CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

EC Number: -

CAS Number: 98886-44-3

Index Number: 015-168-00-0

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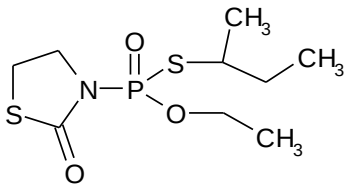
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	S-sec-butyl O-ethyl (2-oxo-1,3-thiazolidin-3-yl)phosphonothioate (*RS*)-[*S*-(*RS*)-*sec*-Butyl *O*-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphonothioate] or (3*RS*)-3-[(*RS*)-*sec*-Butylthio(ethoxy)phosphinoyl]-1,3-thiazolidin-2-one
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	Fosthiazate
EC number (if available and appropriate)	-
EC name (if available and appropriate)	-
CAS number (if available)	98886-44-3
Other identity code (if available)	CIPAC 585
Molecular formula	$C_9H_{18}NO_3PS_2$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	283.35 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 93

Fosthiazate is a racemic mixture of four stereoisomers. Due to the manufacturing process of fosthiazate, an equimolar ratio of the stereoisomers is expected.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Fosthiazate	≥ 93	Acute Tox. 3*; H301 Acute Tox. 4*; H312 Acute Tox. 3*, H331 Skin Sens. 1, H317	Acute Tox. 3; H301 Acute Tox. 3; H311 Acute Tox. 3, H331 Skin Sens. 1, H317

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
		Aquatic Acute 1, H400 Aquatic Chronic 1, H410 EUH070	Aquatic Acute 1, H400 Aquatic Chronic 1, H410 EUH070

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
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Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
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2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling according to the CLP criteria

Chemical name	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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					**Modify:** Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 **Add:** Eye Irrit. 2 Repr. 2 Lact. STOT SE 1 STOT RE 2	**Retain:** H331 H301 **Modify:** H311 **Add:** H319 H361fd H362 H370 (nervous system) H373 (adrenals)	**Add:** GHS08	**Retain:** H331 H301 **Modify:** H311 **Add:** H319 H361fd H362 H370 (nervous system) H373 (adrenals)	**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	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission					Repr. 2 Lact. Acute Tox. 3 Acute Tox. 3 Acute Tox. 3 STOT SE 1 STOT RE 2 Eye Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361fd H362 H331 H311 H301 H370 (nervous system) H373 (adrenals) H319 H317 H400 H410	GHS06 GHS08 GHS09 Dgr	H361fd H362 H331 H311 H301 H370 (nervous system) H373 (adrenals) H319 H317 H410	EU070	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	

Table 7: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	data conclusive but not sufficient for classification	**Yes**
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases		
Gases under pressure		
Flammable liquids	data conclusive but not sufficient for classification	**Yes**
Flammable solids	hazard class not applicable	No
Self-reactive substances	data lacking	**Yes**
Pyrophoric liquids	data conclusive but not sufficient for classification	**Yes**
Pyrophoric solids	hazard class not applicable	No
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids	hazard class not applicable	No
Organic peroxides	data conclusive but not sufficient for classification	**Yes**
Corrosive to metals	data lacking	**Yes**
Desensitised explosives	hazard class not applicable	No
Acute toxicity via oral route	Harmonised classification proposed	**Yes**
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Harmonised classification proposed	**Yes**
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation		
Germ cell mutagenicity		
Carcinogenicity		
Reproductive toxicity	Harmonised classification proposed	**Yes**
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure		
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Harmonised classification proposed	**Yes**
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Fosthiazate is an active substance approved in accordance Directive 91/414/EEC. In the CLP-regulation (EC) No 1272/2008 fosthiazate was introduced as Acute Tox 3* (oral), Acute Tox 4* (dermal), Acute Tox 3* (inhalation), Skin Sens. 1, Aquatic Acute 1, Aquatic Chronic 1. The approval expires on 31.10.2021. A Renewal Assessment Report (RAR) has been prepared by the DS and was submitted to EFSA on 30.09.2020.

In accordance with the alignment process with the renewal of the active substance under Regulation (EU) 1107/2009, it is necessary to prepare a targeted CLP report taking into account few new data and the re-evaluation of the existing data.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. However, it should be acknowledged that renewal of approval of fosthiazate as an active ingredient in plant protection products is under discussion in the EU.

5 IDENTIFIED USES

Fosthiazate is an organophosphate (Mode of Action Group 1B) with nematicidal and insecticidal activity. When applied to the soil, fosthiazate is systemic in plants with absorption occurring via the roots.

Registration in potatoes is for control of potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*) at an application rate of 30 kg/ha formulated product (10 % fosthiazate) to be applied immediately before planting. In some countries the product is registered as well for wireworm control (*Agriotes spp*.) at an application rate of 20 kg/ha formulated product (10 % fosthiazate).

Registration is also for tomato against root knot nematodes (Meloidogyne sp.) at a rate of 30 kg/ha formulated product (10 % fosthiazate). Single yearly application is recommended for the use on tomatoes.

6 DATA SOURCES

Main data source for the evaluation of the toxicological properties of fosthiazate were Volumes 1 and 3 of the revised Renewal Assessment Report (RAR) dated 22 September 2020, which was prepared for the pesticides procedure and submitted to EFSA but was not subject to peer review by MS authorities yet.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	clear liquid	Wojcieck (1994)	Visual assessment (Purity 93.6 %)
	clear liquid	Malone and Sweetapple (1995)	Visual Assessment (Purity 99.0 %)
	straw	Wojcieck (1994)	Munsell system (Purity 93.6 %)
	colourless	Malone and Sweetapple (1995),	Munsell system (Purity 99.0 %)
Melting/freezing point	No evidence of freezing or solidification detected by DSC when fosthiazate was cooled from 27 °C to -163 °C.	Malone and Sweetapple (1995)	Measured EC A.1 (capillary method) (Purity 99.0 %)
Boiling point	198.0 °C at 0.067 kPa.	Koyanagi (1989a)	Measured EC A.2 (Purity 92.2 %)
Relative density	1.234	Miyaji, 1989	Measured Method equivalent to EEC method A.3.
Vapour pressure	5.6 x 10^{-4} Pa (25 °C) 2.1 x 10^{-3} Pa (35 °C) 5.1 x 10^{-3} Pa (45 °C)	Kauppila and Lorence (1995)	Measured Gas saturation method (equivalent to EC A.4) (Purity 98.4 %)
	3.6 x 10–4 Pa (25 °C, calculated) 2.4 x 10–2 Pa (60 °C) 2.1 x 10–1 Pa (80 °C) 1.1 Pa (100 °C)	Katayama (1989b)	Measured Gas saturation method (equivalent to EC A.4) (Purity 98.8 %)
Surface tension	-		Study not acceptable
Water solubility	9.88 g/L at 25 °C (pH 5) 9.00 g/L at 25 °C (pH 7) 9.46 g/L at 25 °C (pH 9) There was evidence of instability of fosthiazate in solution at pH 7 and 9. After three days at pH 7 levels were reduced by ca 8 %. After seven days at pH 9 levels were reduced at ca 50 %.	Lorence and Yoder (1993)	Measured Shake flask method (equivalent to EC A.6) (Purity 99.3 %)
Partition coefficient n-octanol/water	At 25 °C log Kow: 1.68 The pH was not reported.	Lorence (1993)	Measured shake flask method (Purity 99.3 %)
	At 25 °C log Kow: 1.75 The pH was not reported.	Kanza (1989)	Measured shake flask method (Purity 98.8 %)
Flash point	Mean flash point was 139 °C (corrected to 1.01 kPa).	Wojcieck (1994)	Measured EC A.9 (closed cup) (Purity 93.6 %)
	Mean flash point was 127 °C	Kamiyama (1989)	Measured EC A.9 (closed cup)

Property	Value	Reference	Comment (e.g. measured or estimated)
			(Purity 94.8 %)
Flammability	Flammability stated to be not applicable since fosthiazate does not cause the rapid formation of heat or highly flammable gases on contact with water, as supported by data on water solubility, Kow, hydrolysis and oxidation data.	Malone and Sweetapple (1995)	Statement EC A.15 (Purity 93.6 %)
Explosive properties	Insensitive to shock. Friction sensitivity not required for liquids.	Muranaga (1989)	Measured EC A.14 (Purity 93.6 %)
	Insensitive to shock. Thermal sensitivity: refer to B.2.1.20 and B.2.1.3. Friction sensitivity not required for liquids.	Wojcieck (1994)	Measured EC A.14 (Purity 93.6 %)
Self-ignition temperature	Auto flammability: the minimum auto-ignition temperature was 256 °C at 99 kPa and a 56 second reaction delay time.	Malone and Sweetapple (1995)	Measured EC A.15 (Purity 93.6 %)
Oxidising properties	Fosthiazate technical is not an oxidising substance.	Cage (2014)	Statement EC A.21 (Purity 97.4 %)
Granulometry			data lacking
Stability in organic solvents and identity of relevant degradation products			data lacking
Dissociation constant	UV/VIS spectra of fosthiazate in aqueous buffer at pH 2, 4, 6 and 8 were similar. There was evidence of spectral shift for those at pH 10 and 12, which was accompanied by a 'foul' odour. The pH of these solutions was adjusted back to pH 6; the spectral shift remained. On this basis it has been stated that no pKa activity could be determined between pH 2 and 8; no pKa could be determined > pH 8 due to decomposition.	Gallacher (1992)	Measured OECD Test Guideline 112 (Purity 99.7 %)
Viscosity	80 cPs at 1.5 rpm 83 cPs at 3 rpm 83 cPs at 6 rpm	Wojcieck (1994)	measured

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC method A.14	Insensitive to shock. Not explosive		Wojcieck, 1994
EEC method A.14	Insensitive to shock. Thermal sensitivity. Not explosive		Wojcieck, 1994

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Fosthiazate was tested for explosive properties using the EC Method A.14 and was found not to be explosive.

8.1.2 Comparison with the CLP criteria

Method EC A.14 is not sufficient on its own to conclude on explosive properties.

Fosthiazate does not carry functional groups listed in tables A6.1 in Annex 6 (Screening Procedures) of the Manual of Tests and Criteria associated with explosive properties. Therefore, the acceptance procedure for explosives need not to be applied and Fosthiazate does not meet the criteria for classification as explosive.

8.1.3 Conclusion on classification and labelling for explosive properties

Data conclusive but not sufficient for classification.

Fosthiazate is not classified as explosive.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable.

8.3 Oxidizing gases

Hazard class not applicable.

8.4 Gases under Pressure

Hazard class not applicable.

8.5 Flammable liquids

Table 10: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
EC A.9 (closed cup)	Mean flash point was 139 °C (corrected to 1.01 kPa).		Wojcieck (1994)
EC A.9 (closed cup)	Mean flash point was 127 °C.		Kamiyama (1989)

8.5.1 Short summary and overall relevance of the provided information on 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.

8.5.2 Comparison with the CLP criteria

The CLP Regulation defines flammable liquids as a "liquid having a flash point of not more than 60 °C". In this case, the flash point is well above 120 °C.

Fosthiazate does not meet the criteria for classification as a flammable liquid.

8.5.3 Conclusion on classification and labelling for flammable liquids

Data conclusive but not sufficient for classification.

Fosthiazate is not classified as flammable liquid.

8.6 Flammable solids

Hazard class not applicable.

8.7 Self-reactive substances

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Fosthiazate does carry functional groups (P-O moiety) listed in tables A6.3 in Annex 6 (Screening Procedures) of the Manual of Tests and Criteria associated with self-reactive properties.

8.7.2 Comparison with the CLP criteria

According to CLP Regulation, self-reactive properties are tested using UN test series A to H; the hazard class can be assessed also based on the criteria in CLP Annex I, 2.8.4.2. Since no corresponding UN test results are available and Fosthiazate does contain the above mentioned groups, it cannot be excluded that the substance has self-reactive properties.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification due to lack of data.

8.8 Pyrophoric liquids

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Experience in manufacture and handling shows that the liquid does not ignite spontaneously on coming into contact with air at normal temperatures.

8.8.2 Comparison with the CLP criteria

According to the CLP Regulation, based on experience in handling, the classification procedure for pyrophoric liquids does not need to be applied.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Data conclusive but not sufficient for classification.

Fosthiazate is not classified as pyrophoric liquid.

8.9 Pyrophoric solids

Hazard class not applicable.

8.10 Self-heating substances

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

Fosthiazate is a liquid with a melting point below -163 °C.

8.10.2 Comparison with the CLP criteria

According to the CLP Guidance 2.11.4.2, Substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class. Therefore, Fosthiazate does not meet the criteria for classification as self-heating substance.

8.10.3 Conclusion on classification and labelling for self heating substances

Data conclusive but not sufficient for classification.

Fosthiazate is not classified as a self-heating substance.

