Committee for Risk Assessment
RAC

Annex 1

Background document

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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted
30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Hexythiazox

EC Number:
CAS Number: 78587-05-0
Index Number: 613-125-00-6

Contact details for dossier submitter: Finnish Competent Authority, Finnish Safety and Chemicals Agency (Tukes), Finland

Version number: 2 Date: 19.10.2017

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Hexythiazox***
EC number:	
CAS number:	***78587-05-0***
Annex VI Index number:	***613-125-00-6***
Degree of purity:	minimum purity 97.6 % (1:1 mixture of (4R, 5R) and (4S, 5S)
Impurities:	Confidential, no impurity is considered relevant for the classification of hexythiazox.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Aquatic Acute 1; H400 Aquatic Chronic 1; H410
Current proposal for consideration by RAC	Aquatic Acute 1; H400, M-factor of 1 Aquatic Chronic 1; H410, M-factor of 1
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1; H400, M-factor of 1 Aquatic Chronic 1; H410, M-factor of 1

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

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CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives			Not classified	Hazard class not assessed in this report
2.2.	Flammable gases			Not classified	Hazard class not assessed in this report
2.3.	Flammable aerosols			Not classified	Hazard class not assessed in this report
2.4.	Oxidising gases			Not classified	Hazard class not assessed in this report
2.5.	Gases under pressure			Not classified	Hazard class not assessed in this report
2.6.	Flammable liquids			Not classified	Hazard class not assessed in this report
2.7.	Flammable solids			Not classified	Hazard class not assessed in this report
2.8.	Self-reactive substances and mixtures			Not classified	Hazard class not assessed in this report
2.9.	Pyrophoric liquids			Not classified	Hazard class not assessed in this report
2.10.	Pyrophoric solids			Not classified	Hazard class not assessed in this report
2.11.	Self-heating substances and mixtures			Not classified	Hazard class not assessed in this report
2.12.	Substances and mixtures which in contact with water emit flammable gases			Not classified	Hazard class not assessed in this report
2.13.	Oxidising liquids			Not classified	Hazard class not assessed in this report
2.14.	Oxidising solids			Not classified	Hazard class not assessed in this report
2.15.	Organic peroxides			Not classified	Hazard class not assessed in this report
2.16.	Substance and mixtures corrosive to metals			Not classified	Hazard class not assessed in this report
3.1.	Acute toxicity - oral			Not classified	Hazard class not assessed in this report
	Acute toxicity - dermal			Not classified	Hazard class not assessed in this report
	Acute toxicity - inhalation			Not classified	Hazard class not assessed in this report
3.2.	Skin corrosion / irritation			Not classified	Hazard class not assessed in this report
3.3.	Serious eye damage / eye irritation			Not classified	Hazard class not assessed in this report
3.4.	Respiratory sensitisation			Not classified	Hazard class not assessed in this report

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CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.4.	Skin sensitisation			Not classified	Hazard class not assessed in this report
3.5.	Germ cell mutagenicity			Not classified	Hazard class not assessed in this report
3.6.	Carcinogenicity	No classification	Not applicable	Not classified	Data conclusive but not sufficient for classification
3.7.	Reproductive toxicity			Not classified	Hazard class not assessed in this report
3.8.	Specific target organ toxicity –single exposure			Not classified	Hazard class not assessed in this report
3.9.	Specific target organ toxicity – repeated exposure			Not classified	Hazard class not assessed in this report
3.10.	Aspiration hazard			Not classified	Hazard class not assessed in this report
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1 H400** **Aquatic Chronic 1 H410**	**M-factor of 1** **M-factor of 1**	**Aquatic Acute 1 H400** **Aquatic Chronic 1 H410**	
5.1.	Hazardous to the ozone layer			Not classified	Hazard class not assessed in this report

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Inconclusive, conclusive but not sufficient for classification, hazard class not assessed in this report or hazard class not applicable

Labelling:

Signal word: Warning
Pictogram: GHS09
Hazard statements: H400: Very toxic to aquatic life, H410: Very toxic to aquatic life with long lasting effects
Precautionary statements: No precautionary statements are proposed since they are not included in Annex VI of Regulation (EC) No 1272/2008
Proposed notes assigned to an entry: None

2 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

2.1 History of the previous classification and labelling

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 not to classify the substance with Xn; R20 but 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 Aquatic Acute 1: H400 and Aquatic Chronic 1: H410 in Annex VI of CLP. At the time of submission the substance is not registered under the REACH Regulation (1907/2006).

2.2 Short summary of the scientific justification for the CLH proposal

Hexythiazox was evaluated by Finland as an existing active substance in a review program covered by the Community legislation on placing plant protection products on the market (in the scope of Regulation EC 1107/2009 repealing Directive 91/414/EEC). During the review program (2009-2010) Finland did not propose to classify hexythiazox for carcinogenicity but concerns were raised by some Member states with regard to the potential classification of hexythiazox for carcinogenicity. Thus, EFSA concluded that the final decision has to be taken by ECHA (EFSA 2010). Carcinogenicity and aquatic toxicity are evaluated in this CLH dossier.

This classification proposal is based on the Draft Assessment Report (DAR; Finland 2006), Addendum (2007), Additional report to the DAR (Finland 2009), Final Addendum to Additional report (Finland 2010) and EFSA conclusion on the peer review (2010) and on scientific peer-reviewed open literature.

There is one carcinogenicity study available in the rat and one carcinogenicity study available in the mice. In the rat (F344), a two-year dietary administration of hexythiazox resulted in slightly but not statistically significantly increased incidence of thyroid parafollicular cell adenoma in high dose (163 mg/kg bw/d) males compared to concurrent controls. Moreover, the incidences of mammary gland fibroadenoma were increased in hexythiazox treated male rats compared to concurrent controls at the end of the study and the incidence of testicular interstitial cell (Leydig cell) adenoma was slightly increased in high and mid dose (23 mg/kg bw/d) males at 12 months' interim sacrifice. There are no fully acceptable historical control data available for these tumours from the performing laboratory. There was no clear dose response in the incidences of thyroid parafollicular cell adenoma. Mammary gland fibroadenoma is relatively common tumour in F344 rats and the dataset revealed no remarkable morphological or histological findings that would indicate hormonal mechanism of mammary tumour formation in males only. Therefore, it remains unclear whether the slightly increased incidences of parathyroid and mammary gland adenomas are related to the hexythiazox treatment. It is also considered unlikely that the slight increases in the incidences of these benign tumour types in high dose male rats only would be toxicologically significant. Since Leydig cell adenoma is a common spontaneous tumour in F344 rats and there were no differences in the incidences of this tumour type at the end of the study, we do not consider the findings at 12 month interval relevant for carcinogenicity classification.

In the mice (B6C3F1), two years treatment with high hexythiazox dose (267/318 mg/kg bw/day) resulted in statistically significant increase in the total number of hepatocellular tumours, including both benign and malignant tumours, in both sexes. No acceptable historical

control data (HCD) from the performing laboratory is available, but the observed incidences of hepatic tumours are within or slightly over the incidence ranges of these tumour types in the performing laboratory at different time frames and similar incidences have been observed also in other laboratories in B6C3F1 mice. The data indicates that the increased incidences of liver tumours in high dose mice are presumably caused by hepatoxicity and proliferative stimulus on the liver. However, the alterations of tumour incidences occurred only after 18 months treatment 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. B6C3F1 mice strain is exceptionally sensitive to promotion of liver tumours with high spontaneous incidences. Taken also into account the fact that in spite of signs of hepatoxicity, there were no effect on hepatic tumour incidences in hexythiazox treated rats, we conclude that the increased incidence of liver tumours after two years high dose hexythiazox treatment in B6C3F1 mice strain is not sufficient evidence for carcinogenicity classification. The *in vitro* and *in vivo* studies revealed no evidence of mutagenic potential for hexythiazox.

Altogether the findings in hexythiazox treated rats and mice are considered as weak and inconsistent evidence and not sufficient to warrant carcinogenicity classification. Therefore, no classification is proposed for carcinogenicity.

It is recognised that the current Annex VI entry for Hexythiazox includes a classification with Aquatic Acute 1; H400 and Aquatic Chronic; H410 Aquatic acute 1. According to CLP, respective M-factors should be allocated.

Hexythiazox is not rapidly degradable according to the CLP criteria. This conclusion is based on hydrolysis test as well as water/sediment and soil simulation tests. The hydrolysis half-lives were ≥370 days at environmentally relevant temperature. Regarding the water/sediment simulation tests, as it is not possible to differentiate the degradation rate in water from that in sediment, the dissipation half-life in water cannot be used to evaluate rapid degradation. In the absence of biodegradation data for the surface water compartment the half-lives in water/sediment systems and in soil were compared to CLP criteria. The primary degradation half-lives were above 16 days in sediments (geometric mean 39 days), water-sediment-systems (geometric mean 72 days), and in four of the five tested soils (geometric mean 23.7 days).

A measured octanol-water partition coefficient is available and revealed a logPow of 2.67 at 25°C. The mean whole fish bioconcentration factor (BCF) was determined as 975 for bluegill sunfish which is above the trigger value of ≥ 500 according to the CLP. Based on the measured BCF value and the criteria set in CLP hexythiazox has the potential to bioaccumulate.

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 is based solely on the ecotoxicity of the parent compound.

Available data show that aquatic invertebrates were the most sensitive species. Based on acute aquatic toxicity data (on measured concentrations) with L(E)C50 values below 1 mg/L, classification with Aquatic Acute 1 is applicable. An acute M-factor of 1 is applicable based on 0.1 < L(E)C50 ≤ 1 mg/L considering the various *Daphnia magna* EC50 data (0.36 mg/L to > 0.47 mg/L) in this range for hexythiazox.

Since no adequate chronic data is available for all three trophic levels, the classification of hexythiazox in a chronic category is assessed using two approaches according to CLP (2nd ATP, Regulation No 286/2011):

1. In the case of non-rapidly degradable substances for which there are adequate chronic toxicity data available classification of Aquatic Chronic 1 is applicable for hexythiazox based on NOEC value of 0.0418 mg/l for *Daphnia magna* (≤ 0.1 mg/l) with a chronic M-factor of 1 (0.01 < NOEC ≤ 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; Aquatic Chronic category 2 is applicable for hexythiazox based on 96 h LC50 of 3.2 mg /l for Rainbow trout (1< L(E)C50 ≤ 10 mg/l).

The most stringent outcome shall be chosen and therefore hexythiazox shall be classified as Aquatic Chronic 1 with M-factor of 1 according to Regulation EC 1272/2008.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Table 4: Current classification and labelling of hexythiazox in Annex VI of CLP

Classification		Labelling	
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Pictogram, Signal Word (Code(s)	Hazard Statement Code(s)
Aquatic acute 1 Aquatic chronic 1	H400 H410	GHS09 Wng	H410

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Classification and labelling of hexythiazox is according to its current entry in Annex VI to CLP.

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.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Hexythiazox is a pesticidal active substance in the meaning of Directive 91/414/EEC (subsequently replaced by EU Regulation 1107/2009). In accordance with Article 36(2) of the CLP-regulation, hexythiazox should be subject to harmonized classification and labelling. Hexythiazox already has an entry in Annex VI to CLP; therefore, the present CLH report proposes to revise the existing Annex VI entry and therefore does not address all hazard classes.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

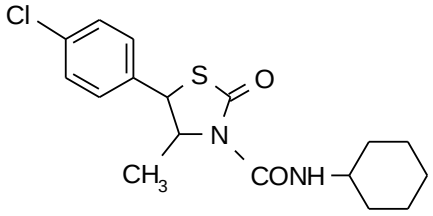
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	
EC name:	
CAS number (EC inventory):	78587-05-0
CAS number:	78587-05-0
CAS name:	3-Thiazolidinecarboxamide, 5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-, (4*R*,5*R*)-*rel*-
IUPAC name:	(4*RS*,5*RS*)-5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide
CLP Annex VI Index number:	613-125-00-6
Molecular formula:	$C_{17}H_{21}ClN_2O_2S$
Molecular weight range:	352.9 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Hexythiazox	≥ 97.6% (w/w)	97.6 – 99.43% (w/w)	1:1 mixture of 4R, 5R and 4S, 5S Minimum purity of the active substance as manufactured

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are no known relevant impurities. Details on the impurities are considered to be confidential and further information is provided in the IUCLID file and flagged confidential.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The purity of hexythiazox tested in the studies ranged from 96.8% to >99.9%. Information on the actual purity used is provided in the relevant tables of this report. The tested material in all cases is considered to be equivalent to and representative of that specified above.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101.3 kPa	Pure material: Pale yellow odourless powder	DAR Additional Report IIA 2.4.1 & IIA 2.4.2	Visual assessment, Organoleptic
Melting/freezing point	105.4 °C (>99.9 %)	DAR Additional Report IIA 2.1.1	OECD 102, Capillary tube method, GLP
Boiling point	222 °C (99.9 %) Slow vaporization starts at ca. 160–165 °C. Temperature of decomposition: > 300 °C (99.9%)	DAR Additional Report IIA 2.1.2	OECD 103, DSC method, GLP
Relative density	1.2829 g/ml at 20°C (density) (> 99.9 %)	DAR IIA 2.2	EEC A3, Pyknometer method, GLP
Vapour pressure	$<1.33 \cdot 10^{-6}$ Pa at 25 °C (99.8%)	DAR Additional Report IIA 2.3.1	OECD 104, Gas saturation method, GLP
Surface tension	71.8 mN/m (90 % saturated aq. solution, 20 °C) (99.7 %)	DAR IIA 2.14	OECD 115, Ring tensiometer method, GLP
Water solubility	0.12 mg/l at 25 °C, deionized water (98.9 %) pH 5: 0.10 mg/l at 20°C (99.6 %) pH 7: 0.10 mg/l at 20 °C (99.6 %) pH 9: 0.11 mg/l at 20 °C (99.6 %)	DAR Additional Report IIA 2.3.1 DAR IIA 2.6	OECD 105, Column generator method, GLP OECD 105, Column elution method, non GLP
Partition coefficient n-octanol/water	log P_{ow} = 2.67 at 25°C, (conc.: 0.23 mg/l) log P_{ow} = 2.82 at 25°C, (conc.: 0.45 mg/l) Unbuffered solution, (>99 %) Effect of pH (4 to 10) not required, does not dissociate because hexythiazox is neither an acid nor a base	DAR IIA 2.8	U.S.EPA guidelines, subdivision D, product chemistry, non GLP
Flash point	Not applicable for solid substances		Expert statement/waiver

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Flammability	Not flammable (99.7 %) Hexythiazox was not able to be ignited but it emitted yellow sparks and melted in contact with the ignition source. No more sparks were observed after removing the ignition source. Molten hexythiazox turned light yellow.	DAR Additional Report IIA 2.11.1	EEC A 10, Flammability (solids), GLP
Explosive properties	From examination of the structural formula, hexythiazox does not contain any chemically unstable or highly energetic groups that might lead to an explosion. Not explosive under the condition of the test (thermal stress, mechanical stress by shock and by friction). Non explosive (99.7%)	DAR Additional Report IIA 2.13.2 DAR Additional Report IIA, 2.13.1	EEC A 14, GLP EEC A 14, GLP Expert statement
Self-ignition temperature	Hexythiazox did not self-ignite prior to termination of the test at 400 °C. Not self-ignitable (99.7%)	DAR Additional Report IIA 2.11.2	EEC A 16, Relative self-ignition temperature for solids, GLP
Oxidising properties	From examination of the structural formula, it can be concluded that hexythiazox has no oxidising properties.	DAR IIA 2.15	EEC A 17, GLP
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	Hexythiazox is expected to be stable in organic solvents.		Expert statement/ waiver

Property	Value	Reference	Comment (e.g. measured or estimated)
Dissociation constant	No apparent spectrum intersections showing dissociation were observed in the pH range 1.28 - 13.22. It was concluded that hexythiazox does not dissociate in aqueous solutions. Alternative methods were unsuitable because of low solubility of hexythiazox.	DAR Additional Report IIA 2.9.4	OECD 112, Spectrometric Method, GLP
Viscosity	Not applicable or solids		Expert statement/ waiver

2 MANUFACTURE AND USES

2.1 Manufacture

Hexythiazox is manufactured outside of the EU.

2.2 Identified uses

Hexythiazox is placed on the market in the EU as an acaricide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification is proposed based on the evaluated data.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Following studies and references are taken from the Draft Assessment Report (DAR) of hexythiazox.

4.1.1 Non-human information

Metabolism studies in rats using hexythiazox ^{14}C labelled in [thiazolidine-5-^{14}C]-moiety are available. No metabolism studies using hexythiazox labelled in the cyclohexane-moiety were

conducted. The position paper, which covers the issue concerning the metabolic fate of cyclohexane-moiety in mammals was prepared in 2009.

Summary of toxicokinetics studies of hexythiazox is presented in **Table 10** .

Table 10: Studies on absorption, distribution, metabolism and excretion of hexythiazox.

Route **Guideline** **GLP**	**Species** **Strain** **Sex** **No of animals**	**Administration**	**Reference**
Oral In accordance with EPA FIFRA 85-1, 1982 non-GLP	Rat F344 Fischer Male/ Female 5 animals / sex / group	Single dose of 10 mg/kg bw of [Thiazolidine-5-^{14}C]-hexythiazox in DMSO (Groups B, C) or 880 mg/kg bw hexythiazox in olive oil (Group D)	DAR IIA 5.1/01
Oral In accordance with EPA FIFRA 85-1, 1982 non-GLP	Rat F344 Fischer Male/ Female 5 animals / sex	Single dose of 880 mg/kg bw [Thiazolidine-5-^{14}C]-hexythiazox in olive oil (Group D)	DAR IIA 5.1/02
Oral In accordance with EPA FIFRA 85-1, 1982 non-GLP	Rat F344 Fischer Male/ Female 5 animals / sex	Single dose of 10 mg/kg bw of [Thiazolidine-5-^{14}C]-hexythiazox in DMSO after a 14-day pre-treatment with 10 mg/kg/day of unlabeled hexythiazox (Group C)	DAR IIA 5.1/03
Oral In accordance with EPA FIFRA 85-1, 1982 non-GLP	Rat F344 Fischer Male/ Female 5 animals / sex	Single dose of 10 mg/kg bw of [Thiazolidine-5-^{14}C]-hexythiazox in DMSO (Group B)	DAR IIA 5.1/04

Route **Guideline** **GLP**	**Species** **Strain** **Sex** **No of animals**	**Administration**	**Reference**
Oral In accordance with EPA FIFRA 85-1, 1982 non-GLP	Rat F344 Fischer Male 3 animals	Single dose of 10 mg/kg bw of [Thiazolidine-5-^{14}C]-hexythiazox in DMSO	DAR IIA 5.1/05
Oral Supplemental information on metabolism of hexythiazox in rat. Re-analysis of samples from DAR IIA 5.1/04 study. non-GLP	Rat F344 Fischer Male/ Female	10 or 100 mg/kg bw of [Thiazolidine-5-^{14}C]-hexythiazox	DAR Additional Report IIA 5.1/07
Oral (gavage) In compliance with the OECD 417 GLP	Rat F344 Fischer Female 15 animals	Single dose of 10 mg/kg bw of [Thiazolidine-5-^{14}C]-hexythiazox in 1 % methyl cellulose solution containing 0.5 % Tween 80. Groups of 3 rats were sacrificed at 5, 12, 24, 48 and 96 hours after dosing.	DAR Additional Report IIA 5.1/08
91/414/EEC Review of Hexythiazox - Mammalian metabolism and excretion of cyclohexane-derived compounds. non-GLP			DAR Additional Report IIA 5.1/09

Metabolism of hexythiazox in rats was studied using radioactively labelled hexythiazox in three different treatment protocols and one preliminary experiment (IIA 5.1/01-05). Summary of the dose groups and analyzed samples is presented in **Table 11**.

Table 11: Summary of the study design in different groups (IIA 5.1/01-05).

Experiment No. **Dose Group**	**Preliminary Group**	**Group B** **1**	**Group B** **2**	**Group C** **1**	**Group C** **2**	**Group D** **1**	**Group D** **2**
Nominal dose level **(mg/kg bw)**	10	10	10	10 (14-day pre-treatment 10 mg/kg/d)	10 (14-day pre-treatment 10 mg/kg/d)	880	880
Vehicle	DMSO	DMSO	DMSO	DMSO	DMSO	Olive oil	Olive oil
No. of animals (male/female)	3/-	5/-	-/5	5/-	-/5	5/-	-/5
Duration [h]	48	72	72	96	96	96	96
Samples	Exhaled air, urine, faeces	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues

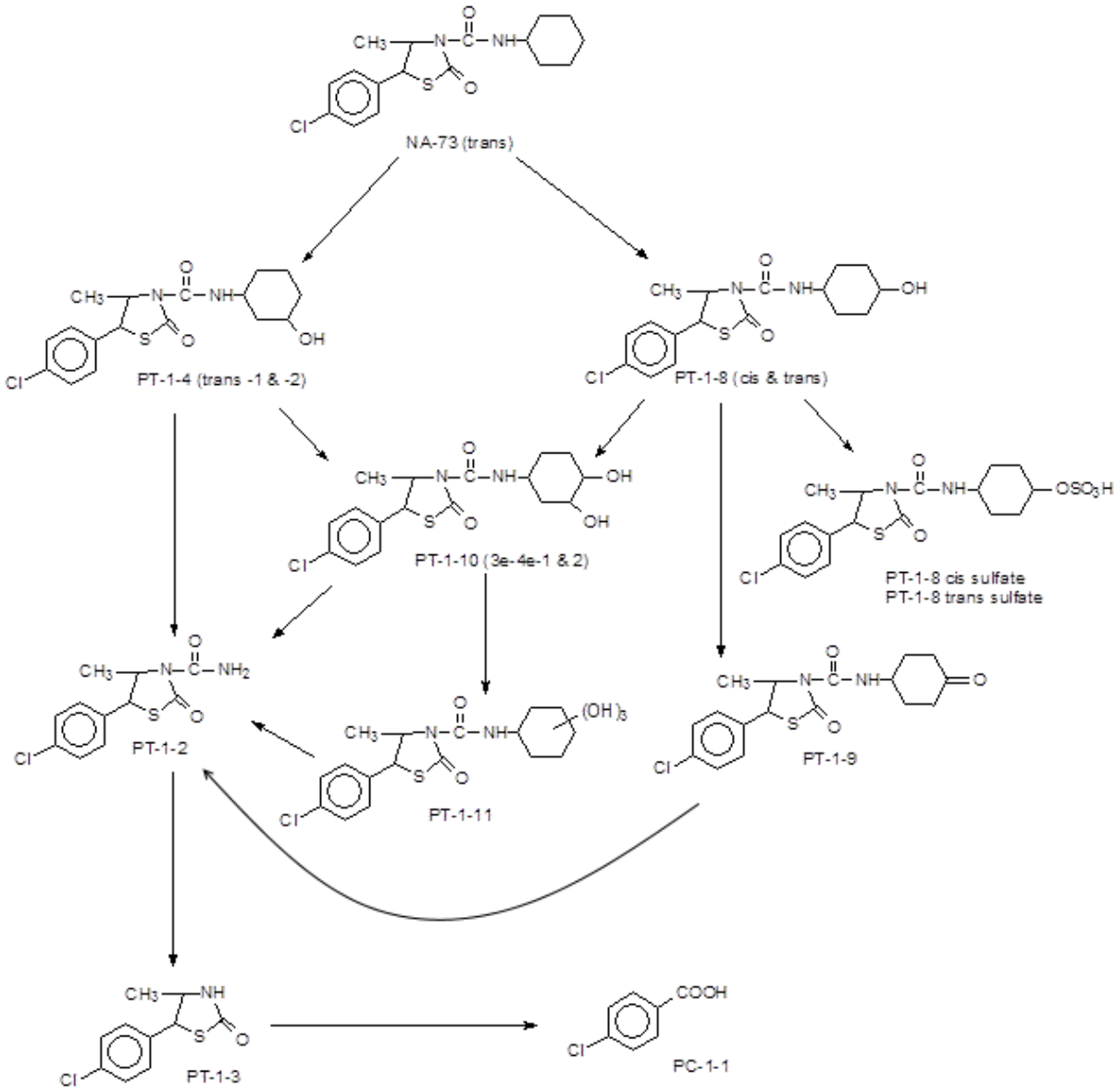
In rats (IIA 5.1/01-05), absorption of hexythiazox decreased with a higher dose. The maximum concentrations in plasma were observed about 3-4 hours after administration in the low dose groups (B and C) and 12 hours after administration in the high dose group (D). At those times the average concentrations observed in the groups B and C were 1.8-2.2 ppm for males and 2.3-2.6 ppm for females, and in the group D 37 ppm for males and 27 ppm for females. 72 or 96 hours after administration ca. 0.1 ppm of hexythiazox remained in plasma in the groups B and C; the levels in plasma in the group D decreased to ca. 2.6 ppm. Oral absorption was estimated at 30 % (EFSA 2010).

1.1-10.1 % of the administered dose was associated with tissues. The highest concentrations of radioactivity were found in fat, adrenal, liver, ovary and digestive organs and their contents. The highest levels of radioactivity of tissues were observed in fat, reaching approximately 2.3, 1.2 and 76 ppm in males and 5.4, 3.3 and 129 ppm in females (groups B, C and D). Residue levels in fat were generally two-fold higher in females than in males. Differences between males and females in the levels of radioactivity occurred in all tissues and organs analysed. (IIA 5.1/01-05)

The proposed metabolic pathway of hexythiazox (NA-73) in rats (IIA 5.1/07, IIA 5.1/09) is presented in **Figure 1**.

Figure 1: Metabolic pathway of hexythiazox in rats

(Isomeric indication of NA-73 is for the thiazolidine ring, and the others are for the cyclohexane ring)



The major radiolabelled metabolite was PT-1-8 (cis), which comprised 8-12 % of the administered radioactivity in excreta in the low dose groups and 2-4 % in the high dose group. The other metabolites included PT-1-2, PT-1-3, PT-1-4 (trans-1), PT-1-4 (trans-2), PT-1-8 (trans), PT-1-9, PT-1-10 (3e-4e-1), PT-1-10 (3e-4e-2) and PC-1-1. Neither of these metabolites exceeded 2 % of the dosed radioactivity. About 11-20 % of the dosed radioactivity in the groups B and C, and 65-69 % in the group D, was excreted as intact hexythiazox. Approximately 37-71 % of the radioactivity in liver and a maximum of 9 % of the radioactivity in fat remained as bound ^{14}C in the extracted tissues. A major radiolabelled component in fat was the parent compound. (IIA 5.1/01-05)

PT-1-3, a minor metabolite in rat, was also found in abiotic and biotic degradation studies (Chapter 5.1.). It is also a metabolite in processed commodities. In the rat study, PT-1-3 appeared in very low amounts (< 1% of the administered dose) in urine and faeces of males and females (IIA 5.1/04). According to oral acute toxicity study PT-1-3 (LD_{50} = 341 mg/kg bw) appears to be more toxic than the parent hexythiazox (LD_{50} > 5000 mg/kg bw). PT-1-3 further metabolises into PT-1-1 which is also found in low amounts. Nevertheless, based on the data available, no conclusion can be drawn on the relative toxicity of PT-1-3. (EFSA 2010)

Supplemental investigation on unknown metabolites found in urine and faeces of group B rats (IIA 5.1/04) was conducted (IIA 5.1/07), because many minor polar metabolites (> 42 compounds) remained unidentified. Only one compound occurred at an amount > 10 % of administered radioactivity. The amount of all other compounds was < 5 % each. The investigations on the identification of these unknown metabolites were carried out using the urine and faeces of the rats administered with 100 and 10 mg/kg bw ^{14}C-hexythiazox. A metabolic compound (> 10 %) was determined as a mixture of two metabolites, PT-1-8 cis sulfate and PT-1-8 trans sulfate. A minor unknown metabolite was speculated as tri-hydroxylated compound (PT-1-11) of the cyclohexane ring of hexythiazox. Thus, all metabolites, which occurred in faeces and urine in amounts of > 10 % of administered radioactivity, were identified. Some of the unknown compounds remained unidentified, but they were speculated to be conjugates of hydroxylated compounds.

The metabolic fate of cyclohexane-moiety in radiolabeled hexythiazox in mammals was discussed in the position paper (IIA 5.1/09). Same types of metabolites of hexythiazox were formed during environmental biodegradation and physiological biotransformation. Based on the data of a soil metabolism and a water/sediment study, where no cyclohexane-moiety derived compounds have been identified as metabolites of hexythiazox it was assumed that in mammalian metabolism a similar behaviour occurs and that cyclohexane-moiety derived metabolites are transient compounds characterized by short retention times in the mammalian organism. Assuming a theoretical formation of related compounds like cyclohexanone or cyclohexanol, originated from the cyclohexane-moiety of hexythiazox, the writer states that these compounds are apparently quickly metabolized in mammals (e.g. hydroxylation, glucuronidation) and rapidly excreted. Due to the chemical nature of the metabolites, the toxicity of both compounds, cyclohexanol and cyclohexanone, is evaluated by available toxicity tests performed with hexythiazox.

Bioaccumulation potential of hexythiazox in female rats was studied in a separate study (IIA 5.1/08). Distribution and elimination of radioactively labelled hexythiazox was analysed in 24 tissues/ organs. The high concentrations of radioactivity were found in adrenal, bladder, bone marrow, fat, gastrointestinal tract (including the contents), liver and pancreas after 5 hours from dosing and then decreased with time, except in gastrointestinal tract (including the contents) where the highest concentration of radioactivity was observed after 12 h. Elimination half-life values of radioactivity were in the range of 10 to 47 hours. The shortest half-life was found in brain (10 hours). The longest half-life was found in fat (47 hours) and was more than twice that of plasma (21 hours).

In the rat study (IIA 5.1/01-05) 24.5-30.1 % of the administered radioactivity was excreted in urine in the groups B and C. In these groups 66.5-73.4 % of the administered radioactivity was

recovered in the faeces of males and 59.8-67.0 % in the faeces of females. In the group D (average of both sexes) 9.5 % of the radioactivity was found in urine and 89.1 % in faeces.

4.1.2 Human information

No information available.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution and excretion of hexythiazox were rapid in rats. Oral absorption was estimated at 30 %. There was no evidence for accumulation of hexythiazox in the body. The main metabolic pathway identified was oxidation of the cyclohexane ring to form the major metabolite PT-1-8 (cis).

4.2 Acute toxicity

Not evaluated in this dossier.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

Not evaluated in this dossier.

4.7 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

The data on repeated dose toxicity is included here only as supporting evidence for the carcinogenicity endpoint. No classification is discussed or proposed for this endpoint for hexythiazox. Four repeated dose toxicity studies are available by oral route (dietary) in mouse, rat and dog.

Table 12. Summary table of short-term toxicity studies

Method Species, Strain No/sex/group	**Doses Exposure**	**NOAEL (males/females)**	**LOAEL (males/females) Effects at LOAEL**	**Reference**
28-day oral, mouse, B6C3F1 (C57BL/6XC/3H/He)	50, 300, 1800 and 10800 ppm	300 ppm (55.1/62.9 mg/kg bw/day)	1800 ppm (319.1/388.2 mg/kg bw/day)	DAR IIA 5.3/01

Method Species, Strain No/sex/group	**Doses Exposure**	**NOAEL (males/females)**	**LOAEL (males/females) Effects at LOAEL**	**Reference**
OECD 407, no GLP 10/sex/group Acceptable	Continuous in diet Purity: 98.3%		Body weight gain ↓ (M) Absolute and relative liver weights ↑ Hepatocellular hypertrophy Total cholesterol ↓ (M)	
28-day oral, dog no guideline, no GLP 2/sex/group Acceptable as a range finding study	125, 500, 2000 and 8000 ppm Continuous in diet	2000 ppm (89.4/78.9 mg/kg bw/day)	8000 ppm (324/346 mg/kg bw/day) Body weight gain ↓ (F) Slight irregular heart rhythm (F)	DAR IIA 5.3/02
90-day oral, rat, Fischer OECD 408, no GLP 20/sex/group Acceptable	10, 70, 500 and 3500 ppm Continuous in diet Purity: 98.3%	70 ppm (8.1/5.4 mg/kg bw/day)	500 ppm (58.6/38.1 mg/kg bw/day) Body weight and body weight gain ↓ (F) Changes in haematology and clinical chemistry Absolute and relative liver weights ↑ Fatty degeneration of adrenal cortex	DAR IIA 5.3/03
One-year oral, dog OECD 409, GLP 4/sex/group Acceptable	100, 500 and 5000 ppm Continuous in diet Purity:97.7%	100 ppm (2.87/3.17 mg/kg bw/day)	500 ppm (13.1/13.9 mg/kg bw/day) Adrenocortical hypertrophy Phosphorus ↓ (F)	DAR IIA 5.3/04

28-day study in mouse (DAR IIA 5.3/01)

Hexythiazox (purity 98.3 %) was administered orally via diet to 6-week old B6C3F1 (C57BL/6XC3H/He) mice (10/sex/group) for a period of 4 weeks. Test substance was dissolved in acetone and added to powder mouse diet in concentrations of 50, 300, 1800 and 10800 ppm. Throughout the study, mean daily intake of hexythiazox was 9.9/13.2, 55.1/62.9, 319.1/388.2 and 1908.4/2045.0 mg/kg bw/day for males and females in the four treatment groups, respectively. Diets were supplied biweekly and stored in a deep freezer (-20 °C) until used. Control animals received basal diet. Haematological tests were carried out on 8 males and 8 females in each group at the termination of the study. Blood samples for the determination of sodium, potassium, glucose, urea nitrogen, total cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase, glutamic pyruvic transaminase and glutamic oxaloacetic transaminase were taken from all surviving mice after overnight fasting at the termination. Each two samples from the same sex in the same group were pooled and examined. Urine samples were collected from 8 males and 8 females in each group at 3 weeks. Gross post-mortem examinations were performed, organ weights determined and histopathological examination of liver, kidneys, heart, lung and spleen performed on all animals. Histopathological evaluation for a larger variety of tissues was conducted on the control and high dose animals.

