, and no evidence of malignancy. Trace interstitial hyperplasia was noted in all animal groups, with no clear dose response between untreated and treated groups (incidences: 5/10, 3/10, 3/10 and 6/11 for groups 0, 3.2, 23.1 and 163 mg/kg bw/day respectively).

In the limited investigation conducted after 12-months, Leydig cell adenoma were seen in both the mid- and high-dose groups (2/10 and 3/11 respectively). No such tumours were seen in the low dose and control animals autopsied at this time point. The significance of this reduced onset time for this very common tumour in a small number of animals is unclear, given that at the end of the study it appears that all these tumours had occurred spontaneously, and were not treatment-related. The observation of comparable levels of hyperplasia (trace) and testicular degeneration in control and treated animals at interim sacrifice further indicate the absence of a treatment-related effect.

The performing laboratory provided historical control data (HCD) from 7 studies conducted between 1982 – 1988 for 12 month interval data (year of hexythiazox carcinogenicity study: 1981). The HCD showed that the incidences of interstitial cell adenoma in control male F344 rats varied between 0 – 15%. However, although the incidence rates seen after 12-month treatment with hexythiazox were slightly above this range, no firm conclusions can be drawn given the absence of contemporary historical control data. Ideally, HCD data should be considered within a period including 5 years before and 5 after the study of interest.

Table: *Historical control data (12-month interim sacrifice) of testicular interstitial cell adenoma in male F344 rats (conducted from 11/83 – 11/88)*

	Study A	Study B	Study C	Study D	Study E	Study F	Study G
No. Animals	11	20	11	20	20	11	20
Incidence	0	3	0	0	1	1	0
% Incidence	0	15	0	0	5	9.1	0

In conclusion, RAC is in support of the Dossier Submitter's assessment that the findings in the testes of male rats do not indicate a carcinogenic effect of hexythiazox.

Thyroid

Table: *Tumour and related findings in the thyroid of F344 rats*

	Dose (mg/kg bw/day)							
	Males				**Females**			
Finding	**0**	**3.2**	**23.1**	**163**	**0**	**4.02**	**29.3**	**207**
Parafollicular cell hyperplasia	3/70 (4.3%)	8/69 (11.6%)	3/70 (4.3%)	1/68 (1.5%)	8/70 (11.5%)	16/69 (23.2%)	14/69 (20.3%)	14/70 (20%)
Parafollicular cell adenoma	**3/70 (4.3%)**	**3/69 (4.3%)**	**2/70 (2.9%)**	**7/68 (10.3%)**	3/70 (4.3%)	3/69 (4.3%)	3/69 (4.3%)	3/70 (4.3%)
Follicular cell carcinoma	0/70	0/69	1/70 (1.4%)	1/68 (1.5%)	0/70	0/69	0/69	1/70 (1.4%)
Parafollicular cell carcinoma	0/70	0/69	1/70 (1.4%)	0/68	0/70	0/69	0/69	0/70

At study termination, thyroid parafollicular cell adenoma was observed in all male and female dose groups (both treated and untreated). However, there was a slight increase in these benign tumours in top dose males only. This increase was observed in the absence of any statistical significance and there was no clear dose-response (4.3, 4.3, 2.9 and 10.3% in the control, low, mid and high dose groups respectively). Apart from a single incidence in the mid dose group of males, parafollicular cell carcinoma was not evident.

Parafollicular cell hyperplasia was noted in all treated and untreated groups but in the absence of any kind of dose response either in incidence or severity.

In general, spontaneous formation of parafollicular cell adenoma is rare in F344 rats. The US National Toxicology Program (NTP) historical control database reports incidences of 0 – 4.2% in F344 males and 0 – 2.2% in F344 females during the period of 1984 – 1994 from 20 dietary studies. HCD were provided by the laboratory that tested hexythiazox from 10 studies conducted during 1986 – 1998: mean incidence of parafollicular cell adenoma was 2.7% (range 0 – 12%). As the carcinogenicity study with hexythiazox was conducted in 1981, these laboratory data are outside of the preferred 5 year period, and therefore of reduced relevance and reliability. However, the incidence of parafollicular cell adenoma in both male and female control animals in the hexythiazox study is above the mean incidence of 2.7% from the laboratory HCD suggesting that there was a higher than usual spontaneous incidence of parafollicular cell adenoma occurring in this batch of animals. This, together with the absence of malignancy, a lack of statistical significance to the increase in adenoma and no relevant pre-neoplastic changes in treated animals suggests that the limited tumour findings in the thyroid gland of male rats only were not treatment-related.

There was also one incidence of follicular cell carcinoma noted in mid dosed males, in top dose males and in top dose females. No tumours of this type were noted in control animals or in any other dose groups. HCD provided by the laboratory from studies conducted between 1986-1998 (outside of the preferred 5 year period) indicated that isolated incidences of follicular cell carcinoma do occur spontaneously in male F344 rats. No HCD was provided for female F344 rats. As the follicular cell carcinoma observed in the current study occurred only as single incidences in the absence of a dose-response and without statistical significance, and as single incidences have been found to occur spontaneously in this strain of rat, they do not provide convincing evidence of a carcinogenic effect following treatment with hexythiazox.