8.11 Substances which in contact with water emit flammable gases

8.11.1 Short summary and overall relevance of the provided information on 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.

8.11.2 Comparison with the CLP criteria

Since the chemical structure of the substance does not contain metals or metalloids and since the experience in handling shows that the substance does not react with water, the classification procedure for this class need not be applied to Fosthiazate.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Data conclusive but not sufficient for classification.

Fosthiazate is not to be classified as substance, which in contact with water emits flammable gases.

8.12 Oxidising liquids

Table 11: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
EC A.21	Mean pressure rise time from 690 kPa to 2070 kPa (gauge) for 1:1 w/w mixture of 65% nitric acid and cellulose: 7.54 s. 1:1 w/w mixture of test substance and cellulose): 26.8 s. Not oxidising.		Cage (2014)

8.12.1 Short summary and overall relevance of the provided information on 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 RTGD MTC for oxidising liquids category 3 and the result is negative, Fosthiazate can be regarded as not oxidising.

8.12.2 Comparison with the CLP criteria

Fosthiazate does not meet the criteria for classification as an oxidising liquid.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Data conclusive but not sufficient for classification.

Fosthiazate is not classified as oxidising liquid.

8.13 Oxidising solids

Hazard class not applicable.

8.14 Organic peroxides

Fosthiazate does not contain the peroxide group (-O-O-) and therefore does not meet the criteria for classification as an organic peroxide.

Data conclusive but not sufficient for classification.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on substances which are corrosive to metals

No test data is available. Fosthiazate is a liquid with a low melting point.

8.15.2 Comparison with the CLP criteria

According to CLP, corrosivity to metals is tested using UN test series C.1. Since no corresponding UN test results are available and Fosthiazate is a liquid, it cannot be excluded that the substance is corrosive to metals.

8.15.3 Conclusion on classification and labelling for substances which are corrosive to metals

No classification due to lack of data.

8.16 Desensitised explosives

Fosthiazate does not meet the criteria for classification for desensitised explosives as it is not an explosive substance which is phlecmatised to suppress the explosive properties.

The hazard class is not applicable for classification and labelling purposes.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The metabolism of fosthiazate (RS)-S-sec-butyl O-ethyl 2-oxo-1,3-thiazolidin-3-yl phosphonothionate was investigated in the rat. The absorption, distribution, excretion and metabolism of fosthiazate was conducted using different labelling of fosthiazate:

- 14C in position 2 of the butyl chain (B-labelled)
- 14C in positions 4 and 5 of the thiazolidone ring (R-labelled)
- 14C in the carbonylcarbon of the thiazolidone ring (T-labelled)

In all the metabolism studies, corn oil was the solvent used for the administered test compounds.

Two sets of studies were conducted by Daiichi Pure Chemicals in Japan (1986-1990) and by Ricerca Inc in the US (1991).

Table 12: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Metabolism studies on IKI-1145 (Fosthiazate) metabolic fate in rats No guideline, No GLP	Absorption: Majority of an oral dose of either 2 or 20 mg/kg was absorbed. Single dose data showed that elimination via urine and expired air suggest the oral absorption of 88.4 % of the applied dose regardless of dose level, sex or label position. Data from a biliary excretion experiment were in agreement with this value. Metabolism: Samples of plasma, urine, bile and faeces obtained after oral administration of 2 or 20 mg/kg bw ^{14}C(T)-fosthiazate and ^{14}C(B)-fosthiazate were assayed for metabolites using thin layer chromatography and co-chromatography against 10 known standards including the plant metabolites TZO, DETO and DTTO. TZO, DETO were key metabolites in plasma and DETO, TZO and DTTO in urine of animals administered ^{14}C (T)-fosthiazate Results have not been confirmed in subsequent studies where the thiazolidone ring has been labelled, i.e. ^{14}C (R)-fosthiazate. Likewise after administration of ^{14}C (B)-fosthiazate, DETO and DTTO were identified in the urine of male and female rats. These findings were not confirmed in the subsequent study using ^{14}C (B)-fosthiazate.	Deviations: Tentative identification of a number of metabolites has been made based on chromatographic behaviour. Unequivocal identification of metabolites by mass spectroscopy has been made in subsequent studies. Supplementary	Anonymous 20 (1990)
Study to evaluate the distribution and excretion of 14C(B)-IKI-1145 in rats	Absorption: rapidly absorbed at dose levels of 2 and 20 mg/kg bw. Distribution/Excretion: rapid. Urine as major route of excretion. Faeces as a relatively minor route of elimination when compared to amounts in urine.	Acceptable	Anonymous 26, 1992a, amended 1994

Method	Results	Remarks	Reference
Comparable to OECD TG 417 (2010), GLP	Elimination also via the expired air, accounting for up to 9.5 % of the administered radioactivity. Only minor differences related to sex or dose level. Fosthiazate was not accumulated after 168 hours in any of the tissues analysed		
Study to Evaluate the Distribution and Excretion of 14C(B)-IKI-1145 in Rats Following Repeated Dosing Comparable to OECD TG 417 (2010), GLP	Absorption: Rapid. Recovery of radiolabel approx. 95 % of the administered dose. Distribution: At 24 hours, approximately 3.6 % of the administered dose present in blood, organs, and carcass; at 168 hours, approximately 1.5 % of the administered dose was present. At 24 hours, higher concentration of radiolabel in lung, kidney, liver and adrenals than in blood. At 168 hours, only in the adrenals a concentration equal to or greater than that in blood. Excretion: Urine as major route of excretion with 72 % of the administered dose. Approximately 9.6 % of the administered dose excreted in expired air and approximately 7 % of the administered dose eliminated in the faeces. Rapid excretion: complete in 24 hours. No major differences related to sex. Repeated dosing of fosthiazate at 2 mg/kg for 14 days had no effect on the absorption, metabolism, excretion, or distribution of ^{14}C (B)-fosthiazate as compared with a single dose study.	Acceptable	Anonymous 27, 1992b
Study to evaluate the distribution and excretion of 14C(R)-IKI-1145 in rats Comparable to OECD TG 417 (2010), GLP	Absorption: Recovery of radiolabel approx. 97 % of the administered dose. Distribution: About 6.73 % of the administered dose for the low dose group and 6.35 % of the administered dose for the high dose group was present in the carcass 168 hours after dosing. The liver of the low dose group contained 1.46 % of the administered dose; the liver of the high dose group contained 0.69 % of the administered dose. No other organ contained greater than about 0.1 % of the administered dose. No major differences related to sex or dose level were observed. Excretion: Urine was the major route of excretion with 69 % of the administered dose. Approximately 5.8 % of the administered dose was excreted in expired air as CO_2. Approximately 11.6 % of the administered dose was eliminated in faeces. About 8.9 % of the administered dose was retained in the carcass and tissues after 168 hours. Absorption, metabolism and excretion were very rapid. About 40.5 % of the administered dose was excreted in urine by 6 hours post-dosing; about 60.7 % of administered dose was excreted by 24 hours. An average of 1.07 to 2.02 % the administered dose was expired as CO2 by 4 hours post-dosing. Expiration of radiolabelled CO2 was about 79 % the administered dose complete by 24 hours. Elimination in faeces was nearly complete in 24 hours for the low dose group; in 48 hours for the high dose group.	Acceptable	Anonymous 28; 1992

Method	Results	Remarks	Reference
Study to measure the pharmacokinetics of 14C-(B)-IKI-1145 (14C-Fosthiazate) in the blood of rats Comparable to OECD TG 417 (2010), GLP	The median peak time for both sexes at both dose levels was 20 minutes. For the half-lives up to 12 hours, no difference related to sex was observed for either dose level. The half-lives were 4.9 and 5.1 hours for the low dose males and females. For the high dose, the values were 5.6 hours for the males and 6.1 hours for the females. The median peak concentration for the high dose group was about 7 times that for the low dose group. There were sex differences in the average peak concentration. The area under the curve (AUC) for the high dose group was about 11 times that of the low dose group. The ratio of the AUC values was proportionate to the ratio of the doses. At the time of peak concentration, the radioactivity in blood represented about 3 % of the administered dose for the low dose level group and about 2.2 % of the administered dose for the high dose group. By 168 hours postdosing, about 0.18 % of the administered dose was present in blood for both sexes at both dose levels.	Acceptable	Anonymous 31, 1993
Study to Evaluate Distribution and Excretion of 14C(R)-IKI-1145 (14C-Fosthiazate) in Rats Following Repeated Dosing Comparable to OECD TG 417 (2010), GLP	There were no clear differences in the distribution and excretion of radiolabel on the basis of sex or dose in this study. The major route of excretion was via urine. Between 69.1 and 75.8 % of the radiolabel in urine and faeces were excreted within 24 hours. Expired air contained approximately 3.1-5.1 % of the applied radiolabel at 24 hours. Excretion in excreta was essentially complete by 24 hours postdose. Individual tissues contained an average of greater than 0.10 % applied dose. Significant amounts of the radiolabel were present in some tissues and remained 7 days after final dose administration. There was a small reduction in radiolabel in muscle after 7 days compared with 24 hours that suggested a retention of the radiolabel in muscle tissue. Organs with greater amounts than in circulating blood after 7 days were the liver, bone and GI tract. Compared with the single dose study using the same radiolabel there were no distinct differences in the pattern of elimination or distribution of radiolabel in excreta or tissues.	Acceptable	Anonymous 30,1993
Study to Measure the Pharmacokinetics of 14C(R)-IKI-1145 in the Blood of Rats Comparable to OECD TG 417 (2010), GLP	The median peak time for both sexes at both dose levels was 1 hour. The median peak concentrations differed by dose level. The ratios of the concentrations were proportional to the dose ratio. The peak concentration, the radioactivity in blood is comparable to that in the single dose study. Half-life (t1/2) in blood was biphasic at both dose levels for both sexes. The t1/2 values for the β (peak time to 12 hours postdosing) and α (18 hours to 168 hours) phase did not differ between sexes at either dose level.	Acceptable	Anonymous 31, 1993
Study to identify the metabolites of 14C(B)-IKI-1145 in the urine of rats	77 % of the administered dose of ^{14}C-(T)-fosthiazate had been expired in air as $^{14}CO_2$, indicating that the ring was extensively metabolized. The data obtained from use of the B-labelled and R-labelled fosthiazate provided evidence for main processes, which	Acceptable	Anonymous 32, 1994

Method	Results	Remarks	Reference
Comparable to OECD TG 417 (2010), GLP	involved oxidation, methylation, hydrolysis and glutathione conjugation in the metabolism of fosthiazate and allowed for proposal of pathways specifically for metabolism of each label separately and an overall pathway for the metabolism of fosthiazate.		
Study to identify the metabolites of 14C(R)-IKI-1145 in the urine of rats Comparable to OECD TG 417 (2010), GLP	Fosthiazate is rapidly and extensively metabolized in rats. Metabolism appears to involve several parallel processes that include hydrolysis, oxidation, and methylation converting the fosthiazate to small fragments, including CO_2. The large very polar metabolic fraction is believed to represent further reactions of the labelled $–CH_2-CH_2$-fraction of the thiazolidine ring. The carbon atoms of the thiazolidine ring appear to be reincorporated into tissues. Extensive conjugation via the glutathione pathway appears to occur.	Acceptable	Magee, T. A.; Anonymous 33, 1994