A male mouse in the 50 ppm group died from bite wound at 22 days. Toxic signs were not observed in any of the mice treated with hexythiazox throughout 4 weeks. Body weight and/or

body weight gain were decreased significantly in males in the 50, 1800 and 10800 ppm groups at 3 and/or 4 weeks. There was no change in food consumption. Total cholesterol was decreased significantly in both sexes at 10800 ppm and in males at 1800 ppm. Lactic dehydrogenase was decreased in males at 1800 ppm and blood glucose increased in females at 1800 ppm. Specific gravity of urine was increased in males at 10800 ppm. Dose-dependent increase in liver weight and liver/body weight ratio was observed at 1800 ppm and 10800 ppm, in both sexes. Brain weight was decreased in 10800 ppm males and lung weight decreased in 1800 ppm females. Microscopic changes were seen in lung (inflammation), liver (inflammation, lipoid degeneration and swelling), kidney (lipoid degeneration), thymus (cyst), ovary (ganglioneuroma), Harderian gland (inflammation) and eye (necrosis). However, these lesions appeared sporadically without dose relationship except for the swelling of liver. Swollen liver cells located in the central zone were frequently observed in both sexes at 10800 and 1800 ppm. These cells were characterised by slightly enlarged nuclei and eosinophilic cytoplasma, not stainable by PAS. Fat droplets, which usually appeared in the control liver, disappeared in these swollen cells. A ganglioneuroma of the ovary was found in a 10800 ppm group female.
Based on decreased body weight gain and decreased total cholesterol in males and increased absolute and relative liver weights associated with hepatocellular hypertrophy in males and females at 1800 ppm, the NOAEL was 300 ppm (55.1 mg/kg bw/day for males and 62.9 mg/kg bw/day for females).

28-day study in dog (DAR IIA 5.3/02)

Hexythiazox (purity not reported) was administered via diet to groups of 2 male and 2 female purebred beagle dogs for 4 weeks at dose levels of 125, 500, 2000 and 8000 ppm, which correspond to mean daily intakes of 5.58/5.54, 23.1/21.6, 89.4/78.9 and 324/346 mg/kg bw/day for males and females in the four treatment groups, respectively. Control animals received basal diet. Fresh batches of control and test diet were prepared each week. The weights of brain, liver, kidney, heart, spleen, testis, ovary, pituitary, thyroid/parathyroid and adrenal were recorded. Liver and adrenal were prepared from all animals and examined microscopically.
No deaths occurred during the course of the study. Body weight gain was decreased in 8000 ppm females. Relative liver weights were increased in 8000 ppm males and females and in 2000 ppm females. A slight irregular heart rhythm was present in an 8000 ppm female. There were no test substance related changes in gross necropsy and histopathology. Due to adaptive weight increase in the liver at 2000 ppm but without concomitant histopathological liver findings, the NOEL was 500 ppm and the NOAEL 2000 ppm in this range-finding study. The recommended dosage levels for the subsequent study are 100, 500 and 5000 ppm.

90-day study in rat (DAR IIA 5.3/03)

Hexythiazox (purity 98.3 %) was administered via diet to groups of 20 male and 20 female Fischer strain rats for 13 weeks at dose levels of 10, 70, 500 and 3500 ppm, which correspond to mean daily intakes of 1.2/0.8, 8.1/5.4, 58.6/38.1 and 397.5/257.6 mg/kg bw/day for males and females, respectively. Test substance was dissolved in acetone and added to powder rat diet. Control animals received basal diet which contained acetone only. Diets containing hexythiazox were supplied biweekly and stored in a deep freezer until used.
Haematological tests were done without fasting on 10 males and 10 females before administration and 10 males and 10 females from each group after 1.5 and 3 months of feeding. Blood chemistry determinations were done on 10 males and 10 females after overnight fasting at 0, 2 and 3 months of feeding. Untreated animals were examined to provide the results for month 0 and discarded. At the interim determination, animals from each satellite group were examined and discarded. At termination, all surviving animals from treatment groups were

tested. Parameters of sodium, potassium, glucose, blood urea nitrogen, total cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase and total calcium were determined. Cholinesterase activities were determined after overnight fasting at 0, 1, 2 and 3 months of feeding. Plasma and erythrocyte cholinesterase activities were determined on 10 untreated males and females to get the 0 month´s data after which the animals were discarded. At the interim determinations, animals from satellite groups were examined and discarded. At termination, plasma, erythrocyte and brain cholinesterase activities were determined on 10 males and 10 females from each treatment group. Urinalysis was performed on 10 males and 10 females from each treatment group after 1.5 and 3 months of feeding. Gross necropsy and histopathology were done on all animals after study termination.

All animals survived until study termination without any clinical signs of toxicity. Body weight and body weight gain were reduced in 3500 ppm males and females and 500 ppm females. Body weight gain was reduced in both sexes at 3500 ppm for almost the entire study period and slightly in females at 500 ppm after 11 weeks of feeding. There was a slight reduction (< 10 %) in food consumption in both sexes at 3500 ppm; during a few weeks the reduction was statistically significant compared to control. Food efficiency in 3500 ppm females was decreased at 1 week of feeding. Body weights and body weight gains at study termination are shown in **Table 13**.

Table 13. Body weights and body weight gains at termination

Dose level (ppm)	Males		Females	
	Body weight (g)	Body weight gain (g)	Body weight (g)	Body weight gain (g)
0	326.5	190.0	186.7	82.2
10	323.8	187.2	185.3	80.9
70	322.9	185.9	184.3	79.2
500	322.7	186.0	181.1* (97.0%)	76.1** (92.6%)
3500	315.2* (96.5%)	178.4* (93.9%)	172.1*** (92.2%)	67.5*** (82.1%)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (student's t-test)

Haemoglobin and MCV were decreased in 3500 ppm males at 1.5 and 3 months. MCH was decreased at 1.5 months and red blood cell count and PCV at 3 months in 3500 ppm males. The number of platelets was increased at 1.5 and 3 months in 3500 ppm males. MCH was decreased in 3500 ppm females at 1.5 and 3 months. A slight decrease in total leukocyte count was observed in 500 ppm and 3500 ppm males at 1.5 months but not at 3 months. Neutrophil count was decreased and lymphocyte count increased in 3500 ppm females at 1.5 months.

Table 14. Altered haematological parameters in male rats after 3 months

Dose level (ppm) / Parameter	0	10	70	500	3500
Erythrocytes (10^6/mm^3)	8.27	8.28	8.19	8.17	7.93**
PCV (%)	51.0	51.1	50.7	50.4	48.4**
Haemoglobin (g/dl)	16.8	16.8	16.8	16.6	16.0**
MCV	61.6	61.7	61.9	61.7	61.0*
Platelets (10^6/mm^3)	0.618	0.641	0.641	0.630	0.650*
Leukocytes (10^3/mm^3) **1.5 months**	8.0	8.0	7.8	7.2*	7.0*
Leukocytes (10^3/mm^3) **3 months**	6.9	6.8	7.1	6.8	6.7

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (student's t-test)

Total cholesterol, total protein, albumin and calcium were increased in both sexes at 3500 ppm at study termination. All of these parameters except calcium in females were increased also at 2 months in 3500 ppm animals. Total protein, albumin and calcium were also increased in 500 ppm males at 2 months and albumin in 500 ppm males at 3 months. Alkaline phosphatase was decreased in 3500 ppm males and females at 2 months and in 3500 ppm females at study termination. Lactic dehydrogenase was increased in 3500 ppm females at 2 months. Glutamic oxaloacetic transaminase was decreased in 3500 ppm males at 2 months and in 3500 ppm females at 3 months. Erythrocyte and brain acetylcholinesterase activities were not significantly changed. Plasma cholinesterase activity was increased in 3500 ppm males at 3 months and decreased in 3500 ppm females at 1, 2 and 3 months as well as in 500 ppm males at 1 month and in 500 ppm females at 2 and 3 months. Urinary protein was increased in 3500 ppm males at 1.5 and 3 months. Altered parameters of blood chemistry determinations and urinalysis at study termination are shown in **Table 15**.

Table 15. Altered clinical chemistry and urinalysis parameters after 3 months

Dose level (ppm) / Parameter	**0**	**10**	**70**	**500**	**3500**
Cholesterol (mg%)					
- Males	22	20	21	24	30***
- Females	33	32	31	32	41***
Total protein (g%)					
- Males	5.9	5.8	5.9	6.0	6.6***
- Females	6.0	5.9	5.9	6.1	6.6***
Albumin (g%)					
- Males	3.8	3.8	3.8	3.9*	4.2***
- Females	3.9	3.8	3.8	3.9	4.2***
Calcium (mg%)					
- Males	9.8	9.8	9.9	9.9	10.1**
- Females	9.5	9.4	9.5	9.6	9.8**
Alkaline phosphatase (mU/mL)					
- Females	88	86	85	85	71***
Plasma cholinesterase (U/mL)					
- Males	0.29	0.29	0.30	0.28	0.34**
- Females	3.09	2.82	2.91	2.70*	2.36***
Number of animals with 300 mg protein/dL urine					
- Males	0	0	0	0	4

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (student's t-test)

Absolute liver and relative liver/body weights were increased in both sexes at 3500 and 500 ppm. In addition, liver/body weight was slightly increased in 70 ppm females. At 3500 ppm, absolute spleen weight and spleen/body weight were decreased in both sexes, kidney/body weight, adrenal/body weight and gonad/body weight ratios were increased in both sexes, absolute adrenal weight was increased in males, absolute brain, thymus and lung weights were decreased in females and brain/body weight ratios increased in females. Brain/body weight, kidney/body weight and gonad/body weight ratios were increased also in 500 ppm females. Changes in organ weights are shown in **Table 16**.

Table 16. Organ weights at termination in a 90-day study in rat

Dose level (ppm) / Parameter	0	10	70	500	3500
Absolute liver weight (g)					
- Males	8.21	8.22	8.13	8.77**	11.53***
- Females	4.40	4.46	4.45	4.79***	6.11***
Relative liver weight					
- Males	2.64	2.66	2.69	2.85***	3.82***
- Females	2.52	2.59	2.60*	2.85***	3.82***
Absolute spleen weight (g)					
- Males	0.631	0.643	0.626	0.609	0.580***
- Females	0.437	0.435	0.430	0.419	0.363***
Relative spleen weight					
- Males	0.203	0.209	0.207	0.199	0.192**
- Females	0.250	0.252	0.251	0.250	0.228***
Absolute adrenal weight (g)	0.0251/	0.0258/	0.0261/	0.0258/	0.0289***/
- males (right/left)	0.0258	0.0287	0.0281	0.0281	0.0316*
- females (right/left)	0.0286/ 0.0320	0.0296/ 0.0308	0.0277/ 0.0297	0.0284/ 0.0307	0.0276/ 0.0314
Relative adrenal weight	0.0081/	0.0084/	0.0087/	0.0084/	0.0096***/
- males (right/left)	0.0093	0.0093	0.0093	0.0092	0.0105*
- females (right/left)	0.0164/ 0.0183	0.0172/ 0.0179	0.0163/ 0.0174	0.0169/ 0.0182	0.0174/ 0.0197*
Absolute kidney weight	1.056/	1.068/	1.051/	1.066/	1.082/
- males (right/left)	1.070	1.069	1.053	1.063	1.095
- females (right/left)	0.674/ 0.660	0.676/ 0.680	0.672/ 0.672	0.669/ 0.677	0.658/ 0.666
Relative kidney weight	0.339/	0.346/	0.348/	0.347/	0.358**/
- males (right/left)	0.344	0.347	0.349	0.346	0.363**
- females (right/left)	0.386/ 0.378	0.392/ 0.394	0.392/ 0.393	0.398/ 0.403*	0.412***/ 0.417***
Absolute testis weight (right/left)	1.437/ 1.478	1.442/ 1.474	1.439/ 1.389	1.452/ 1.500	1.456/ 1.524
Relative testis weight (right/left)	0.462/ 0.475	0.468/ 0.478	0.477/ 0.460	0.473/ 0.489	0.483/ 0.506**
Absolute ovary weight (right/left)	0.0322/ 0.0347	0.0378/ 0.0339	0.0343 /0.0350	0.0354/ 0.0366	0.0368*/ 0.0354
Relative ovary weight (right/left)	0.0184/ 0.0199	0.0220/ 0.0196	0.0200/ 0.0205	0.0211*/ 0.0218*	0.0231***/ 0.0222*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (student's t-test)

Some changes were observed in gross necropsy but regarded incidental. All male animals at 3500 ppm suffered from glomerulonephritis and 60 % of males (12/20) in other groups, including control group. Renal tubular calcification was observed in all females in all groups. Hepatocellular hypertrophy was observed in all 3500 ppm males and females. Fatty degeneration of adrenal cortex was found in all males and 65 % (13/20) of females at 3500 ppm and in all males and 20 % (4/20) of females at 500 ppm. Other lesions were low in number and they were not related to the amount of dose.

One-year study in dog (DAR IIA 5.3/04)

Hexythiazox (purity 97.7 %) was administered via diet to purebred beagle dogs (4/sex/group) at dose levels of 100, 500 and 5000 ppm, which correspond to mean daily intakes of 2.87/3.17, 13.1/13.9 and 153/148 mg/kg bw in males and females, respectively. Body weights and food

consumption were recorded weekly. Dogs were observed for moribundity and mortality and overt toxicity twice daily throughout the study. Detailed observations of appearance and condition, behaviour and activity, excretory functions, respiration, orifices, eyes and palpable masses were conducted at least once a week. Physical examinations including an inspection for general condition which consisted of an examination of head and neck, thorax, abdomen, external reproductive organs, skin and extremities as well as heart and lung sounds were conducted once during the pretest period and at 3, 6, 9 and 12 months of study. Ophthalmoscopic examinations were conducted on all dogs once during a pretest period and at 27 and 51 weeks of study. Blood biochemical, haematological and urinalysis parameters were determined prior to study initiation and at 3, 6 and 12 months of study. Cholinesterase activities of erythrocytes and serum were determined. Gross necropsy was performed on all animals. A full tissue complement for histopathology was prepared for all animals.

All dogs survived to study termination. Ptyalism was dose-related increased in incidence in treated animals but did not occur throughout the study. This phenomenon was absent in control dogs. There were no statistically significant differences in mean body weight values in any treated group when compared to control group. However, body weight gain was decreased in high dose males and all treated females when compared to controls. Food consumption was decreased in all treated groups generally throughout the study. Statistically significant decreases in food consumption were observed in all treated groups and most frequently at the mid dose level. Both negative and positive food efficiency values were seen in all treated groups and control groups.

Values for erythrocytes, haemoglobin and hematocrit were reduced statistically significantly in 500 and 5000 ppm males at 3 months. Alkaline phosphatase was increased in males and females at 5000 ppm throughout the study; statistically significant differences from controls were noticed in males at 12 months and in females at 3 and 12 months. Decreased alkaline phosphatase in 100 and 500 ppm females is considered incidental. Alanine aminotransferase was increased at 5000 ppm in males and females throughout the study but only in females at study termination difference to control was statistically significant. There were significant reductions in total protein at 12 months at 5000 ppm in males and females and at 500 ppm in males; however, the values were constant for these groups at all intervals. Hence, the biological significance is doubtful. Phosphorus was decreased in 500 and 5000 ppm females and in 5000 ppm males at study termination but only in females difference to control was statistically significant. There were no significant changes in serum or red blood cell cholinesterases.

Absolute adrenal and adrenal/body weights were significantly ($p<0.01$) increased at 5000 ppm in males and females. This finding was attributed to adrenocortical hypertrophy seen in histopathology. Cortical cells were enlarged and the number of lipid vacuoles in cells was increased. All three zones of the cortex were affected. Increase in adrenal weight was apparent at 500 ppm but the values were not statistically significant. In histopathology, trace changes were consistently seen at this dose level. Liver/body weight was significantly ($p<0.05$) increased in 5000 ppm males. Absolute liver weight increase was apparent at 5000 ppm in males and females but the values did not achieve statistical significance. Liver weight increase was related to hepatocellular hypertrophy of trace to mild in severity and serum enzyme changes. In gross necropsy, enlarged (mild or moderate) thyroid was found at 5000 ppm in 2 males and at 100, 500 and 5000 ppm in 1 female at each concentration. Thyroid + parathyroid weights were increased in 5000 ppm males and in 500 and 5000 ppm females, but the difference to control was not statistically significant. Changes in adrenal, liver and thyroid weights are shown in **Table 17** and incidences of histopathological findings in **Table 18**. There were no test substance related changes in urinalysis and ophthalmoscopy.

Table 17. Adrenal, liver and thyroid weights in the 1-year dog study

Parameter	Dose level 0 ppm	100 ppm	500 ppm	5000 ppm
Absolute adrenal weight (g)				
Males	1.10	1.16	1.41	1.76 **
Females	1.28	1.19	1.59	2.26 **
Adrenal/body weight				
Males	9.0	9.5	11.0	16.1 **
Females	12.6	12.5	16.1	21.4 **
Absolute liver weight (g)				
Males	332.84	303.75	360.14	386.21
Females	308.46	249.05	271.44	353.21
Liver/body weight				
Males	2.71	2.44	2.82	3.51 *
Females	3.03	2.60	2.72	3.35
Absolute thyroid/parathyroid weight (g)				
Males	1.21	0.96	1.38	1.58
Females	0.97	0.85	1.29	1.46
Thyroid/parathyroid / body weight				
Males	10.09	7.83	10.39	14.30
Females	9.36	8.67	12.80	14.00

* $p < 0.05$, ** $p < 0.01$ (t-test, Dunnet's Test)

Table 18. Incidence of some microscopic observations in a 1-year dog study

Tissue / Observation	Dose level 0 ppm	100 ppm	500 ppm	5000 ppm
Adrenal, cortex Males	(4)	(4)	(4)	(4)
Hypertrophy, cortical	0	0	4	4
-trace	0	0	4	0
-mild	0	0	0	4
Within normal limits	4	4	0	0
Adrenal, cortex Females	(4)	(4)	(4)	(4)
Lymphocytic infiltration, mild	0	0	1	0
Hypertrophy, cortical	0	0	4	4
-trace	0	0	4	0
-mild	0	0	0	4
Within normal limits	4	4	0	0
Liver Males	(4)	(4)	(4)	(4)
Hypertrophy, mild	0	0	0	4
Within normal limits	4	4	4	0
Liver Females	(4)	(4)	(4)	(4)
Hypertrophy	0	0	0	4
-trace	0	0	0	1
-mild	0	0	0	3
Within normal limits	4	4	4	0
Thyroid Males	(4)	(4)	(4)	(4)
Parafollicular cell hyperplasia, mild	1	3	1	3
Lymphocytic infiltration	1	1	0	0

Tissue Observation	Dose level 0 ppm	100 ppm	500 ppm	5000 ppm
-mild	0	1	0	0
-moderate	1	0	0	0
Within normal limits	2	1	3	1
Thyroid Females	(4)	(4)	(4)	(4)
Parafollicular cell hyperplasia, mild	3	1	0	2
Lymphocytic infiltration, moderate	0	0	1	0
Within normal limits	1	3	3	2

( ) = number of animals examined

Summary and discussion of repeated dose toxicity

Four dietary repeated dose toxicity studies are 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 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 were observed at intermediate (500 ppm) and high (5000 ppm) hexythiazox doses (**Table 18**). 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 (**Table 18**). 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.

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.

4.8 Germ cell mutagenicity (Mutagenicity)

The data on mutagenicity is included only as supporting evidence for the carcinogenicity endpoint. No classification is discussed or proposed for this endpoint for hexythiazox.

4.8.1 Non-human information

A wide range of *in vitro* and *in vivo* mutagenicity studies on hexythiazox are available and reported in the DAR. Only studies considered as acceptable by the reporting member state FI during PPP review are included in the CLH proposal and listed in **Table 19**. The remaining mutagenicity studies evaluated during PPP review (e.g. inadequately conducted ones) are available in the DAR.

Table 19: Summary of acceptable *in vitro* and *in vivo* mutagenicity studies on hexythiazox

Method	Results and remarks	Reference
***In vitro* studies**		
Mammalian cell gene mutation test in compliance with the OECD 476 Chinese hamster V79 cells, HPRT gene locus 9.38, 18.8, 37.5, 75.0 and 150 µg/ml of hexythiazox in DMSO With and without S9 mix Negative control: DMSO Positive controls: Ethylmethanesulphonate 5 mM and 10 mM in the absence of S9 mix, dimethylnitrosamine 5 mM and 10 mM in the presence of S9 mix Purity of the test substance 98.6% non-GLP	Test result: Negative, with and without metabolic activation Cytotoxicity: Yes Hexythiazox did not increase mutation frequency either in the absence or presence of metabolic activation compared to controls.	DAR IIA 5.4/03 Acceptable
Chromosome aberration test in compliance with OECD 473 Chinese hamster ovary (CHO) cells 5, 20, 35 and 50 µg/ml of hexythiazox in DMSO without S9 mix 35, 50, 200, 350 and 500 µg/ml of hexythiazox in DMSO with S9 mix Vehicle control: DMSO 10 µl/ml Negative control: McCoy's 5a Positive controls: Mitomycin C 80 ng/ml in the absence of S9 mix and cyclophosphamide 25, 17.5 µg/ml in the presence of S9 mix Purity of the test substance not reported non-GLP	Test result: Negative, with and without metabolic activation There was no significant increase in the percentage of chromosomally aberrant cells at the concentrations tested. The difference in harvest times (10, 20 and 30 hours) did not significantly alter the results from the solvent control and thus did not change the result of this experiment.	DAR IIA 5.4/05 Acceptable

Method	**Results and remarks**	**Reference**
Bacterial recombination assay Test method not specified *Bacillus subtilis* strains H17 (Rec+) and M45 (Rec-) 400, 800, 1600 and 3200 µg of hexythiazox (vehicle DMSO) /disk Vehicle control: DMSO 50 µg/disk Negative control: Kanamycin 10 µg/disk Positive control: Mitomycin C 0.1 µg/disk Purity of the test substance 97.7% non-GLP	Test result: Negative	DAR IIA 5.4/06 Supplemental information
Unscheduled DNA synthesis (UDS) in compliance with OECD 482 Rat primary hepatocytes 2.5, 5, 10, 25, 50, 100 and 250 µg/ml of hexythiazox in DMSO Vehicle control: DMSO 1% Positive control: 2-acetyl aminofluorene 0,1 µg/ml Purity of the test substance 98.4% non-GLP	Test result: Negative Cytotoxicity: Hexythiazox was totally cytotoxic at 250 µg/ml, high toxicity (21 % of the cells survived) was observed at 100 µg/ml, moderate toxicity was noted (62.5 % of the cells survived) at 50 µg/ml but no precipitate was apparent. None of the treatments with the test material caused nuclear labelling significantly different from the vehicle control.	DAR IIA 5.4/07 Acceptable

Method	Results and remarks	Reference
Bacterial mutation assay (Ames test) in compliance with OECD 471 *Salmonella typhimurium* (TA 98, TA 100, TA 1535 and TA 1537) and *Escherichia coli* (WP2uvrA/pKM101) 313, 625, 1250, 2500 and 5000 µg of hexythiazox (vehicle DMSO) / plate, 3 plates/ dose With and without S9 mix Negative control: DMSO Positive controls in the absence of S9 mix: N-ethyl-N'-nitro-N-nitrosoguanidine, 2-nitrofluorene, 9-aminoacridine hydrochloride Positive control in the presence of S9 mix: 2-aminoanthracene Purity of the test substance > 99.9% GLP	Test result: Negative with and without metabolic activation Cytotoxicity: Yes. Crystallization of the test substance at all concentrations.	DAR additional report IIA 5.4/12 Acceptable
***In vivo* study**		
Micronucleus assay in compliance with OECD 474 Mouse bone marrow Single intraperitoneal injection of 500, 1000 or 2000 mg/kg bw of hexythiazox in corn oil Vehicle control: corn oil 20 ml/kg bw Positive control: cyclophosphamide 50 mg/kg bw Purity of the test substance 99.0% GLP	Test result: Negative Results suggest that there was bioavailability of the test substance to the bone marrow.	DAR IIA 5.4/11 Acceptable

4.8.2 Human information

No information available.

4.8.3 Other relevant information

No other relevant information is available.

4.8.4 Summary and discussion of mutagenicity

The reverse mutation test with bacterial cells (*Salmonella typhimurium* and *Escherichia coli*) reveals that hexythiazox did not induce gene mutations either in the presence or absence of metabolic activation. In eucaryotic cells (Chinese Hamster V79), hexythiazox did not induce point mutation (at the HPRT locus), either in the presence or absence of metabolic activation. In the cytogenetic test in Chinese hamster ovary (CHO) cells, hexythiazox did not induce chromosomal aberrations *in vitro*. The bacterial recombination assay (supplemental study) was also negative for hexythiazox. Hexythiazox did not induce DNA damage and repair in *in vitro* UDS test with rat primary hepatocytes. Hexythiazox was also negative in mouse micronucleus test after single dose levels of up to 2000 mg/kg bw.

From the results of adequately performed tests it can be concluded that hexythiazox is not mutagenic and does not cause DNA damage when tested *in vitro* in bacterial and mammalian cells. No micronuclei induction has been observed in *vivo* in mice.

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.

4.9 Carcinogenicity

There are two combined long-term/carcinogenicity studies available, one in the rat and one in the mouse.

Table 20. Summary table of relevant carcinogenicity studies

Method **Species, Strain** **No./sex/group**	**Doses** **Exposure**	**Results**			**Reference**
		NOAEL	**LOAEL**	**Effects**	
24-month dietary toxicity and carcinogenicity study in rats OECD 453 (1981), no GLP Rat, Charles River Fischer 344 80/sex/group Acceptable	0, 60, 430 and 3000 ppm Continuous in diet over 24 months Purity: 98.2 %	NOAEL for chronic toxicity: 60 ppm (3.2/4.0 mg/kg bw/day in males/ females) NOAEL for oncogenicity: 430 ppm (23.1/29.3 mg/kg bw/day in males/females)	430 ppm (23.1/29.3 mg/kg bw/day)	430 ppm: A slight ↓ in body weight (F). Adrenal fatty changes in females. 3000 ppm (163/207 mg/kg bw/day): Adrenal fatty changes in females, ↑ liver weights in both sexes, slight increases in incidences of mammary gland fibroadenoma and thyroid parafolliccular cell adenoma in males.	DAR IIA 5.5/01 and 5.5/08 Key Study
24-month dietary toxicity and carcinogenicity study in mice OECD 453 (1981), no GLP Mice, B6C3F1 80/sex/group Acceptable	0, 40, 250 and 1500 ppm Continuous in diet over 24 months Purity: 98.2 %	NOAEL for chronic toxicity: 40 ppm (6.7/8.4 mg/kg bw/day) NOAEL for oncogenicity: 250 ppm (41.6/51.2 mg/kg bw/day)	250 ppm (41.6/51.2 mg/kg bw/day)	250 ppm: body weight gain ↓ in males, changes in haematological and clinical chemistry parameters in both sexes, absolute and relative adrenal weights ↑ in males, relative kidney weight ↑ and proteinaceous casts in kidney ↑ in both sexes 1500 ppm (267/318 mg/kg bw/day): increased incidence of hepatic adenoma in females	DAR IIA 5.5/03-07 Key Study

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

In a 24-month dietary toxicity and oncogenicity study in rats (DAR IIA 5.5/01 and 5.5/08) hexythiazox was administered in the diet to 80 male and 80 female Fischer 344 rats per test group at dietary dose levels of 0, 60, 430 and 3000 ppm. 30 animals per sex and dose level were designated as satellite animals. Animals for water consumption measurements, clinical chemistry, haematology, and for interim necropsy after 12 months (10 rats/sex/group) were selected from these satellite groups.

Animals were observed twice per day for overt signs of toxicity. Feed consumption was determined once a week. Body weights were measured weekly for the first 14 weeks and every other week thereafter. Water consumption was determined for a 7 day period during study weeks 25, 51, 77 and 103. Clinical examinations, including palpation for masses were performed weekly throughout the study. Ophthalmoscopic examinations were conducted on all rats pretest and on all rats in the control and high dose groups (excluding those in the satellite groups) at 6, 13, 25, 52, 78 and 104 weeks of study. Haematological and biochemical measurements and urinalysis determinations were performed on 10 rats/sex twice before study initiation and on 10 rats/sex/group at 26, 52, 78 and 104 weeks of study (according to OECD 453 haematological measurements should have been performed on 20 rats/sex/group and also at 3 months). Biochemical measurements on serum included determinations of sodium, potassium, chloride, calcium, phosphorus, alkaline phosphatase, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH), urea nitrogen, creatinine, total protein, albumin, globulin (calculated), cholesterol and glucose. Urinalysis determinations included colour, appearance, microscopic examination of sediment, specific gravity, volume, pH, protein, glucose, occult blood, nitrites, bilirubin, ketones and urobilinogen.

Necropsy was performed to all rats which died spontaneously or were sacrificed in extremis. Interim necropsy was done at 12 months on 10 rats/sex/group and terminal necropsy on all survivors at 24 months. Organ weights for adrenals, brain (with stem), heart, kidneys, liver, lung, ovaries, spleen and testes were determined. Samples of protocol-designated tissues (except cecum and rectum) were collected from all animals for microscopic examination. In addition, 3 transverse sections through the head, including tongue, nasal cavity, turbinates, paranasal sinuses, nasopharynx, portions of oral cavity and middle ear, were examined on 10 animals/sex/group. If tumour occurred at one of these sites, all animals were examined microscopically at that particular site.

Results:

The stability of the test substance over the study period and the stability of the test substance in diet were analytically confirmed. The correctness of the dietary concentrations was also analytically confirmed for the 430 and 3000 ppm groups. At the 60 ppm concentration, there was occasional variability in the concentration and homogeneity during the initial phases of the study, but validity of the study was not compromised. Mean test substance consumption was 3.20/4.02, 23.1/29.3 and 163/207 mg/kg bw/day for males and females in the three treatment groups, respectively.

There were no major differences in mortality among the groups; overall survival was > 70% in all groups at week 104. Apart from few exceptions (two high dose males and one low dose female) spontaneous deaths occurred during the last 52 weeks of the study. Approximately

from week 82 there was a high incidence of internal abdominal masses in all of the male groups including the controls with the mid and high dose groups being affected to a greater degree. Beginning at approximately form week 79, swollen testicles and non-descended testicles were observed among all the male groups with more mid and high dose animals affected. There were no other treatment related clinical findings.

Table 21. Survival of animals at 104 weeks of the study

Dose level (ppm)	Number of survivors/Number at beginning of the study	
	Male	Female
0	50/70	57/70
60	57/70	49/70
430	59/70	61/70
3000	53/70	56/70
Administration of the test substance was initiated with 80 animals/sex/group. 10 animals/sex/group were sacrificed at 12 months.		

Body weights were statistically significantly lower in males and females of the 3000 ppm group when compared to controls throughout the study. At 430 and 60 ppm, body weights were slightly decreased both in males and females; in females, difference to control was statistically significant at most of the intervals but in males only occasionally. However, the decrease in body weight gain was considered biologically significant only in 3000 ppm females throughout the study and in 3000 ppm males at study termination. The slight decreases in body weights in the low and mid dose group were considered to reflect normal biological variation. There seemed to be, at least occasionally, a dose related increase in food consumption in all treated groups. Yet, this effect was statistically significant only in males and in females of the 3000 ppm group and in 430 ppm females. Moreover, food consumption achieved the biologically significant increase of 10 % (on a g/kg bw/day basis) only in 3000 ppm females in many occasions and at study termination in 3000 ppm males. Water consumption was comparable to controls in all treatment groups at most of the measurement intervals, except an increase that was observed in 430 and 3000 ppm males at the final interval and in 3000 ppm females at the last 2 intervals (weeks 77 and 103). There were no treatment related findings in haematological, biochemical or urinalysis examinations. No test substance related ophthalmoscopic findings were observed.