No other adverse effects to the thyroid were reported in this study or in repeated dose studies carried out in rats, mice or dogs.

Mammary gland

***Table**: Tumour and related findings in the mammary gland of F344 rats*

Finding	Dose (mg/kg bw/day)							
	Males				Females			
	0	**3.2**	**23.1**	**163**	**0**	**4.02**	**29.3**	**207**
Fibroadenoma	0/70	1/69 (1.4%)	**2/70 (2.9%)**	**6/67 (9.0%)**	6/70 (8.6%)	3/68 (4.4%)	1/70 (4.3%)	5/70 (7.1%)
Fibroma	0/70	0/69	0/70	1/67 (1.5%)	0/70	0/68	0/70	0/70
Adenocarcinoma	0/70	0/69	0/70	1/67 (1.5%)	0/70	0/68	1/70 (1.4%)	0/70

There was a clear dose-related increase in incidence of mammary gland fibroadenoma in male rats at the end of the study: tumour frequencies were 0, 1.4, 2.9 and 9.0% in control, low, mid and high dose males, respectively. This increase was found to be statistically significant in tests for unadjusted and adjusted trend and cox analysis. In a pair-wise comparison of control and high dose groups, no statistical significance was found. Mammary gland fibradenoma was seen in all female groups. In contrast to the males, there was no dose-related effect in females. The tumour rate in control females (8.6%) was similar to that in high dose males (9%).

Also observed was an isolated incidence of adenocarcinoma in top dose males and in mid dose females. These single incidences were not found to have any statistical significance and they lacked a dose-response. In RAC's opinion they do not provide evidence of a carcinogenic effect of hexythiazox and are not considered further in this assessment.

Laboratory historical control data (HCD) was submitted for 6 studies conducted between the years 1986 – 1995. As the study with hexythiazox was conducted between 1981-1983, this was outside of the preferred 5 year period for such data. In these studies the incidence of mammary gland fibroadenoma in male F344 rats ranged between 0 – 6%. HCD was also submitted by the Applicant from other laboratories (NTP, US), showing that the incidence of fibroadenoma in the mammary gland of male F344 rats is more variable – range 0 – 12%. Although it is noted that these HCD are not ideal for comparative purposes, they do at least show that this type of tumour occurs spontaneously in this strain of rat and that the incidence of 9% observed in top dose male rats is not outside the range observed in the open literature.

Further, there were no non-neoplastic findings in the mammary glands of males or females in the current or in the available repeated dose studies. Both the DS and one of the commenting MSCA questioned whether there might be a hormonal influence to the observed increase in fibroadenoma in male rats. No studies designed to specifically assess a hormonal mechanism of action were carried out. Although study summaries were not presented in the CLH report, the DS commented that there were no effects in standard rat reproductive toxicity studies with hexythiazox that would indicate hormonal influence on fertility. However, the DS noted that pups of both the F1B and the F2 generation in a rat 2-generation study were observed to have lower body weights throughout the lactation period. The DS considered this might indicate a potential functional impact of hexythiazox on the mammary gland. As mammary gland tumours were found only in males, the RAC does not consider this finding alone evidence of a relevant hormonal effect.

The DS also highlighted that there were effects to the pituitary, adrenal glands and effects on testis and ovary weights in repeated dose studies and the current carcinogenicity study.

In the current carcinogenicity study, pituitary hyperplasia was noted in all treated groups (0/70, 5/70, 4/70, 3/70 control, low, mid and high dose groups). However, there was no dose response and the details provided show that there was no increase in severity with increasing dose. The RAC does not consider these small increases in incidence of hyperplasia a treatment-related effect.

Small increases in adrenal weight were noted in rats and in dogs in repeated dose studies. In the current carcinogenicity study in rats, relative adrenal weights were increased in top dose males and females (116% and 109% respectively compared to controls). There was no clear dose-response in males. Testis weights were increased in males of the top dose group only (123% compared to controls) and relative ovary weight was increased in top dose females (117% compared to controls).

In a 90-day repeated dose study in rats, increases in relative adrenal weight were noted at the top dose of 397 mg/kg bw/day in males only (120% compared to controls). In the same dose group, the testis weight was noted to be slightly increased (relative weight: 106% compared to controls) and the ovary weight was increased in top dosed females (dose: 257 mg/kg bw/day, increase in relative weight: 126% compared to controls).

In dogs, dosed for 1 year with 153 mg/kg bw/day, adrenal gland weight was increased by 160% (absolute) and 180% (relative) when compared to controls. No effects to the testis or ovaries were noted.

Increases in adrenal gland weight were reported in the 24-month carcinogenicity study in mice; however the magnitude of these changes was not discussed.

The increased adrenal gland, testis and ovary weights in rats and dogs were not associated with any histopathological findings in any of the studies. Therefore, the biological significance is unclear.

Overall, there is no obvious mechanistic basis to support a possible treatment-related effect of hexythiazox on the mammary gland of male rats that could lead to the development of a carcinogenic response, but no studies designed to specifically assess a mechanism of action were carried out. Given that this benign finding is a relatively common tumour type in the F344 rat, RAC agrees with the DS that the increase above controls of fibroadenoma in the top dose group males was most likely an incidental finding and does not provide sufficient evidence of hexythiazox carcinogenicity to justify classification.