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

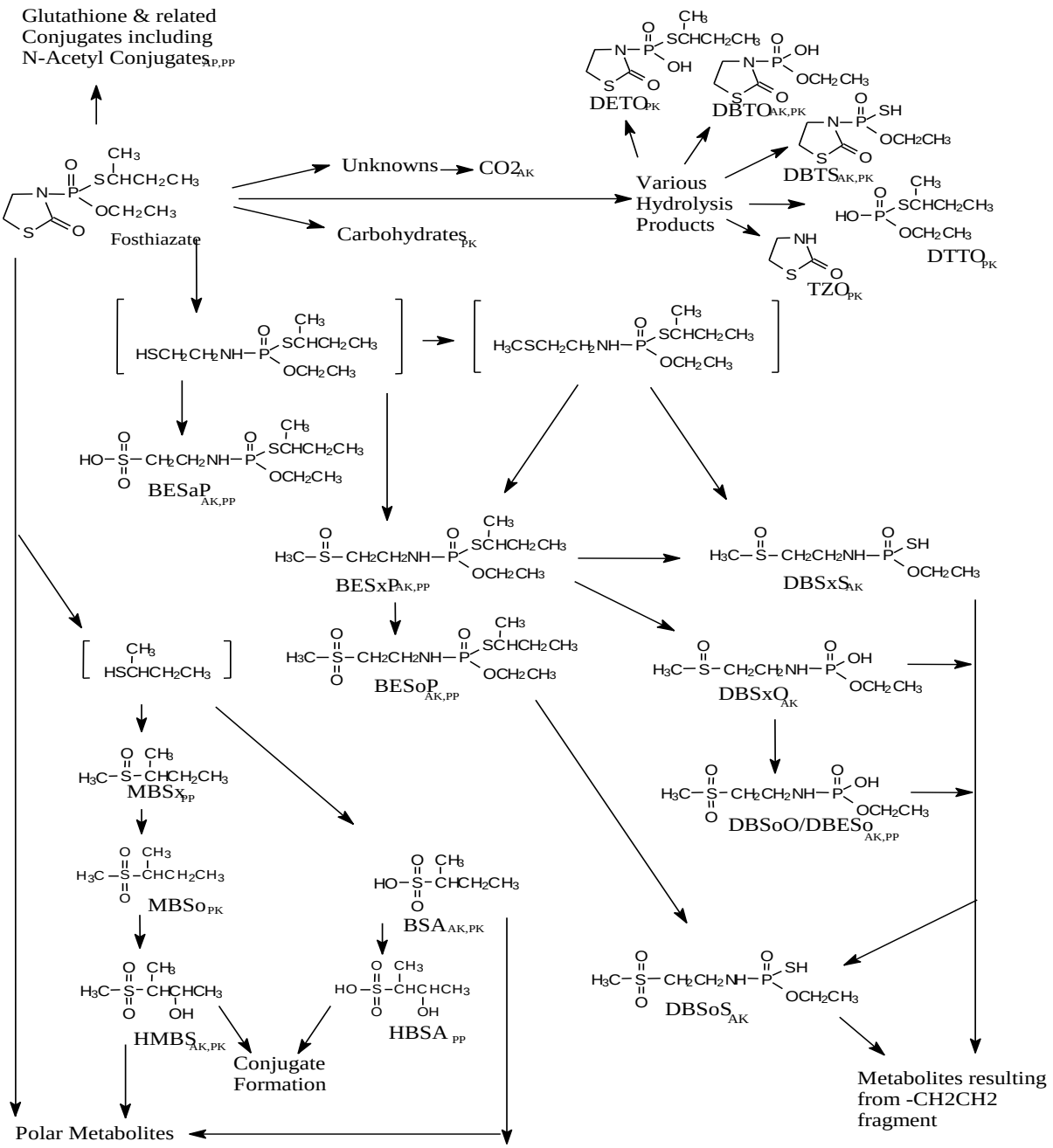
Studies on the metabolism of fosthiazate, with (RS)-S-sec-butyl O-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphonothioate (fosthiazate), labelled with 14C in position 2 of the butyl chain (B-labelled fosthiazate), in positions 4 and 5 of the thiazolidinone ring (R-labelled fosthiazate), in the carbonyl carbon of the thiazolidinone ring (T-labelled fosthiazate) and/or with 13C in positions 1 and 4 of the butyl chain were conducted because of the extensive nature of the metabolism of fosthiazate.

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

With one exception, urine is the major route for excretion of the metabolites of fosthiazate from an orally administered dose and, in general, contains at least 70 % of the administered dose regardless of sex, dose level and single or multiple dose administrations. The exception is that expired air is the major excretory route for the T-labelled test material (carbonyl carbon of the thiazolidinone ring is labelled with 14C) for which 70 % or more of the administered dose is expired as radiolabelled CO_2. Biliary excretion data indicated that there is extensive reabsorption of radiolabel from bile. Further metabolism of the absorbed biliary metabolites was followed by excretion in urine.

Absorption of radiolabel into blood is rapid. Peak concentrations of radiolabel are reached in blood 15 minutes to 2 hours after oral dose administration. The maximum concentrations in blood and the areas under the curve of blood concentration versus time are dose related and somewhat related to the position of the radiolabel in the test material. The elimination of radiolabel from blood is biphasic with an initial, rapid (α-phase) half-life followed by a subsequent, slower (β-phase) half-life. 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. In general, the pattern of distribution appears to be independent of sex, dose level, label position and whether a single or multiple doses are administered.

Fosthiazate is extensively metabolised in rats by a combination of hydrolytic and oxidative processes that rapidly convert the parent molecule to small fragments, including CO_2. The carbon atoms of the thiazolidine ring appear to be reincorporated into tissues based on the levels found in carcasses at termination. Extensive conjugation via the glutathione pathway appears to occur.



AK = known rat metabolite, PK = known plant metabolite, AP = proposed rat metabolite, PP = proposed plant metabolite; other metabolites are logical intermediates in the scheme.

Figure 1: Metabolism of fosthiazate in the rat

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

There is a current harmonised classification and labelling of fosthiazate as Acute Tox. 3* (H301), Acute Tox. 4* (H312), Acute Tox. 3* (H331). This is based on "translation" of a former classification as "Harmful" (Xn, R21, R41, R43) and "Toxic" (T, R23/25-39) to the Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation). Hence, the acute toxicity of fosthiazate is addressed in the present CLH dossier.

10.1 Acute toxicity - oral route

The acute oral toxicity of fosthiazate was investigated in two studies conducted in rat and mice. The studies are compiled in Table 13.

Table 13: Summary table of acute oral toxicity studies

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD50	Reference
Oral (gavage) Meets the essential criteria of 92/69/EEC Method B1. Body weights of 2 animals from the 64 or 81 mg/kg bw dose groups deviated significantly from their group mean body weight. The achieved concentrations of the tested dose formulations were 90% and 77% of the nominal values, 128 and 41 mg/kg bw respectively. GLP Acceptable	Rat, CD (remote Sprague-Dawley), 5M & 5 F	Fosthiazate (93.4 % purity)	nominal: 41, 51, 64, 81, 128 mg/kg bw in maize oil, single dose	M: 73 mg/kg bw F:57 mg/kg bw in females.	Anonymous 14, 1989b
Oral (gavage) Meets the essential criteria of 92/69/EEC Method B1 but uncertainty in concentration dosing solutions. GLP Supplementary	Mouse, CD-1 5M & 5 F	Fosthiazate (93.4 % purity)	nominal: 51, 81, 102, 128, 161 mg/kg bw in maize oil, single dose	M: 104 mg/kg bw F: 91 mg/kg bw in females.	Anonymous 13, 1989a

No human data on acute oral toxicity is available.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In the acute oral toxicity study by Anonymous 14, 1989b in rats deaths occurred at dose levels of ≥ 64 mg/kg bw within 3 days after treatment (see Table 14). 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. Deviations in test concentration were reported and the achieved concentrations of the tested dose formulations were 90 % (highest dose) and 77 % (lowest dose) of the nominal values. For other dose levels no information on achieved concentrations is available. Another deviation was a significant difference of the body weights of 2 animals from the 64 or 81 mg/kg bw dose in comparison to the group mean body weight. Nevertheless, the results of the study were considered to be valid, as the deviations didn't influenced the scope of the study.

Table 14: Mortality incidences in rats (oral exposure)

Dosage (mg/kg bw)	Male	Female	Combined
41	0/5	0/5	0/10
51	0/5	0/5	0/10
64	2/5	5/5	7/10
81	3/5	5/5	8/10
128	5/5	5/5	10/10

The acute oral LD50 of fosthiazate in the rat was 73 mg/kg bw (males), 57 mg/kg bw (females) or 65 mg/kg bw (both sexes).

In mice all the animals given dose of ≥102 mg/kg bw fosthiazate died within 4 days of treatment except 3/5 males at 128 mg/kg bw (see Table 15). At dose level of ≤ 81 mg/kg bw no signs of toxicity were reported. At ≥ 102 mg/kg bw decreased motor activity, hunched posture, ataxia and muscle tremor were observed. Less frequent observations were lethargy, irregular breathing, prone posture and closed eyes. The achieved dosage levels of the two dosages assayed, 51 and 161 mg/kg bw were 73 % and 70 % respectively of the nominal dose. Due to uncertainty of dosing solutions the study was considered as supplementary information.

Table 15: Mortality incidences in mice (oral exposure)

Dosage (mg/kg bw)	Male	Female	Combined
51	0/5	0/5	0/10
81	0/5	0/5	0/10
102	5/5	5/5	10/10
128	2/5	5/5	7/10
161	5/5	5/5	10/10

The acute oral LD50 of fosthiazate in mice was 104 mg/kg bw in males and 91 mg/kg bw in females.

10.1.2 Comparison with the CLP criteria

Table 16 presents the result of the acute oral toxicity studies conducted in mice and rats in comparison with the CLP criteria for oral acute toxicity.

Table 16: Results of acute oral toxicity in comparison with CLP criteria

Toxicological result	CLP criteria
Oral LD_{50}, rat: 73/57 mg/kg bw (m/f) Oral LD_{50}, mouse: 104/91 mg/kg bw (m/f)	Cat 4 (H302): 300 < LD_{50} ≤ 2000 mg/kg (oral) **Cat. 3 (H301): 50 < LD_{50} ≤ 300 mg/kg (oral)** Cat. 2 (H300): 5 < LD_{50} ≤ 50 mg/kg (oral) Cat. 1 (H300): LD_{50} ≤ 5 mg/kg (oral)

The acute oral LD_{50} of fosthiazate in rats was 73 mg/kg bw in male and 57 mg/kg bw in female animals, the

acute oral LD_{50} of fosthiazate in mice was 104 mg/kg bw in male and 91 mg/kg bw in female animals. According to CLP Regulation the acute toxicity estimate is derived using the LD_{50}. Since the lowest LD_{50} is 57 mg/kg bw for female rats, the ATE (oral exposure) for fosthiazate can be set at 57 mg/kg bw. Based on the available data fosthiazate meets the criteria to be classified for acute oral toxicity (Acute Tox. 3, H301).

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification as Acute Tox. 3 (H301) is considered appropriate. The proposed ATE value (oral exposure) is 57 mg/kg bw.

10.2 Acute toxicity - dermal route

The acute dermal toxicity of fosthiazate was investigated in one study conducted in rats. The study is compiled in Table 17.

Table 17: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
Dermal Meets the essential criteria of 92/69/EEC Method B3. GLP Acceptable	Rat, CD, 5M & 5 F	Fosthiazate (93.4 % purity)	M: 1965, 2472, 3115, 3918 mg/kg bw, F: 309, 494, 779, 1236, 1557, 1965, 2472 mg/kg bw	M: 2396 mg/kg bw F:861 mg/kg bw in females.	Anonymous 15

No human data on acute dermal toxicity available.

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In the acute dermal toxicity study by Anonymous 15, 1989c mortality occurred within 8 days at dose levels of ≥ 1965 mg/kg bw in males and ≥ 779 mg/kg bw in females. At dose levels of ≥ 3115 mg/kg bw in males and ≥ 1236 mg/kg bw in females, all animals died. 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. Body weight loss was observed in most surviving animals during the first week. Post-mortem examination of the animals which died showed general fur staining, altered gastrointestinal contents, isolated cases of uterine distension, darkened lungs, darkened glandular stomach mucosa and dark encrustation at the treatment site.

10.2.2 Comparison with the CLP criteria

Table 18: Results of acute dermal toxicity in comparison with CLP criteria

Toxicological result	CLP criteria	
Dermal LD_{50}, rat: 2396/861 mg/kg bw (m/f)	Cat. 4 (H312):	$1000 < LD_{50} \leq 2000$ mg/kg (dermal)
	Cat. 3 (H311):	**$200 < LD_{50} \leq 1000$ mg/kg (dermal)**
	Cat. 2 (H310):	$50 < LD_{50} \leq 200$ mg/kg (dermal)
	Cat. 1 (H310):	$LD_{50} \leq 50$ mg/kg (dermal)

Table 18 presents the result of the acceptable dermal toxicity study (Anonymous 15, 1989c) in comparison with the CLP criteria for dermal acute toxicity.

After acute dermal exposure, the treated skin sites were not affected. The acute dermal LD50 of fosthiazate in rats was 2396 mg/kg bw in male and 861 mg/kg bw in female animals. According to CLP Regulation the acute toxicity estimate is derived using the LD_{50}. Since the lowest LD_{50} is 861 mg/kg bw for female rats, the ATE (dermal exposure) for fosthiazate can be set at 861 mg/kg bw. Based on the available data fosthiazate meets the criteria to be classified for acute toxicity (Acute Tox. 3, H311).

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Classification as Acute Tox. 3 (H311) is considered appropriate for dermal effects. The proposed ATE value (dermal exposure) is 861 mg/kg bw.

10.3 Acute toxicity - inhalation route

The acute toxicity of fosthiazate via inhalation was investigated in one study conducted in rats. The study is compiled in Table 19.