Body and organ weights after 24 months are shown in **Table 22**. Biologically and statistically significant increases in liver weights were observed in both males and females of the 3000 ppm group in comparison to controls. Absolute and relative liver weights were increased in 3000 ppm males and females at study termination and liver/body weight also in interim sacrifice. Absolute and relative adrenal weights were increased in 60 and 3000 ppm males at study termination. Absolute and relative kidney weights were increased in 3000 ppm females at study termination. Increased absolute and relative testis weights were observed at 3000 ppm in interim sacrifice and increased testis/body weight at study termination. Ovary/body weight was increased in 3000 ppm females at study termination. Other statistically significant differences in organ weights between groups were considered sporadic findings or in case of relative changes, to reflect decreased body weights.

Table 22. Mean body and organ weights after 24 months

Dietary dose level	0 ppm	60 ppm	430 ppm	3000 ppm
Males				
Body weight (g)	379	378	367	347 **
Brain weight (g)	2.01	2.00	2.00	1.99
Brain / body weight (%x10)	5.35	5.38	5.58*	5.83**
Adrenal (mg)	64	70 *	64	68 *
Adrenal / body weight (%x10^3)	17.1	19.0 *	17.8	19.8 **
Adrenal / brain weight (%)	3.21	3.54 *	3.21	3.41 *
Heart (g)	1.27	1.29	1.28	1.27
Heart / body weight (%x10)	3.37	3.47	3.57 *	3.73 **
Heart / brain weight (%x10^{-1})	6.35	6.48	6.39	6.40
Kidney (g)	3.57	3.48	3.53	3.63
Kidney / body weight (%x10)	9.50	9.34	9.83	10.63 **
Kidney / brain weight (%x10^{-2})	1.78	1.74	1.77	1.83
Liver (g)	15.67	15.58	15.81	18.09 **
Liver / body weight (%)	4.15	4.17	4.40	5.29 **
Liver / brain weight (%x10^{-2})	7.81	7.80	7.91	9.14 **
Lung / mainstem bronchi (g)	2.06	1.98	2.13	2.21
Lung / mainstem bronchi / body weight (%x10)	5.49	5.33	5.96 *	6.51 **
Lung / mainstem bronchi / brain weight (%x10^{-1})	10.28	9.92	10.67	11.14
Testis (g)	5.91	5.50	5.88	6.62
Testis / body weight (%x10)	15.79	14.73	16.27	19.41 **
Testis / brain weight (%x10^{-2})	2.94	2.75	2.93	3.32
Females				
Body weight (g)	282	279	273*	247 **
Brain weight (g)	1.83	1.84	1.84	1.82
Brain / body weight (%x10)	6.58	6.64	6.79	7.44**
Adrenal (mg)	75	68 **	70 *	73
Adrenal / body weight (%x10^3)	26.9	24.6 **	25.9	29.5 **
Adrenal / brain weight (%)	4.09	3.71 **	3.82 *	3.98
Heart (g)	1.04	1.04	1.04	1.08
Heart / body weight (%x10)	3.76	3.74	3.83	4.39 **
Heart / brain weight (%x10^{-1})	5.72	5.63	5.65	5.91
Kidney (g)	2.61	2.60	2.66	2.81 **
Kidney / body weight (%x10)	9.42	9.39	9.81	11.48 **
Kidney / brain weight (%x10^{-2})	1.43	1.42	1.45	1.55 **
Liver (g)	11.91	11.89	11.80	13.14 **
Liver / body weight (%)	4.30	4.29	4.35	5.36 **
Liver / brain weight (%x10^{-2})	6.53	6.47	6.44	7.21 *

Dietary dose level	0 ppm	60 ppm	430 ppm	3000 ppm
Lung / mainstem bronchi (g)	1.54	1.46	1.52	1.56
Lung / mainstem bronchi / body weight (%x10)	5.60	5.26	5.62	6.35 *
Lung /mainstem bronchi / brain weight (%x10^{-1})	8.45	7.93	8.28	8.54
Ovary (mg)	128	122	130	134
Ovary / body weight (%x10^{2})	4.61	4.42	4.79	5.38 **
Ovary / brain weight (%)	7.00	6.66	7.07	7.32

* Statistically significant p<0.05;
** Statistically significant p<0.01

Incidences of microscopic findings in interim sacrifice are shown in **Table 23**, and incidences of microscopic findings at study termination in **Table 24**. Vacuolar, fatty changes in adrenal were slightly increased in the incidence and in severity in 430 and 3000 ppm females and 3000 ppm males. However, there was no significant evidence of hyperplasia or hypoplasia. Chronic nephritis was a very common finding in all groups being more severe in hexythiazox-treated rats. Cytoplasmic alteration in 3000 ppm males was the only histopathological liver finding which was increased when compared to control animals. Seminal vesicle vesiculitis was slightly increased in 3000 ppm males.

Table 23. Incidences of microscopic observations in interim (12 months) sacrifice

Tissue observation	0 ppm	60 ppm	430 ppm	3000 ppm
MALES				
Adrenal				
- vacuolar change, fatty	2/10	1/10	5/10	**9/11***
- trace	2/10	1/10	5/10	1/11
- mild	0/10	0/10	0/10	8/11
- pheochromocytoma	0/10	0/10	1/10	0/11
Kidney				
- chronic nephritis	7/10	6/10	9/10	8/11
- trace	6/10	5/10	5/10	2/11
- mild	1/10	1/10	4/10	**6/11***
Testis				
- interstitial cell tumour, benign	0/10	0/10	**2/10**	**3/11**
- hyperplasia (trace)	5/10	3/10	3/10	6/11
- testicular degeneration, moderate	1/10	2/10	0/10	0/10
,within normal limits	4/10	5/10	5/10	2/10
FEMALES				
Eye				
- degeneration	0/10	2/11	1/10	2/10
- trace	0/10	2/11	1/10	1/10
- mild	0/10	0/11	0/10	1/10
- atrophy and degeneration, moderate	0/10	0/11	0/10	1/10

*** p<0.05, Fisher exact test**

Incidences of testicular interstitial cell (Leydig cell) adenoma were slightly but not statistically significantly increased in males of the 430 and 3000 ppm groups at interim sacrifice (incidences

2/10, and 3/11, at 430 ppm and 3000 ppm, respectively, compared to 0/10 in controls, **Table 23**). At the termination of the study there were no significant differences in indicences of this tumour type between the different groups (**Table 24**). The incidence of thyroid parafollicular cell adenoma was slightly but not statistically significantly increased in 3000 ppm males at the termination of the study (10.3% vs. 4.3% in controls). In addition, one parafollicular cell carcinoma in 430 ppm treated group, (incidence 1.4% vs. 0 % in controls) and two follicular carcinomas in 430 ppm and 3000 ppm groups (0%, 1.4% and 1.5%, at 0 ppm, 430 ppm and 3000 ppm, respectively) were observed. In females there were no differences in the incidences of thyroid tumours between the treated groups and the controls (**Table 24**).

Fibroadenoma of mammary gland was increased in 3000 ppm males (9.0% vs. 0% in controls) at the end of the study. Tests for unadjusted trend ($p<0.01$), adjusted trend ($p<0.01$), and homogeneity of life table data for the cox analysis ($p<0.05$) revealed statistically significant differences in the incidences of these tumours between different groups (0, 1.4%, 2.9% and 9.0% at 0 ppm, 60 ppm, 430 ppm and 3000 ppm, respectively). Yet, the Kruskal-Wallis analysis of life table data and the pairwise comparison of control and high dose groups revealed no statistical significances. In females, there were no significant differences in incidences of mammary gland tumours 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).

There were no other remarkable differences in microscopic findings between the control and hexythiazox-treated groups. There were numerous uterine polyps (endometrial stromal tumours, **Table 24**) and monocytic leukaemias (mononuclear cell leukaemia, **Table 24**) in all groups. These findings were considered typical for Fischer 344 rats. The two most common causes for death during this study were monocytic leukaemia (slightly higher incidence in male rats) and pituitary adenomas (**Table 24**).

Table 24. Incidences of microscopic findings in terminal (24 months) sacrifice

Tissue observation	0 ppm		60 ppm		430 ppm		3000 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
MALES								
Adrenal, cortex								
- vacuolar change	2/20	11/50	3/12	9/57	3/11	14/59	6/16	18/53
- trace	0/20	3/50	0/12	1/57	0/11	2/59	0/16	9/53
- mild	2/20	8/50	3/12	8/57	3/11	11/59	5/16	8/53
- moderate	0/20	0/50	0/12	0/57	0/11	1/59	1/16	1/53
- hyperplasia	0/20	0/50	0/12	0/57	0/11	1/59	2/16	1/53
- hemolymphoreticular neoplasm present	2/20	1/50	0/12	1/57	1/11	4/59	4/16	2/53
Adrenal, medulla								
- pheochromocytoma	1/20	4/50	3/12	6/57	0/11	9/59	2/16	5/53
- pheochromocytoma, malignant	0/20	0/50	0/12	0/57	0/11	0/59	1/16	0/53
- hemolymphoreticular neoplasm present	0/20	0/50	0/12	0/57	1/11	0/59	2/16	2/53
Kidney								
- chronic nephritis	18/20	50/50	9/13	57/57	11/11	59/59	16/16	53/53
- trace	0/20	3/50	2/13	0/57	6/11	2/59	1/16	0/53
- mild	8/20	27/50	2/13	16/57	4/11	24/59	8/16	10/53
- moderate	10/20	18/50	5/13	41/57	1/11	33/59	7/16	43/53

Tissue observation	**0 ppm**		**60 ppm**		**430 ppm**		**3000 ppm**	
	DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
- severe	0/20	2/50	0/13	0/57	0/11	0/59	0/16	0/53
- hemolymphoreticular neoplasm present	1/20	0/50	2/13	0/57	0/11	1/59	6/16	1/53
Hemolymphoreticular neoplasm present								
- lymph node, abdominal	8/20	0/50	3/13	4/57	2/11	5/59	8/16	6/53
- lymph node, mandibular	9/20	1/50	2/13	2/57	2/11	5/59	6/15	7/53
- lymph node, regional	0/1	0/3	0/0	1/4	0/3	1/10	3/5	1/8
- lymph node, thoracic	8/20	0/50	1/13	2/57	1/11	4/57	7/16	4/53
Lymph node, thoracic								
- congestion	0/20	1/50	0/13	5/57	1/11	6/57	1/16	4/53
- mild	0/20	1/50	0/13	2/57	0/11	0/57	0/16	3/53
- moderate	0/20	0/50	0/13	3/57	1/11	6/57	1/16	1/53
Liver								
- cytoplasmic alteration, clear	0/20	3/50	0/13	4/57	0/11	4/59	0/16	7/53
- trace	0/20	2/50	0/13	0/57	0/11	0/59	0/16	1/53
- mild	0/20	1/50	0/13	4/57	0/11	4/59	0/16	4/53
- moderate	0/20	0/50	0/13	0/57	0/11	0/59	0/16	2/53
Liver								
- Hepatocellular carcinoma	0/70		2/57	0/11	0/11	0/59	0/16	1/53
- Neoplastic nodule	1/20	2/50	0/13	1/57	0/11	1/59	2/16	1/53
Lymphoreticular system								
- monocytic leukaemia	13/14	6/6	7/7	10/10	5/5	20/20	12/12	15/15
Mammary gland								
- fibroadenoma	0/20	0/50	0/13	1/56	1/10	1/59	0/14	**6/53**
- fibroma	0/20	0/50	0/13	0/56	0/10	0/59	**1/14**	0/53
- adenocarcinoma	0/20	0/50	0/13	0/56	0/10	0/59	0/14	**1/53**
Pituitary								
- hyperplasia	0/20	0/50	0/13	5/57	0/11	4/59	0/15	3/53
- trace			0/13	1/57	0/11	0/59	0/15	1/53
- mild	…….		0/13	2/57	0/11	3/59	0/15	2/53
- moderate			0/13	2/57	0/11	1/59	0/15	0/53
- -adenoma	4/20	18/50	3/13	20/57	5/11	13/59	4/15	11/53
Seminal vesicle								
- vesiculitis	0/20	5/50	1/13	1/57	1/11	5/58	1/16	11/53
- trace	0/20	0/50	0/13	0/57	0/11	0/58	0/16	1/53
- mild	0/20	5/50	1/13	1/57	1/11	5/58	1/16	7/53
- moderate	0/20	0/50	0/13	0/57	0/11	0/58	0/16	3/53
Testis								
- interstitial cell tumour, benign	90%	98%	77%	98%	73%	98%	100%	98%
	18/20	49/50	10/13	56/57	8/11	58/59	16/16	52/53
Thyroid								
- parafollicular cell hyperplasia	0/20	3/50	0/13	8/56	0/11	3/59	0/15	1/53
- parafollicular cell adenoma								

Tissue observation	**0 ppm**		**60 ppm**		**430 ppm**		**3000 ppm**	
	DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
- follicular carcinoma	1/20	2/50	0/13	3/56	0/11	2/59	**1/15**	**6/53**
- parafollicular cell	0/20	0/50	0/13	0/56	0/11	1/59	0/15	**1/53**
carcinoma	0/20	0/50	0/13	0/56	0/11	1/59	0/15	0/53
FEMALES								
Adrenal, cortex								
- vacuolar change	3/13	15/57	3/20	17/49	5/9	25/61	7/14	21/56
- trace	0/13	1/57	0/20	2/49	0/9	4/61	0/14	0/56
- mild	3/13	13/57	3/20	15/49	5/9	19/61	6/14	20/56
- moderate	0/13	1/57	0/20	0/49	0/9	2/61	1/14	1/56
Adrenal, medulla								
- pheochromocytoma	0/13	0/57	1/20	2/49	0/9	0/61	0/14	2/56
- pheochromocytoma,								
malignant	0/13	0/57	0/20	0/49	0/9	0/61	1/14	0/56
Kidney								
- chronic nephritis	9/13	52/57	11/20	49/49	7/9	60/61	10/14	56/56
- trace	2/13	22/57	3/20	10/49	3/9	11/61	4/14	4/56
- mild	5/13	21/57	3/20	33/49	4/9	44/61	5/14	39/56
- moderate	2/13	9/57	4/20	6/49	0/9	5/61	1/14	13/56
- severe	0/13	0/57	1/20	0/49	0/9	0/61	0/14	0/56
Pituitary								
- hyperplasia	0/13	0/57	2/20	3/48	0/9	4/61	0/14	4/56
- trace			0/20	1/48	0/9	1/61	0/14	1/56
- mild			1/20	1/48	0/9	3/61	0/14	1/56
- moderate			1/20	1/48	0/9	0/61	0/14	2/56
- adenoma	5/13	27/57	9/20	23/48	3/9	24/61	3/14	23/56
Thyroid								
- parafollicular cell								
hyperplasia	0/13	4/57	0/20	8/49	0/8	7/61	2/14	5/56
- trace	0/13	0/57	0/20	2/49	0/8	4/61	0/14	0/56
- mild	0/13	1/57	0/20	6/49	0/8	3/61	2/14	5/56
- moderate	0/13	3/57	0/20	0/49	0/8	0/61	0/14	0/56
- parafollicular cell adenoma	1/13	2/57	0/20	3/49	0/8	3/61	0/14	3/56
- follicular carcinoma	0/13	0/57	0/20	0/49	0/8	0/61	0/14	1/56
- parafollicular carcinoma	0/13	0/57	0/20	0/49	0/8	0/61	0/14	0/56
Liver								
- Hepatocellular carcinoma	070		0/69		1/9	0/61	0/70	
- Neoplastic nodule	0/70		0/69		0/9	2/61	0/14	2/56
Lymphoreticular system								
- monocytic leukaemia	9/9	7/7	11/11	6/6	6/6	10/10	9/9	8/8

Tissue observation	0 ppm		60 ppm		430 ppm		3000 ppm	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Mammary gland								
- Fibroadenoma	1/13	5/57	0/19	3/49	0/9	1/61	1/14	4/56
- Fibroma	0/13	0/57	0/19	0/49	0/9	0/61	0/14	0/56
- Adenocarcinoma	0/13	1/57	0/19	0/49	0/9	1/61	0/14	0/56
Uterus								
- Polyp (endometrial stromal tumour)	4/13	11/57	3/20	14/49	2/9	13/61	4/14	14/56

DOS = Died on study

SAC = Terminal sacrifice

Interstitial cell adenomas

Slightly, but not statistically significantly increased incidences of testicular interstitial cell (Leydig cell) adenoma were observed in males of the 430 and 3000 ppm groups at interim sacrifice (**2/10, 20% and 3/11, 27%** at 430 and 3000 ppm, respectively, compared to **0/10** in controls, **Table 23**). At final sacrifice (24 months) the incidences of the testicular interstitial cell adenoma were >98% in all groups including the control (**Table 24**).

According to the notifiers statement the incidences at interim sacrifice were slightly higher than historical control incidences of the laboratory. The in-life phase of the rat carcinogenicity study was conducted November 1981 - November 1983. The performing laboratory (IRDC) had limited background control data for 12 months in this rat strain, but in another study conducted in the same laboratory, an isolated incidence of two cases of interstitial cell adenoma (in 10 animals) was seen in a treated group with no cases in the high dose group. The notifier has also submitted historical control data for 12 months interval in F344 rats from the performing laboratory (7 studies conducted 1982 - 1988) in IRDC/MPI research[1]). At 12 months interval the incidences of interstial cell adenoma varied between **0 and 15 %** in these studies (**Table 25**). We note that these studies were conducted in a different time frame and thus the data is not considered valid.

Focal interstitial cell hyperplasia commonly precedes interstitial cell adenoma. However, in the present study 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 respectively, **Table 23**). Adenoma induced clinical signs: increase in swollen testes/not descended testes were observed in mid and high dose groups approximately after week 66 until the termination of the study. These signs could be related to the size of the tumours, but not to the number of adenoma. Necropsy at month 12 revealed testicular mass and red focus in 430 ppm group (1/10 and 1/10) but no changes in other groups including controls indicating that there were no hypertrophy or early adenoma. Significantly increased testes weights observed at month 12 were not related to adenomas (or to mass and focus findings in the 430 ppm group).

[1] MPI research was founded when it took over IRDC´s research business on November 1995

Table 25. Historical control data (12-month interval) of testicular interstitial cell adenoma and non-neoplastic findings in male F344 rats (MPI Research studies/IRDC conducted from 11/83 to 11/88, DAR IIA 5.5/15)

Parameter	Study A	Study B	Study C	Study D	Study E	Study F	Study G
No. of animals	11	20	11	20	20	11	20
	Interstitial cell adenoma, benign						
Incidence	0	3	0	0	1	1	0
%incidence	0.0	15.0	0.0	0.0	5.0	9.1	0.0
	Atrophy and degeneration						
	0	1	0	0	0	0	0
- moderate	0	1	0	0	0	0	0
	Interstitial cell hyperplasia						
	4	0	6	4	10	2	3
-trace	4	0	5	1	8	0	0
- mild	0	0	1	2	2	2	3
-moderate	0	0	0	1	0	0	0
	Mineralisation						
	0	0	0	0	0	0	1
- mild	0	0	0	0	0	0	1
	Testicular degeneration						
	0	0	0	0	2	0	1
- moderate	0	0	0	0	1	0	0
-severe	0	0	0	0	1	0	1

Thyroid and parafollicular cell tumours

The incidence of thyroid parafollicular cell adenoma was slightly, but not statistically significantly, increased in 3000 ppm males compared to concurrent controls at the termination of the study (**10.3% vs. 4.3%** in controls). In addition, one parafollicular cell carcinoma in 430 ppm treated group, (incidence **1.4% vs. 0 %** in controls) and two follicular carcinomas in 430 ppm and 3000 ppm groups (**0%, 1.4% and 1.5%**, at 0 ppm, 430 ppm and 3000 ppm, respectively) were observed. There were no significant differences in the incidences of parafollicular cell hyperplasia in male rats. There were no differences in parafollicular cell tumour incidences in female rats, but thyroid parafollicular cell hyperplasia was slightly increased in all test substance treated females (**Table 24**). However, no clear dose-response relationship could be observed in the increase of the incidence or in severity in test substance

treated animals. One follicular cell carcinoma was observed in high dose females compared to zero incidences in other groups.

The in-life phase of the rat carcinogenicity study was conducted November 1981 - November 1983. The notifier has submitted historical control data from the performing laboratory (IRDC/MPI Research, 10 studies conducted during 1986 - 1998, DAR IIA 5.5/14, **Table 27**). We note that none of the studies in the submitted historical control dataset has been conducted over acceptable time frame (within 2-3 years to study) and therefore the data is not considered valid. However, this data reveals that in most of the studies of the performing laboratory control F344 males had no parafollicular cell tumours but few parafollicular cell tumours were observed in control animals of three different studies (14/513, mean 2.7%, s.d 4.4 **Table 27**). In general, parafollicular cell tumours are rare in F344 rats. According to the NTP (National toxicological program, U.S) historical control database incidences of parafollicular cell adenoma ranged **0-4.2% and 0-2.2%** in F344 males and females respectively during 1984-1994 in 20 dietary studies (total incidence for males 6/924, 0.6%, mean (sd) 0.6% (1.2)). In the present study (DAR IIA 5.5/01) also control animals had parafollicular cell adenomas. This suggests that slightly increased incidence of this tumour type in high dose males would not be related to hexythiazox treatment.

Table 26. Thyroid tumour incidences in Fischer 344 rats after 24 months (DAR IIA 5.5/01)

Sex	Tumour type	Hexythiazox dose			
		0 ppm	60 ppm	430 ppm	3000 ppm
Male	Parafollicular cell adenoma	3/70 (4.3 %)	3/69 (4.3 %)	2/70 (2.9 %)	7/68 (10.3 %)
	Parafollicular cell carcinoma	0/70 (0 %)	0/69 (0 %)	1/70 (1.4 %)	0/68 (0 %)
	Follicular carcinoma	0/70 (0 %)	0/69 (0 %)	1/70 (1.4 %)	1/68 (1.5 %)
Female	Parafollicular cell adenoma	3/70 (4.3 %)	3/69 (4.3 %)	3/69 (4.3 %)	3/70 (4.3 %)
	Parafollicular cell carcinoma	0/70 (0 %)	0/69 (0 %)	0/69 (0 %)	0/70 (0 %)
	Follicular carcinoma	0/70 (0 %)	0/69 (0 %)	0/69 (0 %)	1/70 (1.4 %)

Table 27. Historical control data (24-month interval) of thyroid tumours in male F344 rats (MPI Research/IRDC studies conducted 1986 – 1998, DAR IIA 5.5/14)

Parameter	Study 1996-1998	Study 1993-1995	Study 1992-1994	Study 1989-1991	Study 1988-1990	Study 1986-1988	Study 1988-1990	Study 1988-1990	Study 1986-1988	Study 1987-1989
No. of animals	60	50	50	44	50	60	50	50	49	50
Parafollicular cell adenoma										
Incidence	0	0	0	0	0	4	0	0	4	6
%incidence	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	8.0	12.0
Parafollicular cell carcinoma										
Incidence	0	0	0	0	0	1	0	0	0	3
%incidence	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	6.0
Follicular carcinoma										
Incidence	1	1	0	0	0	0	0	1	0	0
%incidence	1.7	2.0	0.0	0.0	0	0	0	2.0	0	0

Mammary gland fibroadenomas

Incidence of mammary gland fibroadenoma was increased in high dose (3000 ppm) males when compared to concurrent controls at the end of the study (**0%, 1.4%, 2.9% and 9.0%**, at 0 ppm, 60 ppm, 430 ppm and 3000 ppm, respectively). In females, there were no significant differences in the incidences of mammary gland tumours between the treated groups and controls (**Table 28**). The in-life phase of the rat carcinogenicity study (DAR IIA 5.5/01) was conducted from November 1981 to November 1983. The notifier has submitted historical control data from the performing laboratory (IRDC, 6 studies conducted during 1986-1995,**Table 29**, DAR IIA 5.5/14). In these studies the incidences of mammary gland fibroadenoma in F344 males ranged **0-6%**. We note that none of the studies in the submitted historical control dataset has been conducted over acceptable time frame (within 2-3 years to study) and therefore the data is not considered valid. The notifier has also submitted historical control data from other laboratory (National Toxicological Program, U.S), which shows more variable incidences of fibroadenoma of the mammary gland in F344 males (**0-12 %**, **Table 29**.).

Table 28. Mammary gland tumour incidences in Fisher 344 rats after 24 months (DAR IIA 5.5/01)

Tumour type	Hexythiazox dose			
Males	**0 ppm**	**60 ppm**	**430 ppm**	**3000 ppm**
Fibroadenoma	0/70 (0 %)	1/69 (1.4 %)	2/69 (2.9 %)	6/67 (9.0 %)
Fibroma	0/70 (0%)	0/69 (0 %)	0/69 (0 %)	1/67 (1.5 %)
Adenocarcinoma	0/70 (0 %)	0/69 (0 %)	0/69 (0 %)	1/67 (1.5 %)
Females	**0 ppm**	**60 ppm**	**430 ppm**	**3000 ppm**
Fibroadenoma	6/70 (8.6 %)	3/68 (4.4 %)	1/70 (1.4 %)	5/70 (7.1 %)
Fibroma	0/70 (0 %)	0/68 (0 %)	0/70 (0 %)	0/70 (0 %)

Adenocarcinoma	1/70 (1.4 %)	0/68 (0 %)	1/70 (1.4 %)	0/70 (0 %)

Table 29. Historical control incidences of mammary gland tumours in F344 rats

Laboratory and Period	Number of studies and animals	Tumour type	Incidence (range)	Reference
IRDC 1986-1995*	6 studies, 50 or 60 rats/study	Fibroadenoma Adenoma Adenocarcinoma	males 0-6% (avg 2%) females 0-14% (avg 10.6%) males 0% females 0-4% (avg.1.3%) females 0-2% (avg. 1%)	DAR IIA 5.5/14 *
NTP form 1 January 1977 – 29 April 1987	40 studies	Fibroadenoma Adenoma Carcinoma	males 0-12% 0-2% 0-2%	Haseman et al. 1990
NTP All studies prior to 1 July 1994	194 studies	Mammary gland neoplasms	**5%**	Haseman and Elwell 1996
NTP 1990-1997	27 feeding studies	Fibroadenoma Adenoma Carcinoma	males 0-12% 0-2% 0-2%	Haseman et al. 1998

*Historical control data of the performing laboratory

Conclusions

Body weight gain was decreased statistically and biologically significantly in 430 and 3000 ppm males and 3000 ppm females. Absolute and/or relative liver, adrenal, testis, kidney and ovary weights in 3000 ppm males and females were increased at study termination and often also in interim sacrifice at 12 months. Cytoplasmic alteration of liver cells was slightly increased in 3000 ppm males. Fatty changes in adrenal were slightly increased in the incidence and in severity in 430 and 3000 ppm females and 3000 ppm males. The incidence of testes interstial cell adenoma was slightly increased in 430 and 3000 ppm males at 12 month interval and the incidences of thyroid parafollicular cell adenoma and fibroadenoma of mammary gland were slightly increased in 3000 ppm males at the end of the study (24 months). Based on significant decrease in body weight gain at 430 ppm in males and fatty changes in adrenal in females, the NOAEL for chronic toxicity was 60 ppm (3.20 mg/kg bw/day for males and 4.02 mg/kg bw/day for females). The NOAEL for oncogenicity was 430 ppm (23.1 mg/kg/day for males and 29.3 mg/kg bw/day for females).

In a 24-month dietary toxicity and carcinogenicity study in mice (DAR IIA 5.5/03-07) hexythiazox (NA-73; batch no. SAF-25, purity 98.2 %) was administered via diet to specific pathogen free (SPF) mice of the B6C3F1 strain (80/sex/group) at dose levels of 0, 40, 250 and 1500 ppm. The test substance was administered to 50 animals/sex/group for 104 weeks. Satellite groups of 10 animals/sex/group were scheduled for interim sacrifices at 26, 52 and 78 weeks.

Body weight was determined weekly during the first 26 weeks and bi-weekly thereafter. Individual food consumption was determined weekly. Haematological and biochemistry examination and urinalysis were performed on 8-10 animals/sex/group at 26, 52, 78 and 104 weeks. Haematological and biochemistry examination were also performed before dosing (biochemistry on 10 animals/sex, the number of animals in haematology not reported).

Hematological examination should have been performed on 20 animals/sex/group and also at 3 months. Biochemical measurements on serum included determinations of inorganic phosphate, glucose, blood urea nitrogen, uric acid, total cholesterol, total protein, albumin, alkaline phosphatase, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase and cholinesterase. Urinalysis measurements included determinations of pH, occult blood, ketone, glucose, protein, urobilinogen and bilirubin. Necropsy was performed on all mice. Organ weights of brain, heart, lung, liver, kidneys, adrenals, spleen, testes and ovaries were determined. Samples of protocol-designated tissues were collected from all animals and examined microscopically. Histopathological examinations were performed on mice sacrificed at weeks 52 and on all mice survived until study termination as well as on all animals died or sacrificed in moribund condition during the study. In addition, histopathology of the liver was performed on mice sacrificed at 78 weeks.

Results

The stability of the test substance in the diet and the correctness of the dietary concentrations and homogeneity were analytically confirmed. The mean test substance consumption was 6.72/8.38, 41.6/51.2 and 267/318 mg/kg bw/day for males and females in the three treatment groups respectively.

Survival in all groups was > 90% at week 78 and > 70% at week 104. There was no difference in mortality and in the incidence of clinical findings among the control and test substance treated groups. Clinical findings included abnormal teeth, piloerection, wasting, sluggish behaviour, tumour mass, dirty hair, loss of hair, eye discharge and anemia.

In male control group the body weights were abnormally high and there was large intergroup variation. Therefore the male body weight data was also compared with historical control data from the same laboratory (

Table 30). In comparison to both concurrent controls and historical controls body weights of 1500 ppm males were decreased from week 15 until end of the study (week 104). The body weights of 250 ppm males were slightly decreased from week 17 to week 46 when compared to both concurrent controls and historical controls. The body weights of 40 ppm males were consistent with those of the historical controls and thus the observed reduction was not considered treatment related. Body weights of females receiving hexythiazox were comparable to those of females in the control groups.

Food consumption of 1500 ppm females was increased during the first 13 weeks. Food efficiency was slightly decreased in 1500 ppm females until week 13 and in 250 ppm and 1500 ppm males from week 26 to 52.

Table 30. Mean body weights (g) of male mice given diets containing hexythiazox for up to 104 weeks

Time point	Mean body weight (g)				
		Hexythiazox dietary concentration (ppm)			
	Historical control groups[a]	0 (concurrent control group)	40	250	1500
Week 0	21.3	22.1	22.2	22.1	22.0
Week 52	45.2	47.1	45.4	43.9**	42.7**
Week 78	45.4	48.2	45.7	44.0**	42.9**

Week 86	n.a.	47.9	44.9	44.5*	42.3**
Week 104	42.7	46.0	41.0**	42.3*	37.8**

* $p < 0.05$ (Dunnett's test)
** $p < 0.01$ (Dunnett's test)
[a] nine studies with 650 mice at initiation, n.a. = not applicable

There were several changes in haematological parameters (**Table 31**). Red blood cell (RBC) counts were decreased statistically significantly in 1500 ppm males at 26, 52 and 78 weeks, in 1500 ppm females at 26 and 78 weeks and in 40 and 250 ppm males at 52 weeks. Hematocrit values of hexythiazox treated males were decreased similarly to RBC counts. In females, hematocrit was decreased statistically significantly at 1500 ppm at 26 weeks. The statistically significant reduction of red blood cell count and haematocrit in the 40 ppm males at week 52 was not considered to be treatment related as this change occurred only once and because the values were within the normal range of the background data of the laboratory. Hemoglobin was decreased statistically significantly in 1500 ppm males at 78 weeks and in 1500 ppm females at 26 weeks. Mean corpuscular volume was increased in 1500 ppm males at 52 and 78 weeks and in all test substance treated females at 52 weeks and in 250 and 1500 ppm females at 104 weeks. Mean corpuscular haemoglobin was increased statistically significantly in 1500 ppm males at 26, 52 and 78 weeks and in 40 and 250 ppm males at 52 weeks. Mean corpuscular haemoglobin was increased in 1500 ppm females at 26 weeks and in 250 and 1500 ppm females at 52, 78 and 104 weeks, although the difference from control was not statistically significant at 1500 ppm at 78 and 104 weeks. WBC counts were decreased dose-dependently and statistically significantly in 250 and 1500 ppm males at 52, 78 and 104 weeks. Platelet counts were increased in 1500 ppm males and females at 52 weeks and in 1500 ppm males at 104 weeks. Reticulocyte counts were increased at 26 weeks in all test substance treated males and in 1500 ppm females and at 52 weeks in 1500 ppm males. Based on the statistically significantly reduced WBC counts at the 250 and 1500 ppm dose levels the NOAEL for haematological parameters is 40 ppm.

