Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

fosthiazate (ISO); *S-sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate

EC Number: -
CAS Number: 98886-44-3

CLH-O-0000007388-63-01/F

Adopted

30 November 2023

30 November 2023

CLH-O-0000007388-63-01/F

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

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on **30 November 2023 by consensus** on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **fosthiazate (ISO); *S-sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate**

EC Number: **-**

CAS Number: **98886-44-3**

Rapporteur, appointed by RAC: **Kirsten E. Rakkestad**

Co-Rapporteur, appointed by RAC: **Manuel Facchin**

Admnistrative information on the opinion

Germany has submitted on **23 November 2022** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **30 January 2023**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 March 2023**.

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

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	015-168-00-0	fosthiazate (ISO); *S*-*sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate	-	98886-44-3	Acute Tox. 3* Acute Tox. 3* Acute Tox. 4* Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H331 H301 H312 H317 H400 H410	GHS06 GHS09 Dgr	H331 H301 H312 H317 H410	EUH070		
Dossier submitters proposal	015-168-00-0	fosthiazate (ISO); *S*-*sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate	-	98886-44-3	**Modify:** Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 **Add:** Eye Irrit. 2 Repr. 2 Lact. STOT SE 1 STOT RE 2 **Retain:** Aquatic Acute 1 Aquatic Chronic 1	**Retain:** H331 H301 **Modify:** H311 **Add:** H319 H361fd H362 H370 (nervous system) H373 (adrenals) **Retain:** H400 H410	**Add:** GHS08 **Retain:** GHS09 Dgr	**Retain:** H331 H301 **Modify:** H311 **Add:** H319 H361fd H362 H370 (nervous system) H373 (adrenals) **Retain:** H410	**Retain:** EUH070	**Add:** inhalation: ATE = 0,53 mg/L (dusts or mists) dermal: ATE = 861 mg/kg bw oral: ATE = 57 mg/kg bw STOT SE 1; H370 (nervous system): C ≥ 1 % STOT SE 2; H371 (nervous system): 0.2 % ≤ C < 1 % M = 1 M = 1	
RAC opinion	015-168-00-0	fosthiazate (ISO); *S*-*sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate	-	98886-44-3	**Modify:** Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 **Add:** Eye Irrit. 2 Repr. 1B Lact. STOT RE 1 **Retain:** Aquatic Acute 1 Aquatic Chronic 1	**Retain:** H331 H301 **Modify:** H312 **Add:** H319 H360Df H362 H372 (nervous system, adrenals) **Retain:** H400 H410	**Add:** GHS08 **Retain:** GHS09 Dgr	**Retain:** H331 H301 **Modify:** H312 **Add:** H319 H360Df H362 H372 (nervous system, adrenals) **Retain:** H410	**Retain:** EUH070	**Add:** inhalation: ATE = 0,56 mg/L (dusts or mists) dermal: ATE = 860 mg/kg bw oral: ATE = 57 mg/kg bw M = 1 M = 1	

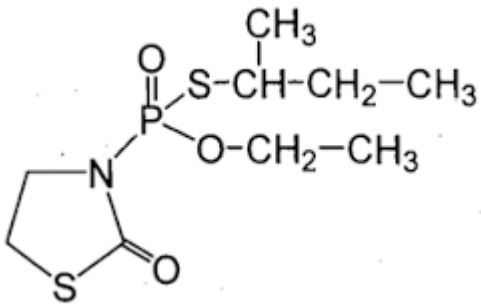
Resulting Annex VI entry if agreed by COM	015-168-00-0	fosthiazate (ISO); *S*-*sec*-butyl *O*-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate	-	98886-44-3	Repr. 1B Lact. Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 STOT RE 1 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360Df H362 H331 H311 H301 H372 (nervous system, adrenals) H317 H319 H400 H410	GHS06 GHS08 GHS09 Dgr	H360Df H362 H331 H311 H301 H372 (nervous system, adrenals) H317 H319 H410	EUH070	inhalation: ATE = 0,56 mg/L (dusts or mists) dermal: ATE = 860 mg/kg bw oral: ATE = 57 mg/kg bw M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Fosthiazate is an organophosphate with nematicidal and insecticidal activity. It is registered for control of potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*). In some countries, the product is also registered for wireworm control (*Agriotes spp.*). It is also registered for use on tomato against root knot nematodes (*Meloidogyne sp.*).

Fosthiazate is a racemic mixture of four stereoisomers. Due to the manufacturing process of fosthiazate, an equimolar ratio of the stereoisomers is expected. Technical fosthiazate is a light gold liquid with a boiling point of 198°C at 0.067 Pa, a low vapour pressure and a log K_{ow} of 1.68-1.75. Fosthiazate has a solubility of above 9 g/L in water and is soluble in methyl-2-pyrrolidinone (NMP), isopropyl alcohol, and xylene.



In the CLP Annex VI, fosthiazate has currently a harmonized classification as Acute Tox 3* (oral), Acute Tox 4* (dermal), Acute Tox 3* (inhalation), Skin Sens. 1, Aquatic Acute 1, Aquatic Chronic 1.

Fosthiazate is an active substance approved in accordance Directive 91/414/EEC (repealed by Regulation (EC) No 1107/2009). The approval expired on 31.10.2021. A Renewal Assessment Report (RAR) has been prepared by the DS and was submitted to EFSA on 30.09.2020.

Toxicokinetics

Fosthiazate is rapidly and extensively absorbed, distributed to organs and tissues, extensively metabolised and rapidly excreted after oral administration to rats.

Urine is the major route for excretion of fosthiazate metabolites from an orally administered dose, as shown in several studies. In general, at least 70 % of the administered dose regardless of sex, dose level and single or multiple dose administrations is excreted via the urine. The only exception is a study where the test material has been labelled (14C) in the carbonyl carbon of the thiazolidinone ring. In this study, 70 % or more of the administered dose is expired as radiolabelled CO_2, indicating that the ring was extensively metabolised and that air is a major excretory route.

The excretion data indicate that 90 % or more of the administered dose is absorbed and excreted within 48 hours regardless of sex, dose level, label position or the number of dose administrations.

All the toxicokinetic studies were performed in the rat.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Explosive substances

Fosthiazate was tested for explosive properties using the EC Method A.14 and was found not to be explosive. Additionally, the DS provided an assessment of the chemical structure to fully exclude explosive properties.

Flammable liquids

The flash point of fosthiazate was determined using the EC Method A.9 (closed cup) and was found to be above 120°C. The DS proposed no classification of fosthiazate as flammable liquid.

Self-reactive substances

An assessment of the chemical structure by the DS shows that fosthiazate carries functional groups (P-O) listed in tables A6.3 in Annex 6 (Screening Procedures) of the UN Manual of Tests and Criteria (UN MTC), which can be associated with self-reactive properties. As no relevant tests are available, the DS proposed that fosthiazate is not classified due to lack of data.

Pyrophoric liquids

Experience in manufacture and handling shows that the fosthiazate does not ignite spontaneously on coming into contact with air at normal temperatures. Based on the CLP criteria, the DS proposed no classification of fosthiazate as a pyrophoric liquid.

Self-heating substances

Fosthiazate is a liquid with a melting point below -163°C. Based on the Guidance on the Application of the CLP Criteria (CLP Guidance), the DS proposed no classification as a self-heating substance.

Substances which in contact with water emit flammable gases

The chemical structure of the substance does not contain metals or metalloids and the experience in handling shows that the substance does not react with water. Therefore, the DS proposed no classification of fosthiazate as a substance which in contact with water emits flammable gases.

Oxidising liquids

The mean pressure rise time due to reaction with cellulose for the test substance is significantly lower than for the reference (65 % aqueous nitric acid). Since test method EC A.21 is equivalent to method O.2 of the UN MTC, when the result is negative, as in this case, it can be regarded as not oxidising. Therefore, the DS proposed no classification of fosthiazate as an oxidising liquid.

Organic peroxides

Fosthiazate does not contain the peroxide group (-O-O-) and, therefore, does not meet the criteria for classification as an organic peroxide. The DS proposed that the data is conclusive but not sufficient for classification as organic peroxide.

Corrosive to metals

No test data is available. Fosthiazate is a liquid with a low melting point and thus, corrosive to metals properties are relevant. Therefore, the DS proposed no classification due to lack of data.

Comments received during consultation

Only one comment was received by a Member State merely stating that physical hazards have not been reviewed.

Assessment and comparison with the classification criteria

Explosive substances

The EC method A.14 results for fosthiazate were not sufficient for classification as explosive. However, this test method is not conclusive for classification as indicated by the DS in the CLH report. Therefore, the assessment based on CLP screening procedure was provided by the DS. Fosthiazate,does not contain chemical groups commonly associated with explosive properties as listed in table A6.1 in Annex 6 to the UN MTC.

RAC agrees with the DS that fosthiazate is not classified as explosive, based on the screening procedure indicated in CLP Annex I, 2.1.4.3.

Flammable liquids

Two closed cup methods according to EC method A.9 are available. Therein, flashpoints of 139°C and 127°C are stated. Both values are well above the threshold value of 60°C for classification as flammable liquid. Nevertheless, the results are considered reliable and conclusive by RAC and not warranting classification.

Therefore, RAC concludes that fosthiazate **does not warrant classification as flammable liquid**, according to the criteria in CLP Annex I, 2.6.2.1 (in agreement with the DS proposal).

Self-reactive substances

An assessment of the chemical structure by the DS shows that fosthiazate contains chemical groups, which can be associated with self-reactive properties. The P-O group is listed in table A6.3 in Annex 6 of the UN MTC. Therefore, further testing would be necessary to conclude on the self-reactive properties. Since no further tests according to UN test series A to Hare available, fosthiazate self-reactive properties cannot be fully excluded.

RAC agrees with the DS that fosthiazate **does not warrant classification as self-reactive**, due to the lack of data.

Pyrophoric liquids

Experience in manufacture and handling shows that the liquid does not ignite spontaneously on coming into contact with air at normal temperatures. This is in line with the screening procedure indicated in CLP Annex I, 2.9.4.1. Therefore, it can be concluded that fosthiazate is not classified as pyrophoric liquid.

RAC agrees with the DS that fosthiazate **does not warrant classification as pyrophoric liquid**, according to the screening procedure in CLP Annex I, 2.9.4.1.

Self-heating substances

Fosthiazate is a liquid at room temperature and does not contain any solid or is adsorbed on a solid. According to the CLP guidance, substances with a melting point below 160°C should not be considered for classification in this class, as for liquids the substance-air surface is not large enough to exhibit self-heating properties. The melting point of fosthiazate is reported below -163°C and is well below 160°C. Therefore, it can be concluded that fosthiazate is not classified as self-heating substance.

RAC agrees with the DS that fosthiazate **does not warrant classification as self-heating substance**, according to CLP Annex I, 2.11.4.2.

Substances which in contact with water emit flammable gases

Fosthiazate does not contain metals or metalloids and the experience in handling shows that the substance does not react with water. Therefore, according to CLP Annex I, 2.12.4.1, it can be concluded that fosthiazate is not classified as substance which in contact with water emit flammable gases.

RAC agrees with the DS that fosthiazate **does not warrant classification as substance which in contact with water emit flammable gases**, according to CLP Annex I, 2.12.4.1.

Oxidising liquids

A test according to EC method A.21 is available. A negative result from this test is considered equivalent to the method O.2 in the UN MTC. The test results show a significantly lower mean pressure rise of fosthiazate when compared to the reference test item. Therefore, it can be concluded that fosthiazate is not classified as oxidising liquid in accordance with CLP, Annex I, 2.13.

RAC agrees with the DS that fosthiazate **does not warrant classification as oxidising liquid**, according to CLP, Annex I, 2.13.

Organic peroxides

Fosthiazate does not contain an organic peroxide group (-O-O-) in accordance with the criteria set out in CLP, Annex I, 2.15.1. Therefore, fosthiazate is not classified as organic peroxide.

RAC agrees with the DS that fosthiazate **does not warrant classification as organic peroxide**, according to CLP, Annex I, 2.15.1.

Corrosive to metals

No test data and no waiving justification are available. Substances with acidic and basic functional groups, halogens and the potential to form metal complexes should be considered for classification according to the CLP Guidance, 2.16.4.1. In fosthiazate no halogens, but acidic and basic functional groups are present. Neither information on the pH of dilutions with water nor on metal complex building properties are available. Therefore, the screening procedure cannot exclude corrosive to metals properties. In accordance with the CLP criteria, UN test C.1 is recommended to determine corrosive to metals properties of liquids. Due to lack of data it is not possible to conclude on corrosive to metals properties of fosthiazate.

RAC agrees with the DS that fosthiazate **does not warrant classification as corrosive to metals**, due to lack of data.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity, oral route

The potential acute toxicity of fosthiazate via oral route was investigated in two GLP-compliant studies, one in rat and one in mouse. The rat study (Anonymous 14, 1989b) was considered acceptable, while the mouse study (Anonymous 13, 1989a) was considered supplementary (due to uncertainty of dosing solutions).

In the acute oral toxicity study in rats, 5 animals/sex/dose were exposed to fosthiazate. The doses were 41, 51, 64, 81 and 128 mg/kg bw. Deaths occurred at ≥64 mg/kg bw within 3 days after treatment. The mortality incidences are shown in the table below.

***Table**: Mortality incidences in rats (oral exposure)*

Dosage (mg/kg bw)	Males	Females
41	0/5	0/5
51	0/5	0/5
64	2/5	5/5
81	3/5	5/5
128	5/5	5/5

Signs of toxicity were observed in both sexes at all dose levels and included decreased motor activity, prone or hunched posture, muscle tremor, irregular breathing and piloerection. Less frequent signs were ataxia, muscle spasms, pigmented orbital secretions, irritability, blanching, salivation, pigmented staining of the snout and diarrhoea.

The acute oral LD_{50} of fosthiazate in rats was 73 mg/kg bw for males, and 57 mg/kg bw for females. LD_{50} of fosthiazate combined for both sexes was 65 mg/kg bw.

In the acute oral toxicity study in mice, 5 animals/sex/dose were exposed to fosthiazate. The doses were 51, 81, 102, 128 and 161 mg/kg bw. Deaths occurred at ≥102 mg/kg bw within 4 days of treatment. Signs of toxicity were observed at ≥102 mg/kg bw. These were decreased motor activity, hunched posture, ataxia and muscle tremor. Less frequent observations were lethargy, irregular breathing, prone posture and closed eyes.

The acute oral LD_{50} of fosthiazate in mice was 104 mg/kg bw in males and 91 mg/kg bw in females.

The DS concluded that oral LD_{50} values in both rat and mouse, in both sexes, were between 50 – 300 mg/kg bw which, according to CLP criteria, warrant classification in Category 3 (H301). Further, according to CLP, the acute toxicity estimate (ATE) is derived using the lowest LD_{50}. The lowest LD_{50} in these studies was 57 mg/kg bw for female rats, and hence the DS proposed an ATE value for oral exposure at 57 mg/kg bw.

Acute toxicity, dermal route

The potential acute toxicity of fosthiazate via the dermal route was investigated in one study. The study (Anonymous 15, 1989c) was performed in rats, was GLP-compliant, and was considered to be of acceptable quality.

In the acute dermal toxicity study in rats, 5 animals/sex/dose were exposed to fosthiazate. The doses were 1956, 2472, 3115 and 3918 mg/kg bw in males, and 309, 494, 779, 1236, 1557 and 2472 mg/kg bw in females. Deaths occurred within 8 days at ≥1965 mg/kg bw in males, and ≥779 mg/kg bw in females. Observed signs of toxicity consisted of decreased motor activity, prone posture, muscle tremor, ungroomed appearance, pigmented orbital secretion and thin body conformation. The signs persisted for up to 12 days in surviving animals. Less frequently irregular breathing, salivation, opisthotonus, hunched posture, ataxia, bulging eyes and blanching was observed. Local dermal reactions at the treated skin site were not reported.

The acute dermal LD_{50} of fosthiazate in rat was 2396 mg/kg bw in males, and 861 mg/kg bw in females.

The DS concluded that dermal LD_{50} values in female rats were within the range of 50 – 1000 mg/kg bw, which according to CLP criteria warrants classification in Category 3 (H311). Further, according to CLP, the ATE is derived using the lowest LD_{50}. The lowest LD_{50} in this study was 861 mg/kg bw for female rats, and hence the DS proposed an ATE value of 861 mg/kg bw for dermal exposure.

Acute toxicity, inhalation route

The potential acute toxicity of fosthiazate via the inhalation route was investigated in one study. The study (Anonymous 21, 1989) was conducted in rats, was GLP-compliant, and considered to be of acceptable quality.

In the acute inhalation toxicity study in rats, 8 animals/sex/dose were exposed. Exposure was via nose-only (aerosol). The doses were 0, 0.53, 0.8, 0.9 and 1.23 mg/L. Mass median aerodynamic diameter ranged between 2.04-2.29 µm, and exposure lasted for 4 hours. Deaths occurred at ≥0.53 mg/L within 2 days for males, and within 4 days for females. Observed signs of toxicity included reduction in spontaneous motor activity, decrease in respiratory rate, salivation, limb paralysis, adoption of prone position, nasal bleedings, red tears, and lacrimation. The mortality incidences are shown in the table below.

***Table**: Mortality incidences in rats (exposure via inhalation)*

Dosage (mg/L)	Males	Females
0 (air control)	0/8	0/8
0.53	1/8	4/8
0.8	3/8	5/8
0.9	5/8	8/8
1.23	7/8	8/8

The acute inhalation LC_{50} of fosthiazate in rats was 0.83 mg/L in males, and 0.56 mg/L in females.

The DS concluded that the LC_{50} values for inhalation route in both male and female rats were within the range of 0.5 – 1 mg/L which according to CLP criteria for acute inhalation toxicity of dusts and mists, warrant classification in Category 3 (H331). Further, according to CLP, the ATE

is derived using the LC_{50}. The lowest LC_{50} in this study was 0.56 mg/L for female rats, and hence the DS proposed an ATE value of 0.56 mg/L for exposure via inhalation route.

Comments received during consultation

One Member State Competent Authority (MSCA) commented, agreeing with the DS' classification proposal, including the suggested ATE values for the oral, dermal and inhalation route.

Assessment and comparison with the classification criteria

The DS presented acceptable *in vivo* studies in rats for acute toxicity of fosthiazate via oral, dermal and inhalation route. For exposure via the oral route, also a supplementary study in mice was presented. For fosthiazate, all studies, for all exposure routes, have LD_{50}/LC_{50} values that fall into the ranges for a Category 3 classification according to CLP Annex I Par. 3.1.2.1.. The only exception is for dermal exposure, where the LD_{50} for male rats is almost three times higher than that in females, and does not fall within a range for any Category. However, the LD_{50} for female rats is clearly in the range for Category 3 classification (LD_{50} is 861 mg/kg bw, the range for Category 3 is <200 – 1000 mg/kg bw). RAC supports the DS' proposal to use the LD_{50} for females as the ATE value and as the basis for classification.