Table 19: Summary table of acute toxicity study in rats, route: inhalation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LC_{50}	Reference
Inhalation (nose-only) In accordance with US EPA FIFRA Pesticide Assessment guidelines and satisfies the essential criteria of 92/96/EEC. GLP Acceptable	Rat, Sprague-Dawley, 8M & 8 F	Fosthiazate (92 % purity)	0, 0.53, 0.8, 0.9 and 1.23 mg/L; mass median aerodynamic diameter (MMAD) ranged from 2.04-2.29 µm 4 hours exposure to an aerosol of fosthiazate	M: 0.83 mg/L F: 0.56 mg/L	Anonymous 21, 1989

10.3.1 Short summary and overall relevance of the provided information on toxicity via inhalation

At all test dose levels mortality occurred (see Table 20) within 2 days (males) or 4 days (females). 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. Necropsy of the rats which died showed hyperaemia and/or haemorrhage in the lung, stomach filled with gas, haemorrhage of the mucous membrane in the glandular stomach and cloudy eyeball.

Table 20: Mortality incidences in treated rats (after exposure via inhalation)

Analytical concentration (mg/L)	Males	Females
0 (air control)	0/8	0/8
0.53	1/8	4/8
0.80	3/8	5/8
0.90	5/8	8/8
1.23	7/8	8/8

The median lethal concentration (LC_{50}) of fosthiazate in the rat was 0.83 mg/L in male rats and 0.56 mg/L in female rats.

10.3.2 Comparison with the CLP criteria

Table 21 presents the result of the acute inhalation toxicity study in comparison with the CLP criteria for acute toxicity via inhalation.

Table 21: Results of acute inhalation toxicity in comparison with CLP criteria

Toxicological result	CLP criteria	
Inhalation LC_{50}, rat: 0.83/0.56 mg/L (nose-only, aerosol, 4-h)	Cat. 4 (H332):	$10.0 < LC_{50} \leq 20.0$ mg/L (vapours)
		$1.0 < LC_{50} \leq 5.0$ (dusts and mists)
	Cat. 3 (H331):	$2.0 < LC_{50} \leq 10.0$ mg/L (vapours)
		$0.5 < LC_{50} \leq 1.0$ (dusts and mists)
	Cat. 2 (H330):	$0.5 < LC_{50} \leq 2.0$ mg/L (vapours)
		$0.05 < LC_{50} \leq 0.5$ (dusts and mists)
	Cat. 1 (H330):	$LC_{50} \leq 0.5$ mg/L (vapours)
		$LC_{50} \leq 0.05$ (dusts and mists)

The acute LC_{50} of fosthiazate via the inhalation route in rats was 0.83 mg/L in male and 0.56 mg/L in female animals. According to CLP Regulation the acute toxicity estimate is derived using the LC_{50}. Since the lowest LC_{50} is 0.56 mg/L for female rats, the proposed ATE (exposure via inhalation; dusts and mists) for fosthiazate is 0.56 mg/L. Based on the available data fosthiazate meets the criteria to be classified for acute toxicity (Acute Tox. 3, H331) via the inhalation route.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification as Acute Tox. 3, H331 is considered appropriate. The proposed ATE value (inhalation route; dusts and mists) is 0.56 mg/L

10.4 Skin corrosion/irritation

This endpoint is not addressed in this CLH report and is outside the scope of the consultation.

10.5 Serious eye damage/eye irritation

The serious eye damage/eye irritation potential of fosthiazate has been investigated in two studies. The studies are compiled in

Table 22.

Table 22: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
- Observations and time point of onset
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>Comparable to 92/69/EEC Method B5
GLP
Acceptable</td><td>6 (4 male and 2 female) albino New Zealand White rabbits</td><td>Fosthiazate, 0.1 mL (93.4 % purity)</td><td>1, 24, 48, 72 hours and 7 days</td><td>Eye irritation scores (mean scores reported at 24h, 48h, 72h) in rabbit
<table>
<tr><th>Animal</th><th>Corneal opacity</th><th>Iritis</th><th>Conjunctival redness</th><th>Conjunctival oedema (chemosis)</th></tr>
<tr><td>24TN 675M</td><td colspan="4">Animal killed in extremis after 5 h</td></tr>
<tr><td>24TN 676M</td><td>0</td><td>0.3</td><td>2</td><td>0.3</td></tr>
<tr><td>24TN 677M</td><td colspan="4">Animal found dead after 5 h</td></tr>
<tr><td>24TN 688F</td><td>0</td><td>0</td><td>2.3</td><td>0</td></tr>
<tr><td>24TN 705M</td><td colspan="4">Animal killed in extremis after 5 h</td></tr>
<tr><td>24TN 715F</td><td>0</td><td>0</td><td>2.6</td><td>0</td></tr>
</table>
Score for conjunctival redness ≥ 2 in the 3 surviving animals</td><td>Anonymous 17, 1989e</td></tr>
<tr><td>Comparable to 92/69/EEC Method B5 with deviation: washing of the eyes 2-3 minutes after instillation instead after approx. 30 minutes
GLP
Supplementary</td><td>3 female albino New Zealand White rabbits</td><td>Fosthiazate, 0.1 mL, (93.4% purity)</td><td>1, 24, 48, 72 hours and 7 days</td><td>Eye irritation scores (mean scores reported at 24 h, 48h, 72h) in rabbits
<table>
<tr><th>Animal</th><th>Corneal opacity</th><th>Iritis</th><th>Conjunctival redness</th><th>Conjunctival oedema (chemosis)</th></tr>
<tr><td>24TN 738F</td><td>0</td><td>0</td><td>2</td><td>0</td></tr>
<tr><td>24TN 739F</td><td>0</td><td>0</td><td>1.3</td><td>0</td></tr>
<tr><td>24TN 740F</td><td>0.6</td><td>0</td><td>1.3</td><td>0.3</td></tr>
</table>
score for conjunctival redness ≥ 1.3 in all treated animals</td><td>Anonymous 18, 1989f</td></tr>
</table>

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The serious eye damage/eye irritation potential of fosthiazate has been investigated in two studies by Anonymous 17 and 18, 1989e and 1989f (0.1 mL of fosthiazate, 93.4 % purity) was instilled into the conjunctival sac of the right eye of albino New Zealand White rabbits. In the first study fosthiazate was toxic by the ocular route to the rabbit: one male animal was found dead and further two males showed signs of systemic toxicity (reduced activity, prone position, muscle tremor, respiratory distress and/or pupil constriction) and were killed in extremis. Necropsy showed multiple dark punctate foci and areas of change on the thymus of two rabbits and multiple dark areas on all lobes of the lungs of the rabbits. The surviving male rabbit showed irritation of the eye with a mean score for conjunctival redness of 2, slight iritis (score 0.3)

and chemosis (score: 0.3). Also in the two female rabbits conjunctival redness, (score of 2.3 and 2.6) was reported. The effects had resolved 7 days after treatment. The second study (1989) was a supplement for the first study and was conducted with the deviation of washing the eyes already after 2-3 minutes after instillation. Due to this deviation the study is considered as supplementary for classification and labelling. No mortalities were observed. For the three female animals signs of eye irritation were reported with a mean score for conjunctival redness of ≥ 1.3. One of the females also showed slight corneal opacity (score: 0.6) and chemosis (score: 0.3). All ocular lesions had resolved by day 8.

10.5.2 Comparison with the CLP criteria

Table 23 presents the results of the acceptable eye damage/eye irritation study (Anonymous 17, 1989e) in comparison with the CLP criteria for eye irritation.

Table 23: Results of serious eye damage/eye irritation test in comparison with CLP criteria

Toxicological result	CLP criteria
Mean score (24-72 h): corneal opacity: no animal ≥ 1 iris lesion: no animal ≥ 1 conjunctival redness: 3 animal ≥ 2 oedema of the conjunctivae (chemosis): no animal ≥ 2 all ocular effects had fully resolved by day 8 after treatment	Irritating to eyes (Category 2, H319): at least in 2/3 tested animal a positive response of: corneal opacity: ≥ 1 and/or iritis: ≥ 1 and/or **conjunctival redness: ≥ 2** and/or conjunctival oedema (chemosis): ≥ 2 Calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within an observation period of 21 days.

The study gave positive response in three of three surviving (out of six) animals with a score of ≥ 2 for conjunctival redness. Based on the available data fosthiazate meets the criteria to be classified as Eye Irrit. 2 (H319).

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Classification as Eye Irrit. 2, H319 is considered appropriate for eye effects.

10.6 Respiratory sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the consultation.

10.7 Skin sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the consultation.

10.8 Germ cell mutagenicity

This endpoint is not addressed in this CLH report and is outside the scope of the consultation.

10.9 Carcinogenicity

This endpoint is not addressed in this CLH report and is outside the scope of the consultation.

10.10 Reproductive toxicity

10.10.1Adverse effects on sexual function and fertility

The reproductive toxicity of fosthiazate was assessed in a two-generation study and a range-finding study in rats. Results of these studies are summarised in Table 24. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in the text below. For additional information, reference is made to Volume 3 Chapter B.6 of the RAR.

Table 24: Summary table of animal studies on adverse effects on sexual function and fertility

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Dose range-finding generation study
(5 month)

Range-finding study, no guideline
Oral (dietary)
Rat, CD, groups of 10 M and 10 F
GLP
Supplementary</td>
<td>Fosthiazate (93.3 % purity)
Doses: 0, 10, 30, 100 or 300 ppm.
Equivalent to: Males: 0, 0.68, 2.03, 7.09 and 22.64 mg/kg bw/day; females: 0, 0.81, 2.48, 8.93 and 28.19 mg/kg bw/day
90 days of treatment prior to mating.
Observations on offspring from day 1 after birth. On day 4 post-partum, litter size was reduced to 8 (4 males and 4 females) where possible. Offspring culled at day 4 were killed and grossly</td>
<td>Parental toxicity:
≥ 30 ppm: isolated clinical signs of toxicity in F0 (tremor, hunched posture, hair loss),
≥ 100 ppm: maternal bw gain↓, inactive mammary tissue

Effects on sexual function and fertility: 300 ppm: number of pregnant animals↓, number of implantation sites↓, litter size↓,
Summary of selected findings
<table>
<tr><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><th></th><th>0</th><th>10</th><th>30</th><th>100</th><th>300</th></tr>
<tr><td>Mean body weight of females before pairing (F0), week 0</td><td>148</td><td>147</td><td>146</td><td>149</td><td>148</td></tr>
<tr><td>Mean body weight of females before pairing (F0), week 1</td><td>177</td><td>175</td><td>179</td><td>174</td><td>151 (-15%)</td></tr>
<tr><td>Mean body weight of females before pairing (F0), week 6</td><td>264</td><td>265</td><td>271</td><td>272</td><td>247 (-6%)</td></tr>
<tr><td>Mean body weight of females before pairing (F0), week 13</td><td>305</td><td>303</td><td>322</td><td>317</td><td>293 (-4%)</td></tr>
<tr><td>Number of mated animals [a]</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Percentage mating</td><td>100</td><td>100</td><td>100</td><td>100</td><td>100</td></tr>
<tr><td>Number pregnant</td><td>9</td><td>10</td><td>6</td><td>10</td><td>7</td></tr>
<tr><td>Conception rate (%)</td><td>90</td><td>100</td><td>60</td><td>100</td><td>70</td></tr>
<tr><td>Fertility index (%)</td><td>90</td><td>100</td><td>60</td><td>100</td><td>70</td></tr>
<tr><td>Mean bodyweight of females, day 0 post coitum</td><td>305</td><td>304</td><td>325</td><td>315</td><td>283[NS]</td></tr>
<tr><td>Mean bodyweight of females, day 6 post coitum</td><td>339</td><td>333</td><td>357</td><td>343</td><td>295</td></tr>
<tr><td>Mean bodyweight of females, day 13 post coitum</td><td>381</td><td>370</td><td>397</td><td>375**</td><td>311</td></tr>
<tr><td>Mean bodyweight of females, day 20 post coitum</td><td>439</td><td>440</td><td>473</td><td>435</td><td>338***</td></tr>
</table>
</td>
<td>Anonymous 25, 1990</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>examined for abnormalities. Litters surviving to day 21 were also killed and necropsied.</td><td>
<table>
<tr><td>Mean bodyweight of females, day 1 post partum</td><td>343</td><td>336</td><td>352</td><td>338</td><td>270**</td></tr>
<tr><td>Mean implantation sites (number of animals)</td><td>14.3 ± 3.8 (9)</td><td>14.9 ± 3.6 (10)</td><td>17.2 ± 2.3 (6)</td><td>14.1 ± 3.8 (10)</td><td>7.6 ± 4.3 (7) (53%)</td></tr>
<tr><td>Mean litter size (number of animals) on day 1</td><td>12.8 ± 3.6 (9)</td><td>14.3 ± 3.5 (10)</td><td>15.3 ± 2.3 (6)</td><td>12.2 ± 2.6 (10)</td><td>6.2 ± 2.5 (5) (48%)</td></tr>
<tr><td>Mean litter size (number of animals) on day 4 pre culling</td><td>11.0 ± 4.3 (9)</td><td>12.8 ± 3.9 (10)</td><td>13.4 ± 2.2 (5)</td><td>3.8 ± 5.5 (4)</td><td>No surviving offspring</td></tr>
</table>
[a]: following pairing and check for copulation plugs vaginal smears were prepared to prove mating by presence of spermatozoa, NS: bodyweight not significantly different from Controls (Student’s t-test), ** Bodyweight change day 0 to day 13 and at day 1 post partum significantly different from controls, P < 0.01 (Student’s t-test), ** * Bodyweight change day 0 to day 21 significantly different from controls, P < 0.01 (Student’s t-test)
</td><td></td></tr>
<tr><td>Multigeneration study
Oral (dietary)
Rats, CD, groups of 25 male and 25 female
Method is comparable to OECD Test Guidelines 416 (26 May 1983; 22 January 2001 and satisfies the essential requirements of EC guidelines for a multigeneration study (87/302/EEC Part B)
Stability in the vehicle was not</td><td>Fosthiazate (93.3% purity)
0, 3, 10, 30 or 100 ppm. Equivalent to: Males: 0, 0.21, 0.69, 2.09 and 7.21 mg/kg bw/day, females: 0, 0.26, 0.86, 2.62 and 9.34 mg/kg bw/day
Six-week-old F0 rats received fosthiazate for at least 99 days before mating, throughout mating, gestation and the lactation periods. Four</td><td>Parental toxicity:
100 ppm: absolute adrenal weight↑ and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams,
Effects on sexual function and fertility:
≥ 10 ppm: altered oestrus cycle, 100 ppm: gestation length prolonged