Table 31. Some haematological parameters in the 104-week mouse study

Parameter	Week	0 ppm	40 ppm	250 ppm	1500 ppm
RBC (10^6/mm^3) – males	26	8.94	8.99	8.82	8.66**
	52	8.87	8.45**	8.43**	8.24***
	78	8.80	8.66	8.33	8.01**
	104	10.35	9.97	9.03	9.47
RBC (10^6/mm^3) – females	26	9.31	9.34	9.15	8.91***
	52	8.82	8.86	8.65	8.74
	78	8.77	8.80	8.72	8.41*
	104	8.84	8.77	8.54	8.23
Haematocrit (%) – males	26	42.3	42.1	41.3	41.1*
	52	40.8	39.1*	39.1*	38.6**
	78	39.3	38.5	37.9	37.2*
Haematocrit (%) – females	26	43.4	43.3	43.0	42.0**
Haemoglobin (g/dl) – males	78	14.4	14.1	13.9	13.6*
Reticulocytes (%) – females	26	17	19	17	24*
White blood cells (10^3/mm^3) – males	52	3.0	2.6	1.8*	1.2**
	78	3.0	2.5	1.7**	1.6**
	104	4.2	2.6	2.1*	1.6**

*** p < 0.05 (Student t-test);**
**** p < 0.01 (Student t-test);**
***** p < 0.001 (Student t-test)**

Clinical chemistry values did not show consistent changes during the administration period, but the following statistically significant differences were observed. Inorganic phosphate was increased dose-dependently and statistically significantly in 1500 ppm males and females at 26 weeks and in all treated females at 78 weeks. SGPT was increased dose-dependently and statistically significantly in 1500 ppm males at 104 weeks. Total cholesterol was decreased statistically significantly in 1500 ppm males at 52 and 78 weeks and in 250 ppm males at 52 weeks, when the decrease was dose-dependent. In females, total cholesterol was increased statistically significantly at 1500 ppm at 78 and 104 weeks. Glucose was increased statistically significantly and dose-dependently in 250 and 1500 ppm females at 104 weeks. Uric acid was decreased statistically significantly in 1500 ppm males and females and 250 ppm females at 52 weeks and in 1500 ppm females at 78 weeks. Albumin was decreased statistically significantly in 1500 ppm males at 52 weeks and in 250 and 1500 ppm females at 104 weeks. The NOAEL for clinical chemistry parameters was 40 ppm. The results of the urinalysis were comparable with the control group.

Absolute liver and/or liver/body weights were statistically significantly increased in males and females at 1500 ppm throughout the study (**Table 32**). Testes/body as well as brain/body and kidney/body weights in males were statistically significantly increased at 1500 ppm at 52, 78 and 104 weeks, at 250 ppm at 78 and 104 weeks and at 40 ppm at 104 weeks. The changes in

absolute testes and brain weights were very small and the increase in the relative testes and brain weights appeared to be due to decreases in body weights. Although, the changes in absolute kidney weights were also rather small the increase in kidney/body weight was considered treatment related in the presence of histopathological findings at 250 and 1500 ppm. Absolute adrenal and adrenal/body weights of 1500 ppm males were statistically significantly increased at 104 weeks and adrenal/body weight also at 52 weeks. Adrenal/body weight was increased in 250 ppm males at 104 weeks. Absolute ovaries and ovaries/body weights were statistically significantly decreased in 1500 ppm females at 26 weeks. Spleen/body weight was increased in 1500 ppm males at 26 weeks and in 1500 ppm females at 78 weeks. Heart/body weight was increased in 1500 ppm males and females at 52 weeks and in 1500 ppm males at 104 weeks. Lungs/body weight was increased in 1500 ppm females at 52 weeks.

Table 32. Liver weights in a 24 months carcinogenicity study in mice

Parameter	Week	0 ppm	40 ppm	250 ppm	1500 ppm
Absolute liver weight (g) – males	26	1.25	1.21	1.31	1.43**
	52	1.60	1.60	1.55	1.49
	78	1.63	1.68	1.45	1.66
	104	1.95	2.02	1.87	2.59*
Absolute liver weight (g) – females	26	1.10	1.09	1.12	1.26*
	52	1.12	1.12	1.12	1.22*
	78	1.17	1.27	1.25	1.39***
	104	1.46	1.61	1.51	1.69*
Liver weight/body weight (%) – males	26	3.493	3.504	3.604	4.214***
	52	3.360	3.416	3.499	3.914***
	78	3.491	3.530	3.410	4.014
	104	4.455	5.159	4.593	7.002**
Liver weight/body weight (%) – females	26	3.868	3.683	3.734	4.048
	52	3.288	3.427	3.390	4.031***
	78	3.501	3.333	3.526	4.036**
	104	4.146	4.775	4.178	4.954***

*** p < 0.05 (Student t-test);**
**** p < 0.01 (Student t-test);**
***** p < 0.001 (Student t-test)**

The histopathology of the study was subsequently re-evaluated according to pathological evaluation criteria for liver tumours by Maronpot et al., 1987 (DAR IIA 5.5/04-07). The outcome of these evaluations is included below.

Ovarian atrophy was increased in the frequency at study termination in the treated females compared to controls. Hyaline body formation in central nervous system was increased in the frequency in 1500 ppm males and in all test substance treated groups of females at study termination. However, there was no clear dose-response relationship in the incidences of ovarian atrophy and hyaline body formation and these effects are known to be age-related. Hence, the toxicological significance of these phenomenona remains obscure. Proteinaceous casts in kidney were increased in the frequency in 250 and 1500 ppm males and females at

study termination. Gross and histological examination revealed no differences in adrenals between control and hexythiazox treated groups.

Anisonucleosis with the occasional appearance of the fatty change or swelling of the liver cells was slightly increased in hexythiazox treated male mice (**Table 33**). These non-neoplastic lesions were observed at 52 weeks of the experimental period or in the mice of dead or moribund sacrifice and were considered to be possibly related to treatment by the study rapporteur. In females, cytological alterations including nuclear polymorphism and fatty degeneration of liver cells were slightly increased in 1500 ppm group compared to controls at week 78. An increased incidence of hepatic nodules in males and females at 1500 ppm was observed at terminal sacrifice (**Table 33**). These were defined as non-neoplastic hepatoproliferative lesions including focus/area of cellular alteration of both basophilic and eosinophilic natures and hyperplastic change. Liver necrosis was increased in males at 1500 ppm at study termination but not in mice dying or killed earlier in the study.

At terminal sacrifice the incidence of hepatocellular adenoma was statistically significantly increased in 1500 ppm females (**Table 33**). In addition, three hepatocellular carcinomas were observed in each of the hexythiazox treated groups compared to zero incidence in the control group and one hepatoblastoma in 1500 ppm group compared to zero incidence in the control group (**Table 33**). In 1500 ppm males, the incidences of hepatocellular adenoma and carcinoma were slightly, but not statistically significantly increased at terminal sacrifice and three hepatoblastomas were observed compared to zero incidence in controls (**Table 33**). Two of the three mice with hepatoblastoma also had hepatocellular adenoma and carcinoma. Hepatoblastomas were characterised by the irregularly proliferating, closely packed spindle-shaped or polygonal tumour cells with the vascular (capillary) stroma. Mitotic figures were abundant. Hepatoblastomas were defined as poorly differentiated and malignant although no metastases were found. The total incidence of hepatic tumours was statistically significantly increased in both males and females of the 1500 ppm group compared to control groups. There were no other differences in the tumour incidences between the different groups.

Table 33. Summary of microscopic liver findings in a 24 months carcinogenicity study in mice

	Dietary concentration of hexythiazox							
	Males				**Females**			
	0 ppm	**40 ppm**	**250 ppm**	**1500 ppm**	**0 ppm**	**40 ppm**	**250 ppm**	**1500 ppm**
Week 52 [#]								
No. examined [##]	11	10	10	11	12	10	10	10
Cytological alterations - slight	0	5	5	9	0	0	1	0
Fatty metamorphosis - slight - moderate	8 2	3 5	5 2	3 3	4 0	9 0	7 0	5 0
Hepatic nodule	0	0	0	0	1	0	0	0
Adenoma	2	0	1	0	1	0	0	0
Week 78								
No. examined [##]	13	13	11	11	12	11	10	11
Cytological alterations - slight - moderate	9 0	8 1	8 0	8 1	4 0	5 0	7 0	8 0
Fatty metamorphosis - slight	9	7	1	7	1	3	3	7
Hepatic nodule	5	1	2	5	0	1	1	4
Adenoma	7	3	2	2	1	0	0	0
Carcinoma	0	1	1	0	0	0	0	0
Week 104								
No. examined [##]	46	47	49	48	46	49	50	49
Cytological alterations - slight	36	41	47	47	3	4	2	2
Fatty metamorphosis - slight - moderate	28 0	20 0	21 1	25 0	12 2	16 2	24 1	10 2
Necrosis								

- slight	4	5	5	8	2	3	3	5
Hepatic nodule	10	8	14	30	5	5	2	15
Adenoma	11 (24%)	20 (42%)	14 (29%)	25 (52%)	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	3 (6%)	0	0	0	1 (2%)
Total								
No. examined ##	70	70	70	70	70	70	70	70
Hepatic nodule	15	9	16	**35*****	6	6	3	**19****
Adenoma	20 (28.6%)	23 (32.9%)	17 (24.3%)	27 (38.6%)	7 (10%)	1* (1.4%)	5 (7.1%)	**16* (22.9%)**
Carcinoma	11 (15.7%)	9 (12.9%)	10 (14.3%)	14 (20.0%)	0	3 (4.3%)	3 (4.3%)	3 (4.3%)
Hepatoblastoma	0	0	0	3 (4.3%)	0	0	0	1 (1.4%)
Total no. hepatic tumours	31	32	27	**44***	7	4	8	**20****
No. mice with hepatic tumour	29	30	25	37	7	4	8	**20****

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Fisher's exact test)

Histopathology was not performed at 26 weeks ## Includes decedents,

The data includes re-evaluations (DAR IIA 5.5/04-07)

The in-life phase of the mouse carcinogenicity study (DAR IIA 5.5/03) was conducted December 1981 - November 1983. According to the notifier histopathology data were not recorded electronically in the performing laboratory over this time.. Thus, there are no historical control data of the performing laboratory on liver neoplastic changes in B6C3F1:Slc mouse from the respective time frame.

Instead, the notifier has submitted historical control data on liver neoplastic changes from studies conducted during 1988-1992 in the performing laboratory (11 studies, 500 males and 550 females, **Table 34**, DAR IIA 5.5/16) and spontaneous liver tumour rates from studies of the NTP from 1980 until March 1983 (**Table 35**, Haseman et al. 1984). The later data is compiled from 5 different CROs with more than 5 studies from the NTP program (**Table 35**). The notifier has also submitted historical control data of hepatoblastoma from six independent studies conducted during 1985-1991 in the performing laboratory (AN-PYO Center) with a total of 551 animals (251 males, 300 femals, DAR IIA 5.5/17). The data reveals hepatoblastoma incidence range **0-2%** (mean incidence 0.4%) in males and **0%** incidence in females. We note that this data was gathered in a different time frame (the mouse carcinogenity study was terminated in November 1983) and thus can not be considered valid. The NTP database reveals hepatoblastoma control incidence ranges **0-2%** for both male and female B6C3F1 mice (mean incidences 0.2%, 950 animals in 19 studies during 1984-1994). The

observed incidences of hepatoblastoma in high dose males (**4.3%**) and females (**1.4%)** are slightly over these incidence ranges.

Table 34. B6C3F1:Slc mouse (109 weeks) historical control data (1988 – 1992) of pre-neoplastic and neoplastic liver changes of AN-PYO Center (DAR IIA 5.5/16)

Sex	No. of animals sacrificed	Classification of tumours	Incidence	Rate (%)	Range (%)
Males	500	Focus of cellular alteration*	97	19.4	8-42
		Adenoma, hepatocellular	235	47.0	22-76
		Carcinoma, hepatocellular	102	20.4	12-28
Females	550	Focus of cellular alteration*	41	7.5	2-12
		Adenoma, hepatocellular	81	14.7	8-32
		Carcinoma, hepatocellular	35	6.4	2-12

* Hepatic nodule in the study is expressed as “focus of cellular alteration” in this paper due to change of technical term

Table 35. B6C3F1:Slc mouse (104 weeks) historical control data (1980 – 1983) of neoplastic liver changes of NTP studies (Haseman et al. 1984)

	Males mice						Female mice					
	No. of studies	No. of Animals sacrificed	Liver adenoma		Liver carcinoma		No. of studies	No. of animals sacrificed	Liver adenoma		Liver carcinoma	
Laboratory			Rate (%)	Range (%)	Rate (%)	Range %			Rate (%)	Range (%)	Rate (%)	Range (%)
A	9	448	9	6-14	23	12-30	9	446	3	0-14	4	0-6
B	5	248	6	2-10	22	10-32	5	247	4	0-8	4	0-10
C	15	745	12	0-22	21	10-36	15	748	6	0-18	4	0-8
D	8	398	9	2-14	22	8-28	8	400	2	0-4	6	2-15
E	10	280	9	2-17	19	13-27	7	371	6	2-9	4	0-9

Conlusion

Dietary administration of hexythiazox to mice resulted in a reduction of body weight gain in 250 and 1500 ppm males. The NOAEL for haematological and clinical chemistry parameters was 40 ppm. Absolute and relative liver weights were increased in 1500 ppm males and females. Absolute and relative adrenal weights were increased in 250 and 1500 ppm males. The increase in kidney/body weight was considered treatment related in the presence of increased incidence of proteinaceous casts in 250 and 1500 ppm males and females. Histopathology confirmed liver as the target organ. The liver findings included slight increase of cytological alterations in 1500 ppm males and females at interim sacrifices, increase in liver necrosis in 1500 ppm males and increase of liver nodules in 1500 ppm males and females at the terminal sacrifice. The incidences of hepatocellular adenomas and total hepatocellular tumours were statistically significantly increased in 1500 ppm females. The incidence of total hepatocellular tumours was statistically significantly increased in 1500 ppm males. However, no dose-related hepatoproliferative effect of hexythiazox was observed. Hepatocellular tumours and preneoplastic changes were more common and appeared earlier in the control group than in the treated groups up to 78 weeks of the experiment. The NOAEL for chronic

toxicity was 40 ppm (6.72 mg/kg bw/day for males and 8.38 mg/kg bw/day for females). The NOAEL for oncogenicity was 250 ppm (41.6 mg/kg bw/day for males and 51.2 mg/kg bw/day for females).

4.9.1.2 Carcinogenicity: inhalation

No data are available

4.9.1.3 Carcinogenicity: dermal

No data are available

4.9.2 Human information

No data are available

4.9.3 Other relevant information

No data are available

4.9.4 Summary and discussion of carcinogenicity

The carcinogenicity of hexythiazox has been investigated in oral two-year combined toxicity and carcinogenicity studies in F344 rats and B6C3F1 mice (DAR IIA 5.5/01, DAR IIA 5.5/03-07).

In rats, the incidence of testicular interstitial cell (Leydig cell) adenoma was slightly but not statistically significantly increased in high (3000 ppm corresponding to 163 mg/kg bw/day) and mid dose (430 ppm corresponding to 23 mg/kg bw/day) males compared to concurrent controls and historical controls at 12 months interim sacrifice. At the end of the study (24 months) the incidence of thyroid parafollicular cell adenoma was slightly increased in high dose males compared to concurrent controls and the incidences of mammary gland fibroadenoma were increased in hexythiazox treated male groups compared to control group.

Testicular interstial cell adenoma is a common spontaneous tumour in F344 rat strain. Leydig cell tumourigenesis in the rat is often caused by a prolonged dusruption of the pituitary-gonadal hormone axis, a major impetus considered to be sustained high circulating LH levels. Due to properties of their LH receptor, F344 rats are particularly sensitive to this tumour type and its occurance is not normally considered informative in F344 rats (European Commision Joint Research Centre, Specialized Experts Meeting, January 2004). There were no differences in the adenoma incidences between different groups at the end of the study (at 24 months). There was only trace interstitial cell hyperplasia and no dose response or clear relation in its incidences or in the incidences of other histopathological findings to adenoma incidences. Due to reasons above we do not consider a slight increase in the incidence of this tumour type in mid and high dose F344 males at 12-month interval relevant for carcinogenicity classification.

Following two years hexythiazox treatment the incidence of thyroid parafollicular cell (chief cell, C-cell) adenoma was slightly, but not statistically significantly increased in high dose male rats compared to concurrent controls (incidences **4.3%, 4.3%, 2.9% and 10.3%** at 0, 60 ppm, 430 ppm and 3000 ppm, respectively, **Table 26).** In females, there were no differences in the incidences of parafollicular cell tumours, but parafollicular cell hyperplasia was slightly

increased in all hexythiazox treated groups compared to concurrent controls (**Table 24**, 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). Only hyperplasia graded as trace or mild was observed in hexythiazox treated groups. There are no fully acceptable historical control data available on the spontaneous incidences of thyroid tumours in F344 rats in the performing laboratory at the time of the study (see section 4.9.1.1). Parathyroid gland neoplasms are generally rare in F344 rats as well as in other rat strains. However, occasional spontaneous incidences of parafollicular adenoma in F344 males have been reported in a few studies in the performing laboratory (7-12% in three studies 1986-1989, **Table 27**) and in NTP database (4%). In the rat carcinogenicity study (DAR IIA 5.5/01) parafollicular cell adenomas were observed in all female and male groups including the controls. We further note that there was no clear dose response in the incidences of this tumour type or in the incidences of any other thyroid tumour in either sex. This suggests that the finding is not related to hexythiazox treatment. Moreover, we consider it unlikely that a slight increase in the incidence of thyroid parafollicular cell adenomas in high dose males only would be toxicologically significant.

The incidence of mammary gland fibroadenoma was increased in hexythiazox treated F344 male rats compared to concurrent controls (incidences **0%, 1.4%, 2.9% and 9.0%** at 0, 60 ppm, 430 ppm and 3000 ppm, respectively, **Table 28**). 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 (see 4.9.1.1.). There are no fully acceptable historical control data available from the performing laboratory at the time of the study. The observed incidence in high dose males was slightly above the submitted historical control data range in the performing laboratory at different time frame (**0-6.0%**) but within the historical ranges of NTP database (**0-12%**).

The notifier has stated that the increase in fibroadenomas in the high dose males should be regarded as an incidential finding due to following reasons (DAR IIA 5.5/10):

"- fibroadenomas seen in the high-dose rats were essentially identical morphologically with spontaneously occurring fibroadenomas

- lack of additional compound-related alterations occurring in the mammary tissue

- lack of compound-induced pituitary lesions which might have resulted in mammary alterations

- lack of any similar response in female rats

- Historical data which suggests that the incidence of fibroadenomas in male F344 rats may be quite variable

Based on this study (DAR IIA 5.5/01), it has also been questioned during PPP review whether hexythiazox treatment reduces the incidence of spontaneously occurring mammary fibroadenomas in female rats (incidences **8.6%, 4.4%, 1.4% and 7.1%**, in 0, 60 ppm, 430 ppm and 3000 ppm, respectively, **Table 28**) The notifier has submitted a statement including the following reasoning against this (DAR IIA 5.5/02):

"- There is considerable variation in the spontaneous incidence of mammary tumours in this rat strain and the incidences of this tumour type in females in all dose groups were within the historical control range of the laboratory.

- There were no indications of hormonal influence of hexythiazox on fertility parameters in a two-generation study in rats or in teratogenicity studies in rats and rabbits (DAR IIA 5.6/01, 1984, DAR IIA 5.6/02, DAR IIA 5.6/03). Based on the data of the body weight development of pups during lactation in a two generation study it was deduced that the physiological function of the mammary gland was not affected by hexythiazox treatment. The rate of surviving

newborns in the treated groups during the lactation period was comparable to the control group. No treatment related histopathological changes caused by hormonal reasons were observed in these studies."

We agree that there are no morphological or histological findings in mammary gland that would support hormonal mechanism of mammary tumour formation in males only. Nor there are findings in reproduction toxicity studies that would indicate hormonal influence on fertility. However, regarding function of the mammary gland in females, we note that in a rat two generation reproduction study (DAR IIA 5.6/01) pup weights of high dose F1B litter were statistically significantly reduced over the lactation period, from day 4 to day 28, although there were no difference in pup weights on day 0. This finding was apparent also in litter F2B. Moreover, we note that incidences of pituitary hyperplasia were slightly increased in all hexythiazox treated groups compared to concurrent controls in both males and females at the end of the rat carcinogenicity study DAR IIA 5.3/01, **Table 24**). There are also some consistent findings in the database suggesting that hexythiazox may cause hormonal imbalance. First, following hexythiazox treatment at doses above 150 mg/kg bw increased adrenal weights were observed in several studies in rat, dog and mouse particularly in males (DAR IIA 5.3/03-04, 5.5/01, 5.5/03). This finding was accompanied with fatty degeneration/changes of adrenal cortex in rat (DAR IIA 5.3/03, DAR IIA 5.5/01) and adrenocortical hypertrophy in dog (DAR IIA 5.3/04) which may indicate effect on steroidogenesis. Secondly, increased ovary and testes weights in response to doses comparable to those of adrenal findings were observed in rat (DAR IIA 5.3/03, 5.5/01, 5.6/01). However, there were no histological findings in these hormone sensitive tissues (hormone levels were not measured in the study) or in the mammary gland and thus no clear indication of hormonal mechanism of tumour formation in males only. Taking also into account that mammary gland fibroadenoma is relatively common tumour in F344 rats we conclude that it remains unclear whether these findings, a slight increase in mammary gland fibroadenoma in high dose males and the slightly decreased incidence of these tumours in low and mid dose females, are related to hexythiazox treatment.

In mice, dietary administration of high hexythiazox dose for two years resulted statistically significantly increased total incidence of hepatic tumours (DAR IIA 5.5/03-07). In females, the incidence of hepatocellular adenoma was statistically significantly increased at high dose (1500 ppm, corresponding to 318 mg/kg bw/day) at terminal sacrifice (incidences **10% and 22.9%** in controls and 1500 ppm, respectively). In addition, three hepatocellular carcinomas in each of the hexythiazox treated groups and one hepatoblastoma in high dose group were observed compared to zero incidence in concurrent controls. In high dose (267 mg/kg bw/day) males the incidences of hepatocellular adenoma and carcinoma were slightly, but not statistically significantly increased at terminal sacrifice and three hepatoblastomas were observed compared to zero incidence in controls (**Table 33**). There were no findings suggesting oncogenic effect of hexythiazox on liver or on other organs at lower doses.

B6C3F1 mice have very high spontaneous incidence of liver tumours. In ECHA CLP Guidance 2015, the liver tumours in B6C3F1 mice are given as an example for tumours with a high spontaneous incidence, and it is stated that “where the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories.” (ECHA Guidance CLP Criteria 2015). No acceptable historical control data from the performing laboratory is available. However, the observed incidences of hepatic tumours are within or slightly over the incidence ranges of these tumour types in the performing laboratory at different time frame and observed in other laboratories in B6C3F1 mice (**Table 34** and **Table 35**, Tarone et al. 1981).

The high hexythiazox dose was hepatotoxic in mice indicated by increased liver weights, hepatic nodules, hepatic necrosis and cytological alterations of liver cells. Increased incidence

of hepatic nodules at the end of the study suggests that hexythiazox has proliferative effect on the liver of B6C3F1 mice. Thus, increased incidences of liver tumours in high dose B6C3F1 mice are presumably caused by hepatoxicity and proliferative stimulus on the liver. However, no dose-responses in hepatoproliferative effect or in the incidences of tumours were observed. Moreover, hepatocellular tumours and preneoplastic changes were more common and appeared earlier in the control group than in the treated groups up to 78 weeks of the experiment (**Table 33**). Thus, the alterations of tumour incidences occurred only after 18 months treatment with high hexythiazox dose. 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. Moreover, hexythiazox treatment had no effect on survivability of mice (hepatic tumours were not considered as a cause of death for any of the decedents), and in spite of signs of hepatoxicity, there were no effect on hepatic tumour incidences in hexythiazox treated rats (DAR IIA 5.5/01). Taken together, we do not consider increased incidence of liver tumours after two years high dose hexythiazox treatment in B6C3F1 mice strain sufficient evidence for carcinogenicity classification. No other tumour incidences were increased in mice of either sex and therefore hexythiazox is not considered to be carcinogenic in mice.

4.9.5 Comparison with criteria

According to CLP criteria for the purpose of classification for carcinogenicity substances are allocated to one of two categories (Category 1 and 2) based on strength of evidence and additional considerations (weight of evidence). Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance.

Substances in Category 1 (known or presumed human carcinogens) may be further distinguished as Category 1A (known to have carcinogenic potential for humans) or Category 1B (presumed to have carcinogenic potential for humans) carcinogens. Classification for Category 1A is largely based on human evidence and classification for Category 1B is largely based on animal evidence.

There are no human data available for hexythiazox, thus classification for Category 1A is not possible.

Classification for **Category 1B** should be based on animal experiments (or human studies) for which there is sufficient evidence to demonstrate animal carcinogenicity i.e. **"a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites."**

In rat, two years high hexythiazox dose treatment caused slight increases in the incidences of two benign tumour types in two tissues (parathyroid gland and mammary gland) in males only. The slightly increased incidences of Leydig cell adenoma (benign) in this study in mid and high dose males after 12 months hexythiazox treatment is not considered relevant for carcinogenicity classification. There is no evidence of genotoxic potential for hexythiazox (see

section 4.8) and no clear indication of hormonal mechanism of tumour formation in males only. Mammary gland fibroadenoma is relatively common tumour in F344 rats. It is unclear whether these findings are related to hexythiazox treatment and thus we consider that a causal relationship has not been established between the increased tumour incidences and hexythiazox treatment. In mice, two years treatment with high hexythiazox dose resulted statistically significant increase in total hepatocellular tumours, including both benign and malignant tumours, in both sexes. However, since this mice strain (B6C3F1) is exceptionally sensitive to promotion of hepatic tumours these findings are not considered sufficient evidence to warrant classification for Category 1B.

The placing of a substance in **Category 2** (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. Limited evidence of carcinogenicity in animal studies is defined as: **the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.**

We do not consider the causality between hexythiazox treatment and the slight increases in the incidences of mammary gland fibroadenoma and parafollicular cell adenoma (benign tumours) in high dose F344 male rats credible. It is also considered unlikely that these findings would be toxicologically significant. There is evidence that hexythiazox promotes formation of liver tumours in B6C3F1 mice strain. In principle, this could warrant classification for Category 2. However, significant increases in the total incidence of hepatocellular tumours following high dose hexythiazox treatment in a mice strain exceptionally sensitive to promotion of liver tumours with high spontaneous incidences is considered of low relevance for humans. Despite hepatotoxicity, there were no effect on hepatic tumour incidences in hexythiazox treated rats. These and other factors discussed above considerable decrease the level of concern regarding the hexythiazox carcinogenicity concern for humans (**Table 36**). Altogether these findings are considered weak and inconsistent evidence and as such not sufficient for carcinogenicity classification for Category 2.

Table 36. Some important factors which may be taken into consideration when assessing the overall level of concern of the hexythiazox-induced tumours.

Tumour type:	Liver tumours (the causality and toxicological significance of mammary gland fibroadenomas and parafollicular cell adenomas in male rats only is not considered credible)
Multi-site responses:	No (only liver)
Progression of lesions to malignancy:	Yes, but no metastases were found and hepatic tumours did not cause lethality
Reduced tumour latency:	No, (alterations of tumour incidences occurred only after 18 months' treatment)
Whether responses are in single or both sexes:	Both sexes (in males hepatic tumour incidences were increased only slightly at high dose and

	there were no clear dose response in the incidences of any of the liver tumour types in either females nor males.
Whether responses are in a single species or several species:	Single species (mice). In spite of hepatoxicity no increases in liver tumour incidences were observed in rats.
Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:	Not noted
Routes of exposure:	oral (relevant for human)
Comparison of absorption, distribution, metabolism and excretion between test animals and humans:	Not known
The possibility of a confounding effect of excessive toxicity at test doses:	No
Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity:	The data suggests that increased tumour incidences are caused by hepatotoxicity and proliferative stimulus on the liver of B6C3F1 mice. The data demonstrates promoting activity only in the liver in a mice strain exceptionally sensitive to promotion of liver tumours which is of low relevance for humans.

4.9.6 Conclusions on classification and labelling

No classification.

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	**2/70**	**6/67**	6/70	3/68	1/70	5/70

		(1.4%)	**(2.9%)**	**(9.0%)**	(8.6%)	(4.4%)	(4.3%)	(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**.

4.10 Toxicity for reproduction

Not evaluated in this dossier.

4.11 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 37: Summary of relevant information on degradation

Method	Results	Comment	Reference
Stability in water Hydrolysis rate Purity: 99.8 %, ^{14}C radio-labelled, 6.6 mCi/mmol BBA techn. bulletin No. 55, part 1, non GLP	DT_{50} at 22°C: pH 5, >2917 days (conc. 0.025 and 0.25 mg/l) pH 7, >2917 days (conc. 0.025 and 0.25 mg/l) pH 9, 370 days (conc. 0.025 mg/l), 504 days (conc. 0.25 mg/l) DT_{50} at 50°C: pH 5, 1721 days (conc. 0.025 mg/l), 2608 days (conc. 0.25 mg/l) pH 7, 179 days (conc. 0.025 mg/l), 203 days (conc. 0.25 mg/l) pH 9, 3 days (conc. 0.025 and 0.25 mg/l) DT_{50} at 70°C: pH 5, 194 days (conc. 0.025 mg/l), 315 days (conc. 0.25 mg/l) pH 7, 12 days (conc. 0.025 and 0.25 mg/l) pH 9, 0.2 day (conc. 0.025 and 0.25 mg/l) Hydrolysis study with ^{14}C-Hexythiazox was conducted at 22, 50 and 70 °C at pH 5, 7 and 9 under sterile conditions in absence of light. At all conditions PT-1-3 was the major hydrolysis product.	Hydrolysis constants and half-lives estimated by first-order kinetics.	DAR IIA 7.2/01

Method	Results	Comment	Reference
Soil Photolysis Purity: 99 %, ^{14}C-thiazolidine radio-labelled, 6.6 mCi/mmol US EPAguidelines Subdivision N, Environmental Fate §161-3 (1982) non GLP	**The half-life** (DT50) for photodegradation of Hexythiazox was calculated with the least-squares method assuming first-order degradation kinetics. The DT50 was estimated to be approximately 116 days. Photodegradation is proposed to follow the same degradation pathway as aerobic degradation in soil.	The study was performed in natural conditions, where temperature and luminous intensity had a large variation during the test. For the above reasons, the study does not fulfil the current requirements according to the DAR. However, the DAR also mentions that in spite of the deficiencies, the results of the study suggest that photolysis does not significantly contribute to degradation of Hexythiazox in soil surface and the results can be used in risk assessment.	DAR IIA 7.1/04

Method	Results	Comment	Reference
Photochemical degradation in water DAR IIA 7.2/03 98.8 %, ^{14}C-thiazolidine radio-labelled, 56 mCi/mmol BBA test guideline, Part A EPA Subdivision N, §161-2 GLP compliant DAR Additional report IIA 7.2/12 98.4 %, ^{14}C-thiazolidine radio-labelled, 52.7 mCi/mmol JMAFF no. 13-Seisan-1739, similar to OECD draft Guideline phototransformation of chemicals in water – direct and indirect photolysis GLP compliant	DAR IIA 7.2/03 $t_{½}$ at 25 °C in distilled water under artificial sunlight (Xenon lamp): 51.0 days $t_{½}$ at 25 °C in river water under artificial sunlight (Xenon lamp): 13.8 days The major photolysis product in river water was PT-1-8 (5.7 % in 14 days) The mass balance in distilled and river water irradiated test samples was from 97 % to 102 % and for the dark control samples from 98 % to 103 % of applied radioactivity DAR Additional report IIA 7.2/12 DT_{50}-values: Hexythiazox degraded slowly in distilled water and in river water under artificial sunlight. The DT_{50}-values under continuous irradiation were calculated to be 168 days ($k=4.14\times10^{-3}$ day^{-1}, r^2 0.78) and 147 days ($k=4.73\times10^{-3}$day^{-1}, r^2 0.86) in distilled water and river water, respectively. Several degradation products were detected but none of them exceeded 4.3 % of applied radioactivity. Converted to natural sunlight at 35° Northern latitude (April to June), the half-lives were 1206 and 1056 days in distilled water and river water, respectively.	Hexythiazox did not react with direct phototransfor-mation. Since no absorbance exists > 290 nm photodegradation first order rate constant in water could not be calculated. RMS agrees with the position paper (DAR Additional report IIA 7.2/13), in which the unrealistically high irradiation intensity (leading to formation of radicals) and non sterilised test system (leading to microbial degradation) are suggested to be the reasons for the observed formation of high amount (40.5 % AR) of unidentified metabolites in the study of DAR IIA, 7.2/03assessed in DAR (p. 330).	DAR IIA, 7.2/03, DAR Additional report IIA 7.2/12 and DAR Additional report IIA 7.2/13
Quantum yield 98.8 %, ^{14}C-thiazolidine radio-labelled, 13.6 mCi/mmol BBA test guideline, Part A, GLP PNAP-PYR actino-meter	3.03×10^{-5} molecules degraded per photon in distilled water.		DAR IIA, 7.2/03

Method	Results	Comment	Reference
Stability in air, photochemical oxidative degradation Atkinson calculation, non GLP	DT_{50} = 3.62 hours, k = 35.4×10^{-12} $cm^3 \cdot molecule^{-1} \cdot sec^{-1}$		DAR IIA, 7.2/09
Aerobic degradation in soil DAR IIA 7.1/01 97.4% ^{14}C-thiazolidine radio-labelled 1.95 GBq/mmol OECD Draft Guideline 307 SETAC Guideline 1995 GLP compliant Test temperature: 17-22 °C DAR IIA, 7.1/02 > 99 % [^{14}C] labelled Hexythiazox (NA-73) 6.6 mCi/mmol Regarding guidelines it is only mentioned that "study is similar to SETAC guideline, Part 1, Section 1" GLP: no (study was conducted prior to the implementation of GLP) Test temperature: 15 °C and 25 °C DAR Additional report IIA 7.1/11>99 %, ^{14}C-cyclohexyl radio-labelled, 28.2 mCi/mmol OECD Guideline 307 SETAC Guideline 1995 GLP compliant Test temperature 20 ± 2°C	**DT_{50} at 20°C** (Q_{10} of 2.58 and Walker equation coefficient of 0.7:[a] Sand 32.5 (Shiotani 2002) Sandy loam 27.4 (Shiotani 2002) Clay loam 7.8[a, c] (Anonymous 1984) Light clay 14.4[c] (Anonymous 1984) Clay loam 10.3[b, c] (Anonymous 1984) Light clay 18.9[c] (Anonymous 1984) Silt loam 56.0 (Klöppel 2009a) Geomean: 23.7 (n=5) [a]see DAR Additional Report IIA 7.1/12) [b]DT_{50} derived from the DT_{90} value based on FOMC kinetics divided by the factor of 3.32 (pseudofirst-order DT_{50}) [c]Values derived from the 15 °C and 25 °C tests using temperature correction		DAR IIA, 7.1/01, DAR IIA, 7.1/02, DAR Additional report IIA 7.1/11

Method	Results	Comment	Reference
Soil field studies 10.3% Hexythiazox in 10 WP formulation BBA Guideline Part IV, 4-1 (1986) GLP compliant	**$DisT_{50}$ at 25 -100 cm in Germany (bare soils):** Silty loam n.d. Loamy sand n.d. Sandy loam n.d. Sandy loam n.d. n.d.= Not determined because not enough data points above the detection limit of 0.01 mg/kg were available **$DisT_{90}$ at 25 -100 cm in Germany (bare soils):** Silty loam around 58 days.* Loamy sand around 16 days* Sandy loam around 97 days* Sandy loam around 29 days.* *The estimate of $DisT_{90}$ indicates the sampling time when the Hexythiazox residues in soil were first time detected to be below the detection limit of 0.01 mg/kg		DAR IIA, 7.1/06

Method	Results	Comment	Reference
Water/sediment studies DAR IIA 7.2/05 >97 %; ^{14}C-thiazolidine radio-labelled, 2.07 GBq/mmol and 614 MBq/mmol BBA Guideline part IV, 5-1 (1990) EPA Guidelines, Subdivision N, §162-4 (1982) GLP compliant Test temperature: 20 °C DAR Additional report IIA 7.2/14>99 %; ^{14}C-cyclohexyl radio-labelled, 28.2 mCi/mmol OECD Guideline 308 SETAC – Guideline 1995 GLP compliant Test temperature: 20±2 °C	**DT_{50} - DT_{90} (days)** Sandy loam: Whole system 38 - 128 Water 0.5 - 1.5 Sed. 42 - 138 Sand: Whole system 33 - 109 Water 0.6 – 1.8 Sed. 37 - 123 Sandy: Whole system 156 -519 Water 11 - 38 Sed. n.d. Loamy: Whole system 135 - 449 Water 4 - 13 Sed. n.d. **DT_{50} – DT_{90}: Geo mean (days)** Whole System: 72 - 239 Water 1.9 – 6.0 Sed. 39 - 130	According to the DAR the IIA 7.2/05 study was well performed and generally followed the current OECD 308 guideline. Also the DAR Additional report IIA 7.2/14) study is included in the EFSA Conclusion (EFSA 2010). .	DAR IIA, 7.2/05 and DAR Additional report IIA 7.2/14

5.1.1 Stability

Hydrolysis in water

Under sterile conditions at ambient (22ºC) temperature, hydrolytic degradation of Hexythiazox was slow in alkaline (pH 9) aqueous solutions, whereas at neutral (pH 7) and acidic conditions (pH 5) Hexythiazox was found to be stable. The hydrolytic half-life of Hexythiazox at 22°C and at pH 9 ranged from 370 to 504 days. Only one metabolite - PT-1-3 -was found at ambient temperature, at pH 5, 7 and 9 and it accounted for up to 7 % (DAR IIA 7.2/01). **Based on the hydrolysis half-lives Hexythiazox is not rapidly degradable according to the CLP criteria.**

Soil Photolysis

Hexythiazox degraded slowly by photolytic processes on the soil surface. The photolytical half-life of Hexythiazox was determined to be approximately 116 days. The photolytic degradation of Hexythiazox on soil surface follows the same mechanisms as found for microbial degradation in soil. No metabolite exceeded 4 % during photolytic degradation of Hexythiazox on the soil surface.