Therefore, RAC concluded that fosthiazate:

warrants for oral route a classification in Category 3, H301, with an ATE of 57 mg/kg bw;

warrants for dermal route a classification in Category 3, H311, with a rounded ATE value of 860 mg/kg bw;

warrants for inhalation route a classification in Category 3, H331, with and ATE value (dust and mists) of 0.56 mg/L.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Potential specific target organ toxicity of fosthiazate after single exposure (STOT SE) were assessed in eight studies in total. Six studies in rats, one in mouse and one in hen (*gallus gallus*). All studies were GLP-compliant. The rat studies were: one acute neurotoxicity screening study compliant to OECD TG 424 and of acceptable quality (Anonymous 38, 1997), one age sensitivity study with no applicable guideline but considered to be of acceptable quality (Anonymous 7, 2006), one acute oral toxicity study (Anonymous 14, 1989b), one acute dermal toxicity study (Anonymous 15, 1989c) and one acute inhalation toxicity study (Anonymous 21, 1989). The acute oral, dermal and inhalation studies were also used for acute toxicity classification and were all of acceptable quality. The study set also included a sixth rat study that was performed to determine a NOEL for acetylcholine esterase (AChE) inhibition (Anonymous 37, 1994). This study was not performed in accordance with a guideline and was judged as supplementary only . The mouse study was an acute oral toxicity study (Anonymous 13, 1989a), that was also considered as supplementary. The study in hen (*gallus gallus*) was an acute delayed neurotoxicity study

(Anonymous 19, 1989g). This study satisfied the requirements of OECD TG 418 and was judged as acceptable.

Four of the acute toxicity studies were also used for the acute toxicity classification. Hence, the description of how these studies were conducted is given above in the acute toxicity section, while the results relevant to the assessment of STOT SE are included here.

The results from all eight studies are summarised and discussed in "Supplementary information" in the Appendix to this opinion and in Table 30 in the CLH report.

According to the Dossier submitter, these studies show that fosthiazate is a neurotoxic substance acting via AChE inhibition. According to the CLP criteria, STOT SE Category 1 and 2 is assigned on the basis of findings of 'significant' and/or 'severe' toxic effects and of 'significant' toxic effects in animal studies, respectively. Classification after single exposure covers all significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed effects are included. Both in the acute neurotoxicity screening study, the acute oral toxicity study in rats, the acute oral toxicity study in mice, and in the acute delayed neurotoxicity study in hens, clinical neurotoxic symptoms of cholinergic responses were observed at concentrations below 300 mg/kg bw which is the limit value for Category 1 of STOT SE based on oral rat studies. Neurotoxic effects of cholinergic responses in the acute inhalation toxicity study in rats were observed from the lowest dose on (0.53 mg/L), also corresponding to guidance value (GV) range for Category 1 of STOT SE (inhalation rat: C ≤ 1 mg/L/4h). Neurotoxic effects in the acute dermal toxicity study in rats were observed from 1965 mg/kg bw in males and 779 mg/kg bw in females. While the effect level in females corresponded to the GV range for STOT SE Category 1, effects in males were observed only at a dose corresponding to Category 2 (dermal rat; STOT SE 1: C ≤ 1000 mk/kg bw, STOT SE 2: 2000 ≥ C > 1000 mg/kg bw).

Fosthiazate did not result in pathological or histopathological changes in the nervous system. However, the inhibition of AChE activity together with the observed clinical neurotoxic symptoms were considered by the DS as effects that clearly indicated functional impairment and being of considerably adverse in nature with significant impact on health. Moreover, the inhibition of AChE and the related clinical signs of toxicity are mechanisms and effects that are relevant to humans. For these reasons, the DS concluded that classification for STOT SE Category 1 (H370) was required for fosthiazate with nervous system as the target organ.

Given that the effects were observed after exposure via various routes, the route of exposure was not be specified in the hazard statement.

Setting of specific concentration limits (SCL) for STOT SE

According to the CLP guidance chapter 3.8.2.6 it is recommended to set a SCL if a substance induces relevant effects at doses that are clearly (more than one magnitude) below GV. For fosthiazate, neurotoxicity was seen ≥4-5 mg/kg bw in oral studies, while the GV for STOT SE Category 1 is ≤ 300 mg/kg bw. Hence, the DS proposed a SCL. The SCL was determined in line with the approach recommended in the CLP guidance. The DS proposed SCLs for both Cat. 1 and Cat. 2 as follows:

SCL (Cat.1) = (4 to 5 mg/kg bw) / (300 mg/kg bw) x 100 % = 1.3 – 1.6 %

SCL (Cat.2) = (4 to 5 mg/kg bw) / (2000 mg/kg bw) x 100 % = 0.2 – 0.25 %

Based on the "preferred value approach", the DS proposed a SCL of C≥1 % for Cat. 1, and a SCL of 0.2 %≤C<1 % for Cat. 2.

Comments received during consultation

Only one comment was received during consultation. This was from a MSCA supporting the proposed classification.

Assessment and comparison with the classification criteria

No human data, i.e., data from occupationally or accidentally exposed humans, were available for evaluation of specific target organ toxicity after single exposure (STOT SE) for fosthiazate.

The available animal studies did not indicate respiratory tract irritation. The neurotoxic effects observed after a single dose in animals were compared with the CLP criteria for STOT SE.

Specific target organ toxicity – single exposure means specific, non-lethal toxic effects on target organs occurring after a single exposure to a substance.

According to CLP criteria in Annex I, table 3.8.1, the transient target organ effects include only narcotic effects and respiratory tract irritation. *These are effects which do not meet the criteria to be classified in Categories 1 or 2 and which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alterations of structure or function.* More specifically, as given in Annex I, 3.8.2.2.2 the criteria for narcotic effects cover *(a) central nervous system depression including narcotic effects in humans [...] (b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.*

Decreased motor activity, prone or hunched posture, muscle tremor, irregular breathing and piloerection, ataxia, muscle spasms and salivation were observed in the acute toxicity studies. The effects resolved within 3 (oral) or 4 (inhalation) days and persisted for 12 days after dermal exposure. Muscle tremor and spasms are indicative of nervous system excitation rather than depression, and acetylcholine is typically an excitatory neurotransmitter that operates in many parts of the body, including the autonomic nervous system and neuromuscular junctions. RAC therefore considers these effects are more than transient in nature and do not only represent central nervous system depression. Consequently, classification for STOT SE Category 3, which covers transient effects (respiratory tract irritation and narcotic effects), is not warranted.

For the evaluation of STOT SE 1 and 2, eight relevant single dose studies in animals were evaluated. Most studies were performed in rats, but neurotoxic effects were also studied in mice and hens. Effects of fosthiazate on AChE activity were shown in several of the studies. RBC AChE inhibition started at 5 mg/kg bw, while effects on brain AChE inhibition started at 10 mg/kg bw. Clinical signs of neurotoxicity commonly associated with cholinergic toxicity were also shown, including prone or hunched posture, muscle tremor, irregular breathing and piloerection, ataxia, muscle spasms, salivation, and decreased motor activity. These were seen from 40/20 mg/kg bw (males/females). Altogether, it was demonstrated that fosthiazate is a neurotoxic substance acting via AChE inhibition.

In the acute toxicity studies, increased mortality was also shown, however at higher doses. Neurotoxicity is starting at oral doses of approximately 5 mg/kg bw. The acute oral LD_{50} in rats is 73/57 mg/kg bw for males/females.

According to CLP Annex I, 3.8.2.1.1 classification in Category 1 is appropriate if the substance has produced significant toxicity in humans or, on the basis of evidence from studies in experimental animals, it can be presumed to have the potential to produce significant toxicity in

humans following single exposure. The inhibition of AChE and the related clinical signs of toxicity shown in the animal studies are mechanisms and effects that are considered relevant for humans.

In the CLP Annex I, 3.8.2.1.9.3 guidelines values ranges for the various categories after single dose exposure are given. These include for Category 1 via oral route (rat): C≤300 mg/kg bw. For fosthiazate, as stated by the DS, effects on neurotoxicity were observed at doses that fall within the guidelines values range for Category 1.

However, RAC concludes that only the effects from around 20 mg/kg bw represent significant health effects that impaired function. For fosthiazate, these effects occurred at doses that were only three-fold or less lower than the LD_{50}-values that warrant classification for acute toxicity classification. Therefore, in this case, RAC concludes that it is not necessary to classify the acute neurotoxic effects for STOT SE 1, but that classification for STOT RE for neurotoxic effects is more appropriate because the neurotoxic effects were more severe and occurred at lower doses after repeated exposure than after a single exposure (please see the assessment under the section for STOT RE).

RAC concludes that fosthiazate **does not warrant classification as STOT SE**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The potential of fosthiazate to cause serious eye damage/irritation had been investigated in two studies. Both studies were in rabbits, and both were GLP-compliant. Only one study (Anonymous 17, 1989e) was considered to be of acceptable quality, while the other (Anonymous 18, 1989f) was considered only supplementary (due to washing of the eyes 2 - 3 minutes after instillation instead after approx. 30 minutes).

The results from both studies are summarised in the text and tables below, and in Table 22 in the CLH report.

In the first eye irritation study in rabbits, a total of 6 animals, 4 male and 2 females, were treated with 0.1 mL fosthiazate and scored after 1, 24, 48 and 72 hours, and 7 days. Eye irritation scores and ocular toxicity are shown in the table below.

***Table**: Mean of eye irritation scores reported at 24h, 48h and 72h after exposure to 0.1 mL fosthiazate.*

Animal	Corneal opacity	Iritis	Conjunctival redness	Conjunctival oedema (chemosis)
Male 1	Animal killed in extremis after 5 h			
Male 2	0	0.3	2	0.3
Male 3	Animal found dead after 5 h			
Male 4	Animal killed in extremis after 5 h			
Female 1	0	0	2.3	0
Female 2	0	0	2.6	0

The observed effects had resolved by 7 days after treatment.

In the second eye irritation study in rabbits, three animals, all females, were treated with 0.1 mL fosthiazate and scored after 1, 24, 48 and 72 hours, and 7 days. Eyes were washed 2 – 3 minutes after instillation. Eye irritation scores are shown in the table below.

***Table**: Mean of eye irritation scores reported at 24h, 48h and 72h after exposure to 0.1 mL fosthiazate.*

Animal	Corneal opacity	Iritis	Conjunctival redness	Conjunctival oedema (chemosis)
Female 1	0	0	2	0
Female 2	0	0	1.3	0
Female 3	0.6	0	1.3	0.3

All observed effects had resolved by day 8. No mortalities were observed.

The main study gave positive response in three of three surviving animals with a score of ≥2 for conjunctival redness. According to CLP criteria for eye irritation, conjunctival redness ≥2 calculated as the mean score following grading at 24, 48 and 72 hours after instillation of the substance in at least 2/3 animals, should be classified as irritating to eyes (Category 2). The DS therefore proposed a classification as Eye Irrit. 2, H319. The DS also proposed an additional hazard statement "EUH070 – Toxic by eye contact", because 3 of 6 treated rabbits died/were killed *in extremis* after ocular instillation and according to CLP, EUH070 is allocated for substances or mixtures where an eye irritation test has resulted in overt signs of systemic toxicity or mortality among the animals tested, which is likely to be attributed to absorption of the substance or mixture through the mucous membranes of the eye.

Comments received during consultation

One National Authority (NA) and one MSCA commented. Both supported the DS' proposal for classification as Eye Irrit. 2, H319, as well as the proposed additional hazard statement "EUH070 – Toxic by eye contact".

Assessment and comparison with the classification criteria

The results from the main eye irritation study in rabbits showed mean conjunctival score ≥ 2 in all surviving animals (3 rabbits), and the effects were fully reversible within 21 days. The results are in accordance with the CLP criteria for eye irritation. RAC concludes that fosthiazate **warrants classification as Eye Irrit. 2, H319**.

Additionally, in this eye irritation study in rabbits, systemic toxicity was observed, with 3 animals dying or killed *in extremis*. RAC therefore concludes that the additional hazard statement **"EUH070 – Toxic by eye contact"** is also warranted.

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

Summary of the Dossier Submitter's proposal

To evaluate the possible specific target organ exposure of fosthiazate after repeated exposure, a total of 13 studies were presented. Six were in rat, four in mice and three in dogs. Twelve of the

studies were GLP-compliant, only one histopathological assessment of adrenal glands from mouse (Anonymous 35, 2013) was not in accordance with GLP. Five of the rat studies; one oral 90-day study (Anonymous 9, 1989b), one combined long-term and carcinogenicity study (Anonymous 4, 1990b), one two-generation study (Anonymous 34, 1990), one age-sensitivity study (Anonymous 7, 2006), one dermal 21-day study (Anonymous 10, 1989c); two of the mouse studies; one oral 28-day study (Anonymous 40, 2011), one carcinogenicity study (Anonymous 3, 1990a), and two of the dog studies; one oral 90-day study (Anonymous 8, 1989a/Anonymous 23, 1995), one oral 1 year study (Anonymous 39,1991/Anonymous 23, 1995), were considered acceptable and as key studies. The other four studies were considered supplementary.

A summary or the main results and related considerations by the DS are reported in "Supplementary information" in the Appendix to this opinion.

As described in the Appendix, the DS considered that these repeated-dose studies in rats, mice and dogs showed effects on the nervous system through cholinesterase inhibition, on the adrenal glands, and on haematological markers.

Cholinesterase inhibition was seen in rats and dogs, after both oral and dermal exposure. However, inhibition of cholinesterase was seen also after single exposures, and was identified as an acute toxic effect. Hence, according to the DS, the observed neurotoxic findings in repeat-dose studies were considered to be merely repeated acute effects, and neurotoxic effects were therefore considered covered by the proposed classification for STOT SE.

Haematological changes were seen in oral studies of various exposure times in all three species. However, in the 28-day studies, both in rat and mice, as well as in the subchronic studies in rat and dogs, the changes in the haematology parameter values were below 10 % in comparison to controls. Additionally, the haematological changes observed were not associated with any histopathological or clinical changes. The DS therefore concluded that these effects were of doubtful or minimal toxicological importance, and hence they did not support classification according to the CLP criteria.

Effects on adrenal glands were investigated in eleven studies (eight acceptable key studies, and three supplementary), and in three species (mice, rats and dogs). Organ weights were increased more than 20 %, and histopathology revealed vacuolation of the zona fasciculata and glomerulosa of the adrenal gland in rats. An increase in the incidence of hypertrophy of the zona glomerulosa of the adrenal cortex was observed in female rats. Corticomedullary pigmentation of the adrenal cortex and mineralization of pigmented cells of the adrenal cortex were found in mice, and in dogs an increase in the severity of cytoplasmic hypertrophy and/or pallor of cells in the zona glomerulosa and zona fasciculata were seen.

The DS concluded that adrenal changes were found as consistent and identifiable toxic effects in experimental animals and noted that according to CLP criteria and the CLP guidance, such changes have to be considered as adverse health effects that support classification for specific target organ toxicity after repeated exposure (STOT RE) with the adrenals as target organ.

Three oral and one dermal study in rats showed effects on the adrenals, as well as two oral studies in mice (of the studies that were considered acceptable key studies).

Three studies were performed in dogs; one supplementary oral 28-day study, one oral 90-day study, and one oral 1-year study. In all these studies increased adrenal weight and histopathological changes in the adrenals were observed. The most sensitive species was dog.

The lowest concentration where such effects on adrenals occurred in the 28-day study was ≥0.54 mg/kg bw/day in males, and ≥5.4 mg/kg bw/day in females (histopathological changes). GV to

assist in classification for Category 1 and Category 2 is given in CLP Annex I, 3.9.2.9.6 and 3.9.2.9.7, respectively. These values are given for 90-day studies, but equivalent values for other study lengths shall be calculated. For 28-day oral studies the equivalent GV is ≤30 mg/kg bw/day for Category 1. Hence the findings from this study indicated classification in Cat. 1.

The lowest concentration where adrenal effects occurred in the 90-day study in dogs was also 5.4 mg/kg bw/day (moderate histopathological changes and increased adrenal weight). The GV for oral 90-day studies is ≤10 mg/kg bw/day for Category 1. Hence this result also indicated classification in Cat. 1.

In the 1-year oral study, the lowest concentration where adrenal effects occurred was 5 mg/kg bw/day (histopathological changes). The calculated GV for oral 1-year studies is ≤2.5 mg/kg bw/day for Category 1, and 2.5<C≤25 mg/kg bw/day for Category 2. Hence the results from this study indicated classification in Cat. 2.

In the CLP guidance the following is recommended: "if there are differences in effects at the GV between studies with different duration, then more weight is usually given to studies of longer duration (28 days or more)". The DS therefore concluded that Category 2 (STOT RE 2, H373) was the most appropriate classification for adrenals as the target organ.

Comments received during consultation

One comment was received during consultation. This was from a Company-Manufacturer who did not support the classification of fosthiazate as STOT RE 2, H373, based on changes in the adrenals. The Company-Manufacturer had two lines of argumentation. Firstly, they argued that it was highly plausible that the adrenal effects were secondary to stress induced by AChE inhibition where STOT SE classification was already proposed for nervous system and referred to the Guidance on the CLP criteria where it is stated that "one shall [...] not include secondary effects". Secondly, they argued that the effects on the adrenals had no functional consequence and should therefore not be considered as a "significant toxic effect", again referring to the Guidance on the CLP criteria.

The DS replied that they did not consider the argumentation that the effect on adrenals is a "secondary effect" to neurotoxicity/stress sufficiently supported by the data. If the adrenal effects were related to cholinesterase inhibition, this should be considered as a mechanism of action in the Weight of Evidence analysis. Moreover, the DS considered effects on adrenal weights and histopathological changes as significant toxic effects. The DS therefore kept their proposal for classification as STOT RE 2, H373, with adrenals as the target organ.

Assessment and comparison with the classification criteria

RAC found that no human data, i.e., data from exposure at home, in the workplace or other environment, were available for evaluation of specific target organ toxicity after repeated exposure (STOT RE) for fosthiazate.

The studies on target organ toxicity after repeated exposure showed various effects on cholinesterase activity, adrenals and haematology.

Haematological effects

Haematological effects were reported in the 28-day studies in rats and mice, as well as in the sub-chronic studies in rats and dogs. According to CLP Annex I, 3.9.2.8.1 *"small changes in [..] haematology [..] when such changes or effects are of doubtful or minimal toxicological importance"* do not justify classification. The reported changes in the haematology parameter

values were less than 10 % different from controls, and the haematological changes observed were not associated with any histopathological or clinical changes.

RAC concludes that fosthiazate **does not warrant classification as STOT RE (haematological effects)**.

Effects on the nervous system

Effects on the nervous system through cholinesterase inhibition were reported. Cholinesterase inhibition was seen in rats and dogs, after both oral and dermal exposure. In the DS proposal, STOT SE rather than STOT RE classification was suggested for this effect as effects on nervous system were observed also after a single dose. RAC is however of the opinion that the neurotoxic effects should rather be classified for STOT RE because the neurotoxic effects were more severe and significant neurotoxicity occurred at lower doses after repeated exposure than after a single dose, and as the significant neurotoxic effects after a single dose occurred at doses that were only three-fold, or less, lower than the LD_{50}-values warranting the classification for Acute Tox. 3, H301 (see also assessment under STOT SE).