Mice

B6C3F1 mice were administered hexythiazox in the diet at doses of 0, 40, 250 and 1500 ppm (equivalent to 0, 6.72/8.38, 41.6/51.2 and 267/318 mg/kg bw/day for males and females respectively). Satellite groups of 10 animals/sex/dose were scheduled for interim sacrifices at 26, 52 and 78 weeks and 50/sex/dose were dosed for the main study duration of 24 months.

No differences in mortality or in the incidence of clinical findings were noted among the control and treatment groups. In the male control group, body weights were found to be abnormally high and there was large inter-group variation. Therefore, the male body weight data was also compared with historical control data from the same laboratory performing the study. Mean body weight was consistently reduced in males of the top dose group (267 mg/kg bw/day) and at the end of the study the reduction was 11.4% compared to HCD and 17.8% compared to concurrent control animals. Body weights of treated females were comparable to controls.

Liver

Table: *Hepatocellular tumour incidence in B6C3F1 mice at 104 weeks following dietary administration of hexythiazox*

	Dose (mg/kg bw/day)							
Liver	**Males**				**Females**			
	0	**6.72**	**41.6**	**267**	**0**	**8.38**	**51.2**	**318**
Total no. animals examined	46	47	49	48	46	49	50	49
Hepatic nodule	10	8	14	30	5	5	2	15
Adenoma	11 (24%)	8 (17%)	9 (19%)	14 (29%)	5 (11%)	1 (2%)	5 (11%)	**16* (33%)**
Carcinoma	11 (24%)	8 (17%)	9 (19%)	14 (29%)	0 (0%)	3 (6%)	3 (6%)	3 (6%)
Hepatoblastoma	0 (0%)	0 (0%)	0 (0%)	**3 (6%)**	0 (0%)	0 (0%)	0 (0%)	**1 (2%)**

* $p < 0.05$

At terminal sacrifice the incidence of hepatocellular adenoma was statistically significantly increased in top dose females (11, 2, 11 and 33% for dose groups 0, 8.38, 51.2 and 318 mg/kg bw/day). In males the incidence of hepatocellular adenoma also appeared to be marginally increased in the top dose group but this lacked statistically significance and there was no dose response (24, 17, 19 and 29% for dose groups 0, 6.72, 41.6 and 267 mg/kg bw/day). Hepatocellular carcinoma were observed in all dose groups, aside from the female control animals in the absence of a dose response (in males: 24, 17, 19 and 29% and in females 0, 6, 6 and 6% for dose groups 0, 6.72/8.38, 41.6/51.2 and 267/318 mg/kg bw/day for males and females respectively).

The B6C3F1 mouse strain is very sensitive to the development of liver tumours (Guidance on the Application of CLP Criteria) and, as such, the hepatocellular tumour findings in this study appear of limited toxicological significance. The CLH report included HCD from the open literature for studies of 104 weeks in length conducted between 1980 – 1983 to illustrate this high spontaneous rate of formation of liver adenoma and carcinoma in this strain of mouse. The current study was performed between the years 1981 – 1983.

According to the performing laboratory there are no HCD available within a 5 year period of this study. However, HCD from the same laboratory in the same strain of mice was provided from studies conducted during 1988 – 1992. It is noted that the HCD provided is for study periods of 109 weeks rather than the period of 104 weeks for which this study was carried out.

Table: *Historical control data (1988 – 1992) for studies of 109 weeks carried out in B6C3F1 mice*

	Males			**Females**		
No. of animals examined	500			500		
	Incidence	**Rate**	**Range**	**Incidence**	**Rate**	**Range**
Hepatic nodule[#]	97	19.4%	8 – 42%	41	7.5%	2 – 12%
Hepatocellular adenoma	235	47.0%	22 - 76%	81	14.7%	8 – 32%
Hepatocellular carcinoma	102	20.4%	12 – 28%	35	6.4%	2 – 12%

The term used originally was focus of cellular alteration.

The statistically significant increase of liver adenoma observed in top dose females is almost within the range provided by the laboratory HCD.

In view of the high sensitivity of this mouse strain, RAC agrees with the DS that these tumour findings are of low concern and probably not indicative of hexythiazox carcinogenicity, and thus alone do not support classification.

In males and females of the top dose group another type of liver tumour was also observed. Hepatoblastoma was seen in 3 males (6%) and 1 female (2%). There was no statistical significance to these findings. The hepatoblastoma were defined as poorly differentiated and malignant, although no metastases were found.

No laboratory control data were provided from studies conducted within a 5 year window of the current study for hepatoblastoma incidence. However, laboratory HCD were provided from 6 independent studies conducted during 1985 – 1991 with a total of 251 males and 300 females showing this to be a relatively rare tumour type in control mice. These data show that the hepatoblastoma incidence range in males is 0 - 2% and in females 0%. The NTP database reveals hepatoblastoma incidence ranges of 0 - 2% for both control male and female B6C3F1 mice (950 animals in 19 studies during 1984-1994). Whilst the single incidence in females is within this range, the finding of 3 incidences in top dose males is slightly above.