Summary of parental findings, general toxicity
<table>
<tr><th></th><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><td>Parameter</td><td>Sex</td><td>0</td><td>3</td><td>10</td><td>30</td><td>100</td></tr>
<tr><td>F0 generation</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Total feed intake, g (week 1-14)</td><td>F</td><td>1982</td><td>2020</td><td>2061</td><td>2117**</td><td>2266***</td></tr>
<tr><td>Body weight gain, g (week 1-14)</td><td>F</td><td>303</td><td>301</td><td>314</td><td>319</td><td>329**</td></tr>
<tr><td>Mean compound intake, mg/kg bw/day</td><td>M
F</td><td>0
0</td><td>0.21
0.26</td><td>0.69
0.86</td><td>2.09
2.62</td><td>7.21
9.34</td></tr>
<tr><td>Absolute adrenal weight in F0, g</td><td>F</td><td>0.062</td><td>0.070</td><td>0.071* (114%)</td><td>0.073** (117%)</td><td>0.077** (124%)</td></tr>
<tr><td>Terminal body weight</td><td>F</td><td>335</td><td>337</td><td>348</td><td>361</td><td>367*</td></tr>
<tr><td>F1 generation</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Mean compound intake, mg/kg bw/day</td><td>M
F</td><td>0
0</td><td>0.27
0.31</td><td>0.88
1.02</td><td>2.70
3.14</td><td></td></tr>
</table>
</td><td>Anonymous 34, 1990</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
performed in this study. Analysis of the batch performed in another study at the same laboratory indicated that the test material in rodent diet was stable when stored a 4°C for at least 7 days and at room temperature for up to 4 days. No sperm parameters were analyzed. Mammary glands not evaluated. GLP Acceptable	days after birth the F1 litters were randomly culled to 8 pups/litter (4 males and 4 females) where possible. The F0 dams were allowed to rear the F1 pups before weaning on day 25 post-partum. At 4 weeks old, 25 weanlings/sex were randomly selected from the 0, 3, 10 and 30 ppm dose groups to constitute the F1 parents. The F1 parents were treated for 14 weeks and mated to produce the F2 pups.	* Significantly different from controls at p<0.05, ** Significantly different from controls at p<0.01, *** Significantly different from controls at p<0.001 Summary of findings on sexual function and fertility	

Parameter	Sex	Dose (ppm) 0	3	10	30	100	Historical Controls (%), Range (Mean) [b]
F0							
Number of F_0 with a normal (4 or 5 day) oestrus cycle (% females per group)	F	20 (80)	20 (80)	13* (52)	14 (56)	15 (60)	63-100 (86)
Irregular oestrous cycle (% females per group), F0	F	3 (12)	4 (16)	7 (28)	5 (20)	4 (16)	0-17 (6)
Extended oestrous cycle (% females per group), F0	F	0	1 (4)	4 (16)	3 (12)	0	0-7 (2)
Acyclic/pseudo-pregnant (% females per group), F0	F	2 (8)	0	1 (4)	3 (12)	6 (24)	0-25 (6)
Number mating[a]	F	24	25	25	23	23	
Percentage mating	F	96	100	100	92	92	90-100 (98)
Number pregnant	F	22	20	18	20	20	
Conception rate (%)	F	92	80	72	87	87	72-100 (88)
Fertility index (%)	F	88	80	72	80	80	67-100 (86)
Implantation sites (mean ± SD)	F	14 ± 2.3	13.2 ± 4.7	13.4 ± 2.8	13.8 ± 3.5	12.9 ± 5.2 (92%)	
Litter size at day 1 post-partum pups/litter (mean ± SD)	M/F	12 ± 3	12.5 ± 2.9	11.4 ± 3.5	12.3 ± 3.7	9.8 ± 4.6 (82%)	
F1							
Number of F_1 with a normal (4 or 5 day) oestrus cycle (% females per group)	F	18 (72)	22 (88)	17 (68)	22 (88)		63-100 (86)
Irregular oestrous cycle (% females per group), F1	F	1 (4)	1 (4)	7* (28)	0		0-17 (6)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Extended oestrous cycle (% females per group), F1	F	0	1 (4)	1 (4)	0		0-7 (2)
Acyclic/pseudo-pregnant (% females per group), F1	F	6 (24)	1 (4)	0	3 (12)		0-25 (6)
Number mating	F	25	23	23	25		
Percentage mating	F	100	92	92	100		90-100 (98)
Number pregnant	F	21	17	18	19		
Conception rate (%)	F	84	74	78	76		72-100 (88)
Fertility index (%)	F	84	68	72	76		67-100 (86)
Implantation sites (mean ± SD)	F	13.5 ± 4.2	12.8 ± 2.5	13.4 ± 3.1	13.9 ± 3.8		
Litter size at day 1 post-partum pups/litter (mean ± SD)	M/F	11.4 ± 3.7	12 ± 2.5	11.6 ± 2.9	12.1 ± 3		

[a]: following pairing and check for copulation plugs vaginal smears were prepared to prove mating by presence of spermatozoa, * 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, [b]: background control (30 studies for mating performance and fertility, 18 studies for oestrus data)

Summary of gestation length and index in F0 and F1 females

Diet Level (ppm)	Number Pregnant		Gestation Length (days)						Gestation index (%)
			22	22.5	23	23.5	24	24.5	
F0									
0	22	n (%)	0	9 (41)	12 (55)	1 (5)	0	0	100
3	20	n (%)	1 (5)	9 (47)	8 (42)	1 (5)	0	0	95
10	18	n (%)	0	3 (17)	11 (61)	2 (11)	0	2 (11)	100
30	20	n (%)	1 (5)	5 (25)	10 (50)	4 (20)	0	0	100
100**	20	n (%)	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	17		0	7 (41)	10 (59)	0	0	0	100
10	18		1 (6)	7 (39)	10 (56)	0	0	0	100
30	19		0	6 (32)	12 (63)	1 (5)	0	0	100

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		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	

No human data on adverse effects on sexual function and fertility of fosthiazate are available.

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In a range finding study (Anonymous 25, 1990) rats were dosed up to levels of 300 ppm (22.64 mg/kg bw/day in males, 28.19 mg/kg bw/day in females) and potential effects of fosthiazate on sexual function and fertility were analysed in the parental generation only. At the highest dose signs of toxicity (moderate tremors, hunched posture, hair loss) were observed in females during the first three weeks and tremors recurred after week 16 of treatment in addition to one female showing tremors from the 30 ppm dose group and two females from the 100 ppm dose group. While no marked difference in mean bodyweight gain during the pre-mating period was observed in females, body weight gain was statistically significant reduced during gestation on day 13 post coitum in the 100 ppm group (60 g vs 76 g in ctrl) and on day 21 post coitum in the 300 ppm group (55 g vs 134 g in ctrl). Body weight on day 1 of lactation was significantly reduced in th 300 ppm group (338 g vs 439 g in controls). Reductions in litter size or weight of offspring might contribute to reduced body weight development during gestation. As litter size and weight of offspring was not affected in the 100 ppm group, the reduced body weight seems to be a sign of general toxicity. However, the more pronounced reduction in body weight gain at 300 ppm might be additionally attributable to a reduced litter size (mean litter site day 1: 6.2 vs 12.8 in ctrl; birth weight not affected). The number of pregnant animals was reduced at 30 and 300 ppm: 6 and 7 out of 10 mated animals were pregnant, respectively. As all mated dams were pregnant at 100 ppm a substance related effect is unlikely. The number of implantation sites was markedly reduced at 300 ppm: 7.6 vs 14.3 mean implantation sites in the control group. Observed general toxicity in terms of clinical signs at this dose level didn't influence the mating behaviour (percentage mating: 100 % in all groups) and is not expected to influence the number of implantation sites. Before pairing no significant effect on body weight has been observed. Overall, the observed parental toxicity is not considered to be marked enough to impact sexual function/fertility. Further observed effects on post-implantation survival, live birth index, viability and lactation index, which are relevant for developmental toxicity and/or effects on or via lactation, are assessed in the respective sections (i.e. 10.10.4 & 10.10.8).

Under the conditions of the multigeneration study by Anonymous 34, 1990, no clinical signs of toxicity or reductions in body weight were observed in the parental animals of the F0 generation. In contrast, food intake and body weight gain (also during gestation) were increased in high dose females (100 ppm, corresponding to 9.34 mg/kg bw/d). Food intake was already increased at 30 ppm. Absolute adrenal weights were significantly increased, starting at a dose of 10 ppm in F0-females up to + 24 % at the top dose of 100 ppm and accompanied by an increase of hypertrophy in the zona glomerulosa at the top dose. Oestrus cycle was altered in F0-females and the percentage of females with normal oestrus cycle was lower (no dose-response) in the dose groups of 10, 30 and 100 ppm: 52-60 % showed a normal cycle in these treatment groups while 80 % of the control females showed a normal cycle. Provided historical control data showed a range of 63-100 % for this parameter and a mean of 86%. At 30 and 100 ppm the percentage of acyclic/pseudo-pregnant females was increased: 12 and 24 % respectively. These values were within the range of HCD (0-25 %), but above the mean of 6 % out of 18 background studies. The relevance and reliability of provided HCD for the study is in general low. The information on historical control data given in the original study report for different parameters is limited to the number of studies, values for high and low range and mean of the sum of HCD and the general information "*The Charles River CD rat was used because of background data available on this strain of rat in these laboratories*". No information on e.g. timeframe, performing laboratory, individual data per study is given. Number of pregnant females showed no dose-dependent differences. Slight reductions in conception and fertility rate occurred in all treatment groups. The number of implantation sites was slightly reduced (-8 %) at 100 ppm. The gestation length was significantly prolonged at 100 ppm: 23.5 d in 35 % of females and 24 d in 5 % of females vs 5 and 0% in controls, respectively. The percentage of females with a gestation length of 23.5 days and 24 days was equal or above the high range value of the HCD (23.5 d: 25 %, 24 d: 4 %) and above the mean of the HCD (23.5 d: 7 %, 24 d: 0.4 %) out of 29 background studies. Due to poor offspring survival at 100 ppm in the first generation this dose level was not continued for the second generation.

10.10.3 Comparison with the CLP criteria

Table 25: Results of reproductive studies in comparison with CLP criteria

Toxicological result	CLP criteria
Dose range-finding generation study, rat, (Anonymous 25, 1990): General toxicity: ≥ 30 ppm (2.03/2.48 mg/kg bw/d, male/female): isolated clinical signs of toxicity (tremors, hunched posture, hair loss) Effects on sexual function and fertility: 30 & 300 ppm (2.03/2.48 mg/kg bw/d & 22.64/28.19 mg/kg bw/d, male/female, respectively): number pregnant females ↓ 300 ppm (22.64/28.19 mg/kg bw/d, male/female): number of implantation sites↓, mean litter size day 1↓ **2-generation reproduction study in rats, fosthiazate administered via diet,** (Anonymous 34, 1990): General toxicity: 100 ppm (7.21/9.34 mg/kg bw/d, male/female): absolute adrenal weight↑ and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams Effects on sexual function and fertility:≥ 3 ppm (0.21/0.26 mg/kg bw/d, male/female): fertility index ↓ ≥ 10 ppm (0.69/0.86 mg/kg bw/d, male/female): normal oestrus cycle ↓ (no dose response, but from 10 ppm on below HCD) in F0 females ≥ 30 ppm (2.62 mg/kg bw/d): acyclic/pseudopregnant females ↑100 ppm (7.21/9.34 mg/kg bw/d, male/female): gestation length prolonged, number of implantation sites↓, mean litter size day 1↓	**Category 1A:** Known human reproductive toxicant **Category 1B:** Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects **Category 2:** Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - and where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

No human data on adverse effects on sexual function and fertility are available, hence no classification with Cat. 1A according to CLP regulation is proposed.