In repeated dose studies, significant signs of neurotoxicity (including clinical signs) were reported from 5 mg/kg bw/day. In the 90-day study in rats, brain AChE activity was 77 % and 34 % of controls in the males and females, respectively, at a dose of 4.1/4.7 mg/kg bw/day. The effect in dogs after 90-day exposure to 5.4 mg/kg bw/day was 77 % and 68 % of controls in males and females, respectively. Further, 14-day exposure to 5 mg/kg bw/day in pregnant females inhibited RBC AChE to 0.5 % of controls and brain AChE to 10 % of controls, and caused tremors, unkempt appearance, prostration and red and yellow material on body, repetitive jaw movement, piloerection, gasping and lacrimation on GD 19-20 (last day of exposure). In the 28-day oral study in dogs, neurological examination done only after 3 weeks showed depressed tactile placement from 5.4 mg/kg bw/day. This dose level was also associated with reduced AChE activity being 67 % and 51 % of controls in brain of males and females, respectively, and 22% and 23% of control in RBC of males and females, respectively.

RAC considers that the clinical neurotoxic and neurobehavioral effects, and brain and RBC AChE inhibition of more than 20% occurring after repeated exposure to a dose within GV range for Category 1, justify classification as STOT RE 1 (nervous system); this is because significant neurotoxic effects after repeated exposure occurred at lower doses than after single doses that were also only three-fold or less lower than the LD_{50}-values warranting classification for acute toxicity.

RAC concludes that fosthiazate **warrants classification as STOT RE 1, H372 (nervous system)**.

Effects on adrenals

Adrenal weights were affected in all the three investigated species (mice, rats and dogs). Also, histopathological changes in the adrenals were identified in all species. These included hypertrophy and pallor in rats and dogs and corticomedullary pigment and mineralisation of pigmented cells in the adrenal cortex in mice. RAC agrees with the DS that histopathological changes in adrenals should be regarded as a significant toxic effect. This effect is also relevant for humans. Therefore, fosthiazate should be classified as STOT RE with the adrenals as the target organ. Clinical medicine has shown that the adrenals is a vital organ, and adrenocortical insufficiency may lead to life threatening *"adrenal crisis"*. However, effects other than organ weight and histopathology had not been investigated in any of the studies, and the severity of the effects was considered uncertain. Due to this uncertainty, RAC concludes that fosthiazate **warrants classification as STOT RE 2, H373 (adrenals)**, even though the effects in some studies were seen at doses below GV for Category 1.

Relevant exposure route

Most of the studies that were available were performed by exposure by the oral route. However, there were also effects on the adrenals after dermal exposure to fosthiazate in rats. There were no inhalation studies available for the assessment of STOT RE, but possible effects by inhalation cannot be excluded. Based on this, a specific route of exposure for shall not be specified for fosthiazate.

Overall conclusion on classification and labelling

Substances that are classified as specific target organ toxicants following repeated exposure are placed in one of two categories (CLP Annex I, 3.9.2.1).

Overall, RAC concluded that fosthiazate **warrants a classification as STOT RE 1, H372 (nervous system, adrenals)**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

Potential effects of fosthiazate on sexual function and fertility were assessed in two rat studies; a two-generation reproductive toxicity study (oral, dietary) and a range-finding generation study (oral, dietary). Results of these studies are described in the Appendix and summarised in Table 24 in the CLH report. The dose range-finding study (Anonymous 25, 1990) was GLP-compliant but did not comply to standard test guidelines and was considered supplementary. The two-generation study (Anonymous 34, 1990) was comparable to OECD TG 416 and was considered acceptable (See "Supplementary information" in the Appendix).

In conclusion, different adverse effects on sexual function and fertility were observed in the available dose range-finding study and two-generation study: reduction of number of implantation sites, disturbances in oestrus cycle, prolonged gestation length. Effects on sexual function and fertility were observed in F0 females, mainly in the high dose group. DS did not conclude on possible effects also in F1 females in the high dose group in the main two-generation study due to poor survival, leading to assessment only up to 30 ppm for the F1 generation. In addition, only parental animals (F0) were assessed in the dose range finding study.

The DS concluded that fosthiazate should be classified as Repr. 2, H361f. However, after considering the comments received during the consultation, and partly based on additional analyses performed by the DS in response to the comments received, the DS suggested that Repr. 1B, H360F, should be discussed by RAC.

Adverse effects on development

Potential effects of fosthiazate on development were assessed in seven studies in total. Two of these studies were also used to assess effects on sexual function and fertility (one dose range-finding study and one two-generational study in rats). In addition, there were two teratology studies (one in rats and one in rabbits), and their preliminary/dose-range finding studies (one in rats and two in rabbits). Results of these studies are described in the Appendix and summarised in Table 26 in the CLH report.

In conclusion, several adverse developmental effects were observed in the studies presented. In rats, these included decreased litter sizes, decreased live-birth index, decreased viability index,

decreased lactation index, reduced body weight at birth and weaning, delayed onset of eye opening and tooth eruption. The incidence of small pups was also increased. These effects could not be attributed to parental toxicity. In rabbits, increased number of small pups and slightly reduced foetal body weight were observed. These effects could also not be attributed to maternal toxicity.

In the CLH report, the DS stated that due to the deviations from current guidelines these studies on developmental toxicity were considered supplementary. DS concluded that as there were uncertainties related to the data base, classification in Category 1B was not appropriate. The DS concluded that the evidence sufficed for classification in Category 2, and classification as Repr. 2, H361d was proposed.

However, in response to some of the comments received during the consultation, the DS agreed that the deviations from test guidelines and uncertainties in the data base may not be so relevant, and that classification in Category 1B should be considered by RAC.

Adverse effects on or via lactation

Potential effects of fosthiazate on or via lactation were assessed in two studies. These were the same two studies also used to assess effects on sexual function and fertility, and development (one dose range-finding study and one two-generation study). Both studies were performed in rats. Treatment and dosing, as well as parental toxicity and effects on development, are described above.

In the dose range-finding study the lactation index was significantly reduced at a dose level of 100 ppm (lactation index on PND 7/14/21 (%) in the 0, 10, 30, 100 and 300 ppm dose group: 98/87/97, 91/89/87, 95/83/83, 55/45/45 and 0/0/0). In the study, parental gross necroscopy was performed. Offspring culled at day 4 were killed and grossly examined for abnormalities. Litters surviving to day 21 were also killed and necropsied. Relevant findings for lactation are shown in the table below.

***Table**: Summary of effects on or via lactation in the dose range-finding study in rats – adapted from table 28 in the CLH-report.*

	Dose in ppm (mg/kg bw/day)				
Parental gross necroscopy findings	**0**	**10** (0.68)	**30** (2.03)	**100** (7.09)	**300** (28.19)
Number of animals	10	10	10	10	10
Number of pregnant	9	10	6	10	7
Mammary tissue slightly pale, milk present	-	-	1	-	-
Mammary tissue appears light in colour, no milk present	-	-	-	-	2
Mammary tissue appears inactive	-	-	-	9	3
Observation at necroscopy of offspring (F1) dying or sent to necroscopy before weaning*					
Number of offspring examined (number of litters)	13 (7)	19 (5)	25 (4)	91 (9)	21 (5)
No milk in stomach - % incidence (number of litters)	69.2 (5)	84.2 (3)	100 (4)	94.5 (9)	85.7 (5)

**Excludes missing or severely cannibalized offspring*

In the multigeneration study, the lactation index was significantly reduced at the top dose of 100 ppm from day 7 (lactation index at PND 7/14/25 (%) in the 0, 3, 10, 30 and 100 ppm dose groups: 99/99/99, 99/97/99, 100/99/99, 99/95/94 and 76/52/45). The presence of milk in the stomach was investigated in offspring dying or culled on day 4 post-partum. The results are shown in the table below.

***Table**: Summary of effects on or via lactation in the two-generation study in rats – adapted from table 28 in the CLH-report.*

	Dose in ppm (mg/kg bw/day)				
Parameter	**0**	**3** (0.26)	**10** (0.86)	**30** (2.62)	**100** (9.34)
No milk in stomach, % in offspring dying before terminal kill (F1)	90	55.6	100	83.9	95.2
No milk in stomach, % in offspring culled on day 4 post-partum (F1)	2.7	2.5	0	5.6	25

From the toxicokinetic studies on fosthiazate no information about transfer of fosthiazate in milk was available. Studies on metabolism on livestock revealed only very limited transfer of fosthiazate in milk in lactating goats.

In conclusion, mammary tissue and presence of milk in the stomach of offspring were affected in the presented studies. The DS also considered that significant postnatal effects on offspring viability and development found in these studies were due to effects on or via lactation. Although no cross-fostering study was conducted and the transfer to the rat milk was not demonstrated, effects on mammary tissue were detected at least in the dose range-finding generational study. Based on this, classification for effects on or via lactation was proposed, i.e. Lact. (H362): "May cause harm to breast-fed children".

Comments received during consultation

Sexual function and fertility

Four comments were received during the public consultation. One comment is from a Company-Manufacturer who did not support the proposed classification, stating that there was no clear evidence that the observed effects were treatment-related. Moreover, it was argued that the dose level at which the effect on gestational length was observed probably had induced marked systemic toxicity, and therefore the biological relevance of the observed effect was questioned. The DS did not regard this argumentation as valid. Further response to the arguments in the form of both existing and additional statistical data was given in the RCOM.

One comment was received from a National Authority (NA). The NA questioned if the reduction in implantation sites would be an effect on sexual function and fertility or on development, and asked for information on corpora lutes counts to help discriminate. The DS replied that such information was not available, and that in general, effects prior to implantation were regarded as effects on sexual function and fertility.

The last two comments were from MSCAs, one supporting the proposed classification, the other suggesting that classification as Repr. 1B, H360F should be considered. The argument for Cat. 1B was primarily based on the approximately 50% reduction of mean implantation sites (7.6 ± 4.3 vs. 14.3 ± 3.8 in the control group) and litter size (6.2 ± 2.5 vs. 12.8 ± 3.6) in the 300 ppm dose group in the dose range finding study, in absence of adverse maternal toxicity (Anonymous

25, 1990). This MSCA also pointed out that in the two-generation reproduction toxicity study (Anonymous 34, 1990), the mean number of implantation sites and litter size were reduced at 100 ppm, without adverse maternal toxicity.

Referring back to the first comment from the Company-Manufacturer, the DS re-evaluated the individual data with regard to the reduction in litter size in the dose range-finding study. This resulted in new values for litter sizes. The complete set of new values, and a summary of the statistics can be found in the RCOM, and in the section below (additional key elements). In brief, the DS showed a significantly decreased number of the litter size on PND 1 for the top dose when compared to the control group. However, no statistically significant trend was identified for the reduction in implantation sites and litter size in the main two-generation study (Anonymous 34, 1990). The DS maintained their original classification proposal, but stated that Cat. 1B should be discussed by RAC, in particular with respect to the reduction in implantation sites.

Development

Three comments related to developmental toxicity were received during consultation. One comment was from a Company-Manufacturer who did not support the proposed classification. The Company-Manufacturer argued that, in the main teratogenicity study in rabbit, the reduced foetal bodyweight referred to by the DS was not statistically significant, there was no dose-response, and the reported values for this endpoint were all within the HCD. The Company-Manufacturer further argued that the increased number of small foetuses (in the same study) was not statistically significant, and that the increased occurrence of large litters in the high dose group was the cause of the increase in the parameter 'incidence of small foetus', i.e., the effect was driven by two litters of 14 pups with 8 small foetuses in each. In the multigenerational study in rats, the Company-Manufacturer stated that marked systemic toxicity, in form of reduced cholinesterase activity, must be presumed. This was based on significantly affected cholinesterase levels in several other studies with fosthiazate, however, this parameter was not measured in the study in question. The Company-Manufacturer implied that the observed developmental toxicity effects were presumably secondary to this systemic toxicity.

The DS replied that the observed decrease in birth weight on the top dose in this study was close to the minimum range of the HCD but agreed that there was no dose-response. Further, the DS agreed that a relationship between litter size and foetal body weights could be expected but pointed out that several litters with the same size, or larger, had lower numbers of small foetuses. In addition, the endpoint was statistically re-analysed taking possible litter effects into account. This analysis showed a statistically significant increase in the number of small foetuses in the high dose group compared to the control group. Overall, the DS still considered the increase in the number of small foetuses to be a treatment-related effect. Further the DS did not agree that the described battery of effects in the rat study could be attributed to maternal toxicity in terms of cholinesterase inhibition. Although the DS agreed that cholinesterase inhibition was plausible, it did not result in neurotoxic symptoms and/or in changes in behaviour in the developmental toxicity studies. Moreover, the DS stated that, if cholinesterase inhibition affected biological processes involved in reproduction, this should rather be regarded as a mechanism of action.

The other two comments were received from MSCAs. The first MSCA considered that the effects on development observed in the dose range-finding study, and the multigeneration study, in rats warranted classification as Repr. 1B, H360D. This was primarily based on evidence of effects on prenatal survival such as reduction in post-implantation survival and live birth index, and reduced birth weight, in the absence of significant maternal toxicity. The MSCA also mentioned the increased number of small foetuses and the lower foetal birth weight in the main teratogenicity study in rabbit. The MSCA did not support the argumentation made by the DS that deviations from the guidelines in the presented studies made Category 1B not applicable. The MSCA stated

that, on the contrary, observation of effects already after a shorter treatment period indicated that even more effects could be elicited in studies using the currently required exposure duration. The DS replied that they could agree with this reasoning, and stated that this should be taken into account by RAC when discussing the appropriateness of Category 1B or Category 2.

The other MSCA also highlighted that the pup viability index was severely reduced in the two rat studies (dose range-finding and two-generation), and that there was an increased incidence in small pups in two rabbit studies. This MSCA therefore stated that a discussion regarding a possible classification in Category 1B was warranted, and again the DS agreed with the MSCA that this should be discussed by RAC.

Lactation

Two comments related to effects on lactation were received during the consultation. One comment from a Company-Manufacturer that was of the view that the classification criteria were not met and did not support the proposed classification for effects on or via lactation. The Company-Manufacturer argued that the inactive mammary tissue reported in the rat dose range-finding study was secondary to systemic toxicity due to cholinesterase inhibition, and the effects in pups were a consequence of marked maternal toxicity.

The DS replied that no neurotoxic effects were reported that interfered with nursing behaviour. Further the DS noted that the classification for effects on or via lactation according to the CLP guidance stated that "A substance which does not cause overt toxicity in the mother but which interferes with milk production or quality will normally be classified for effects on or via lactation…". Effects on mammary tissue were observed and absence of milk in the stomach was reported in offspring. Therefore, the DS considered a classification for effects on or via lactation (Lact., H362) still justified.

The second comment was from a MSCA supporting the proposed classification for effects on or via lactation with H362.

Additional key elements

In their response to the comments received from the Company-Manufacturer during the consultation, the DS supplied new statistical analyses for the implantation sites in the dose range-finding study. The DS also re-evaluated the data on litter size from this study, and provided some new values on this parameter, and the results from the statistical analyses performed on these new values. This information is taken into consideration by RAC when formulating the opinion on adverse effects on sexual function and fertility, and therefore it is also included here.

- The parameter "implantation sites" in the range-finder multi-generation study (Anonymous 25, 1990) was analysed with analysis of variance (ANOVA) with the statistical software R (R Core Team, 2022) by the DS. This showed a statistically significant trend (α-level of 0.05, p-value 0.000383). A post-hoc one-sided Dunnett test (with the statistical software R) for a decrease in the number of implantation sites (α-level of 0.05, p-value 0.0014) showed a statistically significant decrease of the number of implantations for the top dose when compared to the control group.
- With regard to the reduction in litter size (dose range-finder study), the DS re-evaluated the individual data and noted that the litter size on PND 1 according to the original study represented the number of born/total pups and not the number of live pups at PND 1 and that two total litter losses were not included at the highest dose. The values for the different groups (0, 10, 30, 100, 300 ppm) therefore were:

- Number of live-born litters (number of pregnant animals): 9(9), 10(10), 6(6), 10(10), 5(7),
- Total on PND 1 (mean ± SD): 12.8 ± 3.6, 14.3 ± 3.5, 15.3 ± 2.3, 12.2 ± 2.6, 4.4 ± 3.6,
- Litter size PND 1 (mean ± SD): 12.2 ± 3.9, 14.2 ± 3.4, 15.3 ± 2.3, 11.3 ± 3.5, 3.7 ± 3.8.

The litter size on PND 1 was analysed with the Kruskal-Wallis-test rank sum test for a trend with the statistical software R (R Core Team, 2022) by the DS. This showed a statistically significant trend (α = 0.05, p = 0.0006626). A post-hoc one-sided Dunnett test (with the statistical software R) for a decrease of the litter size on PND 1 (α = 0.05, p < 1E-04) showed a significantly decreased number of the litter size on PND 1 at the top dose when compared to the control group.

The DS also statistically re-analysed the endpoint 'number of small foetuses' in the main teratogenicity study in rabbits. This information is included in the discussion by RAC in the formulation of the opinion on developmental toxicity, and therefore only a description of the analysis performed, as given by the DS, is included here.

The endpoint 'number of small foetuses' was statistically re-analysed with the statistical software R (R Core Team, 2022). Possible litter effects were taken into account by using generalised estimating equations (GEE) with the foetuses weight as the statistical unit. Significance of treatment effects was analysed with a post-hoc Dunnett test (one-sided) with respect to the results of GEE analysis. This showed a statistically significant increase of the number of 'small foetuses' (alpha level of 0.05, p-value 0.038) for the top dose (300 ppm or 2 mg/kg bw/d) when compared to the control group.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

RAC noted that no human data on adverse effects on sexual function and fertility were available, hence classification with Cat. 1A according to CLP regulation is not justified.

Two animal studies, both in rats, relevant for the assessment of sexual function and fertility were available.

One of the studies is a dose range-finding study with oral exposure to 0, 10, 30, 100 and 300 ppm fosthiazate via diet. The study is supplementary, but GLP-compliant, and RAC considers it to be reliable. The number of animals is however only 10/sex/group, which is lower compared to the two-generation study. Several effects of fosthiazate exposure relevant for sexual function were observed in this study. These were reduced number of pregnant animals, reduced number of implantation sites and reduced litter size. The decrease in the number of implantation sites, and the reduction in litter size showed statistically significant difference from control[1] at the top dose (300 ppm) on PND 1. However, at this dose a significant reduction in the mean body weight of the dams was also observed. Maternal body weight was 23 % lower than in controls at GD 20, and 21 % at PND 1. Reduced body weight could be a sign of general toxicity. This reduction can only partly be explained by reduction in litter size, as it is also present at PND 1.

[1] *Additional statistics performed after PC. Shown in the additional key elements above.*

The other study is the (main) two-generational study with dietary exposure to 0, 3, 10, 30 and 100 ppm fosthiazate. The study is GLP-compliant and reliable. Effects relevant for sexual function and fertility were observed after fosthiazate exposure also in this study. These were reduced fertility index, reduced number of animals with a normal oestrus cycle, increased number of acyclic/pseudopregnant females, and again a reduced number of implantation sites, as well as reduction in the mean litter size on PND 1 in the F0 generation. However, in this study, only the reduction in the number of animals with a normal oestrus cycle showed statistical significance, and this reduction was significant only at 10 ppm, and not at the higher doses. Due to high postnatal mortality in the F1 generation at 100 ppm, the F1 generation was only assessed up to 30 ppm for effects on sexual function and fertility. The only statistically significant effect reported in the F1 generation was an increase in the number of females with irregular oestrus cycle. This effect occurred at 10 ppm and was not seen at 30 ppm. None of the effects observed in this study showed a clear dose-response.