Photochemical degradation in water

Two studies are available to investigate the photolysis in distilled water and river water (**Table 37**). The results and the study design of both studies are compared in the position paper (DAR Additional report IIA 7.2/13)) and the following summary is included in the DAR Additional report (Finland 2009) as follows:

In the photolysis study (DAR IIA, 7.2/03) unrealistic high irradiation intensity and non sterilised test systems were used which resulted in an exaggerated composition of the test compound by photolysis due to formed radicals and by microbial degradation. The unidentified metabolites accounted for 40.5 % AR in the irradiated river water samples. Reasonable efforts have been made to elucidate their identity but the degradation products could not be quantified with the technique used. The unidentified, very polar metabolites obtained only under irradiated river water are considered as not relevant in natural surface waters. In water/sediment study unidentified polar metabolites represented 4-8 % applied radioactivity after 100 days (DAR IIA, 7.2/05, original DAR p. 333). A new photolysis study (DAR Additional report IIA 7.2/12) with river water and distilled water was conducted using lower radiation intensity, sterilised test systems and a more modern analytical technique for the identification of the degradation products. This study confirmed the assumptions already made that any degradation product produced by irradiation are not relevant since in newer study several metabolites were detected but none of them exceeded 5 % of applied radioactivity.

According to the OECD draft guideline "phototransformation of chemicals in water – direct and indirect photolysis" the irradiation period should not exceed 30 days/nights natural sunlight exposure. However, in both studies, unrealistically high irradiation intensity was used resulting in prolonged irradiation periods (e.g. comparable to 147 days sun light days of study described under B.8.4.2) compared to natural conditions and thus the study period exceeded by far the required study duration according to the recommendations of the relevant guidelines. In addition, the half-life of Hexythiazox in the water phase of natural aquatic systems (2 to 11 days) due microbial degradation and adsorption to the sediment phase is much shorter than due to photolysis, and thus the results of both studies at the last sample points are not relevant. It can therefore be concluded that no degradation products of Hexythiazox were formed in relevant amounts and relevant time period by photolysis in natural water as well in distilled water.

Photochemical degradation in water is not considered relevant for the assessment of rapid degradability criterion in the present case. Therefore the information above is not used for classification.

Soil Photolysis

Hexythiazox degraded slowly by photolytic processes on the soil surface. The photolytical half-life of Hexythiazox was determined to be approximately 116 days. No degradation product exceeded 4 % during photolytic degradation of Hexythiazox on the soil surface.

Soil photolysis is not considered relevant for the assessment of rapid degradability criterion in the present case. Therefore, the information above is not used for classification.

Stability in air

The photochemical and oxidative degradation of Hexythiazox in air was evaluated on theoretical grounds by a calculation according to Atkinson. The calculation was performed with the help of the programme AOPWIN, Atmospheric Oxidation Programme v1.90 for Microsoft Windows 3.1, Windows 95/98, Windows NT (© 2000 US Environmental Protection Agency).

The DT_{50} for Hexythiazox in the atmosphere was estimated to be 3.62 hours. As Hexythiazox contains no olefinic carbon-carbon double and acetylic triple bonds, it is not supposed to react with ozone.

Stability in air is not relevant for the assessment of rapid degradability criterion. Therefore the information above is not used for classification.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

No biodegradation screening tests are included in the DAR. Regarding ready biodegradability, it is mentioned in the DAR that "*This test is not required under the provisions of Annex VI to Council Directive 67-548/EEC 18th adaptation to technical progress as amended by Comission Directive 93/21/EEC. Point 5.2.1.3 states that substances can be considered readily degradable if other convincing scientific evidence is available to demonstrate that the substance can be > 70 % degraded (biotically and/or abiotically) in the aquatic environment within a 20 day period. Such evidence is available in the hydrolysis studies in buffered water* [DAR IIA, 7.2/01] *as well as in the water/sediment study* [DAR IIA, 7.2/05] *and they show that hexythiazox is not readily biodegradable.*" It is noted this part of the DAR refers to a directive that has been replaced by the CLP regulation and that the above-mentioned interpretation of water/sediment study as evidence of ready biodegradation is not necessarily applicable to classification under the CLP regulation.

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems

There are two water/sediment studies with Hexythiazox available. In the study from DAR IIA, 7.2/05, ^{14}C-thiazolidine ring labeled Hexythiazox was used, whereas in the study from DAR Additional report IIA 7.2/14, ^{14}C-cyclohexyl ring labeled Hexythiazox was used.

In aerobic water/sediment systems, Hexythiazox was rapidly dissipated from the water phase by degradation to metabolites and by partitioning to the sediment. Partitioning of parent and metabolites from the water phase to the sediment and further metabolism in the sediment led to the formation of bound residues. The dissipation half-life in the water phase was calculated to be in the range from 0.5 to 11 days and the degradation half-life in the entire system in the range from 33 to 156 days assuming simple first order kinetics. In the sediment phase, the dissipation half-life ranged from 37 to 42 days in two systems and in further two systems the half-lives were not calculated due to very slow dissipation.

In the water/sediment study with the thiazolidine ring labelled Hexythiazox, two major degradation products were detected and identified as PT-1-2 and PT-1-9. They accounted for up to 32.2 % and 12.7 % AR in the entire system, respectively. Besides PT-1-2 and PT-1-9, up to 5 metabolites were identified in the water phase and sediment but none of them alone exceeded 8.1 %.

In the water/sediment study with the cyclohexyl labelled Hexythiazox two additional degradation products were identified. PT-1-8-c accounted for up to 9.1 % in water and up to 7.4 % in sediment and PT-1-8-t accounted up to 7.1 % in water and 8.7 % in sediment. One degradation product was identified as the structural isomer of PT-1-10-2 and it accounted up to 7.3 % in water but was not found in sediment.

As it is not possible to differentiate the degradation rate in water from that in sediment, the dissipation half-life in water cannot be used to evaluate rapid degradation. Degradation rate in water/sediment system or in sediment is not among the preferred data to be used for assessing rapid degradability according to the CLP guidance. However, the whole-system primary degradation half-lives in the water-sediment systems as well as the half-lives in sediment are above 16 days and therefore suggest that Hexythiazox is not rapidly degradable according to CLP criteria. Consequently, no further information/assessment regarding the degradation products is needed for assessing the rapid degradability criterion.

Degradation in soil (aerobic conditions)

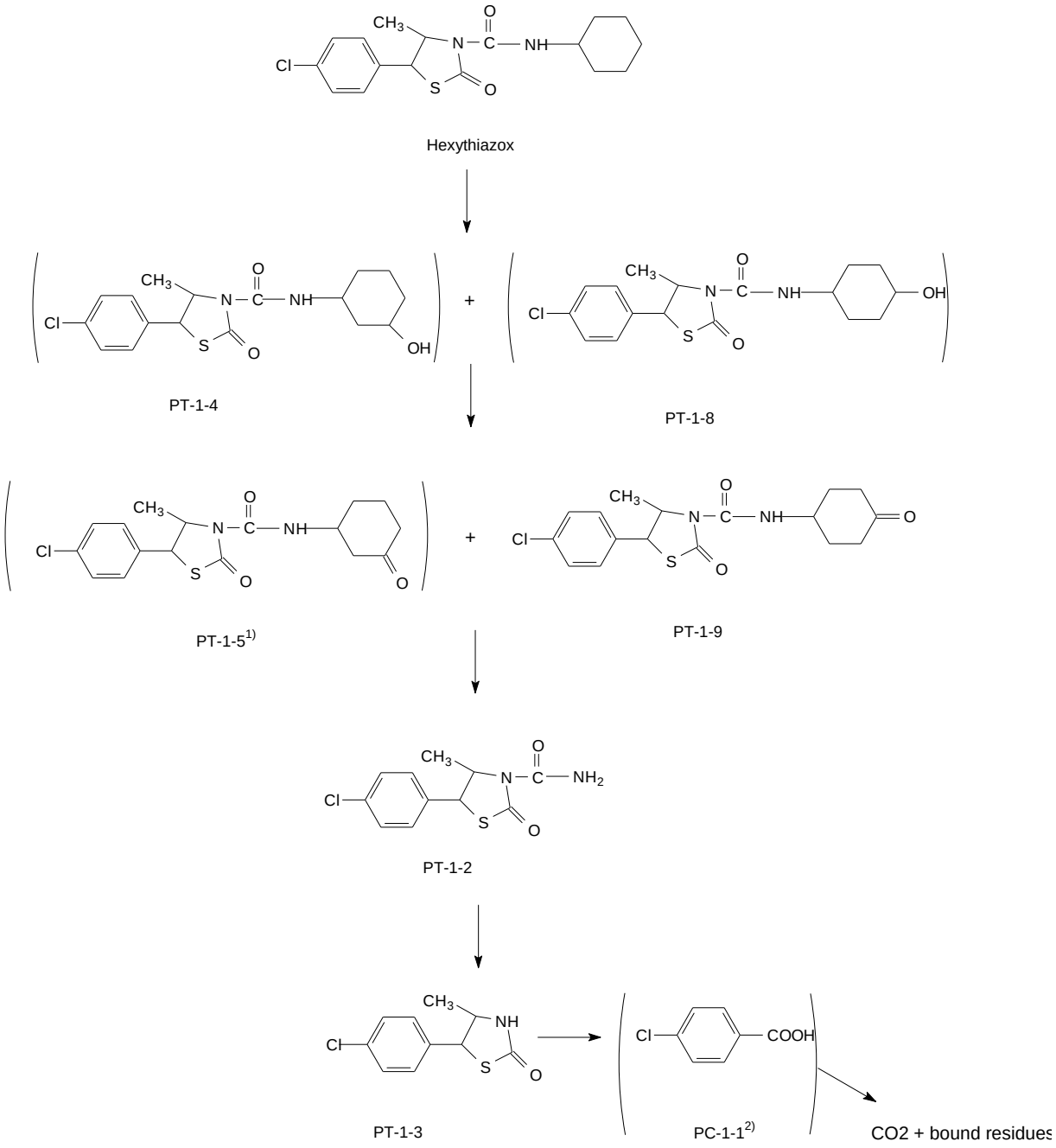
The primary degradation of Hexythiazox in soil was rapid, initially to the metabolites metabolites PT-1-4 and PT-1-8 (sum of both up to 11.4 % and as single up to 2.3 %) by hydroxylation of the cyclohexane ring (**Figure 2**). These degradation products metabolised rapidly further to PT-1-9 (up to 21.1 %) by the oxidation of the cyclohexane ring. PT-1-9 is rapidly metabolised further to PT-1-2 (up to 39.5 %) by cleavage of the two C-N bonds of the urea moiety. PT-1-2 is degraded further to PT-1-3 (up to 9.2 %) by hydrolysis of the amino carbonyl (carbamoyl) substituent in the thiazolidinone moiety of PT-1-2. Final degradation to CO_2 (ranging from 8 to 36 % after 118 to 122 days, 20°C) occurs probably via formation of PC-1-1 (p-chlorobenzoic acid), even though PC-1-1 was not found in the soil metabolism/degradation studies. The formation of non-extractable residues (ranging from 10 to 20 % after 118 to 122 days, 20°C) is another important transformation step in soil. Besides studies with thiazolidine ring labelled Hexythiazox, an additional study with cyclohexyl labelled Hexythiazox was conducted to investigate whether additional degradation products are formed in relevant amounts during the transformation of the cyclohexyl ring moiety of Hexythiazox in soil. According to the DAR the results of this study showed that no additional degradation products were formed in relevant amounts.

The half-life of the parent compound Hexythiazox in soil under aerobic conditions ranged from 7.8 to 56.0 days (geometric mean 23.7 days) at standard conditions of 20ºC and pF 2. The DT_{50} values of the major metabolites of Hexythiazox under aerobic standard (20°C and pF 2) conditions in soil ranged from 6.6 to 25.0 days for PT 1-9, from 9.1 to 264.2 days for PT-1-2 and from 10.7 to 54.1 days for PT 1-3.

The proposed **degradation pathway** of hexythiazox in soil is shown in **Figure 2.** In the DAR the pathway is described as follows: "*Degradation is started by hydroxylation of the cyclohexane ring to form PT-1-4 and PT-1-8, which could not be separated by TLC. It is assumed that neither of the cyclohexane-hydroxylated metabolites alone exceed 10 % of applied radioactivity and therefore PT-1-4 and PT-1-8 are considered as minor metabolites. Degradation proceeds further by oxidation of cyclohexane ring to form PT-1-5 and PT-1-9. The two metabolites can not be separated by TLC but, as PT-1-5 has not been detected in soil metabolism studies, it is assumed that the cyclohexane-oxidated fraction consists mainly of major metabolite PT-1-9. The major metabolite PT-1-2 is formed by cleavage of the two C-N-bonds of the urea moiety of PT-1-9. Hydrolysis of the amino carbonyl substituent in the thiazolidine moiety of PT-1-2 leads to formation of minor metabolite PT-1-3. The final step to CO_2 and bound residues is proposed to take place via p-chlorobenzoic acid (PC-1-1).*"

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Figure 2: Proposed degradation pathway of 14C-hexythiazox in soil under aerobic conditions (reproduced from DAR)



1) not detected DAR IIA, 7.1/01

2) not detected in DAR IIA, 7.1/01 and inDAR IIA 7.1/02

Degradation rate in soil is not among the preferred data to be used for assessing rapid degradability according to the CLP guidance. However, the primary degradation half-lives for the parent substance in the soil tests are mostly above 16 days, suggesting that Hexythiazox is not rapidly degradable. Consequently, no further information/assessment regarding the degradation products is needed for assessing the rapid degradability criterion.

Degradation in soil (anaerobic conditions)

The same degradation pathway is observed under anaerobic conditions (DAR IIA, 7.1/03). The cyclohexane-hydroxylated degradation products of Hexythiazox (PT-1-4, PT-1-6 and PT-1-8) reached levels up to 14.9 % and the cyclohexane-oxidated degradation products (PT-1-5, PT-1-7 and PT-1-9) reached levels up to 7.0 % under anaerobic conditions. The further degradation products, PT-1-2 and PT-1-3, accounted for up to 21.0 % and 3.2 %, respectively.

According to the CLP guidance (ECHA 2015) data regarding anaerobic degradation cannot be used in relation to deciding whether a substance should be regarded as rapidly degradable. Therefore, results from anaerobic degradation are not further included in the CLH report.

Field dissipation

The dissipation of Hexythiazox under realistic outdoor conditions was investigated in four field trials located in geographically and climatically different areas in Germany. It was not possible to quantitatively determine the dissipation half-lives due to rapid dissipation in relation to the used sampling scheme. Estimates for the field $DisT_{90}$ values ranged from around 16 to 97 days. The occurrence of Hexythiazox and its degradation products were generally confined to the uppermost soil layers (0 to 10 cm). According to the DAR, based on the results of the field dissipation studies, it can be concluded that there is no potential for a build-up of Hexythiazox under field conditions.

Field dissipation rates are not among the preferred data to be used for assessing rapid degradability according to the CLP guidance. However, according to the CLP guidance data from such experiments can in principle be used for assessing the potential for a rapid degradation (ECHA 2015). As in the present case half-lives were not determined, the field results cannot be used for assessing rapid degradability.

5.1.3 Summary and discussion of degradation

Under sterile conditions at ambient (22ºC) temperature, **hydrolytic degradation** of Hexythiazox was slow in alkaline (pH 9) aqueous solutions, whereas at neutral (pH 7) and acidic conditions (pH 5) Hexythiazox was found to be stable. The hydrolytic half-live of Hexythiazox at 22°C and at pH 9 ranged from 370 to 504 days. Therefore, based on hydrolysis Hexythiazox is not rapidly degradable according to the CLP criteria.

Photolytic degradation of Hexythiazox was slow **in distilled water and in river water** under artificial sunlight. Under continuous irradiation the DT_{50}-value of Hexythiazox was calculated to be 168 days in distilled water and 147 days in river water. Several metabolites were detected but none of them exceeded 4.3 % of applied radioactivity. No degradation of Hexythiazox was observed in the dark control samples under sterile conditions. As Hexythiazox does not react photolytically under sterile conditions (Hexythiazox shows no UV absorbance in the wave

length range 295 to 800 nm), it is assumed that Hexythiazox is indirectly phototransformed by water radicals generated from irradiation of artificial sunlight.

Photolytic degradation of Hexythiazox was slow **on the soil surface**. The photolytic half-life of Hexythiazox was determined to be 116 days indicating that photolysis does not significantly contribute to the degradation of Hexythiazox in/on soil.

No biodegradation screening tests are available for Hexythiazox.

In aerobic natural **water/sediment systems** (4 systems investigated), Hexythiazox exhibited moderate to high persistence forming the major metabolite PT-1-2, (max. ca. 12.8%AR and 19.4%AR in water and sediment respectively), with this maximum being at the end of the investigations (100 days). The unextractable sediment fraction (not extracted using methanol:water, then methanol or acetone) was a major sink for the thiazolidine ^{14}C ring radiolabel (accounting for 26-28%AR at 100 days) but less significant for cyclohexyl ^{14}C ring radiolabel (accounting for only 2.6-5.4% AR at 100 days). Mineralisation of these radiolabels accounted for only 2.5-6 % AR at 100 days. The available data indicated that sterile hydrolysis or sterile aqueous photolysis are not expected to contribute to the breakdown of Hexythiazox in surface water environments.

Geometric mean	DT_{50}	DT_{90} values are calculated by 1st order kinetics:
Whole system:	72	239
Water:	1.9	6.0
Sediment:	39	130

The single first order half-life (DT_{50}) of Hexythiazox **in soil** under aerobic conditions ranged from 8 days to 56 days (15-25°C) and DT_{90} values at 15°C – 25°C ranged from 32 to 248 days. Under anaerobic conditions the degradation of Hexythiazox was estimated to be slower with a DT_{50} value of 120 days. The single first order DT_{50} values of the major metabolites of Hexythiazox under aerobic conditions in soil at 20ºC ranged from 8 to 39 days for PT-1-9, from 9 to 264 days for PT-1-2 and from 11 to 54 days for PT-1-3.

The behaviour of Hexythiazox under realistic outdoor conditions was investigated in one field trial. The four test sites were located in geographically and climatically different areas in Germany. The dissipation half-lives (DT_{50f}) observed for Hexythiazox in the field ranged from 10 to 29 days and thus were within the same order of magnitude as the half-lives observed under standard laboratory conditions. The occurrence of Hexythiazox and its degradation products was generally confined to the uppermost soil layers (0 to 10 cm).

Overall conclusion concerning biotic and abiotic degradation:

According to the results presented above, **Hexythiazox is not rapidly degradable according to the CLP criteria.**

This conclusion is based on hydrolysis test as well as water/sediment and soil simulation tests. No biodegradation screening tests are available. The hydrolysis half-lives were ≥370 days at environmentally relevant temperature. Regarding the water/sediment simulation tests, as it is not possible to differentiate the degradation rate in water from that in sediment, the dissipation half-life in water cannot be used to evaluate rapid degradation. Degradation rates in water/sediment system, in sediment, or in soil are not among the preferred data to be used for assessing rapid degradability according to the CLP guidance. However, in the absence of biodegradation data for the surface water compartment the half-lives in water/sediment systems and in soil were compared to CLP criteria. The primary degradation half-lives were above 16 days in sediments (geometric mean 39 days), water-sediment-systems (geometric mean 72 days), and in four of the five tested soils (geometric mean 23.7 days). Therefore, the data from the simulation tests suggest that Hexythiazox is not rapidly degradable according to CLP criteria. As the conclusion is clear based on the half-lives of the parent substance, consequently no further information/assessment regarding the degradation products is needed for assessing the rapid degradability criterion**.**

5.2 Environmental distribution

Table 38: Relevant information on Adsorption/Desorption

Method	Results	Comment	Reference
Adsorption /Desorption Purity: >99%, [Cyclohexyl-^{14}C] radio-labelled, 28.2 mCi/mmolOECD Guideline 106: Adsorption – Desorption Using a Batch Equilibrium Method GLP compliant	Soil type / K_f* (mL/g) / K_{foc} (mL/g) / 1/n Sand 128 12823 0.94 Silt loam 134 11165 0.96 Sandy loam 110 9143 1.04 Clay loam 340 8714 0.95 Arithmetric mean 10461 0.98 *K_f = Freundlich adsorption coefficient		DAR Additional report IIA 7.1/14
Column Leaching Purity: >99 %, ^{14}C-thiazolidine radio-labelled, 6.6 mCi/mmol US EPA guidelines, subdivision N; environmental fate, §163-1, non GLP	Soil type* / % AR (0-5cm) / % AR in leachate Sand 92 < 0.1 Silt loam 97.3 < 0.1 Sandy loam 96.5 < 0.1 Sandy loam** 88.6 < 0.1 Clay loam 100.5 < 0.1 > 87 % AR retained in top 5 cm * Japanese soils **Sandy loam aged for 10 days AR = applied radioactivity	In the DAR it is mentioned that due to difficulties in stability of metabolites during the test, a combination of low temperature (10ºC) and sterilized soils were used. It was concluded in the DAR that the study fulfilled the current requirement.	DAR IIA, 7.1/10

5.2.1 Adsorption/Desorption

The adsorption and desorption of Hexythiazox was studied in four different Japanese soils using a batch equilibrium method. The soils covered a wide range of pHs and organic carbon and clay contents, but the characteristics of the soils were not exactly those recommended for European soils in the current guideline (OECD 106). The soils were air-dried and sieved (0.5 mm mesh size) before being used in investigations. Freundlich adsorption coefficients K_F of Hexythiazox in four soils ranged from 110 to 340 mL/g with corresponding K_{foc} values between 8714 and 12823 mL/g. Desorption of Hexythiazox was found to be very low. The results of the adsorption/desorption study indicate a very high degree of adsorption to soil.

In addition, the adsorption and desorption behaviour of PT-1-2, PT-1-3 and PT-1-9 were investigated in three different soils (DAR IIA, 7.1/09). The K_{foc} values ranged from 274 to 561 mL/g for PT-1-2, from 296 to 674 mL/g for PT-1-3 and from 402 to 922 mL/g for PT-1-9 indicating low to medium mobility. The desorption Koc values of the three metabolites were higher than the adsorption Koc values indicating that the strength of adsorption would increase as the time of contact with the soil increases DAR Additional report IIA 7.1/09).

The mobility of Hexythiazox was studied with column leaching method in four Japanese soils (sand, silt loam, sandy loam and clay loam). The soils covered a wide range of pHs and organic carbon and clay contents, but the characteristics of the soils were not exactly those recommended for European soils in the current guideline (OECD 312). The soils were air-dried and sieved (2 mm) prior to use in the experiment. Most of the applied Hexythiazox remained associated with the uppermost (0-5 cm) soil section. The amount of recovered radioactivity in the leachate did not exceed 0.1 % in any soil column. Aged residues of Hexythiazox were also mainly retained in uppermost soil column. Even if there were some deficiencies as compared with OECD guideline 312, the data of the study were included in the official List of endpoints during the EU peer review according to Directive 91/414EEC.

In summary, Hexythiazox showed a strong adsorption to soil particles and is regarded as immobile with K_{Foc} 8714 to 12823 mL/g. The major metabolites of Hexythiazox (PT-1-2, PT-1-3 and PT-1-9), which may be formed under aerobic and anaerobic conditions, showed medium to low mobility with K_{Foc} 274-561 mg/L, 296-674 mL/g and 402-922 mL/g, respectively.

5.2.2 Volatilisation

Table 39: Summary of volatilization

Method	Results	Comment	Reference
Volatilisation from plant surfaces Purity: 96.8%, ^{14}C-thiazolidine radio-labelled, 57 mCi/mmol BBA Guidelines, part IV, 6-1 (phase 2) GLP compliant	Distribution of ^{14}C residues after 24 h volatilization from bush beans : Experim. Extractables (% AR) Non-extractables (% AR) I 83.1 19.2 II 96.2 13.9 AR = applied radioactivity	Volatilisation of ^{14}C-hexy-thiazox from plant surfaces was less than 0.3 % of AR during 24 hour period and nearly the whole amount of AR was present in/on plants.	DAR IIA, 7.2/06

Method	Results	Comment	Reference
Volatilisation from soil surfaces Purity: >99%, ^{14}C-thiazolidine radio-labelled, 5.8MBq/mmol BBA Guidelines, part IV, 6-1 (phase 2) GLP compliant	Distribution of ^{14}C residues after 24 h volatilization from soil: Time (hours) / Extractables (KBq) / Non-extractables (KBq) 0 / 1335.2 / 44.7 1 / 1340.7 / 58.6 3 / 1231.1 / 157.5 6 / 1440.5 / 97.9 24 / 1183.9 / 42.5 AR = applied radioactivity	Volatilisation of ^{14}C-hexy-thiazox from soil surfaces reached only a very low level, which is indirectly suggested by the fact that after 24 hours 90.9 % of AR were found in the soil. In addition it was reported that no volatiles were trapped.	DAR IIA, 7.2/07
Volatilisation from soil and water surfaces Model calculation EPA Guideline: Federal Register, Vol. 40, No. 123, 26890 (1975) Non GLP	Based on methodology described in EPA guidelines the following coefficients were calculated: C_{water}/C_{air} > 10^5 $C_{wet\ soil}/C_{air}$: > 10^6 Hexythiazox is considered as a non-volatile substance from water and soil	-	DAR IIA, 7.2/08

Hexythiazox has a low vapour pressure of < 1.33 x 10^{-6} Pa at 25°C (DAR IIA, 2.3) and due to its intended uses, Hexythaizox is very unlikely to be present in air. According to the results of the volatilisation studies, volatilisation of Hexythiazox from plant surface and soil surface is negligible.

5.2.3 Distribution modelling

Not performed and not necessary for classification purpose.

5.3 Aquatic Bioaccumulation

Table 40: Summary of information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Log Pow Purity: 99% OECD guideline 107 GLP cmpliant	log Pow = 2.67 at 25°C, unbuffered solution, (>99 %)	Effect of pH not required, as hexythiazox does not dissociate	DAR IIA, 2.8
Bioconcentration in Bluegill sunfish Flow-through (28 day exposure) solvent used Purity: 98%, ^{14}C-thiazolidine radiolabelled, 32.4 µCi/mg US EPA Pesticide Assessment Guideline (1982), similar to OECD guideline 305 Non GLP compliant	Associated BCF (based on total ^{14}C residues) in several matrices after exposure of 28 days (low 0.0036 mg/L and high exposure level 0.034mg/L): Low / High Whole fish 1100 / 850 Muscle 250 / 74 Viscera 8400 / 300 Carcass 720 / 410 Elimination of Hexythiazox during a depuration phase of 14 days was 85% and 95 %	Despite some deficiencies, the results are considered to be valid. Bioaccumulation of hexythiazox in bluegill sunfish under flow-through conditions is high. However, most of the accumulated substance is transformed to metabolites in fish tissues and depurated rapidly in clean water. The reported BCFs are for total ^{14}C residues and therefore reflect the sum of hexythiazox, its metabolites and their conjugates.	DAR IIA; 8.2/11
Bioaccumulation in Carp Flow-through (10 weeks) Purity: >99%, Hexythiazox According to Japanese “Law of screening and regulations concerning chemical substances” Non GLP compliant	At low dose group, the mean BCF after 10 weeks was 440 but it is not known whether the plateau was reached. At high dose group the peak mean BCF was 630 and it was reached after 8 weeks exposure	The report of the study was short and the study did not fulfill the current requirements for bioconcentration test in fish and is not regarded as valid	DAR ; 8.2/12

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

A measured octanol-water partition coefficient is available and revealed a logPow of 2.67 at 25°C, which is below the trigger of 4 of the CLP regulation.

5.3.1.2 Measured bioaccumulation data

Two bioconcentration studies with bluegill sunfish (*Lepomis macrochirus*) and carp (*Cyprinus carpio*) were available and the study on bluegill sunfish is presented here although the log Pow of hexythiazox was < 4. The study with carp was assessed as not valid by RMS and EFSA and is therefore not summarized below.