In the range finding generation study, where only the parental generation was analysed for effects on sexual function and fertility (Anonymous 25, 1990), the number of implantation sites (-47 %) and litter size on day 1 (-52 %) was reduced at 300 ppm (22.64 mg/kg bw/day in males, 28.19 mg/kg bw/day in females). Taking into account, that the post implantation survival was markedly reduced at the top dose (58 % vs 89 % in ctrl), the reduced litter size on day 1 is properly caused by effects on sexual function/fertility and development. As the number of pregnant females was reduced at 30 and 300 ppm (6, respectively 7 out of 10 animals vs 9 animals in ctrl), but not at 100 ppm, this effect probably is not related to treatment. The observed effects on sexual function and fertility cannot be attributed to maternal toxicity, which was obvious before mating only in terms of clinical signs (possibly related to neurotoxic potential of fosthiazate) at 300 ppm.

In the multigeneration study (Anonymous 34, 1990) lower doses were applied than in the range finding study. In the F0 generation an increase in the absolute weight of adrenals, accompanied by histopathological findings was reported. Body weight and body weight gain were increased in F0 females at the highest dose of 100 ppm (9.34 mg/kg bw/day). Due to poor offspring survival at 100 ppm in the first generation this dose level was not continued for the second generation. Alterations in oestrus cycles and significantly prolonged gestation length were observed in F0 females. A trend towards an increase in the length of gestation was already noted at 10

ppm and above and reached statistical significance at 100 ppm. As already observed to a greater extent in the high dose group (300 ppm: - 47 %) of the range finding study, the number of implantation sites was reduced at the top dose (100 ppm) about 8 % (12.9 vs 14 in ctrl). Mean litter size on day 1 post-partum, which can additionally be affected by developmental effects, was reduced about 18 % (9.8 vs 12 in ctrl). Observed effects on sexual function cannot be attributed to maternal toxicity, as no marked effects on parental animals were obvious.

According to current guidelines further parameters for sexual function/fertility are required (e.g. AGD, onset of sexual maturation), but have not been examined in the present multigeneration studies. In conclusion, different effects pointing to adversity on sexual function and fertility were observed in the available multigeneration studies: reduction of number of implantation sites, disturbances in oestrus cycle, prolonged gestation length. Therefore, a category 2 classification of reproductive toxicity (Repr. 2, H361f) appears appropriate, since effects on sexual function/fertility were observed in F0 females. It is not possible to conclude on possible effects also in F1 due to poor survival in the main study and assessment of only parental animals (F0)in the range finding study.

Adverse effects on litter size, live-birth index, pup survival, body weight and mammary tissue are relevant for the assessment of developmental and/or lactational toxic effects and are assessed below in the relevant sections.

10.10.4 Adverse effects on development

The developmental toxicity of fosthiazate is assessed based on two teratology studies (one in rats and one in rabbits), their dose-range finding studies and the multigeneration study and its dose range-finding study in rats. The results of these studies are summarised in Table 26. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in Table 26 and the text below. For additional information, reference is made to Volume 3 Chapter B.6 of the RAR. All available developmental toxicity studies were considered supplementary as the treatment period was too short in comparison to the requirements of current test guidelines. Furthermore, selected additional relevant endpoints (AGD in foetuses and thyroid hormones in dams) were included in OECD TG for rats, when the TG 414 was updated in 2018 and were not examined in the present studies.

Table 26: Summary table of animal studies on adverse effects on development

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Dose range-finding generation study (5 month)

Range-finding study, no guideline
Oral (dietary)
Rat, CD, groups of 10 M and 10 F
GLP
Supplementary</td>
<td>Fosthiazate (93.3 % purity)
Doses: 0, 10, 30, 100 or 300 ppm.
Equivalent to: Males: 0, 0.68, 2.03, 7.09 and 22.64 mg/kg bw/day; females: 0, 0.81, 2.48, 8.93 and 28.19 mg/kg bw/day
90 days of treatment prior to mating.
Observations on offspring from day 1 after birth. On day 4 post-partum, litter size was reduced to 8 (4 males and 4 females) where possible. Offspring culled at day 4 were killed and</td>
<td>Parental toxicity:
≥ 30 ppm: isolated clinical signs of toxicity in F0 (tremor, hunched posture, hair loss),
≥ 100 ppm: maternal bw gain↓, inactive mammary tissue

Effects on development:
≥ 30 ppm: viability index↓
≥ 100 ppm: litter size on day 4↓, lactation (day 7, 14, 21)-index ↓
300 ppm: litter size on day 1↓, post implantation survival↓, live birth index ↓

Summary of selected findings
<table>
<tr><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><th></th><th>0</th><th>10</th><th>30</th><th>100</th><th>300</th></tr>
<tr><td>Mean bodyweight of females, day 0 post coitum</td><td>305</td><td>304</td><td>325</td><td>315</td><td>283^{NS}</td></tr>
<tr><td>Mean bodyweight of females, day 6 post coitum</td><td>339</td><td>333</td><td>357</td><td>343</td><td>295</td></tr>
<tr><td>Mean bodyweight of females, day 13 post coitum</td><td>381</td><td>370</td><td>397</td><td>375**</td><td>311</td></tr>
<tr><td>Mean bodyweight of females, day 20 post coitum</td><td>439</td><td>440</td><td>473</td><td>435</td><td>338***</td></tr>
<tr><td>Mean bodyweight of females, day 1 post partum</td><td>343</td><td>336</td><td>352</td><td>338</td><td>270**</td></tr>
<tr><td>Number pregnant</td><td>9</td><td>10</td><td>6</td><td>10</td><td>7</td></tr>
<tr><td>Number of live litter born</td><td>9</td><td>10</td><td>6</td><td>10</td><td>6</td></tr>
<tr><td>Mean implantation sites (number of animals)</td><td>14.3 ± 3.8 (9)</td><td>14.9 ± 3.6 (10)</td><td>17.2 ± 2.3 (6)</td><td>14.1 ± 3.8 (10)</td><td>7.6 ± 4.3 (7)</td></tr>
<tr><td>Mean litter size (number of animals) on day 1</td><td>12.8 ± 3.6 (9)</td><td>14.3 ± 3.5 (10)</td><td>15.3 ± 2.3 (6)</td><td>12.2 ± 2.6 (10)</td><td>6.2 ± 2.5 (5)</td></tr>
<tr><td>Mean litter size (number of animals) on day 4 pre culling</td><td>11.0 ± 4.3 (9)</td><td>12.8 ± 3.9 (10)</td><td>13.4 ± 2.2 (5)</td><td>3.8 ± 5.5 (4)</td><td>No surviving offspring</td></tr>
</table>
</td>
<td>Anonymous 25, 1990</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>grossly examined for abnormalities. Litters surviving to day 21 were also killed and necropsied.</td><td>
<table>
<tr><td>Post implantation survival (%)</td><td>89</td><td>96</td><td>89</td><td>87</td><td>58</td></tr>
<tr><td>Live birth index (%)</td><td>96</td><td>99</td><td>100</td><td>93</td><td>84</td></tr>
<tr><td>Viability index day 4 (%)</td><td>90</td><td>90</td><td>73</td><td>13</td><td>0</td></tr>
<tr><td>Lactation index (%) on Day 7 / 14 / 21</td><td>98 / 87 / 97</td><td>91 / 89 / 87</td><td>95 / 83 / 83</td><td>55 / 45 / 45</td><td>No surviving offspring</td></tr>
<tr><td colspan="6">Parental gross necropsy findings</td></tr>
<tr><td>Number of animals</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Mammary tissue slightly pale, milk present</td><td>-</td><td>-</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td>Mammary tissue appears light in colour, no milk present</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2</td></tr>
<tr><td>Mammary tissue appears inactive</td><td>-</td><td>-</td><td>-</td><td>9</td><td>3</td></tr>
<tr><td colspan="6">Observations at necroscopy of offspring (F1) dying or sent to necroscopy before weaning [A]</td></tr>
<tr><td>Number of offspring (litters) examined</td><td>13 (7)</td><td>19 (5)</td><td>25 (4)</td><td>91 (9)</td><td>21 (5)</td></tr>
<tr><td>No milk in stomach - % incidence, (litters)</td><td>69.2 (5)</td><td>84.2 (3)</td><td>100 (4)</td><td>94.5 (9)</td><td>85.7 (5)</td></tr>
</table>
[a]: following pairing and check for copulation plugs vaginal smears were prepared to prove mating by presence of spermatozoa, NS: bodyweight not significantly different from Controls (Student’s t-test), ** Bodyweight change day 0 to day 13 and at day 1 post partum significantly different from controls, P < 0.01 (Student’s t-test), ** * Bodyweight change day 0 to day 21 significantly different from controls, P < 0.01 (Student’s t-test), A excludes missing or severely cannibalised offspring
</td><td></td></tr>
<tr><td>Multigeneration study
Oral (dietary)
Rats, CD, groups of 25 male and 25 female
Method is comparable to OECD Test Guidelines 416 (26 May 1983; 22 January 2001 and</td><td>Fosthiazate (93.3% purity)
0, 3, 10, 30 or 100 ppm. Equivalent to: Males: 0, 0.21, 0.69, 2.09 and 7.21 mg/kg bw/day, females: 0, 0.26, 0.86,</td><td>Parental toxicity:
100 ppm: absolute adrenal weight↑ and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams,
Effects on development:
≥30 ppm: litter size at weaning ↓, viability index ↓
100 ppm: live birth index ↓, birth weight and weight at weaning of F1↓, lactation index ↓, delay in onset of eye opening and tooth eruption

Summary of parental findings
<table>
<tr><th></th><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><td>Parameter</td><td>Sex</td><td>0</td><td>3</td><td>10</td><td>30</td><td>100</td></tr>
<tr><td>F0 generation</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
</td><td>Anonymous 34, 1990</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

satisfies the essential requirements of EC guidelines for a multigeneration study (87/302/EEC Part B)

Stability in the vehicle was not performed in this study. Analysis of the batch performed in another study at the same laboratory indicated that the test material in rodent diet was stable when stored a 4°C for at least 7 days and at room temperature for up to 4 days.

No sperm parameters were analyzed. Mammary glands not evaluated.

GLP

Acceptable

2.62 and 9.34 mg/kg bw/day

Six-week-old F0 rats received fosthiazate for at least 99 days before mating, throughout mating, gestation and the lactation periods. Four days after birth the F1 litters were randomly culled to 8 pups/litter (4 males and 4 females) where possible. The F0 dams were allowed to rear the F1 pups before weaning on day 25 post-partum. At 4 weeks old, 25 weanlings/sex were randomly selected from the 0, 3, 10 and 30 ppm dose groups to constitute the F1 parents.

Total feed intake, g (week 1-14)	F	1982	2020	2061	2117**	2266***
Body weight gain, g (week 1-14)	F	303	301	314	319	329**
Mean compound intake, mg/kg bw/day	M F	0 0	0.21 0.26	0.69 0.86	2.09 2.62	7.21 9.34
Absolute adrenal weight in F0, g	F	0.062	0.070	0.071* (114%)	0.073** (117%)	0.077** (124%)
Terminal body weight	F	335	337	348	361	367*
F1 generation						
Mean compound intake, mg/kg bw/day	M F	0 0	0.27 0.31	0.88 1.02	2.70 3.14	

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

Summary of developmental findings (F1, F2)