Thus, RAC agrees with the DS that a classification for effects on sexual function and fertility is justified, since effects on relevant endpoints were observed in F0 females. According to CLP Annex I, 3.7.2.1.1. the classification of a substance in Category 1B is based on data providing clear evidence of an adverse effect on sexual function and fertility. Given the absence of a clear dose-response and the lack of statistical significance for most of the effects shown to be affected by fosthiazate at doses without maternal body weight loss, RAC considers this not sufficiently convincing to support classification as Category 1B. Hence, classification in Category 2 seems more appropriate, requiring 'some evidence' of an adverse effect, and 'where the evidence is not sufficiently convincing to place the substance in Category 1'.

Overall, RAC concludes that fosthiazate **warrants classification as Repr. 2, H361f**.

Adverse effects on development

RAC noted that no human data on adverse effects of fosthiazate on development are available, so classification in Category 1A is not justified.

Seven animal studies, five in rats and two in rabbits, relevant to assess possible effects of fosthiazate on developmental toxicity, were presented. Five of the studies reported developmental toxicity. This was mainly related to effects on pup viability/litter size and birth weight/small foetuses. In addition, AChE inhibition in the age-sensitivity study was reported in juveniles.

In the CLH report, the DS proposed classification in Category 2. The reasoning by DS for not proposing Category 1B is, at least partly, was that none of the studies were OECD TG compliant. One of the major shortcomings was that the treatment periods were shorter in comparison to the requirements of the current OECD TG. Furthermore, selected additional relevant endpoints (anogenital distance in foetuses and thyroid hormones in dams) are included in OECD TG 443 (2018) that were not examined in the present studies. This argumentation was however questioned by two MSCAs during the consultation. These MSCAs stated that the observation of effects already after a shorter treatment period indicated that even more effects could be elicited in studies using the currently required exposure duration. RAC agrees with these MSCAs. Moreover, if the additional endpoints had been examined, a negative outcome for these endpoints would not have been sufficient to undermine the severity of the developmental effects of fosthiazate that were reported in the studies that were presented by the DS. In addition, the studies are GLP-compliant, and RAC considers them to be reliable.

In rats a preliminary/range-finding teratogenicity study, and a main teratogenicity study was included by the DS with oral (gavage) exposure to fosthiazate at doses of 0, 1, 2.5, 5 and 10 mg/kg bw/day. No effects on development were reported in any of these studies. However, maternal effects were reported as a statistically significantly decreased body weight gain on GD

16 in the high dose group in both studies. Two of the rat studies are the same as those used to assess effects on sexual function and fertility. In the dose range-finding study in rats several effects relevant for developmental toxicity were seen, i.e., at PND 4 from 30 ppm pup viability were reduced, and at the high dose (300 ppm) all litters were dead. Similar effects were also observed in the two-generation study. These are significantly reduced viability index on PND 4 at 30 and 100 ppm. In addition, statistically significant effects on eye opening and tooth eruption and reduced body weight at weaning (PND 25) were reported.

In the preliminary rabbit study with oral (gavage) exposure to fosthiazate at doses of 0, 1, 2, 2.5 and 5 mg/kg bw/day, incidence of small foetuses and incomplete ossification of the hyoid body were observed. No statistical significance or dose-response were stated, but these are considered as severe effects. Further, the main teratogenicity study in rabbits with oral (gavage) exposure to fosthiazate at doses of 0, 0.5, 1, 1.5 and 2 mg/kg bw/day also showed decreased foetal body weight and increased incidence of small foetuses.

During the consultation, one Company-Manufacturer commented on the observed increase in incidence of small pups, emphasising the link between large litters and small foetuses. When studying the data on individual animals in this study, shown in the table below, RAC also notes that it seems to be consistent that a high number of small foetuses (≥ 4) is found exclusively in connection to large litters (≥ 12 viable pups). However, there were also some large litters that did not result in high numbers of small foetuses, indicating that a clear correlation between litter size and small foetuses was not found. In their reply to the Company-Manufacturer, the DS had performed additional statistics taking possible litter effects into account. The statistic data is included in the additional key elements section above. These calculations showed a statistically significant increase in the number of small foetuses at the top dose compared to control, and the DS held that this effect was treatment related. RAC agrees with the DS and considers the reported increase in small foetuses at the top dose in the rabbit study to be an effect of fosthiazate exposure and not only an effect due to large litter size.

***Table**: Individual data on dams with small pups in the main teratogenicity study in rabbits (Anonymous 6, 1989b).*

Dose level (mg/kg bw/day), Animal number	Number of viable young	Number of small foetus (< 32 g)
0 mg/kg bw/day		
28TD008	10	2
28TD043	9	2
28TD053	8	1
28TD055	11	2
28TD115	12	2
28TD130	5	2
0.5 mg/kg bw/day		
28TD010	15	4
28TD042	10	2
28TD048	12	2
28TD056	13	5
28TD078	10	1
28TD107	10	1
28TD132	13	2

1 mg/kg bw/day		
22TD955	10	2
28TD052	10	2
28TD081	11	1
28TD085	16	8
28TD135	15	7
28TD138	9	2
1.5 mg/kg bw/day		
28TD009	7	3
28TD051	14	6
28TD111	15	5
28TD122	10	2
28TD129	11	2
2 mg/kg bw/day		
28TD045	7	1
28TD049	12	3
28TD101	11	3
28TD118	12	3
28TD123	12	6
28TD133	14	8
28TD153	14	8

In addition to the data presented by the DS under developmental effects, RAC found that the data from the age-sensitivity study in rats, where pups were exposed on PND 11 and PND 21, presented under STOT SE, are relevant for developmental effects. This is in accordance with the RAC Guidance Note on "*developmental neurotoxicity and neurotoxicity under the current CLP hazard classes*" (RAC, 2022). These data show that both brain and RBC AChE are inhibited after a single treatment to 5 mg/kg bw. This includes both animals exposed on PND 11 and those exposed on PND 21. Moreover, animals treated to repeated doses, i.e. daily exposure from PND 11 to PND 21 to 5 mg/kg bw/day, also showed decreased AChE activity. The effects are shown in the table below.

Table: *neurotoxicity in pups from the age-sensitivity study in rats (Anonymous 7, 2006)*

Treatment	Number of animals	Sex	Effect (% of control)	
			Brain AChE	RBC AChE
Acute exposure, 5 mg/kg bw				
PND 11	20	M	85 %	82 %
PND 21	10	M	85 %	63 %
Acute exposure, 5 mg/kg bw				
PND 11	20	F	86 %	56 %
PND 21	10	F	84 %	68 %
Repeat dose, 5 mg/kg bw/day				
PND 11- 21	10	M	50 %	13 %
Repeat dose, 5 mg/kg bw/day				
PND 11- 21	10	F	35 %	7 %

In conclusion, clear adverse effects in pups with poor or no survival, in addition to statistically significant effects on eye opening and tooth eruption, and reduced body weight at weaning, were reported in two reliable rat studies. Supporting evidence in rabbits include an increased number of small foetuses at the highest dose, as shown and discussed above. Additionally, neurotoxic effects were shown in rat pups treated by single or repeated exposure during development. These effects are seen in the absence of marked maternal toxicity and are considered not to be secondary non-specific consequences of other toxicity. According to CLP Annex I, 3.7.2.1.1. the classification of a substance in Category 1B for developmental effects requires data "providing clear evidence of an adverse effect on [..] development in absence of other toxic effects or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects [..]".

Thus, RAC concludes that fosthiazate **warrants classification as Repr. 1B, H360D**.

Adverse effects on or via lactation

According to CLP Annex I: Table 3.7.1 (b) *"substances which are absorbed by women and have been shown to interfere with lactation [..] shall be classified and labelled to indicate this property hazardous to breastfed babies."* This classification can be based on human evidence and/or animal data. No human data on effects on or via lactation are available for fosthiazate. Several effects relevant for lactation have however been investigated in two of the presented rat studies, i.e., the dose range-finding study (Anonymous 25, 1990), and the two-generation study (Anonymous 34, 1990). In the dose range-finding study, gross necroscopy of the mothers showed inactive mammary tissue in 9 of 10 animals in the 100 ppm dose group, and 3 out of 7 animals in the 300 ppm dose group. There were also 2 animals in the latter group with mammary tissue that appeared light on colour, with no milk present. In this study, there were no clear evidence showing that maternal systemic toxicity could have an impact on the development of the inactive mammary tissue. Necroscopy of offspring from treated animals showed an incidence of 'no milk in stomach' ranging from 84 – 100 %, compared to 69 % in the control. It should also be noted that in the high dose group, all of the approximately 30 offspring that were alive on day 1 died before day 4, and in the 100 ppm dose group almost 90 % of the offspring died during this time. Since the pups died early during lactation, around PND 1-4 the effect on lactation is considered to be due to impairment of milk production rather than self-feeding of the pups.

Together with a decreased lactation index at this dose, this indicates that fosthiazate interfere with lactation. This is further supported by findings from the two-generation study where the viability and lactation index are statistically significantly decreased, and the percentage of offspring without milk in the stomach is increased at 100 ppm.

RAC concludes that fosthiazate **warrants classification as effects on or via lactation, H362.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Fosthiazate is an organophosphate with a nematicidal and insecticidal activity. When applied to soil, it acts systematically in plants where uptake is through the roots. Fosthiazate is currently listed in Annex VI of the Regulation (EC) No 1272/2008 as Aquatic Acute 1 and Aquatic Chronic 1. The DS proposes to retain the current classification and to add an acute M-factor of 1 and a chronic M-factor of 1. Information for environmental degradation, bioaccumulation and aquatic toxicity was taken from the CLH Dossier and the Renewal Assessment Report (RAR).

Environmental Degradation

Ready Biodegradability

Based on a ready biodegradability test according to OECD TG 301 F (Manometric Respirometry), fosthiazate is considered as not readily biodegradable (Anonymous, 2012). The biodegradation was 0 % after 28 days.

Hydrolysis

In a first hydrolysis study conducted according to US EPA 161-1 [^{14}C-thiazolidine]-fosthiazate and [^{14}C-sec-butyl]-fosthiazate were added to sterile buffer solutions at pH 5, 7 and 9 at 25°C (Anonymous, 1989). DT_{50} values for fosthiazate were 177, 104 and 3.2 days at pH 5, 7 and 9 respectively. At pH 5 and 7 DETO was detected as major transformation product (10 % respectively 14 % at day 30). At pH 9, DBTO (45 % at day 14), TZO (40 % at day 14) and DTTO (44 % at day 14) were detected as transformation products.

In a second GLP compliant hydrolysis study according to OECD TG 111, DT_{50} values of fosthiazate at 20°C were 312, 255 and 9 days at pH 4, 7 and 9, respectively (Anonymous, 2014). The study indicated that fosthiazate was degraded to DETO, TZO, DTTO and DBTO as well as a volatile transformation product, sec-butyl mercaptan.

Photolysis

One study investigating the photochemical degradation of fosthiazate was available (Anonymous, 1993). Photolysis was examined in buffer solutions at pH 5. Samples were exposed to simulated sunlight (Xenon lamp with filters) in a 12 hours dark/12 hours light cycle over a period of 30 days at 25°C. After test end, fosthiazate accounted for 87 % applied radioactivity (AR) in the dark control and 88 % in light exposed samples. Direct photochemical degradation in aquatic systems was therefore considered as negligible. Fosthiazate degraded only by hydrolysis to DETO, which reached a maximal amount of 9 % AR in the dark control and 8 % AR in light exposed samples after 30 days.

Aerobic degradation

The mineralisation of [^{14}C-R]-fosthiazate and [^{14}C-B]-fosthiazate was investigated in an OECD TG 309 simulation study using surface water from a pond (Schoonrewoerde Wiel, Netherland) (Anonymous, 2014). 10 µg/L (low concentration) and 100 µg/L (high concentration) of [^{14}C-R]-fosthiazate and [^{14}C-B]-fosthiazate was applied and the study was performed for 56 days. The mass balance showed deficiencies at all used labels and concentrations of the test substance (according to OECD TG 309 it should range between 90 – 110 %), but the DS noted that these ranges should be interpreted as targets and should not be used as criteria for acceptance of the test. Mainly primary degradation of fosthiazate was observed to the transformation products TZO (reached a maximum of 36.1 % AR after 31 days at low concentration), DBTO-Li (reached a maximum of 80.3 % AR after 56 days at high concentration) and BSA (reached a maximum of 20.9 % AR after 56 days at high concentration). Further transformation products MB-1, MB-3, MB-4 and Mb-5 were unstable and could not be identified as part of this study. The derived DT_{50} values for [^{14}C-R]-fosthiazate were 15.2 days (low concentration) and 15.5 days (high concentration). For [^{14}C-B]-fosthiazate the derived DT_{50} values were 14.7 days (low dose) and 15.9 days (high dose). Mineralisation to $^{14}CO_2$ was low with 3 to 21 % AR for both, low and high concentration.

Three aerobic water/sediment degradation studies are available. In the first study according to US EPA 162-4, the degradation of [^{14}C-B]-fosthiazate was investigated over a period of 140 days using a US river water/sediment system (Anonymous, 1996). The DT_{50} values were 46 days (water), 214 (sediment) and 70.2 days (total system). Only minor metabolites were detected in the grand river system in concentrations < 10 % AR.

A second study was available conducted according to US EPA 162-4, SETAC Europe Guideline where the degradation of [^{14}C]-fosthiazate was investigated in a pond and river water/sediment system (Anonymous, 1998). The study was performed for 100 days. In the aerobic experiment, the DT_{50} values in the pond system were 30.4 days (water), 62 days (sediment) and 43.7 days (total system). In the river system, the respective DT_{50} values were 19.8 days (water), 172 days (sediment) and 79.8 days (total system). One major transformation product was identified as TZO in the river system, which reached a maximal amount of 35.7 % in the whole system after 30 days. At the end of the study mineralisation ranged from 28 – 36 % for both systems. A mass balance deficiency in the pond water/sediment system was noted from the DS. The mass balance in the pond water/sediment system was < 90 % on day 0.25 and then on day 100. However, the study was accepted as reliable from the DS as the interval between sampling day 0 and 0.25 was very short and the other time with insufficient recovery was only at the end of the study.

The third study was carried out according to OECD TG 308 where the degradation of ^{14}C-fosthiazate was examined in two water/sediment systems (Calwich Abbey Lake and Swiss Lake system) under aerobic conditions (Anonymous, 2013). The test was performed for 100 days. As the mass balance in the Calwich Abbey Lake system was < 90 % AR on more than one sampling day and losses due to volatile radioactivity was not demonstrated, the study was considered as not acceptable for the Calwich Abbey Lake system from the DS. In the Swiss Lake system, the mass balance was only < 90 % AR on day 100 and was therefore accepted. The corresponding DT_{50} values for ^{14}C-fosthiazate were 37.6 days (water), 56.4 days (sediment) and 72.1 days (total system) in the Swiss lake system. Although the Calwich Abbey Lake system was not accepted from the DS due to mass balance deficiencies, it was shown that fosthiazate degraded to the major transformation product DTTO, with a maximal amount of 16.2 % AR in the overall system at day 7. In the Swiss Lake system, the mineralisation was 18.8 % after 100 days.

A study was available investigating the distribution and degradation of fosthiazate stereoisomers in natural water systems (Calwich Abbey Lake and Swiss Lake) as well as in three buffer solutions (pH 4, 7 and 9) (Anonymous, 2014). The study was performed under constant conditions in the

laboratory in the dark where a mixture of ring-labelled and non-labelled fosthiazate was applied at a nominal concentration of 10 mg/L. Four isomers were detected and quantified in the test systems, which were treated with thiazolidine-ring labelled fosthiazate. No significant decrease of radioactivity was observed in the buffer systems at pH 4 and pH 7 as well as in the Swiss lake system. DT_{50} values in the range of 8.4 to 9.2 days were derived for the four isomers in the buffer system at pH 9, whereas DT_{50} values were in the range of 25.7 and 31.7 days for the four isomers in the Calwich Lake system. There was no significant difference between the individual DT_{50} values of each isomer in the buffer system at pH 9 and in the Calwich lake system. Further, it was shown that the detected isomers were equally distributed in the test systems at the individual sampling days.

Overall, the DS considered the available data adequate for classification purposes and concluded that fosthiazate is considered not rapidly degradable in the aquatic environment, according to the CLP criteria.

Bioaccumulation

One experimental bioaccumulation study was available according to US EPA Guideline 165-4 and under GLP (Anonymous, 1989). The study with the bluegill sunfish, *Lepomis macrochirus,* had a 28 days exposure period and 14 days depuration period in a flow-through system. A steady state total ^{14}C-BCF for the whole fish was derived with 3.2 L/kg. Steady state was reached after 1 day of exposure. While the study was conducted with only one test concentration instead of two test concentrations, no growth rate and fish lipid content was given, no information about dissolved or total carbon was given and the overall percent mortality during the whole study was 12 % in the treatment group and 9 % in the control group, the study was considered as valid and reliable by the DS.

There are two studies available dealing with the experimental determination of the log K_{OW} of fosthiazate using the shake flask method. Lorence (1993) reported a log K_{OW} of 1.68 at 25°C and Kanza (1998) a log K_{OW} of 1.75 at 25°C.

Overall, the DS concluded that fosthiazate has a low potential for bioaccumulation in the aquatic environment.

Aquatic Toxicity

The DS indicated that based on available data, degradation products of fosthiazate are less hazardous to the aquatic environment compared to the parent compound and are, therefore, not relevant for classification purposes.

Acute aquatic toxicity

Available test results from acute toxicity studies for all three trophic levels performed on fosthiazate are listed in the table below. Key endpoints in the table are highlighted in bold.

***Table**: Summary of reported studies on acute aquatic toxicity of fosthiazate presented by the DS*

Method	Species	Endpoint	Toxicity value	Reference
Short-term toxicity				
US EPA 72-1	*Oncorhynchus mykiss*	96 h LC50	114 mg a.s./L (nom)	Anonymous (1991a) 91/ISK177/0015
US EPA 72-1	*Lepomis macrochirus*	96 h LC50	171 mg a.s./L (nom)	Anonymous (1991b) 91/0016DP 29462
OECD TG 202	*Daphnia magna*	48 h EC50	**0.28 mg a.s./L (nom)**	Anonymous (1991) 91/ISK179/0017
US EPA 850.1035, 72-3	*Americamysis bahia*	96 h EC50	0.429 mg a.s./L (mm)	Anonymous (2001) 012914
US EPA 122-2	*Raphidocelis subcapitata*	120 h ErC50	> 4.51 mg a.s./L (mm)	Anonymous (1995) 16-01-1

Note: mm – mean measured concentration; n – nominal concentration;

Two acute toxicity studies for fish were available. In the study with *Oncorhynchus mykiss* a 96 h LC_{50} value of 114 mg a.s./L based on nominal concentrations was reported (Anonymous, 1991a). The study with *Lepomis macrochirus* reported a 96 h LC_{50} value of 171 mg a.s./L based on nominal concentrations (Anonymous, 1991b).