The bioconcentration potential of ^{14}C-thiazolidine-ring labelled hexythiazox was studied in Bluegill sunfish (*Lepomis macrochirus*). Two nominal concentrations, 0.05 and 0.005 mg/L corresponding to mean measured concentrations of 0.034 and 0.0036 mg/L, were used in the test and a total of 70 fish were exposed in both concentrations. Seventy fish were also used in control containing well water and in solvent control containing 0.05 mL N,N-dimethylformamide/L. Exposures were performed in four 57-L glass aquaria, which had a water volume of 30 L and a flow-through system to change the whole water volume six times in 24 hours. The fish were exposed for 28 days and the depuration phase in clean water lasted for 14 days. Samples containing four fish were taken after 0, 1, 7, 14, 21 and 28 d exposure and after 1, 3, 7, 10 and 14 d depuration periods. Additional ten fish were sampled for metabolite determination at the end of the exposure period on day 28.

No adverse effects were noted in any of the groups of fish during the entire study. No mortality of fish occurred during the test and the behavior of fish appeared normal. The average length was 4.1±0.5 cm (range 2.7—5.4) and the average weight was 1.82±0.69 (range 0.47-3.73).

The test compound was found to adsorb to debris and scum and, even though effort was made to keep aquaria clean, the material balance (not including debris and scum) showed only 72 % and 65 % recoveries on the low and high exposure level study, respectively. Radioactivity in the scum and debris from the aquaria, which contained significant amounts of radioactivity, were not quantitatively measured and, therefore, could not be included in these material balance calculations.

The recovery of total radioactivity in aqueous stock solutions ranged from 98.1 to 99.4 %. As only a single major peak with the same retention factor as hexythiazox was found in TLC analysis, the content of hexythiazox was determined to be in the range of 95.2 – 100 % of the extracted ^{14}C-residue. In exposure water the content of hexythiazox was 88.7 and 91.6 % of the extracted ^{14}C-residue on day 0 and 85.8 and 80.4 % of the extracted ^{14}C-residue on day 28. The decrease in recovery of radioactivity at day 28 is probably due to transformation products of hexythiazox.

Validity criteria are considered in **Table 41**. It is noted that the study has been conducted in 1984 and the consolidated OECD 305 Test Guideline was adopted only in 1996 (and revised 2012) (OECD 2012). The validity criteria of the revised version (2012) of the OECD 305 Test Guideline are used for comparison and it has not been checked by the dossier submitter whether all of the validity criteria were included in the test guidelines available in 1984. Regarding oxygen levels, there is no oxygen data available and therefore the validity criteria concerning dissolved oxygen cannot be directly evaluated. It is noted that no adverse effects were reported and thus there are no indications of oxygen deficiency. Regarding the possible differences in growth between test and control groups, it is noted that growth results are not available and therefore such comparison cannot be done. In addition, no fish length/weight data are available

for each group but only as average values for all fish. However, as no adverse effects were noted in any of the groups of fish during the entire study, the occurrence of toxicity during the test is unlikely. In general, the observed deficiencies regarding the validity criteria are not considered critical for the use of the test for classification purpose.

Table 41: Bluegill sunfish bioconcentration test (DAR IIA; 8.2/11) - Consideration of the validity criteria of the OECD 305 test guideline (305-I: Aqueous Exposure Bioconcentration Fish Test) (OECD 2012)

Criterion	Fulfilment of criterion	Remarks
Water temperature variation is less than ± 2 ºC in treatment or control groups	Yes	According to the test report, water temperature was maintained at approximately 22±1°C;
Concentration of dissolved oxygen does not fall below 60% of the air saturation value	No data	Dissolved oxygen concentrations are not included in the full study report.
The concentration of the test substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase	Yes	The mean measured hexythiazox concentrations during the exposure were 0.0036±0.0004 mg/L and 0.034±0.004 mg/L in low and high exposure level study, respectively. Regarding the low exposure level, the measured concentration was within ± 20% of the mean in 28 of the 29 measurements and outside of this range in one measurement (day 1: 0.00277 mg/l, corresponding to 76% of the mean). Regarding the high exposure level, the measured concentration was within ± 20% of the mean in 27 of the 29 measurements and outside of this range in two measurements (day 10: 0.0265 mg/l, corresponding to 79% of the mean; day 20: 0.0406 mg/l, corresponding to 121% of the mean).* These minor exceptions are considered acceptable by the dossier submitter.
The concentration of the test substance is below its limit of solubility in water, taking into account the effect that the test water may have on effective solubility	Yes	Two nominal concentrations, 0.05 and 0.005 mg/L corresponding to mean measured concentrations of 0.034 and 0.0036 mg/L, were used in the test. Water solubility of hymexazol is 0.10-0.12 mg/l (**Table 9**)
The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several	Yes	No adverse effects were noted in any of the groups of fish during the entire study. No mortality of fish occurred during the test and the behavior of fish appeared normal.

weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all.		
Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical.	No data	No adverse effects were noted in any of the groups of fish during the entire study. There is no data to estimate fish growth. Average weights and lengths are reported but not separately for test and control groups.

*Calculated by the dossier submitter from data presented in Table II of the full study report (DAR IIA 8.2/11)

An approximate steady-state plateau for ^{14}C residue in the whole fish was achieved after 8 and 2 day exposure at the low and high exposure concentrations, respectively. The highest accumulated concentrations for ^{14}C in whole fish, however, were measured on day 21 and the BCFs of 1600 and 1000 were calculated for the low and high exposure level, respectively. At the end of the exposure period on day 28 the corresponding BCFs were 1100 and 850, for the ^{14}C residue. Accumulation was significantly greater in viscera compared to that in remaining carcass and muscle. Rapid purging of the ^{14}C residue from the fish tissues was seen at the beginning of the depuration period but the decrease in tissue concentrations slowed down later during the depuration phase. After 14 days of depuration, 89 % and 94 % of the total ^{14}C residue measured at day 28 were depurated from the whole fish at low and high exposure concentration, respectively, and the corresponding residues left in the whole tissue were 0.44 ppm and 1.6 ppm.

Table 42: Distribution of hexythiazox equivalent residues in water, whole fish, and tissues of bluegill sunfish after exposure for 28 days and their associated bioconcentration factors

	Low exposure level		High exposure level	
	Total ^{14}C-residue [ppm][a, b]	BCF[c]	Total ^{14}C-residue [ppm][a, b]	BCF[c]
Water (day 28)	0.00358	-	0.0307	-
Water (mean, day 1-28)	0.003642	-	0.03366	-
Whole fish	4.11	1100	28.5	850
Muscle	0.93	250	2.5	74
Viscera	30.5	8400	300	9000
Carcass	2.63	720	13.8	410

[a] residue concentration in test water calculated as ppm ^{14}C-hexythiazox equivalents at day 28 of exposure.

[b] steady-state residue concentration in muscle, viscera, carcass and whole fish calculated as ppm ^{14}C-hexythiazox equivalents at day 28 of exposure

[c] steady-state bioconcentration factors calculated as mean tissue residues at day 28 divided by mean water concentration (days 1-28).

Five different components were found, when the radiolabeled residues extracted from fish tissues at day 28 were characterized with TLC (**Table 43**).

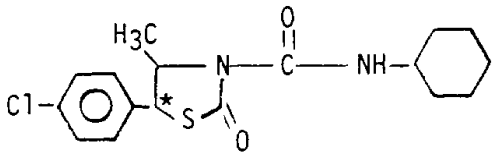
Table 43: Composition of radioactivity in muscle and viscera of bluegill sunfish exposed for 28 days to 0.0036 and 0.034 mg/L of 14C-hexythiazox

Component	TRR distribution in extracts (0.0036 mg/L exposure)**				TRR distribution in extracts (0.034 mg/L exposure)**			
	Muscle		Viscera		Muscle		Viscera	
	% TRR	ppm*	% TRR	ppm*	% TRR	ppm*	% TRR	ppm*
Polar material(s)	87.7	1.81	55.4	33.6	51.9	3.03	26.8	92.2
Conjugates of hydroxy metabolite(s)	3.7	0.08	32.5	19.7	7.1	0.41	56.9	196
Hydroxy metabolites	1.8	0.04	4.3	2.6	14.9	0.87	8.5	29.3
Hexythiazox	4.7	0.10	1.5	0.9	22.6	1.32	3.3	11.5
Bound radioactivity	2.2	0.04	6.2	3.8	3.5	0.21	4.5	15.4
Total	100.1	2.07	99.9	60.6	100	5.84	100	344

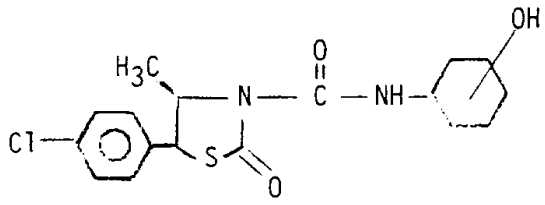
* calculated as ppm of ^{14}C-hexythiazox
** TTR=total residual radioactivity

Of the total radioactive residues, 2 – 6 % was found as unextractable bound radioactivity (**Table 43**). The proportion of unmetabolized hexythiazox of total radioactive residues varied from 1.5 to 22.6 %. Most of the radiolabeled substance, 26.8 – 87.7 %, was analyzed as unidentified polar metabolites, which were not changed by enzyme hydrolysis. The rest of the metabolites comprised conjugates of hydroxy metabolites (possibly glucuronide and/or sulfate ester conjugates) and hydroxy metabolites.

It is noted that no attempts were made to determine the fish lipid contents and therefore the obtained BCFs cannot be normalized to lipids. Rate constants for uptake and depuration have not been determined and no depuration half-lives have been calculated. Despite the deficiencies, results were accepted in DAR. The study shows that bioaccumulation of hexythiazox in bluegill sunfish in flow-through conditions is high. However, most of the accumulated substance is transformed to metabolites in fish tissues and depurated rapidly in clean water.



trans-3-thiazolidinecarboxamide, 5-(4-chlorophenyl)-N-cyclohexyl-4-methyl -2-oxo-



"Cyclohexane ring hydroxylated metabolites"

Figure 3: Chemical structure of hezythiazox and cyclohexane ring hydroxylated metabolites. *The compound is radiolabeled on the number 5 carbon atom of the thiazolidine ring

In the DAR (B.9.2.3 Bioconcentration of metabolites in fish (Annex IIA 8.2.3.)) it is mentioned: “According to PPP-directive (Annex II Point 8.2.3) and the working document "Guidance Document on Aquatic Ecotoxicology" (SANCO/3268/2001) the bioconcentration potential of metabolites, likely to partition into fatty tissues (such as $\log K_{ow} \geq 3$) must be studied, unless it can be justified that exposure leading to bioconcentration is not likely to occur. In water/sediment system two metabolites were formed in total system more than 10 %: PT-1-2 32.2 % and PT-1-9 12.7 %. For PT-1-2 the $\log K_{ow}$ was 2.44 and, consequently, the partition into fatty tissue is not likely. For PT-1-9 the maximum concentration in water phase, where the bioconcentration takes place, was 6.7 % of applied radioactivity and the metabolite is not considered as major metabolite in water. Therefore it is considered that testing bioconcentration with metabolites of hexythiazox is not required.”

The CLP guidance (ECHA 2017) mentions: “The BCF from radio-labelled studies should, preferentially, be based on the parent compound. If these are unavailable, for classification purposes, the BCF based on total radio-labelled residues can be used. If the BCF, in terms of radio-labelled residues, is ≥ 1000, the identification and quantification of degradation products documented to be ≥ 10 % of total residues in fish tissues at steady state, are strongly recommended.”. In the present study, residues exceeding 10% of total residues were observed in fractions that can probably be considered to be less bioaccumulative than hexythiazox (polar materials, hydroxy metabolites, and conjugates of hydroxy metabolites) (**Table 43**). Therefore,

the BCF based on total residues for classification purpose may overestimate the bioaccumulation potential. Only a relatively low proportion (1.5-22.6%) of the total ^{14}C was attributed to the parent substance in the components for which there is data available (muscle, viscera; day 28)) (**Table 43**). If the BCFs are recalculated using the proportion of hexythiazox of the total ^{14}C (i.e., the BCF values in **Table 42** are multiplied by the proportion of hexythiazox (%/100), the values would be: 12 and 17 for muscle and 126 and 297 for viscera at low and high concentration, respectively. Because BCF for viscera was highest of all studied components the whole body BCF (which is used for classification) would be lower than the values reported for viscera. In the present study the identity of the metabolites was not characterized to the compound level. Nevertheless, it is noteworthy that 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 (Chapter 5.5). Therefore it cannot be ruled out that some of the metabolites in fish could potentially be hazardous to the aquatic environment according to CLP criteria. Thus it is warranted to use the BCF based on total ^{14}C residues is used for classification, despite the likely lower bioaccumulation potential of the metabolites.

In summary, based on total ^{14}C residues (BCF 850-1100) the substance has a potential for bioaccumulate for classification purposes. It is noted that the BCF is not lipid normalised and thus BCF could be higher or lower (depending on the test fish lipid content) after lipid normalization. Furthermore, the lack of uptake and depuration rates adds uncertainty.

5.3.2 Summary and discussion of aquatic bioaccumulation

Two bioconcentration studies with bluegill sunfish (*Lepomis macrochirus*) and carp (*Cyprinus carpio*) were conducted although the log Pow of hexythiazox was < 4. The study with carp was assessed as not valid by RMS and EFSA.

The mean whole fish BCF (bioconcentration factor) based on total residual radioactivity was determined as 975 for bluegill sunfish, which is above the trigger value of ≥ 500 of the CLP regulation. Hexythiazox was transformed rapidly into metabolites and the depuration was rapid. 89 % to 94% of the applied radioactivity depurated within 14 days after exposure. Based on the BCF value and the criteria set in CLP hexythiazox has the potential to bioaccumulate. It is noted that the BCF value is associated with the following uncertainties:

-the BCF value is based on total residual radioactivity which is the sum of hexythiazox, its hydroxy metabolites, conjugates of hydroxy metabolite(s), polar material(s) and bound radioactivity

-a significant proportion hexythiazox was metabolized in the studied components (muscle, viscera) but metabolites were not identified to the compound level

-BCF is not lipid normalized

-uptake and depuration rates were not determined

The log Pow of the major metabolite PT-1-2 was 2.44 and therefore no bioconcentration study was triggered.

5.4 Aquatic toxicity

All acute and chronic toxicity data of hexythiazox on fish, aquatic invertebrates and algae which are considered relevant for the classification proposal are summarized in **Table 44**. The data is evaluated and accepted during EU review according to Directive 91/414/EEC. In several studies with hexythiazox, test concentrations far above the water solubility were tested. In these studies, the test media were analyzed and it could be shown that only a minor part of the substance was soluble in the test media. The maximum solubility in the test media was determined to be slightly above the solubility in pure water. In other studies solvents were used in order to increase the solubility of hexythiazox in the test media. In these studies, hexythiazox concentrations above the water solubility could be tested. There were also available studies, in which the concentrations during the exposure were not analytically verified. Those studies are not considered valid for classification and labelling purpose and are therefore omitted from this CLH-proposal.

Available aquatic acute toxicity studies with hexythiazox metabolites PT-1-2, PT-1-3 and PT-1-9 on aquatic organisms are presented in the **Table 45.** Toxicity of PT-1-2 and PT-1-9 for fish (rainbow trout, 1.46 mg/l 96-h and 1.22-2.41 mg/l, respectively, based on mean measured concentration), were on the same range as that of hexythiazox, but in general the metabolites were of lower toxicity to aquatic organisms compared to the parent substance. Therefore, only those acute fish tests are described in more detail in this CLH-proposal.

Table 44: Summary of relevant information on aquatic toxicity

Method		**Results**				**Remarks**	**Reference**
Species	**Guideline and Type of test**	**Concentration of hexythiazox**					
		nominal		**measured**			
		NOEC [mg/L]	**EC_{50} or LC_{50} [mg/L]**	**NOEC [mg/L]**	**EC_{50} or LC_{50} [mg/L]**		
Acute toxicity							
Rainbow trout (*Oncorhynchus mykiss)*	OECD 203 (1992), 92/69/EEC, C.1 (1992) static (96 h) no solvent, stirring for about 48 hours		> 100		> 0.2 Based on mean measured concentrations	No mortality and no signs of toxicity at tested concentrations	DAR IIA; 8.2/01
Rainbow trout (*Oncorhynchus mykiss)*	OECD 203 static (96 h) solvent used		> 9.2		> 4.0 Based on mean measured concentrations	No mortality but signs of toxicity probably, caused by non-dissolved test substance particles	DAR IIA; 8.2/04

Method		**Results**				**Remarks**	**Reference**
Species	**Guideline and Type of test**	**Concentration of hexythiazox**					
		nominal		**measured**			
		NOEC [mg/L]	**EC_{50} or LC_{50} [mg/L]**	**NOEC [mg/L]**	**EC_{50} or LC_{50} [mg/L]**		
Bluegill sunfish (*Lepomis macrochirus)*	OECD 203 static (96 h) solvent used		15		**3.2** Based on mean measured concentrations		DAR IIA; 8.2/05
Carp (*Cyprinus carpio)*	OECD 203 semi-static, medium renewed after 48 h (96 h) solvent used		> 100		> 14.1 Based on mean measured concentrations	Limit test No mortality and no signs of toxicity	DAR Additional report IIA; 8.2/33
Daphnia magna	OECD 202 static (48 h) stirring for 20 h, centrifugation		> 100**		> 0.47 Based on mean measured concentrations	No immobility observed	DAR IIA; 8.2/13
Daphnia magna	OECD 202 static (48 h) solvent and ultrasonic bath used		na		**0.36** Based on mean measured concentrations **Key study**	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
Scenedesmus subspicatus	OECD 201 static (72 h) stirring 20 h and sentrifugation		> 100** ($E_rC_{50;}$ E_bC_{50})		> 0.4 ($E_rC_{50;}$ E_bC_{50}) Based on two measured concentrations and the median analytical recovery rate		DAR IIA; 8.2/25
Pseudokirchneriella subcapitata	OECD 201 static (96 h) solvent and ultrasonic bath		> 100		> 72.0 (E_rC_{50}, E_bC_{50}) Limit test Based on mean measured concentration		DAR Additional report IIA; 8.2/35
Chronic toxicity							

Method		**Results**				**Remarks**	**Reference**
Species	**Guideline and Type of test**	**Concentration of hexythiazox**					
		nominal		**measured**			
		NOEC [mg/L]	**EC_{50} or LC_{50} [mg/L]**	**NOEC [mg/L]**	**EC_{50} or LC_{50} [mg/L]**		
Rainbow trout *Oncorhynchus mykiss*	OECD 204 semi-static (28 d) medium renewed after 48 h	20*		0.04* Based on mean measured concentration		**Prolonged acute test** **Only one test concentration** No mortality and no signs of toxicity at tested concentration	DAR IIA; 8.2/10
Daphnia magna	US EPA pesticide Assessment Guideline, Subdivision E No. 72-4(b), 1982 flow-through (21 d) solvent used	0.050	nr	**0.0277** Based on mean measrued concentrations **Key study**		NOEC based on mean of live young produced per adult Dissolved oxygen saturation dropped intermittently < 60 %, but the dissolved oxygen concentration stayed in all test chambers above 3 mg/l throughout test (the limit indicated in the OECD 211 test guideline).	DAR IIA; 8.2/21
Daphnia magna	OECD 202, Part II (reproduction test) semi-static (21 d) stirring, centrifugation	50**	>100**	**0.0418** Results based on three measured concentrations and the median analytical	>0.0836		DAR IIA; 8.2/22

Method		**Results**				**Remarks**	**Reference**
Species	**Guideline and Type of test**	**Concentration of hexythiazox**					
		nominal		**measured**			
		NOEC [mg/L]	**EC_{50} or LC_{50} [mg/L]**	**NOEC [mg/L]**	**EC_{50} or LC_{50} [mg/L]**		
				recovery rate **Key study**			
Daphnia magna	OECD 202 semi-static (21 d) solvent used	0.120	nr	0.055 Based on mean measured concentrations	nr		DAR IIA; 8.2/24
Scenedesmus subspicatus	OECD 201 static (72 h) stirring 20 h and sentrifugation	100*,** (growth rate) 50** (biomass)		0.4* (growth rate) 0.2 (biomass)			DAR IIA; 8.2/25
Pseudokirchneriella subcapitata	OECD 201 static (96 h) solvent and ultrasonic bath	100		72.0 (cell number)			DAR IIA; 8.2/35
Chironomus riparius	BBA guideline proposal (1995) static (21 d) solvent used	6.4*	> 6.4	1.7*	> 1.7 Based on initial measured concentration	Hexythiazox concentration in overlaying water in the lowest, a medium and the highest concentration were analyzed. Hexythiazox concentration in sediment pore water in the highest test concentration was analyzed. Concentrations were not analyzed	DAR IIA; 8.2/29

Method		Results				Remarks	Reference
Species	Guideline and Type of test	Concentration of hexythiazox					
		nominal		measured			
		NOEC [mg/L]	EC_{50} or LC_{50} [mg/L]	NOEC [mg/L]	EC_{50} or LC_{50} [mg/L]		
						from sediment but the results can be considered valid.	

nr/na not reported/not analysed

* No effects were observed at the highest concentration tested representing the maximum solubility.

** Nominal concentrations do not represent effective exposure concentrations, as the test was performed with an extract obtained by centrifugation.

Table 45: Acute toxicity of the hexythiazox metabolites PT-1-2, PT-1-3 and PT-1-9 to aquatic organisms

Species	Type of test	Concentration of metabolites				Reference
		nominal		measured		
		NOEC [mg/L]	EC_{50} or LC_{50} [mg/L]	NOEC [mg/L]	EC_{50} or LC_{50} [mg/L]	
PT-1-2						
Rainbow trout (*Oncorhynchus mykiss)*	OECD 203 (1992) 92/69/EEC, C.1 (1992) static (96 h) stirring		2.15		1.46 Based on mean measured concentrations	DAR IIA; 8.2/08
Daphnia magna	OECD 202, EC Directive 79/831 Annex V Part C2, EPA 72-2, OPPTS 850.1010 (1996) static (48 h)		nr		14 Based on mean measured concentrations	DAR IIA; 8.2/15
Daphnia magna	OECD 202, EC Directive 92/32EEC C2 static (48 h)		17.7		nr*	DAR IIA; 8.2/16
Pseudo-kirchneriella subcapitata	OECD 201 (1984) static (72 h)	4.95 (E_rC_{10}) 1.46	30.7 (E_rC_{50}) 8.67	nr*	nr*	DAR IIA; 8.2/27

Species	Type of test	Concentration of metabolites				Reference
		nominal		measured		
		NOEC [mg/L]	EC_{50} or LC_{50} [mg/L]	NOEC [mg/L]	EC_{50} or LC_{50} [mg/L]	
		(E_bC_{10})	(E_bC_{50})			
PT-1-3						
Daphnia magna	OECD 202, EC Directive 92/32/EEC C2 static (48 h)		13.6		nr*	DAR IIA; 8.2/17
PT-1-9						
Rainbow trout (*Oncorhynchus mykiss)*	OECD 203 (1992); 92/69/EEC, C1 (1992) static (96 h)		2.15 – 4. 64**		1.22 – 2.41** Based on mean measured concentrations	DAR IIA; 8.2/09
Daphnia magna	OECD 202, EC Directive 79/831 Annex V Part C2, EPA 72-2, OPPTS 850.1010 (1996) static (48 h)		nr		4.2 Based on mean measured concentrations	DAR IIA; 8.2/18
Daphnia magna	OECD 202, EC Directive 92/32/EEC C2 static (48 h)		8.59		nr*	DAR IIA; 8.2/19
Pseudo-kirchneriella subcapitata	OECD 201 (1981) static (72 h)	nr	nr	5.5 (E_rC_{10}) 3.0 (E_bC_{10})	34.6 (E_rC_{50}) 11.9 (E_bC_{50}) Based on initial mean measured concentrations.	DAR IIA; 8.2/28

nr not reported

na no analytical verification of test concentrations

* Measured concentrations were within ± 20 % of nominal values.

** These two consecutive concentrations gave 0 and 100% mortality.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Four valid GLP acute toxicity fish studies using hexythiazox are available. Four further acute toxicity fish studies are available and reported in the DAR. However, due to study deficiencies, principally relating to missing analytical concentration determination, the results are not considered valid and the studies are not reported in this CLH-proposal.

Title:	Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours). DAR IIA, 8.2/01
Guidelines:	OECD 203 (1992) 92/69/EEC, C.1 (1992)
GLP:	Yes; certified laboratory

Materials and methods:

The test substance was identified as follows:

Hexythiazox T.G.A.I.

batch: HA-3026

purity: 99.3 %

solid

Acute toxicity of hexythiazox (purity 99.3%) for the rainbow trout was studied in a static 96-h limit test using nominal concentrations of 0 (control) and 100 mg a.s./L. Municipal dechlorinated tap water with pH 8.0-8.6 was used in the test. Because the water solubility of hexythiazox is low, it was introduced into the aquaria and homogenized by stirring for about 48 hours prior to test by an ultra-turrax stirrer. No solvents were used. The test was performed at 13°C and ten fish were used in both the control and exposure groups. The fish were about 8 months old and their average body lengths and wet weights were 6.07 cm and 2.14 g, respectively. Water samples were taken at the beginning and at the end of the exposure period and the hexythiazox concentration was analyzed by HPLC. Mortality and symptoms of toxicity were observed after 1, 4, 24, 48, 72 and 96 hours and pH, temperature and dissolved oxygen concentration was recorded daily.

Findings

The mean measured concentrations of hexythiazox in water were 0.3 mg/L (0.3 % of nominal) after 1 h and 0.1 mg/L (0.1 % of nominal) after 96 h. Undissolved test compound was visible on the bottom and at the water surface. This reflects the low water solubility of hexythiazox. No mortality or any signs of toxicity was seen during the exposure. Accordingly, the 96-hour LC_{50} of hexythiazox for rainbow trout was determined to be >0.2 mg/L based on mean measured concentrations and >100 mg/L based on nominal concentrations. As no symptoms of toxicity were seen, the no-observed effect concentration of 0.2 mg/L (mean measured) and 100 mg/L (nominal) was determined.

Title:	Static Acute 96-Hour LC_{50} of DPX-Y5893 (NA-73) to Rainbow trout (*Oncorhynchus mykiss*). DAR IIA, 8.2/04
Guidelines:	Not mentioned, study was performed according to OECD guideline 203 and meets good scientific practice.
GLP:	Yes; laboratories in the USA are not certified by any governmental agency, but are subject to official inspections

Materials and methods:

The test substance was identified as follows:

Hexythiazox (DPX-Y5893)

batch: not provided

purity: 98.9 %

The acute toxicity of hexythiazox for the rainbow trout was studied in a static 96-h test using nominal concentrations of 0 (control and solvent control), 0.13, 0.27, 0.47, 0.80, 1.3, 2.1, 3.6, 6.0 and 9.2 mg a.s. /L. Dimethylformamide (DMF) was used as a solvent and its maximum concentration was 0.1 ml/l. Laboratory well water was used in the test and the temperature during the test was maintained between 11 and 13 °C. Total of ten fish per treatment allocated to two replicates of five fish were used in both control groups and in all treatment groups. The average body length and wet weight of fish were 4.5 cm and 1.2 g, respectively. The concentrations of the test compound in water in each replicate were analyzed after 0, 2 and 4 days of exposure with HPLC. Mortality and symptoms of toxicity were observed at 24-hour intervals.

Findings:

No mortality was seen at any of the treatments. Therefore, the 96-h LC_{50} is determined to be greater than 9.2 mg/L based on nominal concentrations. The concentration in water declined during the exposure and the LC_{50} based on the mean measured concentration was determined to be greater than 4.0 mg/L. Symptoms of toxicity, lying on bottom and gasping, were observed in some individuals beginning from the exposure group 2.1 mg/L (nominal). Even though these symptoms are thought to be responses to particulate, non-dissolved hexythiazox, the no-observed effect concentration is determined to be 1.3 mg/L on nominal basis and 0.61 mg/L on mean measured basis.

Analyzed mean concentrations of test compound at the initiation of test were 55 to 120 % of nominal but at the termination of test the corresponding values were dropped to 10 - 47 %. Increasing amount of particulate test compound in water was suggested to cause physical effects on exposed fish.

Title:	Static acute 96-hour LC_{50} of DPX-Y5893 (NA-73) to Bluegill sunfish (*Lepomis macrochirus*). DAR IIA, 8.2/05
Guidelines:	Not mentioned, study was performed according to OECD guideline 203 and meets good scientific practice. Deviations to OECD 203: Length of test fish was in the range of 2.4 – 3.6 cm (OECD 203: 2 ± 1 cm). However, this is not deemed to influence the validity of the study.

GLP: Yes; laboratories in the USA are not certified by any governmental agency, but are subject to official inspections

Materials and methods:

The test substance was identified as follows:

Hexythiazox (DPX-Y5893)

batch: not provided

purity: 98.4 %

Acute toxicity of hexythiazox (purity: 98.4%) for a warm water fish species, bluegill sunfish, was studied in a static 96-h test using nominal concentrations of 0 (control and solvent control), 0.61, 0.86, 1.2, 1.8, 2.5, 3.6, 5.2, 7.4, 11.2 and 15.0 mg ai/L. Dimethylformamide (DMF) was used as a solvent and its maximum concentration was 0.15 mL/L. Laboratory well water was used in the test and the temperature during the test was maintained between 21 and 22°C. Ten fish per group were randomly allocated into both control groups and into all treatment groups. The average body length and wet weight of fish were 2.9 cm and 0.51 g, respectively. The concentration of the test compound in water in each exposure groups was analyzed after 0 (duplicate samples), 2 and 4 (single samples) days of exposure with HPLC. Mortality and symptoms of toxicity were observed at 24-hour intervals.

Findings:

The dissolved oxygen concentration dropped to 58-74% of air saturation at the end of the 96-h test. The mean measured concentrations of test compound during the exposure were 0.14, 0.16, 0.25, 0.43, 0.64, 1.2, 1.0, 1.5, 2.3 and 2.7 mg/L, which represents 18 to 33 % of nominal concentrations.

No mortality was seen in controls or in treatments below the mean measured concentration of 1.5 mg/L, which represents the nominal concentration of 7.4 mg/L. The 96-h LC_{50} was estimated to be 3.2 mg/L (95% C.I. 2.6-5.6 mg/L) based on mean measured exposure concentration and 18 mg/L based on nominal concentration. At mean measured test concentration of 0.64 mg/L and above some individuals were observed to lie on the bottom of the aquaria and be lethargic. Further, at mean measured concentration of 0.25 mg/L and above the color of some individuals was darkened and some individuals were found to come to the surface. Because of these treatment related symptoms of toxicity, the acute no-observed effect concentration was determined to be 0.16 mg/L.

The drop of oxygen concentration during the test was within the accepted range (at minimum 60% of air saturation value) in all but one treatment (3.6 mg/L nominal) at day 4, where the dissolved oxygen was 58% of air saturation. The deviation from the recommended minimum saturation is small and is not deemed to affect the results. The mean measured concentrations during the test were found to range between 18 to 33% of nominal and therefore the toxicity values must be based on the mean measured concentrations.

Title: Hexythiazox: Common carp acute toxicity test. DAR Additional report IIA 8.2/33

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Guidelines: OECD 203 (1992)
US EPA OPPTS 850.1075 (1996), C.1 (1992)

GLP: Yes; Certified laboratory

Materials and methods:

The test substance was identified as follows:
Hexythiazox (technical)
Lot No.: NJH-3424G
purity: 99.7 %

The acute toxicity of hexythiazox (purity 99.7%) to common carp (*Cyprinus carpio)* was determined in a semi-static 96-hour test. Treatments consisted of a blank (containing 0.1 mL/L N,N-dimethylformamide), one nominal concentration of 100 mg hexythiazox/L (limit dose test) and a dilution water control. Ten fish were exposed to the treatment concentration, the blank and the control. The fish loading was 0.74 g (ww)/L. The test solutions were prepared twice, i.e., prior to exposure (0 hour) and after 48 hours exposure, when the medium was renewed. The concentration of test substance in the test solution was analysed at the initiation and termination of the test and during the renewal of the test medium using a HPLC-method. Since the solubility of hexythiazox in water is very low (0.5 mg/L at pH 7, 20°C), an aliquot (2500 mg) of the test item was weighed and dissolved in a portion of the dilution water containing 2.5 mL of N,N-dimethylformamide with aid of ultrasonic. Each solution was introduced to a test water tank and filled up to 25 L with the dilution water containing N,N-dimethylformamide at the rate of 0.1 mL/L to prepare test solutions of 100 mg/L. Carps with average body length of 5.4 cm and body weight of 1.88 g obtained from a fish farm were acclimated for 17 days under the test conditions. No mortality was observed during the acclimatization.

Findings:

From the results of the preliminary tests, a nominal concentration for the definitive test was decided to be 100 mg/L (limit test dose). All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within expected ranges. Since the concentrations of the test substance in the test solution at the initiation of the test (0 hours), 48 hours (before and after medium renewal) and 96 hours ranged from 11 to 18 % of the nominal concentration, the results were based on the mean measured concentrations. The overall mean measured concentration of hexythiazox was 14.1 mg/L (14 % of nominal). The test substance solution was a pale white colour. Therefore, the samples were treated with high-speed centrifuge before being analysed. The measured concentrations are presented in the following table.