The small increases in hepatoblastoma incidence compared to controls were observed alongside the other liver tumour findings and signs of liver toxicity in both male and female mice, especially in males. Absolute and relative liver weights were increased in both males and females of the top dose groups throughout the study. At study termination the increases were 133% (absolute) and 157% (relative) in males and 116% (absolute) and 120% (relative) in females (compared to controls). There was also an increase in the number of males with liver necrosis at the top dose (4, 5, 5 and 8 in the control, low, mid and high dose groups respectively). The number of hepatic nodules (non-neoplastic hepatoproliferative lesions including focus/area of cellular alteration of both basophilic and eosiniophilic natures and hyperplastic change) was increased in males of the top dose group (10, 8, 14 and 30 in control, low, mid and high dose groups). The DS further noted that 2 out of 3 males with hepatoblastoma were also found to have both liver adenoma and carcinoma.

The slight increase in the incidence of liver hepatoblastoma observed in top-dose male and female mice could be viewed as a sign of hexythiazox carcinogenicity. This tumour type was seen above the range of the HCD provided by the DS in male mice and above the HCD range of the performing laboratory but within the range of NTP database in the females, but the increased tumour incidence was not found to be statistically significant. No metastases were found and the overall survival of mice was not affected. Given the increased frequency of adenomas and carcinomas seen in this study, in what is clearly a highly sensitive mouse strain, along with other findings of toxicity that indicated the livers of high dose mice were significantly compromised, RAC is of the opinion that the hepatoblastomas seen in this study are of low toxicological significance. Additionally, no liver tumours were observed in rats, despite the presence of liver toxicity. Therefore, overall, RAC considers the findings of increased adenoma, carcinoma and hepatoblastoma in mice to be insufficient evidence to support classification.

Conclusion regarding carcinogenic hazard

There are no reliable findings in rats or mice to indicate a carcinogenic effect following long-term exposure to hexythiazox. In support of this, hexythiazox is not genotoxic. Therefore, in agreement with the DS, **no classification for carcinogenicity is proposed**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Hexythiazox has currently the following classification for the environment in Annex VI of CLP: Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The hazard classification of hexythiazox according to the Dangerous Substances Directive 67/548/EEC (DSD) was first agreed in the November 1995 meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances (Pesticides). The Working Group agreed to the classification as N; R50-53 (ECBI/94/95 - Rev. 1). The agreed classification was included in Annex I of the DSD in the 24th ATP (98/73/EC) and translated to the CLP Classification as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 in Annex VI of CLP.

The DS's proposal for consideration by RAC was to retain the existing environmental hazard classification and to add an M-factor of 1 for both Aquatic Acute 1 and Chronic 1. The classification is based on the substance being not-rapidly degradable, a BCF ≥ 500 in fish, and the high toxicity observed in *Daphnia magna* (EC_{50} of 0.36 mg/L, NOEC of 0.0277 mg/L).

The three major metabolites of hexythiazox, PT-1-2, PT-1-3 and PT-1-9, had a similar or lower toxicity in aquatic toxicity studies than the parent compound. Therefore, the classification and labelling proposal for hexythiazox was based solely on the ecotoxicity of the parent compound.

Degradation

Hexythiazox was hydrolytically stable at pHs 5 and 7 and had a hydrolysis half-life ranging from 370 to 504 days at pH 9 and at 22°C in a non-GLP study following the guideline BBA technical bulletin No. 55, part 1. Hence, the substance is considered hydrolytically stable for classification purposes.

The CLH dossier includes two studies on photolytic degradation in water but the studies are not considered reliable and relevant for classification purposes. Also a study on soil photolysis is available where the photolytical half-life of hexythiazox was determined to be approximately 116 days. However, the study is not fully reliable and is not considered relevant for classification purposes.

There are no biodegradation screening or surface water simulation tests available for the substance.

Two water-sediment simulation studies were included in the dossier. The GLP-compliant studies were conducted using radiolabelled test material at 20 °C and followed OECD TG 308 or similar guidelines (BBA Guideline part IV, 5-1 (1990), EPA Guidelines, Subdivision N, §162-4 (1982)). The test substance dissipated rapidly from the water phase having a DT_{50} in the range of 0.5-11 days in water. In the sediment phase, the substance dissipated through degradation and formation of non-extractable residues. The sediment DT_{50} for primary degradation ranged from 37 to 42 days in two systems and in other two systems the DT_{50}s were not calculated due to very slow dissipation. The calculated whole system degradation half-lives ranged from 33 to 156 days.

The major degradation products detected and identified in the water-sediment study with thiazolidine ring labelled hexythiazox were PT-1-2, PT-1-9. In the water-sediment study with cyclohexyl labelled hexythiazox two additional degradation products were identified: PT-1-8-c and PT-1-8-t. Mineralisation accounted for 2.5-6 % of the applied radioactivity after 100 days.