Two acute toxicity studies with two different aquatic invertebrates were available. *Daphnia magna* was the most sensitive species tested with a 48 h EC_{50} of 0.28 mg a.s./L, based on nominal concentrations in a study conducted according to OECD TG 202 and under GLP (Anonymous, 1991). The DS noted that measured concentrations showed variation at low exposure concentrations but indicated that in the range of the EC_{50} test concentrations were sufficiently maintained (80 – 120 % of nominal). The study with *Americamysis bahia* which was performed according to US EPA 72-3 and under GLP showed a 96 h EC_{50} of 0.429 mg a.s./L based on mean measured concentrations (Anonymous, 2001).

There was one study available for algae where only one concentration of 4.51 mg a.s./L was tested against the test organism *Raphidocelis subcapitata* (Anonymous, 1995). The study revealed a mean measured 120 h E_rC_{50} of > 4.51 mg a.s./L as no effects were observed at test end. The DS noted that not all validity criteria were met according to OECD TG 201 as cell counts for 24 and 48 hours were not reported and the mean coefficient of variation for section-by-section specific growth rate was 50 %. In addition, the solvent concentration was 0.5 ml/L but should not exceed 0.1 ml/L according to OECD TG 201. However, the DS noted that the study as limit test is regarded as useful to provide information on the toxicity of fosthiazate to algae for classification purposes.

From the available aquatic toxicity data, the invertebrates are the most acutely sensitive trophic level, therefore the acute aquatic classification proposed by the DS was based on a 48 h EC_{50} of 0.28 mg a.s./L with *Daphnia magna*. The DS proposed Aquatic Acute 1 (H400) with an M-factor

of 1. The classification is supported with a second available study with aquatic invertebrates, revealing a 96 h EC_{50} of 0.429 mg a.s./L with *Americamysis bahia*.

Chronic aquatic Toxicity

Available test results from chronic toxicity studies for all three trophic levels performed on fosthiazate are listed in the table below. Key endpoints in the table are highlighted in bold.

***Table**: Summary of reported studies on chronic aquatic toxicity of fosthiazate presented by the DS*

Method	Species	Endpoint	Toxicity value	Reference
Long-term toxicity				
OECD TG 204	*Onchorhynchus mykiss*	28 d NOEC (mortality) 28 d NOEC (body weight)	2.4 mg a.s./L (nom) 7.8 mg a.s./L (nom)	Anonymous (1999) 729156
OECD TG 210	*Pimephales promelas*	EC_{10} (body weight) EC_{10} (length)	1.91 mg a.s./L (nom) 4.0 mg a.s./L (nom)	Anonymous (2013) 96207-662-12-E-6207
OECD TG 211	*Daphnia magna*	21 d NOEC (reproduction) 21 d NOEC (mortality)	**0.06 mg a.s./L (nom)** **0.06 mg a.s./L (nom)**	Anonymous (1994) 123468
US EPA 122-2	*Raphidocelis subcapitata*	120 h NOEC	4.51 mg a.s./L (mm)	Anonymous (1995) 16-01-1
OECD TG 219 Draft (1998)	*Chironomus riparius*	24 d NOEC (development) 24 d NOEC (emergence)	0.1 mg a.s./L (nom) 0.1 mg a.s./L (nom)	Anonymous (1999) 729191

Note: mm – mean measured concentration; n – nominal concentration;

There are two chronic toxicity studies for fish available. The first study conducted according to OECD TG 204 reported a nominal 28 d NOEC (mortality) of 2.4 mg a.s./L and a nominal 28 d NOEC (body weight) of 7.8 mg a.s./L for the test organism *Oncorhynchus mykiss* (Anonymous, 1999). The DS noted that as the OECD TG 204 is not considered as a chronic that is equivalent to the ones indicated in the CLP and REACH Guidance and the endpoints are only used as supportive information. A second study was available with the test organism *Pimephales promelas* revealing a EC_{10} (body weight) of 1.91 mg a.s/L and a EC_{10} (length) of 4.0 mg a.s./L; both are based on nominal concentrations (Anonymous, 2013).

Two long-term toxicity studies with two different aquatic invertebrates were available. The study outcome with *Daphnia magna* as test organism was a 21 d NOEC of 0.06 mg a.s./L referring to reproduction and parental mortality based on nominal concentrations (Anonymous, 1994). The study conducted with the sediment dwelling organism *Chironomus riparius* reported a nominal 24 d NOEC of 0.1 mg a.s./L, which is the highest tested concentration (Anonymous, 1999). The

DS noted a deviation from the OECD TG for the study with *Chironomus riparius* as the sediment phase contained 10 % peat instead of suggested 5 %.

For algae, one toxicity study was available with *Raphidocelis subcapitata* reporting a NOEC of 4.51 mg a.s./L based on mean measured concentrations (Anonymous, 1995).

From the available long-term aquatic toxicity studies, the invertebrates are the most sensitive trophic level, therefore the chronic aquatic classification proposed by the DS was based on a 21 d NOEC of 0.06 mg a.s./L with *Daphnia magna* referring to mortality and reproduction. The DS proposed Aquatic Chronic 1 (H410), with an M-factor of 1 along with the understanding that the substance is not rapidly degradable.

Comments received during consultation

One NA and one MSCA commented on the environmental part of the DS proposal.

The MSCA expressed their support for the proposed environmental classification of fosthiazate.

The NA asked for additional information to support the study endpoint with *Chironomus riparius* including details of tested concentrations (Anonymous, 1999). The DS provided a table presenting the chemical analysis of the overlaying water, pore water and sediment via LSC and HPLC as well as a table presenting the effects on emergence and development (see Tables in RCOM document, comment number 21). The DS confirmed that no statistical effects on emergence and development were observed up to the highest tested concentration (Dunnet-test, α=0.05, one-sided smaller). Further, the DS noted that no reliable EC_{10} value (due to low effects observed leading to high uncertainty) could be yield for this study so the NOEC was the preferred endpoint.

The NA noted that long-term EC_x endpoints with confidence intervals are preferred to NOECs and asked whether an EC_{10} is available from the OECD TG 211 study with *Daphnia magna* (Anonymous, 1994). The DS performed a recalculation and indicated that additional EC_x recalculations showed low reliability due to very wide and overlapping confidence intervals. The estimated EC_{10} (log-logistic model) was 62.89 µg/L (CI: 11.68 – 114.1 µg/L). The NOEC was the preferred endpoint for this study by the DS.

The NA asked if 72/96 hour endpoints are available as the algal endpoints are based on 120 h observations. The DS responded that the E_rC_{50} and E_bC_{50} are also > 4.51 mg/L for 72 and 96 hours as no effects on algal cell numbers were observed at any sampling time.

In answering to these comments, the DS concluded that the provided additional information did not change the proposed classification.

Additional key elements

In the RAR, two additional acute toxicity studies using *Oncorhynchus mykiss* and *Lepomis macrochirus* were available, which were not addressed within the CLH dossier, indicating that the use of Tween 80 in addition to acetone solvent increased the toxicity of fosthiazate. Anonymous (1989a) reported a 96 h LC_{50} of 7.1 mg a.s./L for *Oncorhynchus mykiss* and Anonymous (1989b) a 96 h LC_{50} of 6.7 mg a.s./L for *Lepomis macrochirus*. Both studies were performed according to OECD TG 203 and under GLP. RAC notes that there are no robust summaries of the studies provided to evaluate acceptability and reliability of these studies. Therefore, the revealed values are considered not relevant, as no information on the used methodology was available. Further, Tween 80 is not a co-formulant in the product. In addition, RAC notes that the respective values

for *Oncorhynchus mykiss* and *Lepomis macrochirus* do not trigger a need for an acute classification for the aquatic environment, as they are above 1 mg/L.

In the public literature, a study was available investigating the effects of fosthiazate to test organisms of different trophic levels; *Vibrio fischeri*, *Raphidocelis subcapitata* and *Daphnia magna* (Kungolos *et al.*, 2009). The study was not assessed in the CLH report. Within the study, fosthiazate was used in his commercial formulation of Nemathorin for exposure of the test organisms. After study duration *Vibrio fischeri* was the most sensitive organism against fosthiazate with an IC_{50} of 0.20 (0.17-0.25) mg/L. For *Daphnia magna* an IC_{50} of 0.32 (0.25-0.41) mg/L and for *Raphidocelis subcapitata* an EC_{50} of 1.02 (0.56-1.64) mg/L was reported. RAC notes that no information was given in the study report regarding the co-formulants of the whole formulation (Nemathorin) tested against the test organisms. RAC was also not able to verify whether the study is valid and reliable, as missing information were identified on the methodology and analytical procedure of the study (e.g. results based on nominal or measured concentrations, use of control groups or information on the dissolved oxygen concentration). The study outcome for the bacteria *Vibrio fischeri* and *Daphnia magna* are in the same order of magnitude supporting the proposed classification from the DS as Aquatic Acute 1; however, due to the shortcomings mentioned above and the fact that the study was performed with a formulation instead of the pure substance, the study outcome should only be used as supportive information.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider fosthiazate as **not rapidly degradable**:

- No degradation (0 % after 28 days) was observed in the ready biodegradability test conducted according to OECD TG 301 F, indicating that the substance is not readily biodegradable.
- Although in surface-water simulation test according to OECD TG 309 DT_{50} of < 16 days (14.7 – 15.9 days) was demonstrated, this was mainly due to primary degradation and low mineralization (3-21 %) was observed.
- The DT_{50} values in the total system in three water/sediment system studies were in the range from 30.1 to 72.1 days.
- The longest hydrolysis half-life determined within the pH range 4-9 was > 16 days (312 d, longest half-life within pH 4-9).

Bioaccumulation

RAC agrees with the DS that fosthiazate has a **low potential for bioaccumulation**:

- The experimentally determined steady state total ^{14}C-BCF for the whole fish with 3.2 L/kg is below the CLP criterion of 500. However, it is noted that the study did not meet all relevant validity criteria according to OECD TG 305.
- The measured log K_{OW} values of 1.68 and 1.75 are below the cut-off value of 4.

Aquatic Toxicity

Acute toxicity

Reliable short-term aquatic toxicity data are available for all three trophic levels (fish, invertebrates, algae). Aquatic invertebrates are the most acutely sensitive trophic level, and the lowest result is a nominal 48 h EC_{50} value of 0.28 mg a.s./L for *Daphnia magna* in a study conducted according to OECD TG 202 and under GLP. Although the study showed analytical deficiencies in the lower exposure concentrations, RAC agrees to consider this study valid and

reliable for classification purposes, as the validity criteria according to OECD TG 202 were met (in the control < 10 % of daphnids were immobilized, dissolved oxygen concentration was ≥ 3 mg/L in control and test vessel). RAC notes that although test concentrations showed variability at lower exposure levels, test concentrations were sufficiently maintained (80 – 120 % of nominal) in the range of the EC_{50} value. Based on the key EC_{50} value of 0.28 mg/L, and in line with the DS proposal, RAC is of the opinion that fosthiazate warrants a classification as **Aquatic Acute 1 (H400) with an acute M-factor of 1** ($0.1 < EC_{50} \leq 1$ mg/L). The classification is supported by a 96 h EC_{50} of 0.429 mg a.s./L obtained with *Americamysis bahia* in a study conducted under GLP.

Chronic Toxicity

Reliable chronic toxicity data were available for the three trophic levels (fish, invertebrates and algae). The lowest long-term effect value for fosthiazate was a 21 d NOEC of 0.06 mg a.s./L referring to mortality and reproduction derived in the cladoceran *Daphnia magna*. This value was obtained in an OECD TG 211 test performed in compliance with GLP. The validity criteria according to the test guideline were met.

In addition, the DS provided an EC_{10} (log logistic model) from the same study in the RCOM document. The DS performed a recalculation, which showed low reliability due to very wide and overlapping confidence intervals. The estimated EC_{10} was 62.89 µg /L (CI: 11.68 – 114.1 µg/L). The DS preferred therefore the NOEC as endpoint. RAC acknowledge that Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7b / Chapter R.10) and CLP guidance (Part 4) indicate that generally preference should be given to EC_{10} over NOEC when such a reliable value is available. However, RAC agrees that the NOEC should be given preference in this specific case over the estimated EC_{10}, as the latter value shows low reliability due to the very wide and overlapping confidence interval. Only in the highest tested concentration effects of fosthiazate on reproduction to *Daphnia magna* could be seen, therefore revealing that no meaningful EC_{10} could be derived from the data. RAC notes that both values, EC_{10} and NOEC are in the same range, warranting the same classification.

Overall, RAC agrees with the DS that fosthiazate warrants a classification as **Aquatic Chronic 1 (H410), with a chronic M-factor of 1** ($0.01 < NOEC/EC_{10} \leq 0.1$ mg/L) based on the key (NOEC) value of 0.06 mg/L, for a non rapidly degradable substance.

Additional references

A. Kungolos, C. Emmanouil, V. Tsiridis, N. Tsiropoulos; Evaluation of toxic and interactive toxic effects of three agrochemicals and copper using a battery of microbiotests. Science of The Total Environment, Volume 407, Issue 16, 2009, Pages 4610-4615,ISSN 0048-9697.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter and additional information (if applicable).

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

Appendix

Supplementary information

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

In the acute neurotoxicity screening study, a total of ten rats/sex/dose were treated via oral gavage (single dose). Dosing occurred over four consecutive days: two groups of five males were treated on day 1 and 3, respectively, and similarly two groups of five females were treated on day 2 and 4. The top dose for males was 40 mg/kg bw, and for females 20 mg/kg bw, additional doses for both sexes were 10, 0.4 and 0 mg/kg bw. Functional observational battery (FOB) including open field evaluations, forelimb and hind limb grip strength, landing foot-spread and a motor activity test were performed at baseline, day 0 (3 hours post-dosing; the time of peak effect), and at 7 and 14 days postdosing. AChE activity was measured in plasma, erythrocytes, cerebral cortex, cerebellum and brain stem at day 0, day 7 and day 14 postdosing.

The observed effects relevant for STOT SE are summarised below.

At the top dose, 20/40 mg/kg bw (females/males), anogenital staining, decreased faeces and dried red material on forepaws, mouth and nose was observed in the first and second day following dosing. At the top dose also FOB open field evaluations of mobility, posture, gait, arousal, urination, and righting reflex were different from control on day 0. Mobility was slightly to moderately impaired in all high dose males. Other notations during the 2-minute observation period included hunched posture in five high dose males, splayed hind limbs in one male and a dragging body in one male. Gait abnormalities were considered severe in two high dose males, considerable in one male and slight in seven males. Arousal was considered very low in one high dose male and low in nine other animals. Nine of the ten high dose animals had one or more pools of urine and four control animals had one pool of urine each. The righting reflex was considered slightly impaired in six of the ten high dose males. The number of supported rears was statistically significantly greater for males treated with 0.4 mg/kg bw when compared to control at day 14. Four of the ten animals at low dose had one or more supported rears while none of the controls had supported rears during the 2-minute observation period.

No test item related effects were observed in the grip strength test and the observed differences in foot-spread were not considered to be related to the administration of the test material.

Mean motor activity values were also significantly different from control for the top dose groups at day 0 (3 hours post-dosing). For females, significantly different values could also be seen for the top dose at day 14. This is summarised in the table below (adapted from Table 30 in the CLH report). No significant differences were found at lower doses. No significant differences were found on day 7 for either sex or on day 14 for the males.

***Table**: Mean motor activity data (Acute neurotoxicity screening study, Anonymous 38, 1997).*

Dose level Sex, Day	Distance travelled	Resting time	Ambulatory time	Stereo-typic time	Bursts of stereo-typic	Horizontal counts	Total counts	Vertical counts	Vertical breaks
Males, Base-line[1]									
range (low-high)	2140-2553	456.2-483.4	105.1-121.5	299.1-326.8	951.3-1002	1979-2206	1139-1361	83.6-146.2	23.3-35.2
Males, Day 0									
0 mg/ kg bw	1757	575	83.67	241.4	740.7	1545	976.3	178.4	31.5
40 mg/kg bw	278.9**	748.8**	16.34**	98.9**	261.4**	323.8**	137**	2.4**	0.4**
Females, Baseline									
range (low-high)	2854-3005	413.6-446.7	128.4-139.4	322.3-350.6	980.3-1078	2310-2497	1470-1603	111-200.7	36-51.3
Females, Day 0									
0 mg/ kg bw	2766	492.7	121.4	285.9	906	2330	1588	267.5	51.6
20 mg/kg bw	793.5**	722**	38.16**	139.8**	402.5**	699.1**	402.9**	24.1**	6.1**
Females, Day 14									
0 mg/ kg bw	3802	440	155.8	304.2	1042	3055	2336	632.1	81.7
20 mg/kg bw	2383**	537.6*	105.9**	256.5	815.7*	1934	1356**	340**	58.1

[1]One week prior to exposure. There were no significant differences between groups. *Statistically significant at the 0.05 level, ** Statistically significant at the 0.01 level

At ≥10 mg/kg bw, erythrocyte and brain AChE activities in both males and females were significantly reduced. At the lowest dose (0.4 mg/kg bw) only AChE activity was significantly different from in red blood cells (RBC) at day 0 (statistically significant at 0.01 level). Values for the middle and top doses are shown in the table below (adapted from Table 30 in the CLH report).

***Table**: AChE activity in rat brain and blood samples after fosthiazate exposure at Day 0, 7 and 14. Values for the middle and top doses are shown (Acute neurotoxicity screening study, Anonymous 38, 1997).*

Dose (mg/kg bw)	Day	AChE activity in males (% of control)				
		Plasma	**RBC**	**Brain CC**	**Brain C**	**Brain BS**
10	**0[1]**	26**	72**	79**	76*	72**
	7	87	79**	92	107	92
	14	103	86**	93*	97	96
40	**0[1]**	7**	67**	23**	33**	22**
	7	89	73**	49**	76**	67**
	14	101	82**	63**	92*	80**
		AChE activity in females (% of control)				
10	**0[1]**	14**	75**	70**	69*	65**
	7	89	81**	77**	96	96
	14	100	91**	91*	108	92
20	**0[1]**	7**	67**	35**	38**	34**
	7	103	76**	54**	78**	80**
	14	86	86**	73**	105	90

RBC = erythrocyte, CC = cerebral cortex, C = cerebellum, BS = brain stem, [1] = Day 0, 3 hours postdosing. * Statistically significant difference (at least 10% lower than control for brain and at least 20% lower than control for erythrocyte AChE) at the 0.05 level, ** Statistically significant difference (at least 10% lower than control for brain and at least 20% lower than control for erythrocyte AChE) at the 0.01 level.

The age-sensitivity study was set up to determine the potential of fosthiazate to induce effects on maternal, foetal, neonatal and young adult AChE activity in the blood components and brain after maternal gestational exposure or after acute or short-term repeated exposure at various ages. The tested doses were 0, 0.1, 0.7 and 5 mg/kg bw/d. Exposure was via oral gavage. The study was conducted in different phases and included a high number of animals, see the table below.

***Table**: Overview of the study-phases in the age-sensitivity study in rats (Anonymous 7, 2006).*

Phases	Days of exposure (oral gavage) to: 0, 0.1, 0.7 and 5 mg/kg bw/day	Number of rats
Phase I Gestational exposure	GD 6-20	12 dams and litters
Phase II Peak exposure time determination	PND 11 PND 21	60/sex 30/sex
Phase III Acute exposure	PND 11 PND 21 Young adults	20/sex 10/sex 10/sex
Phase IV Repeated exposure	PND 11 to 21 11 consecutive days from PND 43-46	10/sex 10/sex

GD = gestation day
PND = postnatal day

Animals were examined twice weekly for clinical signs (during the treatment period). Plasma, RBC and brain AChE (peak AChE inhibition) values were evaluated after dose administration. This was based on the peak exposure time determination in Phase II. Relevant findings are summarised in the text and tables below, and in Table 30 in the CLH report.