Table 46: Concentration of test substance during the test period

Nominal concentration [mg/L]	Measured concentration [mg/L]				Mean measured concentration [mg/L]
	0 hours	48 hours		96 hours	
		before medium renewal	after medium renewal		
Blank	< 0.500	< 0.500	< 0.500	< 0.500	< 0.500
Control	< 0.500	< 0.500	< 0.500	< 0.500	< 0.500

100	18.0 (18)	12.4 (12)	15.6 (16)	11.0 (11)	14.1 (14)

Limit of quantification (LOQ) = 0.500 mg/L
( ): Percent of nominal concentration

There was no mortality and no toxic signs at the test concentration. Therefore, the LC_{50}, the maximum concentration with 0 % death and the minimum concentration with 100 % death were considered to be more than 14.1 mg/L (mean measured concentration of hexythiazox).

Acute toxicity of metabolites of hexythiazox to fish

Title:	Metabolite PT-1-2: Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) in a static system (96 hours). DAR IIA, 8.2/08
Guidelines:	OECD 203 (1992); 92/69/EEC, C.1 (1992).
GLP:	Yes, a statement of GLP compliance (OECD) and a quality assurance statement of the laboratory are given.

Materials and Methods:

Metabolite PT-1-2, purity 99.9%.

Acute toxicity of metabolite PT-1-2 to rainbow trout, *Oncorhynchus mykiss*, was studied in a static 96-h test using nominal concentrations of 0 (control), 0.46, 1.0, 2.15, 4.64, 10.0 and 21.5 mg/L. Dechlorinated municipal water with pH 8.0-8.6 was used in the test. The test compound was added to water separately for each concentration and mixed with a wing stirrer for a period of three days. No solvents were used. The test was performed at 12ºC and a loading of ten fish in 50 liter was used in control and all exposure groups. The average body length and wet weight of fish were 5.4 cm and 2.1 g, respectively. The concentration of the test compound in water was analyzed after 0, 1, 24, 48 and 96 hours with HPLC. Mortality and symptoms of toxicity were observed after 1, 4, 24, 48, 72 and 96 hours.

Findings:

The dissolved oxygen concentration remained over 60 % of maximum saturation value during the test. The mean measured concentrations of PT-1-2 during the test were 0.27, 0.67, 1.46, 3.41, 5.88 and 9.07 mg/L, which represents 42 to 74 % of nominal concentrations. At the highest nominal concentration (21.5 mg/L) small amount of undissolved test compound was visible on the bottom of the test system at the beginning of the study. No mortality or any abnormal behavior was seen in water control. The deviation between the nominal and mean measured concentrations of PT-1-2 was greater than 20 % and the toxicity values are based on mean measured concentrations. In PT-1-2 exposed fish no mortality was seen below the mean measured concentration of 1.46 mg/L. The 96-h LC_{50} was estimated to be about 1.50 mg/L based on mean measured concentrations. The first signs of abnormal behavior, restlessness and headstand, was seen after 96 h exposure at mean measured concentration of 0.67 mg/L and consequently the no observed effect concentration (NOEC) was determined to be 0.27 mg/L. In higher exposure concentrations, the observed sublethal effects also included abdominal distensions, convulsions, signs of narcotic state and tumbling.

Title:	Metabolite PT-1-9: Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) in a static system (96 hours). DAR IIA, 8.2/09
Guidelines:	OECD 203 (1992); 92/69/EEC, C.1 (1992).
GLP:	Yes, a statement of GLP compliance (OECD) and a quality assurance statement of the laboratory are given.

Materials and Methods:

Metabolite PT-1-9, purity 99.6%.

Acute toxicity of metabolite PT-1-9 to rainbow trout, *Oncorhynchus mykiss*, was studied in a static 96-h test using nominal concentrations of 0 (control), 0.46, 1.0, 2.15, 4.64 and 10.0 mg/L. Dechlorinated municipal water with pH 8.0-8.6 was used in the test. The test compound was added to water separately for each concentration and mixed with a wing stirrer for a period of three days. No solvents were used. The test was performed at 12ºC and a loading of ten fish in 50 liter was used in control and all exposure groups. The average body length and wet weight of fish were 5.4 cm and 2.1 g, respectively. The concentration of the test compound in water was analyzed after 0, 1, 24, 48 and 96 hours with HPLC. Mortality and symptoms of toxicity were observed after 1, 4, 24, 48, 72 and 96 hours.

Findings:

The dissolved oxygen concentration remained over 60 % of maximum saturation value during the test. The mean measured concentrations of PT-1-9 during the test were 0.15, 0.44, 1.22, 2.41 and 3.81 mg/L, which represents only 32 to 57 % of nominal concentrations. At two highest nominal concentrations (4.64 and 10.0 mg/L) small amount of undissolved test compound was visible on the bottom of the test system at the beginning of the study.

No mortality or any abnormal behavior was seen in water control. The deviation between the nominal and mean measured concentrations of PT-1-2 was greater than 20 % and the toxicity values are based on mean measured concentrations. In PT-1-9 exposed fish no mortality was seen below the mean measured concentration of 2.41 mg/L. The 96-h LC_{50} value was estimated to lie between 1.22 – 2.41 mg/L based on mean measured concentrations. The first sign of abnormal behavior, headstand, was seen after 96 h exposure at mean measured concentration of 0.44 mg/L and consequently the no observed effect concentration (NOEC) was determined to be 0.15 mg/L. In higher exposure concentrations the observed sublethal effects also included apathy, convulsions, signs of narcotic state and tumbling.

5.4.1.2 Long-term toxicity to fish

There is only one long-term fish study available, which is conducted according to OECD 204 guideline. It is noted that OECD 204 test is not considered an adequate chronic toxicity fish test as it is prolonged acute test. However, it is presented here as a supportive study.

Title:	28-day prolonged toxicity study in the Rainbow trout (semi-static). DAR IIA, 8.2/10

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Guidelines:	OECD 204 Deviations: Temperature of test media was in the range of 10.0 - 14.5°C (OECD 204/203: 15 ± 2°C). However, this is not deemed to influence the validity of the study.
GLP:	Yes; certified laboratory

Materials and methods:

The test item was identified as follows:

Hexythiazox

batch: 994

purity: 99.0 %

Toxicity of hexythiazox (purity 99%) was studied according to the OECD guideline 204 by exposing rainbow trout (*Salmo gairdneri*) to a nominal concentration of 20 mg ai/L in a 28-day semi-static test. The test solutions were renewed every 48 hours and tap water was used as test medium. Prior to each renewal, fresh test solution was stirred and aerated for 48 hours at 15±2ºC, filtrated, and then cooled to a temperature of 14ºC or just below. Exposure system consisted of a total of 30 fish divided into eight 16-liter test vessels (one vessel containing two fish in 9 liter) and water control consisted of a total of 12 fish in three test vessels. The average body length and weight of the fish at the beginning of the study were 5.2±0.27 cm and 1.9±0.24 g, respectively. Mortality was observed daily and clinical signs of toxicity were observed every 48 hours during the renewal of medium. Wet weight and body length of all fish were measured at the end of the test. The concentration of the test item in water was analysed with HPLC and the samples were taken at the beginning and at the end of first renewal and at renewals on days 18 and 20, respectively. Samples were also taken from fresh media of the last renewal.

Findings:

Oxygen concentrations and pH varied on the acceptable range during the test, but water temperature was lower (ranging from 10 to 14ºC) than the temperature recommended in the guideline, 15±2ºC. Hexythiazox concentrations in water decreased significantly between the renewals of the test media and the mean measured concentration during the test was 0.04 mg a.s./L, which is only 0.2 % of nominal concentration.

No mortalities were observed during the test in any test vessel. No sublethal signs of toxicity were detected during the test and no significant differences were found in mean wet weights between the control and exposed fish at the end of the test. Therefore, the (28 d) no observed effect concentration (NOEC) is determined to be 0.04 mg hexythiazox/L based on measured concentrations.

Water temperature during the test was occasionally lower than the recommended temperature range but this is not expected to affect the outcome of the study. Due to low solubility to water the deviation between the measured and nominal concentrations is large and the results are based on the mean measured concentrations.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Two acute studies with *Daphnia magna* are available and accepted during EU review according to Directive 91/414/EEC. One further acute toxicity invertebrate study is available and reported in the DAR. However, due to study deficiencies, principally relating to missing analytical concentration determination, the results are not considered valid and the study is not reported here.

Title:	Determination of the acute effect of BAS 9075 I on the swimming ability of the water flea *Daphnia magna* STRAUS. DAR IIA, 8.2/13
Guidelines:	OECD 202 (1984) 92/32/EEC C.2 (1992) OPPTS 850.1010 (1996)
GLP:	Yes; certified laboratory

Materials and methods:

The test substance was identified as follows:

Hexythiazox (BAS 9075 l)

batch: HA-3026

purity: 99.3 %

solid/powder

Acute toxicity of hexythiazox (99.3%) for the aquatic invertebrate (*Daphnia magna*) was studied in a static 48-h test. A study plan included water control containing aerated M4 medium (ISO 10706) and five nominal test concentrations, 6.25, 12.5, 25, 50 and 100 mg/L. Five daphnids (<24 h old) were used per replicate and four replicates were used per test concentration and control. The test concentrations were prepared from aqueous extract, which was attained by stirring 100 mg/L test substance in M4 medium for 20 h and removing the remaining particles by centrifugation. This aqueous extract was then diluted to give desired nominal concentrations. The highest (100 mg/L) and lowest (6.25 mg/L) nominal concentration was analyzed with HPLC at the beginning and at the end of the test. The swimming ability of the daphnids was visually checked after 0, 24 and 48 h.

Findings:

The initial mean measured concentration for the highest and lowest nominal test concentrations were 0.613 mg/L and 0.040 mg/l, and at the end of the test 0.330 mg/L and 0.038 mg/L respectively. In the DAR the mean measured concentrations during the test were found to be 0.47 (nominal 100 mg/L) and 0.03 (nominal 6.25 mg/L) mg/L, i.e. approximately 0.5 % of nominal. No immobility of the daphnids was reported at any concentrations during the test. Therefore, the 48-h EC50 of hexythiazox for *Daphnia magna* is determined to be above 0.47 mg/L based on mean measured concentrations. Dissolved oxygen concentration, pH and temperature were within the accepted range during the test.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Title:	Hexythiazox: Acute toxicity to *Daphnia magna*. DAR Additional report IIA, 8.2/34
Guidelines:	OECD 202 (1984)
	OPPTS 850.1010 (1996)
GLP:	Yes; certified laboratory

Materials and methods:

The test substance was identified as follows:

Hexythiazox (technical)

Lot No.: NJH-3424G

purity: 99.7 %

The acute toxicity of hexythiazox (99.7%) to *Daphnia magna* (< 24 hours old) was determined in a static 48-hour test. Treatments consisted of a control (dilution water (ASTM medium) containing N,N-dimethylformamide (DMF) as a solvent at the rate of 0.1 mL/L), blank (dilution water (ASTM medium)) and five test concentrations. The water solubility of the test substance (hexythiazox) was mentioned to be 0.5 mg/l (20°C) in the test report. An aliquot of the test substance was dissolved in DMF to prepare 12800 mg/L of stock solution. Aliquots of the stock solution were diluted with DMF to prepare dilution solutions of 800, 1600, 3200 and 6400 mg/L. Hundred millilitre of ASTM medium was transferred to 10 µL of the test solutions. The test substance was dissolved by ultrasonic bath in order to prepare the test solutions of 0.08, 0.16, 0.32, 0.64 and 1.28 mg hexythiazox/L concentrations. The selection of the test concentration range in the definitive test was based on results of two preliminary experiments. Twenty daphnids allocated as four groups of five daphnids were used per test concentration, control and blank, which give the loading of 20 mL/individual. The samples of the test medium were analysed at the start and at the end of the test by using a HPLC method. The EC_{50} value was determined using Probit method.

Findings:

All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within expected ranges. Precipitated or undissolved particles were observed in 0.64 and 1.28 mg/L test solutions at the observation period of 48 hours. Mean measured concentrations of hexythiazox were 0.0693, 0.131, 0.276, 0.422 and 0.658 mg/L and ranged from 51 to 87 % of nominal concentrations (**Table 47**). After 3 hours, no immobilisation was observed. After 24 hours, immobilised daphnids were observed at concentrations of 0.422 mg/L and higher. After 48 hours, immobilised daphnids were observed at concentrations of 0.131 mg/L and higher. No daphnids with physical or behavioural abnormality were observed at 0.0693 mg/L and in the controls and blanks during the observation period. Cumulative numbers of immobilised daphnids at every observation time are summarised in **Table 48**.

Table 47: Concentrations of the test substance measured in test vessels

Nominal concentration [mg/L]	Measured concentration of the test substance [mg/L]		Mean measured concentration [mg/L]
	0 hour	48 hours	
Blank	< 0.020	< 0.020	-
Control	< 0.020	< 0.020	-
0.08	0.0687	0.0699	0.0693 (86.6)
0.16	0.140	0.123	0.131 (82.1)
0.32	0.280	0.272	0.276 (86.2)
0.64	0.553	0.313	0.422 (65.9)
1.28	1.040	0.384	0.658 (51.4)

Limit of quantification (LOQ): 0.0200 mg/L
( ): Percent of nominal concentration

Table 48: Cumulative number of immobilised daphnids

Treatment	Mean measured concentration [mg/L]	Cumulative number of immobilised daphnids		
		3 hour	24 hour	48 hour
Blank	-	0	0	0
Control	-	0	0	0
Hexythiazox	0.0693	0	0	0
	0.131	0	0	1(5) *
	0.276	0	0	4(20) *
	0.422	0	3(15) *	11(55) *
	0.658	0	5(25) *	20(100) *

* Figures in parentheses indicate the percentage of immobilisation

EC_{50} values, minimum EC_{100} and NOEC for the acute exposure are given in **Table 49**. All values are based on mean measured concentrations. EC_{50} value of hexythiazox at 48 hours to *Daphnia magna* was determined to be 0.36 mg/l.

Table 49: Acute toxicity of hexythiazox to *Daphnia magna*

	3 hours	24 hours	48 hours
EC_{50} (mg/L)	> 0.658	> 0.658	0.36 (0.31-0.42)*
Minimum EC_{100} (mg/L)	> 0.658	> 0.658	0.658
NOEC (mg/L)	0.658	0.276	0.0693

* Figures in the parentheses indicate 95 % confidence limits.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Three chronic studies with *Daphnia magna* are available and were accepted during EU review according to Directive 91/414/EEC. One further chronic toxicity invertebrate study is available and reported in the DAR. However, due to study deficiencies, principally relating to missing analytical concentration determination, the results are not considered valid and the study is not reported here.

Title:	Hexythiazox technical: Chronic life-cycle toxicity to the water flea, *Daphnia magna*, under flow-through test conditions. DAR IIA, 8.2/21
Guidelines:	EPA FIFRA Guideline No. 72-4(b) Deviations: Dissolved oxygen concentration was observed to be temporarily below 60 %. Therefore, gentle aeration was initiated on day 12 in order to increase dissolved oxygen concentration. However, despite aeration the dissolved oxygen concentration at the end of the test ranged from 43 to 76 % of saturation, but stayed above 3.0 mg/l throughout the test.
GLP:	Yes; laboratories in the USA are not certified by any governmental agency, but are subject to official inspections

Materials and methods:

The test substance was identified as follows:

Hexythiazox

batch: 2232

purity: 99.3 %

light tan powder

Chronic toxicity of hexythiazox (purity 99.3%) for aquatic invertebrates was studied in a 21-day flow-through test using *Daphnia magna* as test species. The test water was aerated, filtrated (5 µm), and active carbon treated town water. Six nominal treatment concentrations, 12.5, 25, 50, 100, 200 and 400 µg hexythiazox/L, a solvent control containing 0.1 mL/L of N,N-dimethylformamide, and a dilution water control were used in the experiment. The flow-through system was adjusted to provide approximately 20 volume additions of test solutions every 24 hours. All treatments consisted of four replicates and each replicate contained ten daphnids (<24 h old at the initiation of test). During the test, daphnids were fed the green algae, *Selenastrum capricornutum*, and YCT (yeast-cereal leaves-trout chow) food. Survival, growth and reproduction were used as endpoints in the test. Survival of the adults was observed daily throughout the test and any dead (=immobile) individuals were removed. The time for the first appearance of young in each treatment and the daily production of live young thereafter were recorded. At the termination of the test, lengths and dry weights of surviving daphnids were measured. Hexythiazox concentrations in test water were analyzed on days 0, 6, 14 and 21 of the experiment with HPLC. Water temperature was monitored hourly and the dissolved oxygen concentration and pH were measured on days 0, 7, 14 and 21. The 7-, 14- and 21-day EC_{50}

values were calculated from the data and statistical difference in survival, growth and reproduction endpoints between the control and hexythiazox treatments was evaluated using Fisher's exact test or Dunnett's multiple comparison test. When significant difference between two controls (control and solvent control groups) was not detected, the control data were pooled and compared for statistical analyses with the test substance groups. If significant difference was detected, only the solvent control data was used for statistical evaluation with the treatments.

Findings:

At the initiation of the test the mean measured hexythiazox concentrations in test chambers ranged from 48 to 72 % of corresponding nominal concentrations. The mean measured concentrations during the test were 6.1, 12.7, 27.7, 53.8, 97.1 and 228 µg/L representing 49 – 57 % of nominal concentrations (see **Table 50**). Undissolved test substance was found in the chemical mixing box but not in the test chambers. Due to solubility problems, the results are based on the mean measured concentrations.

Table 50: Measured concentrations of hexythiazox technical equivalents during a 21-day exposure of *Daphnia magna* under flow-through conditions

Nominal Concentration (µg/L; ppb)	Measured[a] Concentration (µg/L; ppb)						Percent of Nominal
	Day 0	**Day 6**	**Day 14**	**Day 21**	**Mean**	**(± SD)**	
Control	< 0.98	< 1.11	< 0.99	< 1.17	<1.17		--
Solvent Control	<0.98	< 1.11	< 0.99	< 1.17	<1.17		--
12.5	8.98	5.40	3.90	6.00	6.07	(2.13)	49
25.0	14.1	12.1	10.0	14.7	12.7	(2.13)	51
50.0	27.7	27.7	25.0	30.4	27.7	(2.20)	55
100	55.3	55.0	45.6	59.3	53.8	(5.81)	54
200	96.6	102	82.8	107	97.1	(10.4)	49
400	249	203	149	310	228	(68.4)	57

[a] Concentration measured by HPLC and UV detector.

Mean immobility of daphnids in control and solvent control was 15 and 18 %, respectively, during the 21-day test and the acceptance criteria for the control was met. Mean immobility in hexythiazox exposures ranged from 18 % at concentration of 6.07 µg/L to 100 % at concentration of 228 µg/L. The lowest concentration where mean immobility was statistically significantly higher compared to that in control was 12.7 µg/L (see **Table 51**). The EC_{50} values based on immobility were determined to be 164, 122 and 60.3 µg/L for days 7, 14 and 21, respectively.

Table 51: Summary of test endpoints following exposure of *Daphnia magna* to hexythiazox for 21 days

Mean measured concentrations of hexythiazox [µg/L]	Mean adult immobility [%]	Mean live young[b]	Total offspring[c]	Mean adult length [mm]	Mean adult dry weight [mg]
Water control	15	8.81	4994	4.39	0.907
Solvent control	18	8.52	4441	4.55	0.975
6.07	18	8.44	4718	4.47	0.910
12.7	48 [d]	8.57	4227	4.48	0.832
27.7	35 [d]	7.87	4417	4.33	0.745 [d]
53.8	40 [d]	4.78 [d]	2404	4.03 [d]	0.755 [d]
97.1	88 [d]	0.37 [c]	152	3.74 [d]	0.480 [d]
228	100 [d]	0.00	0	- [e]	- [e]

a Percent of dead adult daphnids at the end of the test (immobility was synonymous with death)

b Mean of live young produced per adult reproduction day

c Total number of offspring produced during 21-day chronic exposure

d Statistically significant reduction in comparison to the pooled control.

e All adult animals dead.

First young were observed on day 7 in control, 12.7 and 27.7 µg/L exposures and on days 8 and 9 in other exposure concentrations excluding the highest concentration, where no young were produced.

Day 7 was used to calculate the number of adult reproductive days, which is a sum of days where individual adult daphnids were alive and capable to produce young. Adult reproductive days were used to calculate the number of mean live young. On the average 8.5 – 8.8 live young were produced per adult reproduction day in controls. The lowest exposure concentration where number of mean live young was statistically significantly lower compared to that in control was 53.8 µg/L, where number of mean live young was 4.78 (see **Table 51**). It is noted that number of offspring per adult reproduction day but also total number of offspring during the complete reproduction phase are in the range of the controls in the test groups at measured concentrations of 12.7 µg/L and 27.7 µg/L (only pooled data per replicate are given). At these concentrations, 35 % and 48 % adult daphnids were found dead but reproduction was not adversely affected.

The lowest exposure concentration where mean adult length (helmet-to-spine) at the end of the test was statistically significantly lower compared to that in control was 53.8 µg/L. The lowest concentration where mean adult dry weight at the end of the test was statistically significantly lower compared to that in control was 27.7 µg/L.

As immobility was found to be the most sensitive of the measured endpoints, the study director determined the chronic no-observed-effect concentration (NOEC) of hexythiazox for *Daphnia magna* to be 6.07 µg/L. The lowest observed-effect-concentration (LOEC) was 12.7 µg/L and the maximum acceptable toxic concentration (MATC) as a geometric mean of the NOEC and LOEC was determined to be 8.78 µg/L.

This study has been evaluated during EU review according to Directive 91/414/EEC and it was accepted then to use mortality (NOEC 6.07 µg/L) as the most sensitive endpoint for deriving chronic NOEC for *Daphnia*. However, it is noted that the survival rate did not clearly follow a dose-response pattern (see **Table 51**), and it may be question whether this mortality is substance related or rather a secondary effect, i.e. an effect of low dissolved oxygen content as the dissolved oxygen concentration dropped occasionally below 60 % of the saturation during the study. At the initiation of the test dissolved oxygen concentration was acceptable varying from 63 to 73 percent of saturation. At day 7 the dissolved mean oxygen concentration in all but control had dropped below 60 % of the saturation the lowest value being 44 % at 97.1 µg/L treatment. On day 12 a gentle aeration was initiated and on day 14 the dissolved oxygen concentrations varied from 57 to 76 % of the saturation. Despite aeration the dissolved oxygen concentrations at the end of the test ranged from 43 to 76 % of saturation. Nevertheless, 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 (see **Table 52**). Therefore, the study is considered acceptable also for classification purpose. However, as the mortality did not follow a concentration-response pattern the mean of live young produced per adult reproduction day is chosen as more appropriate endpoint for this classification proposal, giving NOEC of 0.0277 mg/l,

Table 52: Dissolved oxygen concentrations during a 21-day chronic exposure of Daphnia magna to hexythiazox technical

Mean Measured Concentrations (µg/L; ppb)	REP	Dissolved Oxygen Concentration (mg/L)			
		Day 0	**Day 7**	**Day 14**	**Day 21**
Control	A	6.0	6.4	7.4	7.0
	B	6.3	6.9	7.0	7.1
	C	6.1	6.5	6.9	7.1
	D	5.9	6.5	6.4	6.3
Solvent Control	A	6.1	5.2	6.4	4.7
	B	6.2	5.6	6.2	5.1
	C	5.6	5.4	6.0	4.6
	D	6.0	5.0	6.1	4.3
6.07	A	6.8	4.8	5.8	4.3
	B	6.5	4.4	6.0	4.1
	C	6.1	5.8	6.3	5.6
	D	6.6	4.3	6.4	4.1
12.7	A	5.9	5.2	6.3	5.2
	B	6.0	3.8	6.0	4.5
	C	6.6	3.9	6.5	4.4
	D	6.3	4.1	6.2	4.6
27.7	A	6.4	5.1	5.0	4.4
	B	6.6	5.2	5.5	3.9
	C	6.7	4.5	5.1	3.9
	D	6.6	4.6	5.2	3.8
53.8	A	6.8	5.1	5.0	4.2
	B	6.6	4.3	5.5	3.9

	C	6.5	4.4	5.5	3.9
	D	6.5	4.7	5.6	3.6
97.1	A	5.2	4.2	5.6	3.9
	B	6.3	4.4	5.8	3.9
	C	5.3	4.0	5.2	3.9
	D	5.9	3.4	5.3	3.8
228	A	6.0	4.6	5.3	4.9
	B	6.2	5.1	5.8	5.0
	C	5.8	4.1	6.1	4.6
	D	6.2	4.8	6.0	5.3

Title: Determination of the chronic effect of BAS 9075 I on the reproduction of the water flea *Daphnia magna* STRAUS. DAR IIA, 8.2/22

Guidelines: OECD 202 Part II (1981)

EEC Guideline XI/691/86, Draft 4

Deviations: Temperature values were slightly (19.3 - 22.3°C) above the recommended temperature range of 18 - 22°C for two occasions. However, this is not deemed to influence the validity of the study.

GLP: Yes; certified laboratory

Materials and methods:

The test substance was identified as follows:

Hexythiazox (BAS 9075 l)

batch: HA-3026

purity: 99.3 %

powder

Chronic toxicity of hexythiazox (purity 99.3%) for aquatic invertebrates was studied in a 21-day semi-static test using *Daphnia magna* as test species. Synthetic, aerated M4 medium based on demineralized water was used as dilution water. The test concentrations were prepared from aqueous extract, which was attained by stirring 100 mg/L hexythiazox in M4 medium for 20 h and removing the remaining particles by centrifugation. This aqueous extract was then diluted with M4 medium to give desired ten nominal concentrations: 0.2, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L. Semi-static test system was based on renewal of fresh exposure solution three times a week (on Monday, Wednesday and Friday). All hexythiazox treatments and water control consisted of ten replicates but each replicate contained only one daphnid (<24 h old at the initiation of test). Daphnids were fed live green algae (*Scenedesmus subspicatus*) daily during the test. Adult survival and reproduction, i.e. the production of live young and production of immobile young, was used as endpoint in daily observations. Hexythiazox concentrations in water were analyzed from nominal test concentration treatments 1.56, 12.5 and 100 mg/L. Samples for analysis were taken in the 1st, the 2nd and the 3rd week of the test. For each of these three concentrations the freshly prepared test solution (unstocked, on the test days 0, 9, and 19) and the corresponding 48 h or 72 h old test solution (stocked with

daphnids, on the test days 2, 12 and 21) were analysed using HPLC. Water temperature was monitored continuously and pH and oxygen contents were measured on days of renewal of test solution. Lowest-observed-effect-concentration (LOEC) and no-observed-effect-concentration (NOEC) were determined based on Duncan's multiple range test.

Findings:

At the initiation of the test the mean measured concentration was only 0.085 % of nominal concentration. The measured test concentrations during the test ranged from 0.04 to 0.11 % of nominal concentrations. Due to solubility problems, the median analyzed recovery rate, 0.0836 %, was used as basis for calculating the results. Dissolved oxygen concentrations (7.4 – 10.2 mg/L), pH (7.6 – 8.5) and temperature (19.9 – 22.3 ºC) were within acceptable ranges during the test.

Only one dead daphnid was reported and it was found at the highest test concentration. In control and all test concentrations, the first young were found on day 8. Hexythiazox had no effects on reproduction (production of young) at concentration of 0.0418 mg/L and below. At the highest test concentration, 0.0836 mg/L, the mean number of live young produced per female was significantly reduced and the mean number of immobile young produced per female was significantly increased. Accordingly, the 21-day no-observed-effect-concentration (NOEC) for *Daphnia magna* was determined to be 0.0418 mg/L based on median analytical recovery rate.

Table 53: Summary of chronic test endpoints following exposure of Daphnia magna to hexythiazox for 21 days

Nominal concentrations of hexythiazox [mg/L]	Mean concentrations of hexythiazox based on recovery rates [mg/L]	Mean number adult survival of ten exposed daphnids	Mean total live young produced per surviving female	Mean total immobile young produced per surviving female
Water Control	Water Control	10 / 10	165.4	0.0
0.2	0.0002 [a]	10 / 10	151.8	0.0
0.39	0.0004 [a]	10 / 10	152.3	0.0
0.78	0.0008 [a]	10 / 10	158.2	0.6
1.56	0.0015 [a]	10 / 10	166.9	0.0
3.13	0.0030 [b]	10 / 10	165.9	0.0
6.25	0.0060 [b]	10 / 10	165.7	0.0
12.5	0.0119 [b]	10 / 10	160.5	0.0
25	0.0209 [c]	10 / 10	168.6	0.0
50	0.0418 [c]	10 / 10	152.2	0.0
100	0.0836 [c]	9 / 10	24.1	21.6

[a] Based on the median analytical recovery rate of 0.0973 % for the samples of 1.56 mg/L not stocked with daphnia.

[b] Based on the median analytical recovery rate of 0.0955 % for the samples of 12.5 mg/L not stocked with daphnia.

[c] Based on the median analytical recovery rate of 0.0836 % for the samples of 100 mg/L not stocked with daphnia.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Title:	Chronic toxicity of trans-3-thiazolidine-carboxamide, 5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-OXO to *Daphnia magna*. DAR IIA, 8.2/24
Guidelines:	Not mentioned, study was performed according to OECD guideline 202. Deviations: Number of daphnids per treatment was lower than that recommended in the guideline.
GLP:	Yes. A quality assurance statement is given.

Materials and methods:

The test substance was identified as follows:

Hexythiazox (DPX-Y5893)

batch: not provided

purity: 98.9 %

Chronic toxicity of hexythiazox (purity: 98.9%) for aquatic invertebrates was studied in a 21-day semi-static test using *Daphnia magna* as test species. Study was conducted prior to GLP requirements; nevertheless a quality assurance statement is given in the report. Water from large *D. magna* culture aquaria was used as dilution water after it had been filtered (0.45 μm) and aerated (for 24 h). Water control, solvent control containing 0.1 mL N,N-dimethylformamide (DMF) as solvent, and nominal hexythiazox concentrations of 0.03, 0.06, 0.12, 0.25, 0.50 and 1.0 mg/L were used in the test. Fresh, food (trout chow, yeast) containing test solution was renewed three times per week. Two exposure systems were prepared:

Three replicates per treatment with five individuals in each replicate were used to study survival, while reproduction and growth was studied using seven individually exposed daphnids per treatment. Reproduction as an endpoint included observations of the first day of young production, number of reproduction days and number of young (total number and number per reproduction day) produced. The hexythiazox concentrations in water were analyzed using HPLC. Water samples for analyzing were taken on duplicate on days 0, 6, 13 and 21. Temperature was monitored continuously and dissolved oxygen and pH were measured daily. The differences in the endpoints between the control and treatments were tested by Dunnett's test.

Findings:

Mean measured concentrations of hexythiazox were close to nominal concentrations at low exposure concentrations. Limited water solubility of hexythiazox was seen at high nominal concentrations, where mean measured values were only 33 to 67 % of nominal concentrations (see **Table 54**). The results are therefore based on mean measured concentrations.

Table 54: Summary of test endpoints following exposure of Daphnia magna to hexythiazox for 21 days

Nominal concentrations of hexythiazox [mg/L]	Mean measured concentrations of hexythiazox [mg/L]	Mortality [%]	Mean number of reproduction days	Mean number of young produced per reproduction day	Mean length of adult daphnids at day 21 [mm]
Water control	-	0	7.0	14.9	4.4
Solvent control	-	0	8.0	17.4	4.4
0.03	0.033	7	11.0	15.1	4.4
0.06	0.055	0	10.8	13.7	4.3
0.12	0.050	7	9.0	11.7	4.0
0.25*	0.168	20	7.0	2.4	3.4
0.50**	n.s.	100	-	-	-
1.00**	0.330	100	-	-	-

n.s. Not sampled

* Reproduction was observed only in one of seven replicates

** No reproduction was observed

No mortality was reported on controls. Mortality, reproduction and length of adults at mean measured concentration of 0.055 mg/L and below did not differ statistically significantly from those at solvent control. At mean measured concentration of 0.168 mg/L mortality was increased but the difference to control was not statistically significant. At beakers used to monitor reproduction, however, only three of seven daphnids survived and no statistical comparison to control was made, even though reproduction and length of adults were reduced. All daphnids died at mean measured concentrations above 0.168 mg/L.

Based on effects of hexythiazox on reproduction and growth of *D. magna*, the no-observed-effect concentration (NOEC) was determined to be 0.055 mg/L (mean measured concentration).

5.4.3 Algae and aquatic plants

Two valid GLP studies on algal growth using hexythiazox are available. One further study is available and reported in the DAR. However, due to study deficiencies, principally relating to missing analytical concentration determination, the results are not considered valid and the study is not reported here.

Title: Determination of the inhibitory effect of BAS 9075 I on the cell multiplication of unicellular green algae. DAR IIA, 8.2/25

Guidelines: OECD 201 (1984)

92/32/EEC C.2 (1992)

Deviations: The number of controls was identical to that in the test concentrations even though the control replicates should preferably be twice the number of test replicates.