Three soil simulation studies with hexythiazox are available in the dossier. The studies were performed in total with seven different soils at temperatures ranging from 15 to 25° C. Two of the studies were GLP-compliant and followed (draft) OECD TG 307 and SETAC guideline 1995. The third study is mentioned to have followed a guideline "similar to SETAC guideline, Part 1, Section 1". The calculated DT_{50} values for primary degradation at standard conditions of 20°C and pF 2 ranged from 7.8 to 56.0 days and the geometric mean of the DT_{50}s is 23.7 days. The major metabolites detected were PT-1-2, PT-1-3 and PT-1-9. Mineralisation at the end of the tests (after 84-122 days) ranged from 5 % to 36 % of the applied radioactivity.

The DT_{50} calculated for the water phase in the water-sediment studies cannot be used to assess rapid degradability because besides degradation also adsorption to sediment affected the disappearance of the substance from the water phase. The whole water-sediment system degradation half-lives and the sediment DT_{50}s are well above 16 days. In the soil simulation studies the DT_{50} in four out of seven soils were above 16 days and the geometric mean of the available DT_{50} values is 23.7 days. Hence, based on the available water-sediment system and soil data the DS concluded that the substance is not rapidly degradable for classification purposes.

In the CLH dossier data from soil field dissipation studies and anaerobic soil simulation study are also available but these are not considered relevant for the classification purposes.

Bioaccumulation

A log Kow of 2.67 was measured for hexythiazox in a study following OECD TG 107.

Two fish bioconcentration studies were included in the CLH dossier. One of them is not considered valid, as the study report is short and the study did not fulfill current requirements of bioconcentration in fish tests.

The other study followed US EPA Pesticide Assessment Guideline (1982), which is similar to OECD TG 305. In the study, bluegill sunfish were exposed under flow-through conditions to radiolabelled hexythiazox at mean measured concentrations of 0.0036 mg/L and 0.034mg/L during 28 days. The depuration phase was 14 days. 70 fish were used in both test concentration groups as well as in control group. N,N- dimethylformamide was used as solvent and a solvent control containing 0.05 mL N,N- dimethylformamide/L was included in the test.

The highest whole fish BCFs of 1600 and 1000 L/kg based on total ^{14}C for the low and high exposure groups, respectively, were measured on day 21. On day 28 the BCFs were 1100 and 850, respectively. Accumulation was significantly higher in the viscera than in the remaining carcass and muscles. 89 % to 94% of the applied radioactivity was depurated within 14 days after exposure. No uptake or depuration rate constants were calculated. Furthermore, fish lipid content was not measured, and hence, the BCFs cannot be lipid normalised.

On day 28 of the uptake phase the proportion of unmetabolized hexythiazox of total ^{14}C residues varied from 1.5 to 22.6 % in muscle and viscera. Most of the ^{14}C residues were unidentified polar metabolites, hydroxyl metabolites and their conjugates. Hence, the BCF values determined based on total ^{14}C may overestimate the bioconcentration of the parent substance. However, it is noted that the three major metabolites identified for hexythiazox in the degradation studies, PT-1-2, PT-1-3 and PT-1-9, had a similar or lower toxicity in aquatic toxicity studies than the parent compound. Therefore, since the metabolites in fish were not identified to a substance level, it cannot be ruled out that some of them could potentially be hazardous to the aquatic environment according to CLP criteria.

There is some further uncertainty in the results of the study as the results are not lipid normalised, no uptake and depuration rate constants are determined. Therefore, the DS decided to compare the available BCF based on total ^{14}C with the CLP criterion, and hence, the substance may be considered bioaccumulative for classification and labelling purposes.

Aquatic toxicity

The aquatic toxicity studies with hexythiazox included in the CLH dossier are shown in the below table. It is noted that some further studies with fish, daphnia and algae are included in the DAR of hexythiazox. However, the studies are not included in the CLH dossier because the DS considered them not valid due to study deficiencies, *e.g.* lack of analytical determination of the exposure concentrations.

The major metabolites of hexythiazox, PT-1-2, PT-1-3 and PT-1-9, had a similar or lower toxicity to *Oncorhynchus mykiss*, *Daphnia magna* and *Selenastrum capricornutum* (also known as *Pseudokirchneriella subcapitata*) than the parent compound hexythiazox. Therefore, the classification proposal was based only on hexythiazox ecotoxicity.

Method, conditions	**Test organism**	**Endpoint**	**Toxicity value (mg/L)**	**Remarks**	**Reference**
Short-term toxicity to fish					
OECD TG 203 (1992), 92/69/EEC, C.1 (1992) GLP Static	*Oncorhynchus mykiss*	96-h LC_{50}	> 0.2 (meas.) > 100 (nom.)	Limit test. No mortality and no signs of toxicity at tested concentration.	DAR IIA; 8.2/01