No clinical signs of toxicity were observed in Phase II-IV of the study. In Phase I, clinical signs were only observed at the top dose, and no clinical signs were observed during GD 6-18. At GD 19 and/or 20 the following clinical signs were observed in one or more animals in the top dose group: tremors, unkempt appearance, prostration, red and yellow material on various body surfaces, repetitive jaw movement, piloerection, gasping and lacrimation.

In Phase I, no significant changes in AChE values (activity range 96-124% of control) were found in any compartment of either maternal or foetal animals at 0.1 mg/kg bw (at 0.7 mg/kg bw values were statistically significantly lower from control in maternal animals but not in the foetuses, in all compartments. At the top dose of 5 mg/kg bw all values were significantly lower from control. Relevant results are summarised in the table below (adopted from table 30 in the CLH report).

***Table**: Phase I: Mean plasma, RBC and brain AChE values in dams and foetuses on GD 20, in the age-sensitivity study in rats (Anonymous 7, 2006).*

Dosing (mg/kg bw/day)		**0**	**0.7**		**5**	
AChE values		**Mean U/L**	**Mean U/L**	**Activity % of control**	**Mean U/L**	**Activity % of control**
Plasma	Maternal	3147 ± 410	852** ± 139	27 %	142** ± 24.7	4.5 %
	Pooled foetal	491 ± 41	495 ± 51	101 %	329** ± 59	67 %
RBC	Maternal	3931 ± 1475	2193** ± 712	56 %	20[1] ± 0	0.5 %[1]
	Pooled foetal	2644 ± 644	2893 ± 738	109 %	1851* ± 593	70 %
Brain	Maternal	49446 ± 2190	47135** ± 1510	95 %	5152** ± 1719	10 %
	Pooled foetal	6612 ± 6780	6251 ± 650	95 %	5182** ± 685	78 %

***Statistically significant at 0.05, **Statistically significant at 0.01, [1]Values under the limit of detection (LOD), thus the lower limit of quantification (LLOQ) was used for statistical analysis**

In Phase III, mean plasma AChE activity following acute exposure was statistically significantly lower as compared to control at 5 mg/kg bw for all ages in both sexes. RBC AChE activity was statistically significantly lower at 5 mg/kg bw only for PND 11 females, and brain AChE activity was significantly lower at 5 mg/kg bw for PND 11 males and females. The AChE activity values are shown in the table below.

***Table**: Phase III; Mean plasma AChE activity levels for male and female rats at PND 11 and 21, and in young adults, in the age-sensitivity study in rats (Anonymous 7, 2006).*

Age	Males			Females		
	Control	5 mg/kg bw		Control	5 mg/kg bw	
Mean plasma AChE activity						
PND 11	1536 ± 150.8	633** ± 62.2	41%	1482 ± 64.3	641** ± 59.7	43%
PND 21	1094 ± 148.7	432** ± 46.3	39%	1123 ± 157.4	422** ± 65.2	38%
Young adult	888 ± 63.2	519** ± 98.7	58%	1423 ± 399.3	472** ± 96.1	33%
Mean RBC AChE activity						
PND 11	4701 ± 1008.2	3866 ± 3074.8	82%	5558 ± 2940.3	3110* ± 832.2	56%
PND 21	4929 ± 2165.2	3085 ± 1263.6	63%	4058 ± 1576.4	2777 ± 879.3	68%
Young adult	3351 ± 635.6	2582 ± 478.4	77%	4469 ± 2964.6	2387* ± 475.4	53%
Mean brain AChE activity						
PND 11	24360 ± 1177.1	20730** ± 1386.1	85%	24800 ± 932.8	21286** ± 1277.4	86%
PND 21	36661 ± 1971.4	31019 ± 1087.5	85%	36797 ± 2081.1	30784** ± 1843.1	84%
Young adult	47611 ± 2803.8	47783 ± 2016.4	100%	48377 ± 2814.7	47798 ± 3074.9	99%

***Statistically significant at 0.05, **Statistically significant at 0.01**

In Phase IV, short-term repeated exposure, plasma AChE activity was statistically significantly lower from control at 0.7 mg/kg bw/day at PND 21 in males. At 5 mg/kg bw/day AChE activity values were significantly lower from control in all compartments for both female and male PND 21 animals. For the young adults, plasma activity was significantly lower from control at 0.7 mg/kg bw/day in females, and in all compartments for both males and females at 5 mg/kg bw/day. The AChE activity values are shown in the table below.

***Table**: Phase IV: Mean AChE activity values for male and female rats at PND 21 and in young adults, in the age-sensitivity study in rats (Anonymous 7, 2006).*

Age	Males				Females			
	Control	**0.1 mg/kg bw/day**	**0.7 mg/kg bw/day**	**5 mg/kg bw/day**	**Control**	**0.1 mg/kg bw/day**	**0.7 mg/kg bw/day**	**5 mg/kg bw/day**
	Mean plasma AChE activity							
PND 21	1085 ± 109.8	1055 ± 100.1 (97 %)	849** ± 136.9 (78 %)	439** ± 180.3 (40 %)	1019 ± 253.8	1061 ± 151.3 (104 %)	832 ± 193.0 (82 %)	346** ± 104.2 (33 %)
Young adult	773 ± 155.5	828 ± 76.9 (107 %)	756 ± 152.3 (98 %)	325** ± 38.3 (42 %)	1991 ± 433.3	2006 ± 754.3 (101 %)	1152** ± 170.3 (58 %)	253** ± 27.1 (13 %)
	Mean RBC AChE activity							
PND 21	8725 ± 3386.9	7522 ± 2622.6 (86 %)	9528 ± 10383.3 (109 %)	1107* ± 1942.2 (13 %)	7595 ± 3820.6	7595 ± 3820.6 (101 %)	6523 ± 1381.8 (86 %)	508** ± 510.2 (7 %)
Young adult	2941 ± 792.8	2915 ± 950.4 (99 %)	2821 ± 643.6 (96 %)	392** ± 306.0 (13 %)	2548 ± 846.7	3178 ± 1248.9 (125 %)	2196 ± 298.2 (86 %)	20**[1] ± 0.0 (1 %[2])
	Mean brain AChE activity							
PND 21	39177 ± 1109.0	40093 ± 2031.0 (102 %)	37563 ± 825.1 (96 %)	19692** ± 11643.7 (50 %)	37284 ± 8312.6	40936 ± 1081.5 (110 %)	34444 ± 6956.5 (92 %)	13125** ± 1810.5 (35 %)
Young adult	49176 ± 1372.3	49176 ± 1372.3 (104 %)	49595 ± 907.2 (101 %)	38237** ± 4346.9 (78 %)	50227 ± 2570.2	50726 ± 1031.3 (101 %)	48882 ± 1780.8 (97 %)	21476** ± 4545.2 (43 %)

****Statistically significant at 0.01 [1]Values under LOD, thus LLOQ was used for statistical analysis**

In the acute oral toxicity study in rats, increased mortality was seen at ≥64 mg/kg bw (2/5 males, 5/5 females). Clinical signs were seen in both sexes at all dose levels (from 41 to 128 mg/kg bw). Frequent signs were decreased motor activity, prone or hunched posture, muscle tremor, irregular breathing and piloerection. Less frequent signs were ataxia, muscle spasms, pigmented orbital secretions, irritability, blanching, salivation, pigmented staining of the snout and diarrhoea. Clinical signs resolved within 3 days in the surviving animals.

In the acute oral toxicity study in mice, increased mortality was seen at ≥102 mg/kg bw. At this dose 5/5 animals died within four days in both sexes. At 128 mg/kg bw 2/5 males and 5/5 females died within four days. Clinical signs were seen at ≥102 mg/kg bw. Frequent signs were decreased motor activity, hunched posture, ataxia and muscle tremor. Less frequent signs were lethargy, irregular breathing, prone posture, and closed eyes.

In the acute dermal toxicity study in rats, increased mortality was seen in males at ≥1965 mg/kg bw and ≥779 mg/kg bw in females. At ≥3115 (males) and ≥1236 (females) mg/kg bw all animals died within eight days. Clinical signs were decreased motor activity, prone posture, muscle

tremor, ungroomed appearance, pigmental orbital secretion and thin body conformation. Less frequent signs were irregular breathing, salivation, opisthotonos, hunched posture, ataxia, bulging eyes and blanching. Clinical signs persisted for up to 12 days in surviving animals.

In the acute inhalation toxicity study in rats, mortality occurred at all dose levels within two days for males, and within four days for females. Clinical signs were reduced spontaneous motor activity (in all animals of both sexes, all doses including air control, decreased respiratory rate (≥7/8 animals, both sexes, all doses), salivation, limb paralysis (≥7/8 animals, both sexes, all doses), adoption of prone position, nasal bleeding, red tears, and lacrimation. Clinical signs resolved within 4 days in the surviving animals.

Necroscopy showed hyperaemia of the lungs in all dead animals of both sexes at all doses, except one female at 0.9 mg/L. Haemorrhage of the lungs were found in some of the dead animals. Results are shown in the table below (adapted from Table 30 in the CLH report).

***Table**: Mortality and haemorrhage of the lungs (in dead rats) in the acute inhalation study in rats.*

Analytical concentration (mg/L)	Mortality		Haemorrhage, lung (in dead rats)	
	Males	Females	Males	Females
0 (air control)	0/8	0/8	n.a.	n.a.
0.53	1/8	4/8	1/1	4/4
0.8	3/8	5/8	2/3	4/5
0.9	5/8	8/8	2/5	5/8
1.23	7/8	8/8	3/7	3/8

In the acute delayed neurotoxicity study in hen, groups of six animals were exposed via oral gavage to 20 mg/kg bw (in maize oil) at day 1 and day 23, and an additional group was exposed to tri-ortho-cresyl-phosphate (TOCP) as positive control. The animals were observed for 24 days, then killed and necropsied. Sections of the central and peripheral nervous system were examined histopathologically.

Seven out of 18 exposed birds died during the study, but only two were from the treatment group (the other five were exposed to TOCP as a positive control).

Animals treated with fosthiazate exhibited marked cholinergic responses and associated motor impairment. The signs included reduced activity, peripheral vasodilation, unsteadiness, drooped wings, resting on hocks and occasional clonic convulsions. There were no histological findings indicative of delayed neurotoxicity in the fosthiazate treated animals. Such signs were seen in the animals treated with the positive control.

In the rat study performed to determine a NOEL for AChE inhibition, 20 male and 20 females were exposed to a single dose of fosthiazate. The top dose was 4 mg/kg bw, other doses were 0.4, 0.04 and 0 mg/kg bw. Mean AChE activity was measured in plasma, erythrocyte, brain cerebral cortex, cerebellum, and brain stem. Significant reduction in activity was only seen in plasma, and only in the top dose. The results in plasma, and the range between the lowest and the highest values measured in the other compartments, are shown in the tables below.

***Table**: Mean plasma AChE in rats after exposure to a single dose of fosthiazate.*

Dose (mg/kg bw)	Mean plasma AChE (U/L)
Males	
0	422.8
0.04	429.4
0.4	405.8
4	272* (64 % of control)
Females	
0	1145
0.04	1253
0.4	1016
4	219* (19 % of control)

***Statistically significantly different from control, p<0.01**

***Table**: Range of mean AChE in erythrocyte and the brain of rats exposed to a single dose of fosthiazate.*

Compartment	Range of mean AChE (U/L)	
	Control	**Doses: 0.04, 0.4 and 4 mg/kg bw**
Males		
Erythrocyte	139	129-172
Cerebral cortex	238	188-256
Cerebellum	206	202-207
Brain stem	515	503-594
Females		
Erythrocyte	137	123-142
Cerebral cortex	230	226-253
Cerebellum	186	189-220
Brain stem	459	476-572

NOEL for inhibition of plasma AChE was set at 0.4 mg/kg bw. NOEL for inhibition of erythrocyte and brain AChE was set at >4 mg/kg bw.

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

In the oral 90-day rat study, 10 animals/sex/dose were exposed to 0, 0.08, 0.77, 4.12 and 36.4 mg/kg bw/day (males) and 0, 0.09, 0.9, 4.7 and 41 mg/kg bw/day (females), corresponding to 0, 1.07, 10.7, 53.6 and 429 ppm.

Signs of toxicity were observed at the top doses, including hair loss, emaciation (in 5 males) and trachypnoea (in 9 males and one female). Other relevant findings including haematological changes, cholinesterase levels, histopathology and organ weights are shown in the tables below.

***Table**: Effects relevant to organ toxicity in the oral 90-day rat study.*

		Dose in ppm (mg/kg bw/day)				
Parameter	**Sex**	**0**	**1.07 (M: 0.08, F: 0.09)**	**10.7 (M: 0.77, F: 0.9)**	**53.6 (M: 4.12, F: 4.7)**	**429 (M: 36.4, F: 41)**
Feed consumption week 1-13 (% of control)	M F	100 100	101 98	99 102	102 103	100 107
Body weight gain week 1-13 (g)	M F	431 180	445 189	471* 194	434 175	365*** (85%) 157**
PCV week 12 (%)	M F	52 48	50 45	50 42	52 43	47*** (90%) 62
Hb week 12 (g %)	M F	16.2 15.9	16 16	16 15.8	16.4 14.9** (94%)	15.2*** (94%) 14.6*** (92%)
RBC week 12 (mil/cmm)	M F	8.12 7.56	7.95 7.72	7.96 7.39	7.98 7*** (93%)	7.86* (97%) 7.15*** (95%)
MCV week 12 (cμ)	M F	64 64	63 65	64 66*	65* 66*	60*** (94%) 62*** (97%)
ALT (iu/L)	M F	32 26	33 32** (123%)	32 32* (123%)	31 33** (127%)	39*** 34*** (131%)
AST (iu/L)	M F	74 74	77 85**	76 81	65* (88%) 79	86** (116%) 89*** (120%)
Urea	M F	30 40	29 49**	30 46	32 40	32* (107%) 57*** (143%)
Protein	M F	7.2 7.4	7.4 8.3***	7.2 7.8**	7.2 7.4	7.1* 6.9***

Cell AChE (iu/mL)	M F	2052 1946	1810** (88%) 2129**	1867 1625*** (84%)	888*** (43%) 572*** (29%)	214*** (10%) 144*** (7.4%)
Brain AChE (iu/mL)	M F	10000 10000	9900 9600* (96%)	10400 9600* (96%)	7700*** (77%) 3400*** (34%)	1900 (19%) 1000 (10%)
Plasma AChE (iu/mL)	M F	831 3796	888 4136	663*** (80%) 1909*** (50%)	461*** (56%) 834*** (22%)	322*** (39%) 450*** (12%)
Plasma butyryl cholinesterase (iu/mL)	M F	1232 6278	1188 6442	858*** (70%) 2838*** (45%)	736*** (60%) 1372*** (22%)	642*** (52%) 811*** (13%)
Vacuolation of the zona fasciculata	M F	2 0	4 0	5 0	8 2	10 10
Vacuolation of the zona glomerulosa	M F	2 3	0 3	2 3	2 5	10 10

***Significantly different from controls, p<0.05; **Significantly different from controls, p<0.01; ***Significantly different from controls, p<0.001, PCV=packed cell volume, Hb= haemoglobin, RBC=red blood cell, MCV=mean cell volume, ALT=alanine transaminase, AST=aspartate aminotransferase**

***Table**: Organ weights in rats exposed orally to fosthiazate for 90-days.*

Organ weight, Parameter	**Sex**	**Dose in ppm (mg/kg bw/day)**		
		0	**53.6 (M: 4.12, F: 4.7)**	**429 (M: 36.4, F: 41)**
Adrenals (g)	M F	0.05 0.075	0.058 0.071	0.065** 0.085
Adrenals vs body weight (%)	M F	0.0097 0.0246	0.0104 0.0251	0.0134** (138% of control) 0.0319* (130% of control)
Liver (g)	M F	18.7 11.1	20.9 10.7	19.6 11.1
Liver vs. body weight (%)	M F	3.52 3.63	3.77 3.75	4.01 4.14

***Significantly different from controls, p<0.05; **Significantly different from controls, p<0.01**

In the combined long-term and carcinogenicity study in rat, 50 + 10 animals/sex/dose were exposed orally. The additional ten were exposed until interim kills at 12 months, the rest were treated for 24 months. The doses used were 0, 0.042, 0.41, 2.01 and 8.94 mg/kg bw/day for males, and 0. 0.055, 0.54, 2.63 and 12.53 mg/kg bw/day for females. This corresponds to 0, 1.07, 10.7, 53.6 and 214 ppm. Effects on haematology, cholinesterase activity and histopathology were examined. The survival of males in all dose groups and of females in the medium high dose group was slightly below 50 % at the end of the study, not fulfilling the requirement of current OECD test guidelines (OECD TG 453). However, the survival was higher than 50 % till week 92. At the end of the study survival was far above 25% in all groups. All in all, while this study may not meet the OECD test guideline requirements for long term/carcinogenicity testing concerning survival, it is concluded that the results are useful without prejudice to the assessment of carcinogenicity or long-term toxicity.

Haematological observations were not associated with any histopathological changes. They are considered to be of minimal toxicological significance.

Cholinesterase activity were significantly different from control in both sexes at all time points for the two highest doses, see table below. Occasional significantly different values were also seen at lower doses, especially for plasma AChE in females, and cell AChE in both sexes.

***Table**: AChE activity – group mean values (% of control activity) in the combined long-term and carcinogenicity study in rat.*

Sex	Dose level (ppm)[#]	Week 12	Week 24	Week 51	Week 76	Week 102
Plasma butyryl cholinesterase						
Males	**53.6**	51*	64*	44*	31*	37*
	214	45*	48*	37*	28*	27*
Females	**53.6**	19*	18*	19*	26*	23*
	214	12*	12*	15*	21*	18*
Plasma AChE						
Males	**53.6**	60*	57*	43*	35*	33*
	214	45*	44*	32*	24*	33*
Females	**53.6**	20*	19*	19*	26*	25*
	214	13*	12*	13*	20*	18*
Cell AChE						
Males	**53.6**	35*	36*	33*	36*	48*
	214	17*	14*	15*	12*	14*
Females	**53.6**	28*	24*	25*	36*	33*
	214	12*	7*	11*	8*	14*
		Week 53		**Week 104**		
Brain AChE						
Males	**53.6**	51**		51**		
	214	20**		15**		
Females	**53.6**	36**		38**		
	214	12**		10**		

***Significantly different from control, p<0.01; **Significantly different from control, p<0.001. [#] 53.6/214 ppm corresponds to 2.01/8.94 and 2.63/12.53 mg/kg bw/day in males and females, respectively.**

***Table**: Adrenal weights in the combined long-term and carcinogenicity study in rat.*

Dose in ppm (mg/kg bw/day)	Adrenals (g)	Adrenals vs body weight (%)
Males		
0	0.113	0.0074
10.7 (0.41)	0.29	0.0077
53.6 (2.01)	0.12	0.0081
214 (8.94)	0.123	0.0088
Females		
0	0.091	0.0186
10.7 (0.54)	0.076	0.0192
53.6 (2.63)	0.1	0.0153
214 (12.53)	0.12* (132 % of control)	0.0219

*** Significantly different from controls, p<0.05**

Micropathological findings were only significantly different from controls at the high dose, except ovary-foamy interstitial cells where findings were different from control at the two highest doses. The values are presented in the table below.