GLP: Yes; certified laboratory

Materials and methods:

The test substance was identified as follows:

Hexythiazox (BAS 9075 l)

batch: HA-3026

purity: 99.3 %

solid/powder

The effect of hexythiazox (purity 99.3 %) on growth of the freshwater green algae *Scenedesmus subspicatus* was studied in a 72 hours static test. Demineralized water was used as dilution water. The test concentrations were prepared from aqueous extract, which was attained by stirring 125 mg/L hexythiazox in demineralized water for 20 h and removing the remaining particles by centrifugation. This aqueous extract was then diluted with demineralized water to give desired seven nominal concentrations: 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L. Untreated water control containing algal growth medium was run concurrently. Three replicate flasks (250-mL Erlenmeyer) with initial inoculation density of 10^4 cells/mL were used in all treatments and in water control. Algal growth was measured after 0, 24, 48 and 72 h using chlorophyll-a-fluorescence as quantification method. Hexythiazox concentrations in test water in nominal concentrations of 6.25 and 100 mg/L and in stock solution (125 mg/L) were analyzed at the beginning and at the end of the test using HPLC. Algae growth using changes in biomass and growth rate are calculated based on mean measured hexythiazox concentrations (based on the median analytical recovery rates).

Findings:

At the beginning of the test the hexythiazox concentration in stock solution was only 0.29 % of the nominal concentration indicating low solubility to water with the extraction method used. The mean measured concentrations in test water varied between 0.37 and 0.43 % of the nominal concentrations at the beginning, and between 0.13 and 0.46 % at the end of the test. The mean measured hexythiazox concentrations varied between 0.005 – 0.445 mg/L (see **Table 55**).

Inhibition of algae biomass or algae growth rate did not reach 50 % at any hexythiazox concentration during the 72 h exposure, when it was compared to control. Accordingly, no exact 72 h E_bC_{50} or E_rC_{50} values were determined but they were assumed to be >0.4 mg/L, which was the highest mean measured concentration. The 72-h E_bC_{10} was determined to be 0.04 mg/L based on mean measured concentrations.

Table 55: Mean measured hexythiazox concentrations and effects of hexythiazox on Scenedesmus subspicatus growth after 72 hours exposure

Nominal hexythiazox concentration [mg/L]	Mean measured hexythiazox concentration [mg/L]	Inhibition of algal growth compared to control [%]	
		based on algae biomass	based on algae growth rate
Water control	Water control	-	-
1.56	0.005 [a]	2.8	0.5
3.13	0.010 [a]	7.6	2.2
6.25	0.019 [a]	7.7	1.8
12.5	0.056 [b]	12.2	2.9
25	0.111 [b]	16.6	5.1
50	0.223 [b]	10.7	2.3
100	0.445 [b]	18.9	6.0

[a] Based on the median analytical recovery rate of 0.305 % for the samples of 6.25 mg/L without inoculation.

[b] Based on the median analytical recovery rate of 0.445 % for the samples of 100 mg/L without inoculation.

Title: Hexythiazox: Inhibition of growth to alga *Pseudo-kirchneriella subcapitata*. DAR Additional report IIA, 8.2/35

Guidelines: OECD 201 (1984)
EPA OPPTS 850.5400

GLP: Yes; certified laboratory

Materials and methods:

The test substance was identified as follows:
Hexythiazox (technical)
Lot No.: NJH-3424G
purity: 99.7 %

The effect of hexythiazox upon the growth of the freshwater green algae *Pseudokirchneriella subcapitata* at nominal test concentration of 100 mg hexythiazox/L (limit test) was observed over a period of 96 hours under continuously shaking culturing/method. Three replicate flasks at the test concentration, the blank (OECD recommended medium) and three control replicate flasks (OECD recommended medium containing *N,N*-dimethylformamide (DMF) at the rate of 0.1 mL/L) were included in the test. In addition to DMF solvent, an ultrasonic bath was used to promote the dissolution of hexythiazox. The test concentration used in the definitive test was based on the results of the preliminary range-finding test. Cell number was counted at 24-hour intervals by using cell counter. Samples of the test substance solution were analysed at the start and the end of the study by using a HPLC method. In addition, water quality parameters (temperature and pH) were measured. The light intensity during the test was reported to be 4000 to 4050 Lux of continuous illumination. The algal concentration data were evaluated using two statistical methods, areas under the growth curve and average specific growth rate methods.

Findings:

All chemical and physical parameters (pH and temperature) in the definitive test were within expected ranges, although the pH increased during the study from the value of 8.0 to 10.5 due to photosynthesis of the algae. Mean measured concentration of hexythiazox (100 mg/L nominal concentration) was determined to be 72.0 mg/L, which is 72 % of nominal concentration. It is noted that the measured concentration is comparatively high regarding the reported water solubility of hexythiazox. It was not specifically mentioned in the test report whether the test media were checked for undissolved test substance or not. However, any items which possibly would have affected to the test results were not observed during the test. The measured concentrations are presented in **Table 56**.

Table 56: Analytical determination of the hexythiazox concentrations

Nominal Hexythiazox concentration [mg/L]	Measured concentration [mg/L]		Mean measured concentration [mg/L]
	samples taken after		
	0 h (C_0)	96 h (C_{96})	
Blank	< 0.500	< 0.500	< 0.500
Control	< 0.500	< 0.500	< 0.500
100	64.9	79.7	72.0 (72 %)

( ): Percent of nominal concentration

Cell counts of the algae were determined via cell counter. The effects of hexythiazox on cell concentrations and the growth inhibition of *Pseudokirchneriella subcapitata* are shown in **Table 57** and **Table 58**, respectively.

Table 57: Cell concentration of *Pseudokirchneriella subcapitata*

Concentration* [mg/L]	Mean cell concentration [cells/mL]					Increase factor [0-72 h]
	0 h	24 h	48 h	72 h	96 h	

Blank	10644 ±278	47667 ±3786	299333 ±23861	964444 ±13878	1577778 ±67110	91
Control	10333 ±240	47778 ±1711	298667 ±17474	897778 ±61944	1340000 ±65574	87
72.0	10244 ±379	53111 ±3595	320667 ±11015	948889 ±46825	1390000 ±36056	93

* Mean measured concentration of Hexythiazox

Table 58: Growth inhibition of *Pseudokirchneriella subcapitata*

Concentration [mg/L] [1]	Inhibition (0-72 h) [%] [2, *]	Inhibition (0-96 h) [%] [2, *]	Inhibition (24-48 h) [%] [2, **]	Inhibition (24-72 h) [%] [2, **]	Inhibition (24-96 h) [%] [2, **]
Blank	-	-	-	-	-
Control	-	-	-	-	-
72.0	- 6.90	- 5.52	1.79	1.66	2.02

[1] Mean measured concentration of Hexythiazox

[2] Values are the percent inhibition relative to the control

* Percent inhibition based on area under growth curve

** Percent inhibition based on growth rate

The E_bC_{50} and E_rC_{50} values of hexythiazox to the green alga *Pseudokirchneriella subcapitata*, based on the average area under growth curves at 0-72 and 0-96 h, and the specific growth rate at 24-48, 24-72 and 24-96 h, respectively, are summarised below.

Table 59: E_bC_{50} and E_rC_{50} values of the green alga *Pseudokirchneriella subcapitata*

Parameter	Time point				
	0-72 hours	0-96 hours	24-48 hours	24-72 hours	24-96 hours
E_bC_{50}	> 72.0 mg/L	> 72.0 mg/L	-	-	-
E_rC_{50}	-	-	> 72.0 mg/L	>72.0 mg/L	> 72.0 mg/L

Based on the mean measured concentration of hexythiazox, the E_bC_{50} values of hexythiazox at 72 and 96 hours to *Pseudokirchneriella subcapitata* are > 72.0 mg/L, and E_rC_{50} values of hexythiazox at 24-48, 24-72 and 24-96 hours to *Pseudokirchneriella subcapitata* are > 72.0 mg/L.

5.4.4 Other aquatic organisms (including sediment)

Title: Effects of hexythiazox on the development of sediment dwelling larvae of *Chironomus riparius* in a water-sediment system. DAR IIA, 8.2/29

Guidelines: BBA-guideline proposal (1995)

Deviations: pH values were determined to be in the range of 7.01 to 10.08, thus being slightly out of the recommended range of

6 to 9. However, this is not deemed to influence the validity of the study.

GLP: Yes; laboratory certified by the Ministerium für Arbeit, Soziales und Gesundheit, Rheinland-Pfalz, Germany

Materials and methods:

The test substance was identified as follows:

Hexythiazox

batch: L43-275

purity: 99.9 %

Effects of "water-spiked" hexythiazox (purity: 99.9%) on the development of the sediment dwelling larvae of the midge *Chironomus riparius* were studied in a chronic 21-day emergence test. The test was performed under GLP compliant conditions and followed the proposed BBA guideline 1995. The test was considered reliable during the EU review according to Directive 91/414/EEC. Test systems consisted of 2-liter glass beakers containing 2-cm sediment layer and an overlaying, aerated 16-cm water layer. The beakers were covered by a glass plate to reduce evaporation. Standard artificial soil (10 % sphagnum peat, 20 % kaolin clay, 1 % CaCO3 and 69 % quartz sand) described in OECD-guideline 207 was used as sediment and synthetic M4 medium based on ultrapure deionized water was used as test water. Test systems were allowed to stabilize for six days before the treatment. Seven nominal hexythiazox water concentrations, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/L, were used and two replicate beakers were prepared per treatment. Three replicate beakers were used in water control and in solvent control containing 10 mg/L Cremophor RH40. Twenty-five, first instar (less than 3 days old) chironomid larvae were exposed in each beaker and they were introduced into beakers 24 hours prior to treatment. Chironomids were fed commercially available fish feed ground and suspended in M4 water. The amount of feed ranged from 25 to 75 mg/test vessel. Observations on survival, behavior and emergence (the number, time and sex of emerged adults) were made daily. Oxygen, pH and temperature were determined one day before application and on days 7, 14 and 20. Hexythiazox concentrations in overlaying water in the lowest, a medium and the highest concentration were analyzed with HPLC at days 0, 1, 7 and 21. In addition, hexythiazox concentration in sediment pore water in the highest test concentration was analyzed at days 0, 7 and 21.

Findings:

Water temperature varied between 19.5 – 21.1ºC during the test. Oxygen concentration in water was mainly in acceptable range. Only on day 7, two low oxygen concentrations, 3.0 mg/L (solvent control) and 2.4 mg/L (0.1 mg/L), were detected but the concentrations in later measurements were on normal level. pH in water ranged from 7.01 to 10.08. The first midges emerged at day 12 and the emergence was completed at day 19. More than 75 % of the larvae emerged in each treatment. 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.

Based on the results, the 21-d no-observed-effect-concentration (NOEC) is determined to be 1.7 mg hexythiazox/L based on initial measured concentration.

Mean measured hexythiazox concentration in overlaying water were 25 to 42 % of nominal at the beginning of the test. The reason for the low recovery was the filtration of water samples but also the dissipation of hexythiazox from water. Rapid dissipation from water phase was evident as one day after treatment only 6-15 % of nominal initial concentration was analyzed from water and the corresponding percentages at day 7 were 1.5-4.7 % of nominal. At the end of the test no hexythiazox was found from overlaying water. The maximum hexythiazox concentration in sediment pore water, 0.15 mg/L representing 2.4 % of nominal initial concentration in overlaying water, was measured at the end of the test.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Hexythiazox is not rapidly degradable according to the CLP criteria. This conclusion is based on hydrolysis test as well as water/sediment and soil simulation tests. No biodegradation screening tests are available. The hydrolysis half-lives were ≥370 days at environmentally relevant temperature. Regarding the water/sediment simulation tests, as it is not possible to differentiate the degradation rate in water from that in sediment, the dissipation half-life in water cannot be used to evaluate rapid degradation. Degradation rate in water/sediment system, in sediment, or in soil are not among the preferred data to be used for assessing rapid degradability according to the CLP guidance. However, in the absence of biodegradation data for the surface water compartment the half-lives in water/sediment systems and in soil were compared to CLP criteria. The primary degradation half-lives were above 16 days in sediments (geometric mean 39 days), water-sediment-systems (geometric mean 72 days), and in four of the five tested soils (geometric mean 23.7 days). Therefore, the data from the simulation tests suggest that hexythiazox is not rapidly degradable according to CLP criteria. As the conclusion is clear based on the half-lives of the parent substance, consequently no further information/assessment regarding the degradation products is needed for assessing the rapid degradability criterion.

A measured octanol-water partition coefficient is available and revealed a logPow of 2.67 at 25°C. The whole fish mean BCF (bioconcentration factor) was determined as 975 for bluegill sunfish which is above the trigger value of ≥500 L/kg according to the Regulation EC 1272/2008. The BCF is based on total residual radioactivity and is associated with several uncertainties which are discussed in 5.3. However, it is considered appropriate for comparison with the CLP criteria. **Based on the BCF value and the criteria set in CLP hexythiazox has the potential to bioaccumulate.**

The three 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* than the parent compound hexythiazox. LC/EC_{50} values for all tested organism groups were in the range of 1.46 mg/l (LC_{50} of PT-1-2 determined for *O. mykiss*) to 34.6 mg/l (E_rC5_0 of PT-1-9 determined for *S. capricornutum*) based on measured concentrations. Therefore, the classification and labelling proposal for hexythiazox is based solely on hexythiazox ecotoxicity.

There is a full set of valid acute fish, invertebrate and algae data available for hexythiazox. Adequate chronic data is available only for invertebrate and algae as the long-term fish study

is prolonged acute test and not considered adequate chronic study for classification purpose. Thus, the surrogate method is used for assessing the chronic classification for hexythiazox

In the acute toxicity studies on fish with hexythiazox the LC_{50} values ranged from > 0.2 to > 14.1 mg/L based on measured concentrations. In the acute toxicity studies on daphnids with hexythiazox the EC_{50} values ranged from 0.36 to > 0.47 mg/L (based on measured concentrations). In the toxicity studies on algae with hexythiazox, the EC_{50} values ranged from > 0.4 to > 72 mg/L (based on measured concentrations).

For the studies on chronic toxicity to aquatic organisms, the no observed effect concentration (NOEC) for daphnids (21-d) ranged from 0.0277 mg/L to 0.055 mg/L based on measured concentrations. For algae the NOEC values were 0.4 – 72 mg/l. For *Chironomus riparius*, a 21-d NOEC of 1.7 mg/L was found based on filtered samples of overlaying water.

Based on the acute toxicity studies group of daphnids were the more sensitive than the other tested groups of aquatic organisms. Based on the lowest EC_{50} value of 0.36 mg/l (< 1 mg/l), classification of Aquatic Acute 1 is applicable with an acute M-factor of 1 ($0.1 < L(E)C_{50} \leq 1$ mg/L) considering the various *Daphnia magna* EC_{50} data (0.36 mg/L to > 0.47 mg/L) in this range for hexythiazox.

Since no adequate chronic data is available for all three trophic levels, the classification of hexythiazox in to chronic category is assessed using two approaches according to CLP (2nd ATP):

1. In the case of non-rapidly degradable substances for which there are adequate chronic toxicity data available classification of Aquatic Chronic 1 is applicable for hexythiazox based on NOEC value of 0.0277 mg /l for *Daphnia magna* (≤ 0.1 mg/l) with 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; Aquatic Chronic category 2 is applicable for hexythiazox based on 96 h LC50 of 3.2 mg /l for Rainbow trout ($1< L(E)C50 \leq 10$ mg/l).

The most stringent outcome shall be chosen and therefore hexythiazox shall be classified as Aquatic Chronic 1 with M-factor of 1 according to Regulation EC 1272/2008.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Conclusion of environmental classification according to Regulation EC 286/2011 (2nd ATP to EC 1272/2008)

Based on the CLP Regulation, hexythiazox should be classified as:

Classification categories	aquatic **acute category 1, M factor 1**
	aquatic **chronic category 1, M factor 1**

Hazard statement	**H410** 'Very toxic to aquatic life with long lasting effects'

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
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
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	*Pseudokirchneriella subcapitata*[1]	96-h NOEC (cell number)	72.0 (meas.)	Limit test No effects were observed.	DAR Additional report IIA; 8.2/35

Static Solvent used					
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* ($1< L(E)C_{50} \leq 10$ 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**.

6 OTHER INFORMATION

Not relevant

7 REFERENCES

Note: Some of the citations in the text include Annex point (e.g. "DAR IIA 2.2") only and not author names. These sources are included in the reference list with "Confidential" in the column "Author(s)".

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HEXYTHIAZOX (ISO); TRANS-5-(4-CHLOROPHENYL)-N-CYCLOHEXYL-4-METHYL-2-OXO-3-THIAZOLIDINE-CARBOXAMIDE

Author(s)	**Annex point / reference number**	**Year**	**Title** **Source (where different from company)** **GLP or GEP status (where relevant)** **Published or not**	**Data Protection Claimed Y/N**
ECHA		2015	Guidance on the Application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 4.1June 2015.	N
ECHA		2017	Guidance on the Application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0, June 2017.	N
EFSA		2010	European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance hexythiazox. EFSA Journal 2010; 8(10):1722. [78 pp.]. doi:10.2903/j.efsa.2010.1722.	N
European Commission, Joint Research Centre		2004	Summary record of Commission working group of specialized experts in the fields of CMR. ECBI08/04 Rev.2	N
Finland		2006	Draft Assessment Report (DAR) on the active substance hexythiazox. Prepared by the rapporteur Member State Finland in the framework of Directive 91/414/EEC, January 2006.	N
Finland		2009	Additional Report to the Draft Assessment Report on the active substance hexythiazox prepared by the rapporteur Member State Finland in the framework of Commission Regulation (EC) No 33/2008, October 2009.	N
Finland		2010	Final Addendum to the Additional Report on hexythiazox.	N
OECD		2012	OECD Test Guideline 305. OECD guidelines for testing of chemicals. Bioaccumulation in Fish: Aqueous and Dietary Exposure. Adopted 2 October 2012.	N
Confidential	DAR Additional Report IIA 2.4.1	1984	Physical and chemical properties of NA-73 technical. Non GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.4.2	1991	Data concerning the chemical and physical properties of the pure active ingredient. Non GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.1.1	2000	Melting Point of Hexythiazox GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.1.2	2003	Determination of the Boiling Point of Hexythiazox GLP, unpublished	Y

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Confidential	DAR IIA 2.2	2000	Density of Hexythiazox GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.3.1	1995	Series 63 Product Chemistry Determinations of Hexythiazox Technical Grade and Pure Active Ingredient (Water Solubility and Vapor Pressure) GLP, unpublished	Y
Confidential	DAR IIA 2.14	2001	Determination of the surface tension of an aqueous solution of Hexythiazox GLP, unpublished	Y
Confidential	DAR IIA 2.6	1988	Hexythiazox - Solubility in Water Not GLP, unpublished	Y
Confidential	DAR IIA 2.8	1984	Octanol/Water Partition Coefficient of NA-73 Not GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.11.1	2001	Determination of the flammability of Hexythiazox GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.13.2	2002	Determination of the explosive properties of Hexythiazox GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.13.1	2001	Statement on the explosive properties of Hexythiazox GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.11.2	2001	Determination of the relative self-ignition temperature of Hexythiazox GLP, unpublished	Y
Confidential	DAR IIA 2.15	2001	Statement on the oxidizing properties of Hexythiazox GLP, unpublished	Y
Confidential	DAR Additional Report IIA 2.9.4	1999	Dissociation Constant GLP, unpublished	Y
Confidential	DAR IIA 5.1/01	1985	Metabolism of NA-73 in Rats (Group B, C and D). Not GLP, unpublished	Y
Confidential	DAR IIA 5.1/02	1985	Metabolism of NA-73 in Rats (Group D). Not GLP, unpublished	Y
Confidential	DAR IIA 5.1/03	1985	Metabolism of NA-73 in Rats (Group C). Not GLP, unpublished	Y
Confidential	DAR IIA 5.1/04	1985	Metabolism of NA-73 in Rats (Group B)., Not GLP, unpublished	Y
Confidential	DAR IIA 5.1/05	1983	Metabolism of NA-73 in Rats. Not GLP, unpublished	Y

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Confidential	DAR Additional Report IIA 5.1/07	1991	Supplemental Information on Metabolism of Hexythiazox in Rats. Not GLP, unpublished	Y
Confidential	DAR Additional Report IIA 5.1/08	2008	[14C]-Hexythiazox - Tissue Distribution in Rat. GLP, unpublished	Y
Confidential	DAR Additional Report IIA 5.1/09	2009	91/414/EEC Review of Hexythiazox - Mammalian metabolism and excretion of cyclohexane-derived compounds. Not GLP, unpublished	Y
Confidential	DAR IIA 5.3/01	1983	Cumulative toxicity study of NA-73 in mice. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.3/02	1984	Four Week Dietary Range-Finding Study in Dogs with NA-73. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.3/03	1983	Subchronic feeding study of NA-73 in rats. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.3/04	1984	One Year Dietary Toxicity Study of NA-73 in Dogs. GLP, unpublished.	Y
Confidential	DAR IIA 5.4/03	1986	Gene Mutation in Chinese Hamster V79 Cells; Test Substance: NA-73. GLP, unpublished	Y
Confidential	DAR IIA 5.4/05	1986	Clastogenic Evaluation of NA-73 Technical Lot No. SCF-17B in an in vitro Cytogenetic Assay measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells. GLP, unpublished	Y
Confidential	DAR IIA 5.4/06	1983	Rec-Assay with Bacillus subtilis Strains H17 and M45. Not GLP, unpublished	Y
Confidential	DAR IIA 5.4/07	1984	Evaluation of NA-73 Technical Lot No. SCF-05 in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay. Second Amended Final Report. GLP, unpublished	Y
Confidential	DAR IIA 5.4/12	2005	Hexythiazox: Bacterial Mutation Assay. GLP, unpublished	Y
Confidential	DAR IIA 5.4/11	2001	Mammalian erythrocyte micronucleus test. GLP, unpublished	Y
Confidential	DAR IIA 5.5/01	1984	Lifetime (24-Month) Dietary Toxicity and Oncogenicity Study of NA-73 in Rats. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.5/15	2008	Hexythiazox - Historical control data of testicular interstitial cell adenoma in F344 rats (MPI Research) - Supplemental data for the rat chronic/oncogenicity study Not GLP, unpublished	Y

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Confidential	DAR IIA 5.5/03	1984	Chronic Feeding and Oncogenicity Studies in Mice with NA-73. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.5/04	1987	NA-73: Re-examination of the histological findings of B6C3F1 mice from the mouse chronic/oncogenicity study. GLP, unpublished	Y
Confidential	DAR IIA 5.5/05	1987	NA-73: Re-examination of the histological findings of the livers from the mouse chronic/ oncogenicity study. GLP, unpublished	Y
Confidential	DAR IIA 5.5/06	1985	Chronic feeding and oncogenicity studies in mice with NA-73 – Supplement- Histological findings of liver of mice sacrifieced at 78 weeks. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.5/07	1986	Replying Statement to the Inquiry on Histopathological Diagnosis of Hemangiopericytoma in the Liver of B6C3 F1 Mice. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.5/14	2007	NA-73 (Hexythiazox) - IRDC - Audited Historical Control Tumor Data for Fischer 344 Rats Not GLP, unpublished	Y
Haseman et al.		1990	35. Tumor Incidences in Fischer 344 Rats: NTP Historical Data Pathology of the Fischer Rat, 1990, 555-564 Not GLP, published	N
Haseman and Elwell		1996	Evaluation of False Positive and False Negative Outcomes in NTP Long-Term Rodent Carcinogenicity Studies Risk Analysis, 1996, 16 (6), 813-820 Not GLP, published	N
Haseman et al.		1998	Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update Toxicologic Pathology, 1998, 26 (3), 428-441 Not GLP, published	N
Maronpot et al.		1987	Liver lesions in B6C3F1 mice: The National Toxicology Program, experience and position Arch Toxicol Suppl., 1987, 10, 10-26. Not GLP, published.	N
Confidential	DAR IIA 5.5/16	2007	NA-73 (Hexythiazox) - Carcinogenicity Study in Mice Study No. 170 (026-002) Background Data of Histopathological Examination Not GLP, unpublished	Y
Haseman et al.		1984	Use of historical control data in carcinogenicity studies in rodents. Toxicologic pathology, 1984, Vol. 2, No. 2, 126-135. Not GLP, published	N

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Confidential	DAR IIA 5.5/17	2007	Chronic Feeding and Oncogenicity Studies in Mice with NA-73 - Historical Background Data of Hepatoblastoma Not GLP, unpublished	Y
Confidential	DAR IIA 5.5/10	2008	Hexythiazox – Historical control data of mammary fibroadenoma in male F344 rats (NTP studies) and summary of available comments. Not GLP, unpublished	Y
Confidential	DAR IIA 5.5/02	1989	Toxicological statement concerning the question on the influence of mammary tumour rate in male and female rats in a 2-year feeding study with Hexythiazox . Not GLP, unpublished	Y
Confidential	DAR IIA 5.6/01	1984	Two-generation reproduction study in rats with NA-73. GLP, unpublished.	Y
Confidential	DAR IIA 5.6/02	1983	Teratogenicity study of NA-73 in rats. Not GLP, unpublished.	Y
Confidential	DAR IIA 5.6/03	1984	Teratogenicity study of NA-73 in rabbits. Not GLP, unpublished.	Y
Tarone R. E. et al.		1981	Variability in the Rates of Some Common Naturally Occuring in Tumours in Fischer 344 Rats and (C57BL/6N X C3H/HeN)F1 (B6C3F1) Mice JNCI, June 1981, 66, (6) 1175-1181 Not GLP, published	N
Confidential	DAR IIA 7.2/01	1984	Hydrolysis of NA-73 Not GLP, unpublished	Y
Confidential	DAR IIA 7.1/04	1986	Photodegratation of NA-73 on Soil Not GLP, unpublished	Y
Confidential	DAR IIA 7.2/03	1992	Hexythiazox - Photodegradation in Water GLP, unpublished	Y
Confidential	DAR Additional report IIA 7.2/12	2008	Photodegradation of Hexythiazox in Water (No. 2-6-2) GLP, unpublished	Y
Confidential	DAR Additional report IIA 7.2/13	2008	Position Paper - Consideration of photodegradation in water of Hexythiazox Not GLP, unpublished	Y
Confidential	DAR IIA 7.2/09	2003	Estimation of photochemical degradation of Hexythiazox using the Atkinson Calculation Method Not GLP, unpublished	Y
Confidential	DAR IIA 7.1/01	2002	14C-Hexythiazox-Route and Rate of Aerobic Degradation in Two Soils GLP, unpublished	Y

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Confidential	DAR IIA 7.1/02	1984	Fate of NA-73 in Soil Not GLP, Unpublished	Y
Confidential	DAR Additional report IIA 7.1/11	2009	Aerobic soil degradation and metabolism of 14C-Cyclohexyl labelled Hexythiazox according to OECD 307 guideline GLP, unpublished	Y
Confidential	DAR Additional Report IIA 7.1/12	2009	91/414/EEC Review of Hexythiazox - Evaluation of the degradation kinetics of Hexythiazox and its metabolites PT-1-9, PT-1-2 and PT-1-3 in soil and in water/sediment systems	Y
Confidential	DAR IIA 7.1/06	1998	Investigation into the dissipation behaviour of Hexythiazox in the soil under field conditions GLP, unpublished	Y
Confidential	DAR IIA 7.2/05	1994	Degradation of the Test Substance Hexythiazox in Aerobic Aquatic Environment GLP, unpublished	Y
Confidential	DAR Additional report IIA 7.2/14	2009	Aerobic transformation of 14C-Cyclohexyl labelled Hexythiazox in water/sediment systems according to OECD 308 guideline GLP, unpublished	Y
Confidential	DAR IIA 7.1/03	1986	Anaerobic Aquatic Metabolism of NA-73 Not GLP, unpublished	Y
Confidential	DAR Additional report IIA 7.1/14	2009	Determination of Adsorption/Desorption of 14C-Hexythiazox in four soils GLP, unpublished	Y
Confidential	DAR IIA 7.1/10	1985	Soil Column Leaching of NA-73 Not GLP, unpublished	Y
Confidential	DAR IIA 7.1/09	2003	(14C)-PT-1-2, (14C)-PT-1-3, and (14C)-PT-1-9: Adsorption/Desorption in three soils GLP, unpublished	Y
Confidential	DAR IIA 7.2/06	1995	Investigation of the volatilization of 14C-Hexythiazox formulated according to Ordoval from plant surfaces under laboratory conditions Not GLP, unpublished	Y
Confidential	DAR IIA 7.2/07	1992	Investigation of the volatilization of 14C-Hexythiazox formulated according to Ordoval (10%WP) from soil and plant surfaces under standardized conditions Not GLP, unpublished	Y
Confidential	DAR IIA 7.2/08	1987	Hexythiazox - Volatility from Water and Soil Not GLP, unpublished	Y
Confidential	DAR IIA 8.2/11	1984	Laboratory Studies of 14C-Hexythiazox Bioconcentration in Bluegill sunfish Not GLP, unpublished	Y
Confidential	DAR IIA 8.2/12	1984	Bioaccumulation of NA-73 in Carp Not GLP, unpublished	Y

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Confidential	DAR IIA 8.2/01	1999	Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours) GLP, unpublished	Y
Confidential	DAR IIA 8.2/04	1987	Static Acute 96-hour LC50 to the Rainbow Trout GLP, unpublished	Y
Confidential	DAR IIA 8.2/05	1986	Static Acute 96-hour LC50 of DPX-Y5893 (Na-73) to Bluegill sunfish (Lepomis macrochirus) GLP, unpublished	Y
Confidential	DAR Additional report IIA 8.2/33	2003	Hexythiazox - Common Carp Acute Toxicity Test GLP, unpublished	Y
Confidential	DAR IIA 8.2/13	1999	Determination of the acute effect of BAS 9075 I on the swimming ability of the water flea Daphnia magna STRAUS according to OECD 202 GLP, unpublished	Y
Confidential	DAR Additional report IIA 8.2/34	2003	Hexythiazox - Acute toxicity to Daphnia magna GLP, unpublished	Y
Confidential	DAR IIA 8.2/25	1999	Determination of the inhibitory effect of BAS 9075 I on the cell multiplication of unicellular green algae according to OECD 201 GLP, unpublished	Y
Confidential	DAR Additional report IIA 8.2/35	2003	Hexythiazox - Inhibition of Growth to Alga Pseudokirchneriella subcapitata GLP, unpublished	Y
Confidential	DAR IIA 8.2/10	1989	28-Day Prolonged Toxicity Study in the Rainbow Trout GLP, unpublished	Y
Confidential	DAR IIA 8.2/21	1996	Hexythiazox Technical: Chronic life-cycle toxicity to the water flea, Daphnia magna, under flow-through test conditions GLP, unpublished	Y
Confidential	DAR IIA 8.2/22	1999	Determination of the chronic effect of BAS 9075 I on the reproduction of the water flea Daphnia magna STRAUS GLP, unpublished	Y
Confidential	DAR IIA 8.2/24	1986	Chronic Toxicity of trans-3-thiazolidinecarboxamide, 5-(4-Chlorophenyl)-N-Cyclohexyl-4-Methyl-2-OXO- to Daphnia magna Not GLP, unpublished	Y
Confidential	DAR IIA 8.2/29	1998	Effects of Hexythiazox on the development of sediment dwelling larvae of Chironomus riparius in a water-sediment system GLP, unpublished	Y

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Confidential	DAR IIA, 8.2/08	1998	Metabolite PT-1-2: Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours) GLP, unpublished	Y
Confidential	DAR IIA 8.2/15	1998	Effect of the Hexythiazox Metabolite PT-1-2 on Daphnia magnaYy Straus in a 48 hour acute toxicity test GLP, unpublished	Y
Confidential	DAR IIA 8.2/16	2002	PT-1-2 (TRans-5-(4-chlorophenyl)-4-methyl-2-oxothiazolidine-3-carboxamide): Acute toxicity to Daphnia magna GLP, unpublished	Y
Confidential	DAR IIA 8.2/27	1998	Effect of PT-1-2 on the growth of the green alga Pseudokirchneriella subcapitata GLP, unpublished	Y
Confidential	DAR IIA 8.2/17	2002	PT-1-3 (Trans-5-(4-chlorophenyl)-4-methyl-2-oxothiazolidine): Acute toxicity to Daphnia magna GLP, unpublished	Y
Confidential	DAR IIA 8.2/09	1998	Metabolite PT-1-9: Acute toxicity study on the rainbow trout (Oncorhynchus mykiss Walbaum 1792) in a static system (96 hours) GLP, unpublished	Y
Confidential	DAR IIA 8.2/18	1998	Effect of the Hexythiazox Metabolite PT-1-9 on Daphnia magna Straus in a 48 hour acute toxicity test GLP, unpublished	Y
Confidential	DAR IIA 8.2/19	2002	PT-1-9 (Trans-5-(4-chlorophenyl)-4-methyl-N-(4-oxocyclohexyl)-2-oxothiazolidine-3-carboxamide): Acute toxicity to Daphnia magna GLP, unpublished	Y
Confidential	DAR IIA 8.2/28	1998	Effect of PT-1-9 on the Growth of the Green alga pseudokirchneriella subcapitata GLP, unpublished	Y

8 ANNEXES

No additional data/information included.