		Dose (ppm)				
Parameter	**Sex**	**0**	**3**	**10**	**30**	**100**
F1						
Postimplantation survival index (%) (F1)	M/F	86	90	84	90	83
Litter size at day 1 post-partum pups/litter	M/F	12	12.5	11.4	12.3	9.8 (82%)
Litter size at day 25 post-partum pups/litter	M/F	7.8	7.6	7.3	6.9* (88%)	4.6*** (59%)
Live Birth index %	M/F	99	100	100	98	92***
Viability index at day 4 %	M/F	94	97	96	86**	44***
Birth weight of F_1	M/F	6.5	6.4	6.5	6.3	5.8** (89%)
Lactation index at day 7 /14 / 25	M/F	99 / 99 / 99	99 / 97 / 99	100 / 99 / 99	99 / 95 / 94	76*** / 52 ^{NT} / 45***
Weight at weaning (day 25) of F_1	M/F	67.8	66.5	69.5	63.5	46.4*** (68%)
No milk in stomach, % –	M/F	90	55.6	100	83.9	95.2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results							Reference
	The F1 parents were treated for 14 weeks and mated to produce the F2 pups. The study was terminated after weaning of the F2 offspring.	offspring dying before terminal kill (F1)							
		No milk in stomach, % – offspring culled on day 4 post-partum (F1)	M/F	2.7	2.5	0	5.6	25	
		Small pup, % - offspring dying before terminal kill (F1)	M/F	0	22.2	0	3.2	21	
		Small pup, % - offspring culled on day 4 post-partum (F1)	M/F	2.7	10.1	0	19.4	25	
		Small pup, % – at terminal kill (F1)	M/F	0	0	0	5	6.3	
		Eye opening, F1. Onset / completion	M/F	13.4 / 14.5	13.8 / 14.7	13.1 /14.3	13.4/14.5	14.2*/14.9	
		Tooth eruption, F1 Onset/completion	M/F	9.3/11.2	9.5/11.6	9.6/11.2	9.6/11.3	10.6**/11.9	
		F2							
		Postimplantation survival index (%)	M/F	86	93	88	88		
		Litter size at day 1 post-partum pups/litter	M/F	11.4 ± 3.7	12 ± 2.5	11.6 ± 2.9	12.1 ± 3		
		Litter size at day 25 post-partum pups/litter	M/F	7.5 ± 1.4	7.4 ± 1.6	7.7 ± 0.6	7.7 ± 0.7		
		Live Birth index %	M/F	97	100	97	97		
		Viability index at day 4 %	M/F	93	92	85	92		
		Birth weight of	M/F	6.3	6.5	6.5	6.6		
		Lactation index at day 7 /14 / 25	M/F	100 / 100 / 100	99 / 98 / 98	98 / 98 / 97	100 / 99 / 98		
		Weight at weaning (day 25) of	M/F	69	71.1	71.7	71.5		
		No milk in stomach, % – offspring dying before terminal kill	M/F	78.9	55.6	85.7	76.5		
		No milk in stomach, % –	M/F	-	-	-	1.5		

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>offspring culled on day 4 post-partum</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Small pup, % - offspring dying before terminal kill</td><td>M/F</td><td>-</td><td>-</td><td>3.6</td><td>-</td><td></td></tr>
<tr><td>Small pup, % - offspring culled on day 4 post-partum</td><td>M/F</td><td>1.3</td><td>1.7</td><td>-</td><td>4.6</td><td></td></tr>
<tr><td>Small pup, % – at terminal kill</td><td>M/F</td><td>0.7</td><td>0.8</td><td>-</td><td>-</td><td></td></tr>
</table>
* 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. No values available for onset/completion of eye opening and tooth eruption in F2.
</td><td></td></tr>
<tr><td>Preliminary Teratology Study, rat
Oral (gavage)
CD rats
Groups of 12 mated females
Rangefinder, no guideline.
Analysis of the test substances showed that the measured concentrations of the dosing solutions were 52-85 % of the nominal concentrations.
GLP
Supplementary</td><td>Fosthiazate (93.3% purity)
0, 1, 2.5, 5 or 10 mg/kg bw/day
Treatment from day 6 to 15 of presumed gestation. Dams were killed on day 20 of presumed gestation.</td><td>
Maternal toxicity:
10 mg/kg bw/day body weight gain↓
<table>
<tr><th>Dose level (mg/kg bw/day)</th><th colspan="4">Mean body weight gain (g) on Gestation Day</th></tr>
<tr><td></td><td>0</td><td>6</td><td>16</td><td>20</td></tr>
<tr><td>0</td><td>210</td><td>243</td><td>295</td><td>361</td></tr>
<tr><td>1</td><td>212</td><td>241</td><td>290</td><td>355</td></tr>
<tr><td>2.5</td><td>210</td><td>237</td><td>290</td><td>360</td></tr>
<tr><td>5</td><td>212</td><td>244</td><td>302</td><td>373</td></tr>
<tr><td>10</td><td>212</td><td>240</td><td>281* (95%)</td><td>347</td></tr>
</table>
* Significantly different from controls at p<0.05, (%) percent of control
<table>
<tr><th colspan="7">NOAEL maternal toxicity: 5 mg/kg bw/dayLitter data</th></tr>
<tr><th></th><th colspan="5">Dose (mg/kg/d)</th><th>Background data, mean (low / high)*</th></tr>
<tr><td>Parameter</td><td>0</td><td>1</td><td>2.5</td><td>5</td><td>10</td><td></td></tr>
<tr><td>Corpora lutea count</td><td>15.5</td><td>13.9</td><td>15.7</td><td>15.2</td><td>15.1</td><td>16 (13.9 / 19)</td></tr>
<tr><td>Implantations</td><td>14.2</td><td>13.6</td><td>14.8</td><td>14.3</td><td>14.2</td><td>14.6 (12 / 16.7)</td></tr>
<tr><td>Viable young (M&F)</td><td>12.7</td><td>13.1</td><td>14.1</td><td>13.8</td><td>13.3</td><td>13.7 (11.1 / 15.3)</td></tr>
<tr><td>Early resorptions</td><td>1.27</td><td>0.33</td><td>0.67</td><td>0.5</td><td>0.83</td><td>0.68 (0.05 / 1.68)</td></tr>
<tr><td>Late resorptions</td><td>0.18</td><td>0.17</td><td>0</td><td>0</td><td>0.08</td><td>0.18 (0 / 0.58)</td></tr>
<tr><td>Preimplantation loss %</td><td>8.2</td><td>2.4</td><td>5.9</td><td>5.5</td><td>6.1</td><td>8.7 (1.6 / 16.7)</td></tr>
<tr><td>Postimplantation loss %</td><td>10.3</td><td>3.7</td><td>4.5</td><td>3.5</td><td>6.5</td><td>5.9 (1.7 / 12.7)</td></tr>
</table>
</td><td>Anonymous 24, 1989</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Foetal weight (g)</td><td>3.51</td><td>3.54</td><td>3.47</td><td>3.42</td><td>3.61</td><td>3.32 (3 / 3.55)</td></tr>
<tr><td>Placental weight (g)</td><td>0.5</td><td>0.5</td><td>0.49</td><td>0.47</td><td>0.57</td><td>0.5 (0.43 / 0.57)</td></tr>
</table>
* data from 177 studies, no further information (e.g. time frame) given
Developmental effects: No effects up to 10 mg/kg bw/d
NOAEL foetotoxicity and developmental toxicity: 10 mg/kg bw/day
</td><td></td></tr>
<tr><td>Teratogenicity, Wistar rat
Oral (gavage)
CD, rat
Groups of 24 mated female rats
GLP
Supplementary</td><td>Fosthiazate (93.3 % purity)
0, 3, 5 or 10 mg/kg bw/day
Treatment from day 6 to 15 of presu-med gestation. Dams were killed on day 20 of presumed gestation</td><td>
Maternal toxicity:
10 mg/kg bw/day body weight gain↓
<table>
<tr><th>Dose level (mg/kg bw/day)</th><th colspan="4">Mean body weight gain (g) on Gestation Day</th></tr>
<tr><td></td><td>0</td><td>6</td><td>16</td><td>20</td></tr>
<tr><td>0</td><td>210</td><td>240</td><td>307</td><td>365</td></tr>
<tr><td>3</td><td>209</td><td>240</td><td>309</td><td>367</td></tr>
<tr><td>5</td><td>208</td><td>240</td><td>308</td><td>364</td></tr>
<tr><td>10</td><td>209</td><td>240</td><td>292*** (95%)</td><td>350*** (96%)</td></tr>
</table>
*** Significantly different from controls at p<0.001, (%) percent of control
NOAEL maternal toxicity: 5 mg/kg bw/day
<table>
<tr><th colspan="6">Litter data</th></tr>
<tr><th></th><th colspan="4">Dose (mg/kg bw/d)</th><th>Background data, mean (low / high)*</th></tr>
<tr><td>Parameter</td><td>0</td><td>3</td><td>5</td><td>10</td><td></td></tr>
<tr><td>Corpora lutea count</td><td>15</td><td>14.8</td><td>14.6</td><td>14.3</td><td>16 (13.9 / 19)</td></tr>
<tr><td>Implantations</td><td>13.5</td><td>13.5</td><td>13.5</td><td>13.5</td><td>14.6 (12 / 16.7)</td></tr>
<tr><td>Viable young (M&F)</td><td>12.8</td><td>12.6</td><td>12.8</td><td>12.7</td><td>13.7 (11.1 / 15.3)</td></tr>
<tr><td>Early resorptions</td><td>0.71</td><td>0.88</td><td>0.71</td><td>0.79</td><td>0.68 (0.05 / 1.68)</td></tr>
<tr><td>Late resorptions</td><td>0.04</td><td>0.04</td><td>-</td><td>-</td><td>0.18 (0 / 0.58)</td></tr>
<tr><td>Preimplantation loss %</td><td>9.7</td><td>8.7</td><td>8</td><td>5.3</td><td>8.7 (1.6 / 16.7)</td></tr>
<tr><td>Postimplantation loss %</td><td>5.5</td><td>6.8</td><td>5.3</td><td>5.9</td><td>5.9 (1.7 / 12.7)</td></tr>
<tr><td>Foetal weight (g)</td><td>3.47</td><td>3.48</td><td>3.39</td><td>3.45</td><td>3.32 (3 / 3.55)</td></tr>
<tr><td>Placental weight (g)</td><td>0.5</td><td>0.5</td><td>0.5</td><td>0.52</td><td>0.5 (0.43 / 0.57)</td></tr>
</table>
* data from 177 studies, no further information (e.g. time frame) given Developmental effects: No effects
NOAEL foetotoxicity and developmental toxicity: 10 mg/kg bw/day
</td><td>Anonymous 41, 1990</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Tolerance study in the rabbit Oral (gavage) Staircase study and Continuation study each with 2 non-pregnant New Zealand White rabbits Rangefinder, no guideline. GLP Supplementary	Fosthiazate (92.6 % purity) Staircase study: 1 mg/kg bw/d starting dose → 8 mg/kg bw/day (single dose was doubled every 2 days until adverse response) Continuation study: 4 mg/kg bw/day for 7 days	Maternal toxicity: 8 mg/kg bw/day (single dose): bodyweight↓, evidence of gastrointestinal tract disturbance and ataxia No treatment-related effects in 4 mg/kg bw/d group (exposure: 7 days) Developmental effects: Not analysed	Anonymous 36, 1989

<table>
<tr>
<td>Preliminary teratology study, rabbit
Oral (gavage)
Groups of 7 artificially inseminated New Zealand White rabbits
Rangefinder, no guideline.
Low group size.
Treatment only from gestation day 6-19.
Analysis of the test substances showed that the measured concentrations of the dosing solutions were 74 or 75 % of the nominal concentrations of the 1 or 2 mg/kg bw/d dose.
GLP