***Table**: Micropathological findings for animals at terminal sacrifice or those dying during the study period.*

Finding	Dose level (ppm)#		
	0	53.6	214
Males			
Adrenal-vacuolation of zona fasciculate	7	14	27**
Eyes-cataract	6	14	3
Eyes- retinal atrophy	1	0	11*
Pituitary-pars nervosa vacuolation	8	7	24*
Females Pituitary-pars nervosa vacuolation			
Adrenal-vacuolation of zona fasciculate	2	5	12*
Eyes-cataract	0	3	8*
Eyes-retinal atrophy	3	1	27**
Ovary-foamy interstitial cells	9	30**	41**
Pituitary-pars nervosa vacuolation	7	10	26**

***Significantly different from control, p<0.05; **Significantly different from control, p<0.01. # 53.6/214 ppm corresponds to 2.01/8.94 and 2.63/12.53 mg/kg bw/day in males and females, respectively.**

In the two-generation study in rats (comparable to OECD TG 416), 25 animals/sex/dose were exposed orally via diet to 0, 3, 10, 30 or 100 ppm (corresponding to 0, 0.21, 0.69, 2.09, and 7.21 mg/kg bw/day in males, and 0, 0.26, 0.86, 2.62 and 9.34 mg/kg bw/day in females). Six-week-old F0 rats received fosthiazate for at least 99 days covering pre-mating, mating, gestation, and the lactation periods (for more details on this study, see chapter on reproductive toxicity). Relevant effects on organ toxicity in the F0-generation are presented in the table below.

***Table**: Body weights and adrenal effects in the F0-generation in the two-generation rat study of fosthiazate.*

Parameter	Dose in ppm (mg/kg bw/day)				
	0	**3 (0.26)**	**10 (0.86)**	**30 (2.62)**	**100 (9.34)**
Terminal body weight in grams	335	337	348	361	367*
Absolute adrenal weight in grams	0.062	0.070	0.071* (115%)	0.073** (118%)	0.077** (124%)
Relative adrenal weight (%)	0.0188	0.0208	0.0206	0.0207	0.0212
Adrenal Cortex – hypertrophy of zona glomerulosa	0	0	0	3	22***

***Significantly different from controls at p<0.05, **Significantly different from controls at p<0.01, ***Significantly different from controls at p<0.001.**

The age-sensitivity study in rats, was conducted in different phases. Single dose effects are assessed in the chapter on STOT SE, and all the findings are presented in the tables in the chapter on STOT SE. In brief, after 11-day exposure to 5 mg/kg bw/day fosthiazate, plasma AChE activities of young adults were reduced (≥ 25 %), erythrocyte AChE activities of young adults was reduced (13 and 1% of controls in males and females, respectively) and brain AChE activities of young adults were reduced (78 and 43% of controls in males and females, respectively). In pregnant females after 14-day exposure to 5 mg/kg bw/day fosthiazate, erythrocyte AChE activities were reduced (0.5% of controls) and brain AChE activities were reduced (10% of controls). These dams showed also tremors, unkempt appearance, prostration and red and yellow material on body, repetitive jaw movement, piloerection, gasping and lacrimation on GD 19-20.

In the dermal 21-day rat study, 5 animals/sex/dose were exposed for 6-8 hours/day in three weeks. The top dose was 250 mg/kg bw/day, the other doses were 0, 0.5, 2.5 and 25 mg/kg bw/day. Effects relevant for target organ toxicity are summarised in the tables below.

***Table**: Effects related to target organ toxicity in the dermal 21-day rat study.*

Parameter	Sex	Dose (mg/kg bw/day)				
		0	**0.5**	**2.5**	**25**	**250**
Feed consumption week 1-3 (g/rat/week)	M F	544 414	547 418	525 423	505 438	502 388
Body weight gain week 1-3 (g)	M F	62 7	70 5	55 5	49 10	15** ND
Urea concentration (%)	M F	43 48	42 45	41 42	51 43	53 62
Cell AChE (iu/mL) (% of control)	M F	3728 3484	3606 3415	3384 2758* (79%)	1681*** (45%) 830*** (24%)	205*** (5.5%) 361a
Brain AChE (iu(mL) (% of control)	M F	9600 10000	9700 11100*	9500 10700*	7100*** (74%) 3300*** (33%)	1200*** (13%) 800a
Plasma AChE (iu/mL) (% of control)	M F	743 2458	795 2362	817 1568* (64%)	662 559*** (23%)	344*** (46%) 368a
Plasma butyryl cholinesterase (iu/mL) (% of control)	M F	1325 3705	1408 3685	1408 2484* (67%)	1118* (84%) 994*** (27%)	863*** (65%) 828a
Adrenal weight (g)	M F	0.054 0.072	0.053 0.071	0.049 0.074	0.046 0.082	0.057 0.095
Adrenal weight vs body weight (%)	M F	0.0196 0.0322	0.0184 0.0323	0.0183 0.0328	0.0175 0.0361	0.0247 0.0516*
Vacuolization of zona fasciculata (5 animals examined)	M F	0 0	0 0	0 0	0 0	4 2

a: No statistics conducted since only one animal survived; *Significantly different from control, p<0.05; **Significantly different from control, p<0.01; Significantly different from control, p<0.001

In the oral 28-day study in mice, 10 animals/dose were exposed via the diet, in addition to 7 animals in the positive control group, and 3 animals in an extra vehicle control group. All animals were females. The top dose was 400 ppm, the other doses were 0, 50, 100 and 200 ppm (corresponding to 0, 10, 22, 44 and 61 mg/kg bw/day). Duration of exposure for the positive control, 50 and 100 ppm fosthiazate was 35 days. Exposure time for 200 ppm was 28 days, while 400 ppm for 5 consecutive days was not tolerated (mortality). The positive control was

cyclophosphamide monohydrate (CPS), 50 mg/kg bw/day injected intraperitoneally once daily on day 31-34.

In the animals that were treated with 50, 100 and 200 ppm for 28 days, no suppression of the functional humoral immune response (T-cell dependent antibody response to sheep in red blood cells) was observed. Weights of the adrenals gland and liver were slightly higher in all dose groups, and in the 200 ppm group, respectively, but the changes were not statistically significant. The results are shown in the table below.

***Table**: Organ weights (mean) of mice treated with fosthiazate for 28 days.*

Dose in ppm (mg/kg bw/day)	**Adrenal weight (g)**	**Adrenals relative to bw**	**Liver weight (g)**	**Liver relative to bw**
Vehicle control[#]	0.0099	0.036	1.3272	4.772
50 (10)	0.0109 (110 % of control)	0.038 (118 % of control)	1.4058 (106 % of control)	4.969 (104 % of control)
100 (22)	0.0113 (114 % of control)	0.040 (113 % of control)	1.3846 (104 % of control)	4.958 (104 % of control)
200 (44)	0.0116 (117 % of control)	0.044 (124 % of control)	1.4212 (107 % of control)	5.361 (112 % of control)
CPS	0.0091 (92 % of control)	0.034 (96 % of control)	1.4702 (110 % of control)	5.413 (113 % of control)
ANOVA p-value	0.297	0.053	0.681	0.099

[#]Combination of the two vehicle groups.

In the carcinogenicity study in mice, 60 animals/sex/dose were included. The animals were exposed orally via diet for up to 104 weeks. The top dose was 300 ppm, the other doses were 0, 10, 30, and 100 ppm (corresponding to 0, 1.02, 3.1, 10.3 and 30.5 mg/kg bw/day in males, and 0, 1.11, 3.2, 10.4 and 39.2 mg/kg bw/day in females). Effects on organs weights, and histopathological findings are shown in the tables below.

***Table**: Organ weights of mice treated with fosthiazate for up to 104 weeks.*

Parameter	Sex	Dose in ppm (mg/kg bw/day)			
		0 (control group1)	**0 (control group 2)**	**100 (M: 10.3, F: 10.4)**	**300 (M: 30.5, F: 39.2)**
Adrenal weight (g)	M F	0.004 0.007	0.005 0.007	0.004 0.005	0.005 0.010* (143 % of control)
Adrenal weight relative to body weight (g)	M F	0.0094 0.0157	0.0106 0.0158	0.0088 0.0132	0.0125 (133 % of control) 0.0330* (212 % of control)
Kidney weight (g)	M F	0.877 0.535	0.846 0.535	0.850 0.534	0.705** (80 % of control) 0.439* (82 % of control)
Kidney weight relative to body weight (g)	M F	1.8470 1.2910	1.8535 1.2621	1.9143 1.2859	1.9281 (104 % of control) 1.4862* (115 % of control)
Liver weight (g)	M F	2.87 2.07	3.03 1.89	2.91 2.11	2.07** (72 % of control) 1.64** (79 % of control)
Liver weight relative to body weight (g)	M F	2.87 4.657	3.03 4.421	2.91 4.997*	2.07** (72 % of control) 5.535* (119 % of control)

***Significantly different from combined controls, p<0.05; **Significantly different from combined controls, p<0.01**

***Table**: Histopathological changes in mice after 102 weeks treatment with fosthiazate.*

Parameter	Dose in ppm (mg/kg bw/day)				
	0 (control group 1)	**0 (control group 2)**	**30 (M: 3.1, F: 3.2)**	**100 (M: 10.3, F: 10.4)**	**300 (M: 30.5, F: 39.2)**
Males					
Adrenal cortex, cortico-medullary pigment (number examined)	24 (59)	24 (60)	14* (58)	33 (59)	48*** (59)
Adrenal cortex, mineralization of pigmented cells (number examined)	0 (59)	0 (60)	1 (58)	2 (59)	6** (59)
Kidney, papillary mineralization (number examined)	1 (60)	0 (60)	0 (60)	0 (60)	50*** (60)
Pituitary, vacuolation of the pars nervosa (number examined)	3 (27)	5 (25)	3 (27)	5 (34)	31*** (38)
Lungs – pulmonary adenoma	3 (24)	3 (21)	4 (22)	3 (22)	2 (13)
Lungs – pulmonary adenoma	5 (24)	5 (21)	4 (22)	2 (22)	3 (13)

Females					
Adrenal cortex, cortico-medullary pigment (number examined)	2 (60)	4 (58)	8 (60)	20*** (60)	56*** (60)
Adrenal cortex, mineralization of pigmented cells (number examined)	1 (60)	0 (58)	0 (60)	1 (60)	13*** (60)
Kidney, papillary mineralization (number examined)	0 (60)	1 (60)	0 (60)	0 (60)	45*** (60)
Pituitary, vacuolation of the pars nervosa (number examined)	3 (16)	2 (15)	8 (38)	4 (31)	40*** (41)
Lungs – pulmonary adenoma	2 (26)	5 (29)	0 (14)	1 (36)	0 (17)
Lungs – pulmonary adenoma	1 (26)	1 (29)	2 (14)	7 (36)*	1 (17)

***Significantly different from combined controls, p<0.05; *Significantly different from combined controls, p<0.01; ***Significantly different from combined controls, p<0.001.**

In the oral 90-day dog study, 4 animals/sex/dose were exposed. The top dose was 5.4 mg/kg bw/day, the other doses were 0, 0.054, 0.11 and 0.54 mg/kg bw/day.

Haematological changes, cholinesterase levels, adrenal weights and histopathology of the adrenal cortex was examined. The results are presented in the tables below.

***Table**: Haematology and cholinesterase parameters measured in Beagle dogs treated with fosthiazate for 13 weeks.*

Parameter	Sex	Dose (mg/kg bw/day)				
		0	**0.054**	**0.11**	**0.54**	**5.4**
Haematology						
PCV (%) wk 13	M F	39 46	43* 45	44** (113 %) 46	43* (110 %) 43	39 41* (89 %)
Hb (g %) wk13	M F	13.4 15.7	14.9* 15.6	15.0* 15.9	14.7 15.0	13.5 14.1* (89.8 %)
RBC (mil/cmm) wk13	M F	5.66 6.73	6.30* 6.63	6.31 6.58	6.26* 6.21	5.53 5.78* (85.9 %)
Cholinesterase						
Brain AChE (iu/kg)	M F	3100 3800	3800* 3900	3500 3900	3400 3700	2400* (77.4 %) 2600*** (68.4 %)

Plasma butyryl cholinesterase (iu/L)	M F	15791 22501	19424 22803	18414 17405**(77 %)	9081**(58 %) 9535**(42 %)	5348***(34 %) 8072***(34 %)
Plasma AChE (iu/kg)	M F	2916 3984	3486 3858	3270 3132*(79 %)	1842**(63 %) 1842***(46 %	1380***(47 %) 1716***(43 %)
Erythrocyte AChE (iu/L)	M F	3239 3159	3468 2957	2736 2674	3239 2339	1041*** (32.1%) 1068*** (33.8%)

***Significantly different from combined controls, p<0.05; **Significantly different from combined controls, p<0.01; ***Significantly different from combined controls, p<0.001. (%) of control.**

***Table**: Adrenal weights in Beagle dogs treated with fosthiazate for 13 weeks.*

Organ weight, Parameter	**Sex**	**Dose (mg/kg bw/day)**		
		0	**0.54**	**5.4**
Adrenals (g)	M F	1.12 1.11	1.1 1.09	1.41** (126 %) 1.25
Adrenals vs body weight (%)	M F	0.0084 0.0107	0.0086 0.0101	0.0108** (129 %) 0.0114

****Significantly different from combined controls, p<0.01**

***Table**: Incidence and severity of microscopic changes in the adrenal cortex of beagle dogs treated with fosthiazate for 13 weeks. Number of animals per group: 4*

Sex:	**Male**					**Female**				
Dose (mg/kg bw/day):	**0**	**0.054**	**0.11**	**0.54**	**5.4**	**0**	**0.054**	**0.11**	**0.54**	**5.4**
Adrenal cortex, zona fasciculate										
Hypertrophy										
Not remarkable		1				2	3	1	1	
Minimal	4	3	4	4		2	1	3	3	
Slight					2					3
Moderate					2					1
Moderately severe										
Increase in pallor										
Not remarkable							1			
Minimal	3	4	4	3		3	3	4	4	3
Slight	1			1	1	1				1
Moderate					3					
Moderately severe										

Adrenal cortex, zona glomerulosa										
Hypertrophy										
Not remarkable	1						1	1	1	1
Minimal	3	4	4	4		4	3	2	2	1
Slight					2					1
Moderate					2					1
Moderately severe										
Increase in pallor										
Not remarkable							1			
Minimal	4	4	4	4		4	3	3	2	1
Slight					1			1	2	2
Moderate					3					1
Moderately severe										

In the oral 1-year study in dogs, 4 animals/sex/dose were exposed. The top dose was 5 mg/kg bw/day, the other doses were 0, 0.05, 0.1 and 0.5 mg/kg bw/day. Haematological changes, AChE levels, adrenal weights and histopathology of the adrenal cortex were examined. For haematology there were no significant results other than for the top dose. These results are shown in the table below. For AChE activity the only significant result at 0.1 mg/kg bw/day was for female dogs at 9 months where the plasma level was 89 % of control. For erythrocyte and brain AChE there were no significant results at any dose. Results for 0.5 and 5 mg/kg bw/day are presented below, as well as histopathological results.

***Table**: Haematology values in male dogs after fosthiazate exposure for 12 months.*

Parameter	**Interval (month)**	**Dose (mg/kg bw/day)**	
		0	**5**
Erythrocyte count (10^4/mm^3)	3	755	634** (84 %)
	6	804	672* (83.6 %)
	9	794	680** (85.6 %)
	12	773	683** (88.4 %)
Haematocrit concentration (Vol%)	3	51.3	45.8* (89.3 %)
	6	51.6	44.3* (85.5 %)
	9	53.6	48.0* (89.6 %)
	12	49.8	45.5
Hemoglobin concentration (g/dL)	3	16.5	14.7* (89.1 %)
	6	18.5	15.6* (84.3 %)
	9	18.5	16.6* (89.7 %)
	12	17.9	16.1* (89.9 %)

***Significantly different from control, p<0.05; **Significantly different from control, p<0.01; (%) percent of control**

***Table**: Haematology values in female dogs after fosthiazate exposure for 12 months.*

Parameter	Interval (month)	Dose (mg/kg bw/day)	
		0	**5**
Reticulocyte ratio (%)	3	0.4	1.2** (300 %)
	6	0.4	0.9
	9	0.3	1.1** (366 %)
	12	0.3	0.9** (300 %)
Haematocrit concentration (Vol%)	3	50.5	44.1* (87.3 %)
	6	48.6	46.7
	9	50.1	49
	12	48.4	47.3
Mean Corpuscular haemoglobin (pg)	3	21.6	23.5** (109 %)
	6	22.4	22.6
	9	23.2	23.7
	12	21.9	23.4

***Significantly different from control, p<0.05; **Significantly different from control, p<0.01; (%) percent of control**

***Table**: AChE activities in male and female dogs exposed to fosthiazate for 12 months.*

Type	Interval (month)	Sex	Dose (mg/kg bw/day)		
			0	**0.5**	**5**
True plasma (IU/L)	3	M F	3008 2729	1677** (55 %) 2095	1764** (58.6 %) 1688
	6	M F	3925 2929	2395** (61 %) 1861** (63.5 %)	2475** (63 %) 1900** (64.9 %)
	9	M F	3166 3645	2075* (65.5 %) 2595* (71.2 %)	2397* (75.7 %) 2527* (69.3 %)
	12	M F	3802 2729	2534** (66.6 %) 1995* (73.1 %)	2845** (74.8 %) 2041* (74.8 %)
Pseudo plasma (IU/L)	3	M F	4027 4464	1414 1964	1014* (25.2 %) 1094* (24.5 %)
	6	M F	5162 4487	1895** (36.7 %) 1748** (39 %)	1385** (26.8 %) 1616** (36 %)
	9	M F	4545 4059	1597* (35.1 %) 2114** (52.1 %)	1507* (33.2 %) 1660** (40.9 %)
	12	M F	5332 4496	2046** (38.4 %) 2177** (48.4 %)	1785** (33.5 %) 1914** (42.6 %)

***Significantly different from control, p<0.05; **Significantly different from control, p<0.01; (%) percent of control**

***Table**: Incidence and severity of microscopic changes in the adrenal cortex of beagle dogs treated with fosthiazate for 12 months. Number of animals per group: 52*

Sex	**Male**					**Female**				
Dose (mg/kg bw/day)	**0**	**0.05**	**0.1**	**0.5**	**5**	**0**	**0.05**	**0.1**	**0.5**	**5**
Adrenal cortex, zona fasciculate										
Hypertrophy										
Not remarkable	3	3	1	1		5	1	2	4	
Minimal	2		3	4			3	3	1	2
Slight		2	1		2		1			2
Moderate					2					1
Moderately severe					1					
Increase in pallor										
Not remarkable		2				1				
Minimal	5	1	3	2		4	1	4	5	
Slight		2	2	3			3	1		4
Moderate					3		1			1
Moderately severe					2					
Adrenal cortex, zona glomerulosa										
Hypertrophy										
Not remarkable		1				1			1	
Minimal	4	2	3	1		4	4	3	4	
Slight	1	2	2	3	1		1	2		
Moderate				1	4					5
Moderately severe										
Increase in pallor										
Not remarkable						1				
Minimal	4	3	1	1		3		1	3	
Slight	1	1	3	3		1	5	4	2	
Moderate		1	1	1	2					3
Moderately severe					3					2

[2] *Unclear in CLH-report and RAR if the number of animals were 4 or 5 per dose group.*

In addition to these nine studies, four more studies that were all considered to be supplementary were presented. One was an oral 28-day study in rats, showing effects on haematology (Hb, PCV, MCHC (Mean cell haemoglobin concentration calculated as Hb * 100 / PCV) and MCV reduced, alkaline phosphatase and alanine aminotransferase increased), reduction in AChE activity, and increase in adrenal weight at 400 ppm (40.87 and 43.5 mg/kg bw/day for males and females). At concentrations ≥100 ppm changes in the adrenal cortex were also seen (cell enlargement and pallor) in females. An oral 28-day study in mice is also presented. Again, haematological values are affected (Hb and PCV reduced, leukocyte and lymphocyte count increased). There was also an increase in adrenal glands weight in females at 82.4 mg/kg bw/day, as well as an increased liver weight. In a supplementary oral 28-day study in dogs, doses of 0, 0.021, 0.11, 0.54, 5.4 or 26.8 mg/kg bw/day were tested. At the top dose several signs of toxicity were observed in two of the 3 males and one out of 3 females. One female also showed signs of toxicity at 5.4 mg/kg bw/day. Increased adrenal weight was observed at the top dose. Histopathological changes in the adrenals were also observed, both at the top dose and at 5.4 mg/kg bw/day. In addition, at the latter dose, depression in erythrocyte and brain AChE activities were seen. The last supplementary study was a histopathologic assessment of the adrenal glands of selected females from the carcinogenicity study in mice. In this study, a slight increase in incidence and severity of PAS (periodic acid/Schiff staining) positive and LZN (Long Zeihl Neelson stain) positive staining were seen in both left and right adrenals from mice exposed to 300 ppm fosthiazate compared to controls. This was suggested to be a spontaneous age-related finding, which was exacerbated by fosthiazate exposure.