Method, conditions	Test organism	Endpoint	Toxicity value (mg/L)	Remarks	Reference
OECD TG 203 GLP Static Solvent used	*Oncorhynchus mykiss*	96-h LC_{50}	> 4.0 (meas.) > 9.2 (nom.)	No mortality but signs of toxicity possibly caused by non-dissolved test substance particles	DAR IIA; 8.2/04
OECD TG 203 GLP Static Solvent used	*Lepomis macrochirus*	96-h LC_{50}	3.2 (meas.) 18 (nom.)		DAR IIA; 8.2/05
OECD TG 203 GLP Semi-static Solvent used	*Cyprinus carpio*	96-h LC_{50}	> 14.1 (meas.)	Limit test No mortality and no signs of toxicity	DAR Additional report IIA; 8.2/33
OECD TG 204 GLP semi-static	*Oncorhynchus mykiss*	28-d NOEC (mortality)	0.04 (meas.) 20 (nom.)	Prolonged acute test. Only one test concentration. No mortality or other signs of toxicity observed.	DAR IIA; 8.2/10
Short-term toxicity to aquatic invertebrates					
OECD TG 202 GLP Static	*Daphnia magna*	48-h EC_{50}	> 0.47 (meas.)	No immobility observed	DAR IIA; 8.2/13
OECD TG 202 GLP Static solvent used	*Daphnia magna*	48-h EC_{50}	**0.36** (meas.)	Toxic effects observed, Precipitated or undissolved particles were observed at 0.422 and 0.658 mg/L after 48 h	DAR Additional report IIA; 8.2/34
Short-term toxicity to algae					
OECD TG 201 GLP Static	*Scenedesmus subspicatus*	72-h ErC_{50}	> 0.4 (meas.)	Based on two measured concentrations and the median analytical recovery rate	DAR IIA; 8.2/25
OECD TG 201 GLP Static Solvent used	*Pseudokirchneriella subcapitata*[1]	96-h ErC_{50}	> 72.0 (meas.)	Limit test	DAR Additional report IIA; 8.2/35

Method, conditions	Test organism	Endpoint	Toxicity value (mg/L)	Remarks	Reference
Long-term toxicity to aquatic invertebrates					
US EPA pesticide Assessment Guideline, Subdivision E No. 72- 4(b), 1982 GLP flow- through solvent used	*Daphnia magna*	21-d NOEC (reproduction)	**0.0277** (meas.)		DAR IIA; 8.2/21
OECD TG 202, Part II (reproduction test) GLP semi-static	*Daphnia magna*	21-d NOEC (reproduction) 21-d EC_{50}	**0.0418** >0.0836 (meas.)	Results based on three measured concentrations and the median analytical recovery rate	DAR IIA; 8.2/22
OECD TG 202 GLP semi-static solvent used	*Daphnia magna*	21-d NOEC (reproduction)	0.055 (meas.)		DAR IIA; 8.2/24
Long-term toxicity to algae					
OECD TG 201 GLP static	*Scenedesmus subspicatus*	72-h NOErC 72-h NOEbC	0.4 0.2 (meas.)	No effects in growth rate were observed at the highest concentration tested representing the maximum solubility.	DAR IIA; 8.2/25
OECD TG 201 GLP Static Solvent used	*Pseudokirchneriella subcapitata*[1]	96-h NOEC (cell number)	72.0 (meas.)	Limit test No effects were observed.	DAR Additional report IIA; 8.2/35
Other aquatic organisms (including sediments)					
BBA guideline proposal (1995) GLP Static solvent used	*Chironomus riparius*	21-d NOEC 21-d EC_{50}	1.7 > 1.7 (meas.)	Based on initial measured concentration No effects were observed.	DAR IIA; 8.2/29

[1] formerly known as *Selenastrum capricornutum*

Acute toxicity

Four short-term toxicity tests with fish following OECD TG 203 were included in the CLH dossier.

In three of the acute tests with *Oncorhynchus mykiss* and *Cyprinus carpio* no mortality was observed at the tested nominal concentrations (maximum concentrations ranging from 9.2 to 100 mg/L) which were well above the measured water solubility of the substance (0.12 mg/L at 25 °C). The maximum mean measured concentrations were in the range of 0.2 to 14.1 mg/L.

In the acute test with *Lepomis macrochirus* a 96-h LC_{50} of 3.2 mg/L (95% C.I. 2.6-5.6 mg/L) based on mean measured concentration was determined. No mortality was seen in treatments at or below the mean measured concentration of 1.2 mg/L. Abnormal behaviour and/or colouration was observed at mean measured concentration of 0.25 mg/L and above.

Two short-term toxicity tests with *Daphnia magna* following OECD TG 202 were included in the CLH dossier. In one of the studies a 48-h EC_{50} of 0.36 mg/L (95% C.I. 0.31-0.42 mg/L) was determined for immobilisation based on mean measured concentrations. In the other study, no immobilisation of daphnia was observed during 48 hours study duration at the tested concentrations which were up to 0.47 mg/L based on mean measured concentrations.

Two algal toxicity tests with hexythiazox following OECD TG 201 are included in the CLH dossier. In a 72-h static test with *Scenedesmus subspicatus* the inhibition of growth and biomass reached only 6 and 18 %, respectively, at the highest measured concentration of 0.4 mg/L. Therefore, ErC_{50} and EbC_{50} are assumed to be > 0.4 mg/L.

The other algal test was a 96-h limit test with *Pseudokirchneriella subcapitata* using 100 mg/L nominal hexythiazox concentration (mean measured concentration 72.0 mg/L). No statistically significant effects were observed in the algal growth. RAC notes that both the nominal and measured concentrations were well above the water solubility of the substance. However, since no effects were observed, the study can be used as supporting information indicating that hexythiazox shows no toxicity to algae at the limit of its water solubility.

Chronic toxicity

No relevant chronic toxicity tests with fish are available for hexythiazox. The CLH dossier includes a limit test following OECD TG 204 with *Oncorhynchus mykiss* but this is considered a prolonged acute test with mortality as the main endpoint. Hence, the study was considered not relevant by the DS for deciding on the chronic classification.

Three chronic tests with *Daphnia magna* are available. In the key study performed following EPA FIFRA Guideline No. 72-4(b), under flow-through conditions a 21-d NOECs of 0.00607, 0.0277 and 0.0277 mg/L were determined for immobilisation, mean of live young per adult reproduction day and length (growth), respectively. However, the immobilisation did not follow a clear dose-response while the reproduction and growth endpoints did follow. According to the DS it is therefore not clear whether the observed effects in immobilisation were substance related or whether other factors may also have influenced, *e.g.* the lowered dissolved oxygen concentration during the test. The dissolved oxygen concentration occasionally dropped below 60% of the air saturation level in all treatment groups except in the controls. On day 12 a gentle aeration was initiated and the dissolved oxygen concentrations were close to or above 60 % shortly after that but at the end of the test they ranged from 43 to 76 % of saturation. Nevertheless, it is noted that the dissolved oxygen concentration stayed in all test chambers above 3 mg/L throughout the test, which is the limit indicated in the OECD TG 211.

In conclusion, the DS considered the test valid for classification purposes but decided to use the NOEC of 0.0277 mg/L for mean young per adult reproduction day for classification instead of the lower NOEC determined for immobilisation because a clear dose-response was not observed for that endpoint.

In a chronic toxicity study with *Daphnia magna* following OECD TG 202 part II, only one immobile individual was observed, at the highest measured test concentration of 0.0836 mg/L. Statistically significant effects in reproduction (mean number of live/immobile young produced per female) were also only observed at the highest test concentration. Hence, a 21-d NOEC of 0.0418 mg/L was determined for reproduction based on median analytical recovery rate.

In another OECD TG 202 semi-static study a 21-d NOECs of 0.055 mg/L for reproduction and length are reported for *Daphnia magna*. However, it is noted that the number of individuals used to study reproduction and length was lower (seven individually exposed daphnids) than that recommended in the current OECD 211 guideline and no statistical analyses were made. Therefore, RAC considers that the NOECs are not fully reliable. However, the study can be used as supporting information for classification purposes.

Based on the data from the 72-hours static test with *Scenedesmus subspicatus* a 72-h NOErC of > 0.4 mg/L and 72-h NOEbC of 0.2 mg/L are determined. In the other algal test with *Pseudokirchneriella subcapitata* no statistically significant effects were observed in the algal growth at concentrations well above the limit of the water solubility of hexythiazox.

Other aquatic organisms (including sediment)

The CH dossier included a chronic 21-day test following a BBA-guideline proposal (1995) on the effects of "water-spiked" hexythiazox on the development of the sediment dwelling larvae of the midge *Chironomus riparius*. Seven nominal hexythiazox water concentrations in the range of 0.1-6.4 mg/L were used in the test. The lowest emergence rate was found at the highest hexythiazox exposure concentration but the differences in emergence rates were not statistically significant. No statistically significant differences in development rates between control and exposed midges were found either. Hence, a 21-d NOEC is determined to be 1.7 mg/L based on initial measured concentration of hexythiazox in the water layer.

RAC notes that this study is not relevant for classification purposes as it is not a pelagic test and because valid data is available on other aquatic invertebrates (*Daphnia magna*).

Comments received during public consultation

Three MSCAs expressed their support for the DS's proposal for the environmental classification and M-factors. One MSCA commented on the *Daphnia magna* tests. First, they asked whether observation data for animal inspections was included in the acute *Daphnia* test (Additional Report IIA, 8.2/34) used as key study. According to the MSCA this is important to rule out possible physical effects caused by undissolved test substance particles. They also asked whether 24/48-h immobilisation data is available in the chronic studies to support the proposed 48-h EC_{50} value.

Regarding the chronic daphnia study used as key study (DAR IIA; 8.2/21) for the classification proposal, the MSCA commented that additional statistical analysis would be needed to consider if the 21-d immobilisation NOEC is invalid as the oxygen levels were above the limit indicated in similar guidelines. In addition, they asked whether data from other chronic daphnia tests can help in the interpretation of the immobilisation NOEC of the key study.

The DS's responses to the comments are found in the RCOM document. RAC agrees with the DS's responses.

Assessment and comparison with the classification criteria

Degradation

Hexythiazox is hydrolytically stable at pHs 5 and 7 and has a very long hydrolytic half-life (>370) at pH 9. No biodegradation screening tests or surface water simulation tests are available. In the available water-sediment simulation studies the whole system half-lives ranged from 33 to 156 days and the sediment DT_{50}s were above 37 days. The DT_{50}s for the water phase cannot be used for classification purposes as, besides degradation, adsorption from the water phase to sediment is also expected to have influenced these values. In the soil simulation studies the DT_{50} in four out of seven soils were above 16 days and the geometric mean of the available DT_{50} values is 23.7 days. Therefore, RAC agrees with the DS's proposal that hexythiazox is considered not rapidly degradable for the purposes of classification and labelling.