RAC evaluation of reproductive toxicity

In the dose range-finding study the animals were treated for 90 days prior to mating. The top dose was 300 ppm (22.64 mg/kg bw/day in males, 28.19 mg/kg bw/day in females), additional doses were 100, 30, 10 and 0 ppm corresponding to 7.09, 2.03, 0.68 and 0 mg/kg bw/day in males and 8.93, 2.48, 0.81 and 0 mg/kg bw/day in females. Each group included 10 males and 10 females. Effects observed on sexual function and fertility in the parental generation are included in the table below:

***Table**: Summary of selected findings related to sexual function and fertility (and development) in the dose range-finding study in rats (adapted from table 24 in the CLH report).*

Parameter \ Dose in ppm (mg/kg bw/day)	**0**	**10** (0.68)	**30** (2.03)	**100** (7.09)	**300** (28.19)
Mean bw females before pairing (F0), week 0	148	147	146	149	148
Mean bw females before pairing (F0), week 13 (% of controls)	305	303	322	317	293 (-4%)
Number of mated animals/number of pregnant animals	10/9	10/10	10/6	10/10	10/7
Mean bw females, day 6 post coitum	339	333	357	343	295
Mean bw females, day 20 post coitum	439	440	473	435	338*
Mean bw females, PND 1	343	336	352	338	270*
Mean implantation sites (number of animals) (% of controls)	14.3 ± 3.8 (9)	14.9 ± 3.6 (10)	17.2 ± 2.3 (6)	14.1 ± 3.8 (10)	7.6 ± 4.3 (7) (53%)
Mean litter size (number of animals) on PND 1 (% of controls)	12.8 ± 3.6 (9)	14.3 ± 3.5 (10)	15.3 ± 2.3 (6)	12.2 ± 2.6 (10)	6.2 ± 2.5 (5) (48%)
Mean litter size (number of animals) on PND 4 pre culling	11.0 ± 4.3 (9))	12.8 ± 3.9 (10)	13.4 ± 2.2 (5)	3.8 ± 5.5 (4)	No surviving offspring

*** Bodyweight change significantly different from controls, P <0.01 (Student's t-test)**

No information about statistical analysis regarding the two affected parameters in this study was available in the CLH report.

Effects on general toxicity were observed from 30 ppm. These were described as isolated clinicals signs: tremors, hunched posture and hair loss. However, the observed general toxicity did not influence mating behaviour (100% mating in all dose groups), and before pairing there were no

significant effects on body weight. Overall, the general toxicity observed in the parents in this study was not considered to be marked enough to impact sexual function and/or fertility.

In the two-generation reproductive toxicity study the F0 animals (6-week-old) received fosthiazate for at least 99 days before mating, throughout the mating, gestation and lactation periods. Based on the effects reported in the dose-range finding study, the top dose was 100 ppm (7.21 mg/kg bw/day in males, 9.34 mg/kg bw/day in females), additional doses were 30, 10, 3 and 0 ppm (corresponding to 2.09, 0.69, 0.21 and 0 mg/kg bw/day in males and 2.62, 0.86, 0.26 and 0 mg/kg bw/day in females). Each group included 25 males and 25 females.

Four days after birth, the F1 litters were randomly culled to 8 pups/litter before (4 males and 4 females) where possible. The F0 dams were allowed to rear the F1 pups weaning on day 25 post-partum. At the time of being 4 weeks old, 25 weanlings/sex were randomly selected from all dose groups, except the top dose of 100 ppm, which was not continued due to poor offspring survival to constitute the F1 parents. The F1 parents were treated for 14 weeks and then mated to produce the F2 pups.

In this study, potential effects of fosthiazate on sexual function and fertility were analysed in both F0 and F1 animals. The effects observed are included in the table below:

***Table**: Summary of selected findings on sexual function and fertility (and development) in the two-generation study in rats (adapted from table 24 in the CLH report).*

Parameter \ Dose in ppm (mg/kg bw/day)	**0**	**3** (0.26)	**10** (0.86)	**30** (2.62)	**100** (9.34)	**HCD (%) Range (mean)[a]**
F0 (number of females per group)	25	25	25	25	25	
Total feed intake, g (week 1-14) females	1982	2020	2061	2117**	2266***	
BW gain, g (week 1-14) females	303	301	314	319	329**	
Terminal bw	335	337	348	361	367*	
Number of F0 with normal (4 or 5 day) oestrus cycle (% females per group)	20 (80)	20 (80)	13* (52)	14 (56)	15 (60)	
Irregular oestrous cycle (% females/group)	3 (12)	4 (16)	7 (28)	5 (20)	4 (16)	0-17 (6)
Extended oestrous cycle (% females/group)	0	1 (4)	4 (16)	3 (12)	0	0-7 (2)
Acyclic/pseudo-pregnant (% females/group)	2 (8)	0	1 (4)	3 (12)	6 (24)	0-25 (6)
% mating	96	100	100	92	92	90-100 (98)
Number of pregnant	22	20	18	20	20	
Conception rate (%)	92	80	72	87	87	72-100 (88)

Fertility index (%)	88	80	72	80	80	67-100 (86)
Implantation sites (mean ± SD) (% of controls)	14 ± 2.3	13.2 ± 4.7	13.4 ± 2.8	13.8 ± 3.5	12.9 ± 5.2 (92%)	
Litter size at PND 1 pups/litter (mean ± SD) (% of controls)	12 ± 3	12.5 ± 2.9	11.4 ± 3.5	12.3 ± 3.7	9.8 ± 4.6 (82%)	
F1 (number of animals per group)						
Number of F1 with a normal (4 or 5 day) oestrus cycle (% females/group)	18 (72)	22 (88)	17 (68)	22 (88)		63-100 (86)
Number of irregular oestrous cycle (% females/ group)	1 (4)	1 (4)	7* (28)	0		0-17 (6)
Number of extended oestrous cycle (% females/group)	0	1 (4)	1 (4)	0		0-7 (2)
Number of acyclic/pseudo-pregnant (% females/group)	6 (24)	) 1 (4)	0	3 (12)		0-25 (6)
% mating	100	92	92	100		
Number of pregnant	21	17	18	19		
Conception rate (%)	84	74	78	76		67-100 (86)
Fertility index (%)	85	68	72	76		
Implantation sites (mean ± SD)	13.5 ± 4.2	12.8 ± 2.5	13.4 ± 3.1	13.9 ± 3.8		
Litter size at PND 1 pups/litter (mean ± SD)	11.4 ± 3.7	12 ± 2.5	11.6 ± 2.9	12.1 ± 3		

*** Significantly different from controls at p<0.05, ** Significantly different from controls at p<0.01. *** Significantly different from controls at p<0.001. [a]Background control (30 studies for mating performance and fertility, 18 studies for oestrus data).**

***Table**: Summary of gestation length and index in F0 and F1 females in the two-generation study in rats (from table 24 in the CLH report).*

Dose in ppm (mg/kg bw/day)	**Number of pregnant**	**Gestation length (days)**						**Gestation index (%)**
		22	**22.5**	**23**	**23.5**	**24**	**24.5**	
				n (%)				
F0								
0	22	0	9 (41)	12 (55)	1 (5)	0	0	100
3 (0.26)	20	1 (5)	9 (47)	8 (42)	1 (5)	0	0	95
10 (0.86)	18	0	3 (17)	11 (61)	2 (11)	0	2 (11)	100
30 (2.62)	20	1 (5)	5 (25)	10 (50)	4 (20)	0	0	100
100* (9.34)	20	0	2 (10)	10 (50)	7 (35)	1 (5)	0	100
F1								
0	21	0	8 (38)	11 (52)	2 (10)	0	0	100
3 (0.26)	17	0	7 (41)	10 (59)	0	0	0	100
10 (0.86)	18	1 (6)	7 (39)	10 (56)	0	0	0	100
30 (2.62)	19	0	6 (32)	12 (63)	1 (5)	0	0	100
Background control (%), Range, (Mean)[a]		0-24 (9)	14-62 (40)	16-73 (44)	0-35 (7)	0-4 (0.4)	0-5 (0.3)	95-100 (99)

*** Distribution significantly different from controls at p<0.01, a: consisted of 29 studies, n: number of animals in Category.**

The reduced number on F0 animals with a normal oestrus cycle, and the increased number of F1 animals with an irregular oestrus cycle at 10 ppm were statistically significantly different from controls at $p<0.05$. For the prolonged gestational length observed at 100 ppm the distribution was statistically significantly different from controls at $p<0.01$. None of the other observed effects were statistically significant.

Alterations in the oestrus cycle in F0 females manifested in percentages that were outside the historical control range. However, the relevance and reliability of provided historical control data (HCD) for the study was in general low.

The effects on general toxicity in this study were a statistically significant increase in absolute adrenal weight, and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams, observed at 100 ppm. There were no clinical signs of toxicity or reductions in body weight observed in the parental animals of the F0 generation.

For the assessment of developmental toxicity, all the available studies were considered supplementary. This was because the treatment period was shorter in comparison to the requirements of current test guidelines, i.e., OECD TG 414 (exposure GD 6 to 15 compared to current test guideline from GD 6- to the day prior to scheduled caesarean section). Also, several endpoints included in the 2018 version of these guidelines were not examined in the present studies.

Both the preliminary/range-finding teratology (Anonymous 24, 1989) and the main teratogenicity (Anonymous 41, 1990) studies in rats were GLP-compliant, but neither of them fully complied to standard test guidelines. The same held true for the tolerance (Anonymous 36, 1989), the preliminary teratology (Anonymous 5, 1989a), and the main teratogenicity (Anonymous 6, 1989b) studies in rabbit.

The two studies also used to assess effects on sexual function and fertility were described in the previous section. The effects relevant for development observed in the dose range-finding study in rats, and the two-generation study in rats, are summarised in the tables below.

***Table**: Developmental effects in the dose range-finding study in rats (fosthiazate treatment for 90days).*

Parameter	**Dose in ppm** (mg/kg bw/day)				
	0	**10** (0.68)	**30** (2.03)	**100** (7.09)	**300** (28.19)
Mean implantation sites (number of animals)	14.3 ± 3.8 (9)	14.9 ± 3.6 (10)	17.2 ± 2.3 (6)	14.1 ± 3.8 (10)	7.6 ± 4.3 (7)
Number of live litter born	9	10	6	10	6
Mean litter size on day 1 (number of animals)	12.8 ± 3.6 (9)	14.3 ± 3.5 (10)	15.3 ± 2.3 (6)	12.2 ± 2.6 (10)	6.2 ± 2.5 (5)
Mean litter size on day 4 pre culling (number of animals)	11.0 ± 4.3 (9)	12.8 ± 3.9 (10)	13.4 ± 2.2 (5)	3.8 ± 5.5 (4)	No surviving offspring
Post implantation survival (%)	89	96	89	87	58
Live birth index (%)	96	99	100	93	84
Viability index day 4 (%)	90	90	73	13	0
Lactation index on day 7/14/21 (%)	98/87/97	91/89/87	95/83/83	55/45/45	No surviving offspring

The effects relevant for development observed in the two-generation study are summarised in the table below. Values are for F1 animals, both sexes.

***Table**: Developmental effects of fosthiazate treatment in the two-generation study in rats.*

Parameter	**Dose in ppm** (mg/kg bw/day)				
	0	**3** (0.26)	**10** (0.86)	**30** (2.62)	**100** (9.34)
Implantation sites (mean ± SD)	14 ± 2.3	13.2 ± 4.7	13.4 ± 2.8	13.8 ± 3.5	12.9 ± 5.2
Post-implantation survival index (%)	86	90	84	90	83
Litter size at day 1 post-partum pups/litter	12	12.5	11.4	12.3	9.8 (82%)
Litter size at day 25 post-partum pups/litter	7.8	7.6	7.3	6.9* (88%)	4.6*** (59%)
Live birth index (%)	99	100	100	98	92***
Viability index at day 4 (%)	94	97	96	86**	44***
Birth weight	6.5	6.4	6.5	6.3	5.8** (89%)
Weight at weaning (day 25)	67.8	66.5	69.5	63.5	46.4*** (68%)
Lactation index at day 7/14/25	99/99/99	99/97/99	100/99/99	99/95/94	76***/52^{NT}/45***
Eye opening (onset/completion)	13.4/14.5	13.8/14.7	13.1/14.3	13.4/14.5	14.2*/14.9
Tooth eruption (onset/completion)	9.3/11.2	9.5/11.6	9.6/11.2	9.6/11.3	10.6**/11.9

Significantly different from controls at p<0.05. ** Significantly different from controls at p<0.01. Significantly different from controls at p<0.001. NT: Not tested for statistical significance.**

In the preliminary/range-finding teratology study in rats the animals were treated from (presumed) GD 6 to GD 15. The top dose was 10 mg/kg bw/day, additional doses were 5, 2.5, 1 and 0 mg/kg bw/day. Each group included 12 mated females. At 10 mg/kg bw/day maternal body weight gain was lower as compared to controls (significant at GD 16, $p<0.05$). No developmental effects were observed up to the top dose.

In the main teratogenicity study in rats the animals were treated from (presumed) GD 6 to GD 15. The top dose was 10 mg/kg bw/day, additional doses were 5, 2.5, 1 and 0 mg/kg bw/day. Each group included 24 mated females. At 10 mg/kg bw/day maternal body weight gain was lower as compared to controls (significant at GD 16 and 20, $p<0.001$). No developmental effects were observed up to the top dose.

In the tolerance study in rabbits, the animals were treated by oral gavage either in a staircase-manner where the dose was doubled every 2 days until adverse effect, or with a single dose continuously for 7 days. The starting dose in the staircase study was 1 mg/kg bw/day, and in the continuation study the dose was 4 mg/kg bw/day. Each group included two non-pregnant females.

At 8 mg/kg bw/day maternal bodyweight was lower as compared to controls, and they had evidence of gastrointestinal tract disturbance and ataxia. No developmental effects were analysed in this study.

In the preliminary teratology study in rabbits, the animals were treated by oral gavage from (presumed) GD 6 to GD 19. The top dose was 5 mg/kg bw/day, additional doses were 2.5, 2, 1 or 0 mg/kg bw/day. Each group included 7 artificially inseminated females. Effects on maternal toxicity were seen from 2 mg/kg bw/day, where three females were euthanized in extremis due to weight loss. In the top dose group of 5 mg/kg bw/day, increased respiration rate, ataxia and loss of muscular coordination were observed, and no females survived to term in this dose group.

The effects relevant for development observed in the preliminary teratology study in rabbits are summarised in the table below.

***Table**: Developmental effects of fosthiazate treatment from GD6 to GD19 in rabbits (preliminary teratology study).*

Foetal parameter	Dose (mg/kg bw/day)					
	0	1	2	2.5	Background control[1, 2]	
					Mean	Range
Number of foetuses examined at necroscopy (number of litters)	62 (7)	69 (7)	37 (5)	46 (4)		
Small foetus, <32 g. % incidence (number of litters)	18 (3)	7.2 (3)	24 (3)	13 (2)	11	2.7 – 22
Number of foetuses examined at skeletal examination (number of litters)	42 (7)	47 (7)	25 (5)	31 (4)		
Incomplete ossification of hyoid body, % (number of litters)	24 (5)	32 (6)	28 (4)	61 (4)	30	9.6 - 51

[1] Background data from 10 studies for litter data (% abortion loss – placental weight)

[2] Background data based on 7 studies, 606 foetuses for small foetuses and 15 studies, 1079 foetuses for incomplete ossification of hyoid body, no further information available except that it is background data on the same rabbit strain from the conducting laboratory.

In the main teratogenicity study in rabbits the animals were treated by oral gavage from (presumed) GD 6 to GD 19. The top dose was 2 mg/kg bw/day, additional doses were 1.5, 1,

0.5 or 0 mg/kg bw/day. Each group included 15 artificially inseminated females. No effects on maternal toxicity were observed.

The effects relevant for development observed in the main teratology study in rabbits are summarised in the table below.

***Table**: Developmental effects of fosthiazate treatment from GD6 to GD19 in rabbits (main teratogenicity study).*

Foetal parameter	Dose (mg/kg bw/day)						
	0	**0.5**	**1**	**1.5**	**2**	**Background control[1, 2]**	
						Mean	**Range**
Abortion and total litter loss, %	0	0	15	0	7.7	5.6	0 - 15
Pre-implantation loss, %	8.9	18	12	20	18	21	9.1 - 43
Post-implantation loss, %	13	8.1	8.6	12	8.5	13	7.3 – 22
Foetal weight, gram	40.8	42.3	41.5	42.9	38.6	41	38.5 – 45
Incidence of small foetus, <32 g, % (number of litters)	8.7 (6)	14 (6)	21 (6)	16 (5)	28 (7)	11	2.7 - 22

1 Background data from 10 studies for litter data (% abortion loss – placental weight)

2 Background data based on 7 studies, 606 foetuses for small foetuses and 15 studies, 1079 foetuses for incomplete ossification of hyoid body, no further information available except that it is background data on the same rabbit strain from the conducting laboratory.

Slight reductions in foetal birth weights (-5.4 %) and increased number of small foetuses (+19 %) were reported at 2 mg/kg bw/day.