Bioaccumulation

The measured log Kow of hexythiazox is 2.67 at 22°C, which is below the cut-off value of 4 indicated in CLP for bioaccumulation potential. However, measured whole fish BCFs of 850-1100 L/kg are determined for bluegill sunfish, which are above the trigger value of ≥ 500 L/kg indicated in the CLP. RAC notes that the BCFs are based on total radioactive residues and they may overestimate the bioaccumulation of the parent substance since most of the ^{14}C residues found in muscle and viscera at the end of the uptake phase corresponded to metabolites of the parent substance. However, the metabolites were not identified to a compound level and it cannot be ruled out that some of them could be hazardous to aquatic organisms. Further uncertainty is related to the BCF value since it is not lipid-normalised and the uptake and depuration rates were not determined in the study. Therefore, RAC concludes that based on the available information no firm conclusion can be drawn on the bioaccumulation of the parent substance or its metabolites. It is noted that this does not affect the conclusion on the environmental classification as the substance is considered not rapidly degradable for classification purposes.

Aquatic Toxicity

The major metabolites of hexythiazox, PT-1-2, PT-1-3 and PT-1-9, had a similar or lower toxicity to *Oncorhynchus mykiss*, *Daphnia magna* and *Selenastrum capricornutum* (also known as *Pseudokirchneriella subcapitata*) than the parent compound hexythiazox. Therefore, RAC agrees with the DS that the classification proposal is based only on hexythiazox ecotoxicity.

Valid acute toxicity data is available for fish, aquatic invertebrates and algae. Based on the available data, daphnia were the most sensitive group with the lowest 48-h EC_{50} value of 0.36 mg/L for *Daphnia magna*. This is below the classification threshold of 1 mg/L for Aquatic Acute 1 and in the range of $0.1 < L(E)C_{50} \leq 1$ mg/L leading to an acute M-factor of 1.

Valid chronic data is available only for aquatic invertebrates and algae. Hence, the DS assessed the chronic classification of hexythiazox using two approaches according to Figure 4.1.1. of CLP and the most stringent outcome was selected for classification:

1. Based on the available chronic data on aquatic invertebrates and algae, the DS concluded that the lowest valid chronic value is the 21-d NOEC of 0.0277 mg/L for *Daphnia magna* which is below the classification threshold of ≤ 0.1 mg/L for Aquatic Chronic 1 for not rapidly degradable substances and justifies a chronic M-factor of 1 ($0.01 < NOEC \leq 0.1$ mg/L).

2. In case of a substance which is non-rapidly degradable and/or for which the experimentally determined BCF ≥ 500, and for which adequate chronic toxicity data are not available, classification is based on the combination of acute aquatic toxicity data and environmental fate data. Based on this criterion, the DS concluded that Aquatic Chronic category 2 is applicable for hexythiazox based on 96 h LC_{50} of 3.2 mg /L for *Lepomis macrochirus* (1< L(E)C_{50} ≤ 10 mg/L).

Consequently, the DS used the most stringent outcome, which is classification as Aquatic Chronic 1 with an M-factor of 1 based on the available chronic value of *Daphnia magna*.

RAC notes that the LC_{50} of 3.2 mg/L reported for *Lepomis macrochirus* in the CLH dossier is one order of magnitude above the measured water solubility of hexythiazox (0.12 mg/L at 25°C). Furthermore, some of the nominal concentrations (up to 15 mg/L) used in the *Lepomis macrochirus* study were two orders of magnitude higher than the water solubility and there is no information on whether undissolved test material was present in the test solutions. Therefore, it cannot be excluded that some of the observed effects may have been caused by physical effects of undissolved substance particles. Since no mortality was observed at or below the mean measured concentration of 1.2 mg/L in the *Lepomis macrochirus* study, and no effects were observed in the other available acute fish toxicity studies with maximum mean measured concentrations in the range of 0.2 to 14.1 mg/L, RAC considers that the substance shows no acute toxicity to fish at the limit of its water solubility (0.12 mg/L). Therefore, it is not possible to classify the substance for long-term hazard based on the acute fish data by using the surrogate method.

Based on the acute data available for the three trophic levels, RAC considers that aquatic invertebrates are the most sensitive group. Effects on mortality in fish were only observed at concentrations one order of magnitude higher than the water solubility of the substance, in a study where solvent was used to enhance the solubility of the substance. Hence, although RAC considers that the available LC_{50} for *Lepomis macrochirus* is not valid to be used in the surrogate approach, RAC agrees with the conclusion of the DS to base the chronic classification on the available NOEC for *Daphnia magna*.

Conclusion on Classification

Based on the above assessment, RAC agrees with the DS's proposal that hexythiazox meets the classification criteria for **Aquatic Acute 1 (H400)** with an **acute M-factor of 1** and **Aquatic Chronic 1 (H410)** with a **chronic M-factor of 1**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).