Supplementary</td>
<td>Fosthiazate (93.3 % purity)
0, 1, 2, 2.5 or 5 mg/kg bw/day
Treatment from day 6 to 19 of presumed gestation. Dams were killed on day 29 of presumed gestation.</td>
<td>Maternal toxicity:
≥2.0 mg/kg bw/day: bodyweight↓; one (2 mg/kg bw/d) respectively two (2.5 mg/kg bw/d) females euthanized in extremis due to weight loss. 5.0 mg/kg bw/day: mortality↑, respiration rate↑, ataxia and loss of muscular coordination.
<table>
<tr><th colspan="6">Disposition of animals</th></tr>
<tr><th>Diet Level (mg/kg bw/d)</th><th>0</th><th>1</th><th>2</th><th>2.5</th><th>5</th></tr>
<tr><td>Total number inseminated</td><td>7</td><td>7</td><td>7</td><td>7</td><td>7</td></tr>
<tr><td>Mortality</td><td>0</td><td>0</td><td>1</td><td>2</td><td>7</td></tr>
<tr><td>Not pregnant</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Abortion</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>Pregnant at term with viable young</td><td>7</td><td>7</td><td>5</td><td>4</td><td>0</td></tr>
</table>
<table>
<tr><th colspan="7">Group mean bodyweights (kg) of females during gestation</th></tr>
<tr><th></th><th colspan="6">Day of gestation</th></tr>
<tr><th>Diet Level(mg/kg bw/d)</th><th>0</th><th>6</th><th>13</th><th>20</th><th>25</th><th>29</th></tr>
<tr><td>0</td><td>4.4</td><td>4.54</td><td>4.66</td><td>4.55</td><td>4.74</td><td>4.75</td></tr>
<tr><td>1</td><td>3.98</td><td>4.12</td><td>4.23</td><td>4.31</td><td>4.4</td><td>4.5</td></tr>
<tr><td>2</td><td>4.26</td><td>4.42</td><td>4.53</td><td>4.5</td><td>4.54</td><td>4.6</td></tr>
<tr><td>2.5</td><td>4.37</td><td>4.51</td><td>4.66</td><td>4.72</td><td>4.83</td><td>4.87</td></tr>
<tr><td>5</td><td colspan="6">No females survived to term</td></tr>
</table>
NOAEL maternal toxicity: 1.0 mg/kg bw/day
Developmental effects (no females survived to term at the high dose group of 5 mg/kg bw/d):
Incidence of small pups increased above the range of HCD data at 2 mg/kg bw/d. Incomplete ossification of hyoid body increased above the HCD range at 2.5 mg/kg bw/d.
<table>
<tr><th colspan="7">Foetal findings</th></tr>
<tr><th rowspan="2">Diet Level (mg/kg bw/d)</th><th rowspan="2">0</th><th rowspan="2">1</th><th rowspan="2">2</th><th rowspan="2">2.5</th><th colspan="2">Background control*[1,2]</th></tr>
<tr><th>Mean</th><th>Range</th></tr>
<tr><td>Number of pregnant animals</td><td>7</td><td>7</td><td>6</td><td>4</td><td></td><td></td></tr>
<tr><td>% Abortion</td><td>0</td><td>0</td><td>16.7</td><td>0</td><td>2.2</td><td>0 – 20</td></tr>
<tr><td>Corpora lutea count</td><td>11.9</td><td>13.3</td><td>9.2</td><td>14</td><td>10.3</td><td>8.5 – 12.7</td></tr>
<tr><td>Implantations</td><td>9.6</td><td>11.1</td><td>8.2</td><td>12.5</td><td>7.8</td><td>4.7 – 10.3</td></tr>
<tr><td>Viable young (M&F)</td><td>8.9</td><td>9.9</td><td>7.4</td><td>11.5</td><td>6.9</td><td>4.3 – 8.3</td></tr>
<tr><td>Early resorptions</td><td>0.3</td><td>0.7</td><td>0.8</td><td>0.5</td><td>0.5</td><td>0 – 1.4</td></tr>
<tr><td>Late resorptions</td><td>0.4</td><td>0.6</td><td>0</td><td>0.5</td><td>0.4</td><td>0.2 – 0.6</td></tr>
<tr><td>Preimplantation loss %</td><td>19.3</td><td>17</td><td>10.9</td><td>10.7</td><td>24</td><td>13.3 – 46.2</td></tr>
<tr><td>Postimplantation loss %</td><td>7.5</td><td>11.5</td><td>9.8</td><td>8</td><td>11.8</td><td>3.2 – 19.5</td></tr>
<tr><td>Foetal weight (g)</td><td>37.8</td><td>42.1</td><td>40</td><td>41.1</td><td>42.6</td><td>39.2 – 46.9</td></tr>
<tr><td>Placental weight (g)</td><td>5.9</td><td>5.3</td><td>5.7</td><td>5.1</td><td>5.8</td><td>5.2 – 6.6</td></tr>
</table>
</td>
<td>Anonymous 5, 1989a</td>
</tr>
</table>

		Number of foetuses (litters) examined at necroscopy: 62 (7) / 69 (7) / 37 (5) / 46 (4) / /	
		Small foetus (less than 32 g), % incidence (litters): 17.7 (3) / 7.2 (3) / 24.3 (3) / 13 (2) / 11.39 / 2.7 – 21.9	
		Number of foetuses (litters) examined at skeletal examination: 42 (7) / 47 (7) / 25 (5) / 31 (4) / /	
		Incomplete ossification of hyoid body, %: 23.8 (5) / 31.9 (6) / 28.0 (4) / 61.3(4) / 29.62 / 9.6 – 50.7	
		*[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	

Individual data dams with small pups

Diet Level (mg/kg bw/d), Animal number	Body weight (kg) on GD 29	Number of viable young	Number of small foetus (less than 32 g)
0 mg/kg bw/d			
Animal 22TJ762	5.18	10	2
Animal 22TJ786	4.72	11	4
Animal 22TJ797	4.68	7	5
Mean	4.75 ± 0.23	8.9	
1 mg/kg bw/d			
Animal 22TJ456	4.12	10	1
Animal 22TJ463	4.68	10	1
Animal 22TJ469	4.85	12	3
Mean	4.5 ± 0.35	9.9	
2 mg/kg bw/d			
Animal 22TJ746	4.72	9	5
Animal 22TJ795	4.48	10	3
Animal 22TJ802	4.76	7	1
Mean	4.6 ± 0.16	7.4	
2.5 mg/kg bw/d			
Animal 22TJ614	4.51	15	5
Animal 22TJ622	5.27	14	1
Mean	4.87 ± 0.32	11.5	

Teratogenicity, rabbit Oral (gavage)	Fosthiazate (93.3 % purity)	Maternal toxicity: no effects	Anonymous 6, 1989b

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Groups of 15 artificially inseminated New Zealand White rabbits
The study meets the essential criteria of EC guidelines for a rodent or non-rodent teratology study or the OECD Guideline for Testing of Chemicals, 414, 1981/2001. However, the treatment period was gestation days 6–19. Current test guidelines require daily administration from implantation to the day prior to scheduled caesarean section.
GLP
Supplementary</td>
<td>0, 0.5, 1.0, 1.5 or 2.0 mg/kg bw/day
Treatment from day 6 to 19 of presumed gestation. Dams were killed on day 29 of presumed gestation.</td>
<td>
<table>
<tr><th colspan="7">Group mean bodyweights (kg) of females during gestation, mean ± SD</th></tr>
<tr><th></th><th colspan="6">Day of gestation</th></tr>
<tr><th>Dose Level (mg/kg bw/d)</th><th>0</th><th>6</th><th>12</th><th>18</th><th>24</th><th>28</th></tr>
<tr><td>0</td><td>4 ± 0.19</td><td>4.15 ± 0.25</td><td>4.28 ± 0.23</td><td>4.44 ± 0.24</td><td>4.48 ± 0.25</td><td>4.48 ± 0.29</td></tr>
<tr><td>0.5</td><td>4.05 ± 0.33</td><td>4.17 ± 0.33</td><td>4.3 ± 0.32</td><td>4.45 ± 0.32</td><td>4.51 ± 0.31</td><td>4.57 ± 0.34</td></tr>
<tr><td>1</td><td>4.01 ± 0.28</td><td>4.17 ± 0.29</td><td>4.28 ± 0.28</td><td>4.44 ± 0.26</td><td>4.48 ± 0.23</td><td>4.53 ± 0.21</td></tr>
<tr><td>1.5</td><td>4.05 ± 0.35</td><td>4.22 ± 0.34</td><td>4.36 ± 0.35</td><td>4.49 ± 0.35</td><td>4.56 ± 0.37</td><td>4.58 ± 0.39</td></tr>
<tr><td>2</td><td>3.97 ± 0.31</td><td>4.12 ± 0.34</td><td>4.27 ± 0.35</td><td>4.4 ± 0.36</td><td>4.4 ± 0.33</td><td>4.43 ± 0.31</td></tr>
</table>
NOAEL maternal toxicity: 2.0 mg/kg bw/day

Developmental effects:
2 mg/kg bw/day: foetal birth weights↓, number of small foetuses↑
<table>
<tr><th></th><th colspan="5">Litter data</th></tr>
<tr><th></th><th colspan="5">Dose (mg/kg/d)</th><th>Background data, mean (low / high)*</th></tr>
<tr><td>Parameter</td><td>0</td><td>0.5</td><td>1</td><td>1.5</td><td>2</td><td></td></tr>
<tr><td>% Abortion and total litter loss</td><td>0</td><td>0</td><td>15.4</td><td>0</td><td>7.7</td><td>5.6 (0 / 15.4)</td></tr>
<tr><td>Corpora lutea count</td><td>11.3</td><td>12.8</td><td>12.</td><td>12.1</td><td>13.2</td><td>11.6 (9.3 / 13.2)</td></tr>
<tr><td>Implantations</td><td>10.3</td><td>10.5</td><td>10.5</td><td>9.7</td><td>10.8</td><td>9.2 (6.5 / 11.5)</td></tr>
<tr><td>Viable young (M&F)</td><td>9</td><td>9.6</td><td>9.6</td><td>8.5</td><td>9.9</td><td>8 (5.7 / 9.7)</td></tr>
<tr><td>Early resorptions</td><td>0.6</td><td>0.4</td><td>0.8</td><td>0.6</td><td>0.4</td><td>0.5 (0.3 / 1.4)</td></tr>
<tr><td>Late resorptions</td><td>0.6</td><td>0.5</td><td>0.1</td><td>0.5</td><td>0.5</td><td>0.7 (0.2 / 1.6)</td></tr>
<tr><td>Preimplantation loss %</td><td>8.9</td><td>18.1</td><td>12.1</td><td>19.7</td><td>17.7</td><td>20.5 (9.1 / 43)</td></tr>
<tr><td>Postimplantation loss %</td><td>12.5</td><td>8.1</td><td>8.6</td><td>11.9</td><td>8.5</td><td>13.2 (7.3 / 21.7)</td></tr>
<tr><td>Foetal weight (g)</td><td>40.8</td><td>42.3</td><td>41.5</td><td>42.9</td><td>38.6</td><td>41 (38.5 / 45)</td></tr>
<tr><td>Placental weight (g)</td><td>5.4</td><td>5.9</td><td>5.8</td><td>6</td><td>5.4</td><td>6 (4.7 / 6.6)</td></tr>
<tr><td>Incidence of small foetus (less than 32 g) in % (number of affected litter)</td><td>8.7 (6)</td><td>13.6 (7)</td><td>20.8 (6)</td><td>16.2 (5)</td><td>27.7 (7)</td><td>11.39 (2.7 / 21.9)</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

* data from 7 studies, no further information (e.g. time frame) given except that it is background data on the same rabbit strain from the conducting laboratory**Individual data for dams with small pups**

Diet Level (mg/kg bw/d), Animal number	Body weight (kg) on GD 29	Number of viable young	Number of small foetus (less than 32 g)
0 mg/kg bw/d			
28TD008	4.85	10	2
28TD043	4.89	9	2
28TD053	4.32	8	1
28TD055	4.55	11	2
28TD115	4.21	12	2
28TD130	4.35	5	2
Mean	4.48 ± 0.29	9	
0.5 mg/kg bw/d			
28TD010	4.5	15	4
28TD042	4.55	10	2
28TD048	4.88	12	2
28TD056	5.24	13	5
28TD078	4.37	10	1
28TD107	4.3	10	1
28TD132	4.39	13	2
Mean	4.57 ± 0.34	9.6	
1 mg/kg bw/d			
22TD955	4.77	10	2
28TD052	4.37	10	2
28TD081	4.33	11	1
28TD085	4.8	16	8
28TD135	4.42	15	7
28TD138	4.59	9	2
Mean	4.53 ± 0.21	9.6	
1.5 mg/kg bw/d			
28TD009	4.06	7	3
28TD051	5.19	14	6

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results						Reference
		1.5	28TD111	4.55	15	5	NOAEL developmental toxicity: mg/kg bw/day	
			28TD122	4.71	10	2		
			28TD129	4.47	11	2		
			Mean	4.58 ± 0.39	8.5			
			28TD045	4.3	7	1		
			28TD049	4.61	12	3		
			28TD101	4.12	11	3		
			28TD118	4.25	12	3		
			28TD123	4.2	12	6		
			28TD133	4.25	14	8		
			28TD153	4.86	14	8		
			Mean	4.43 ± 0.31	9.9			

No human data on adverse effects of fosthiazate on development are available

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a developmental toxicity study in the rat (Anonymous 41, 1990), the NOAEL for maternal toxicity was 5 mg/kg bw/day based on slight reduction in body weight gain at 10 mg/kg bw/day. The NOAEL for foetotoxicity and teratogenicity was 10 mg/kg bw/day based on the absence of treatment-related effects at any of the test dose levels. There were no treatment-related effects including the number of corpora lutea, pre- and post-implantation losses, and the number of foetal resorptions, and the number of live or dead foetuses. There were no treatment-related changes in foetuses after external, visceral and skeletal examinations. The developmental toxicity study in the rat was based on a range-finding study (Anonymous 24, 1989) in which the NOAEL for maternal toxicity was also 5 mg/kg bw/day based on slight reduction in body weight gain at 10 mg/kg bw/day, but significant only on day 16 p.c. The NOAEL for foetotoxicity and teratogenicity in the preliminary study was 10 mg/kg bw/day based on the absence of changes at any of the highest test dose levels. Fosthiazate was not teratogenic in the rat under the conditions of the test. In both studies the treatment period was not in line with current test guidelines as fosthiazate was only given from day 6 to 15 of presumed gestation. Therefore, the sensitivity of these studies might be impaired and the studies were considered to be supplementary.

In the rabbit developmental toxicity study (Anonymous 6, 1989b), the NOAEL for maternal toxicity and teratogenicity was 2 mg/kg bw/day, the highest test dose level and for developmental toxicity 1.5 mg/kg bw/day based on slight reductions in foetal birth weights (-5.4 %) and increased number of small foetuses (+19 %) at 2 mg/kg bw/day. The incidence of small foetuses (27.7 %) was above the mean (11.39 %) and range (2.7 - 21.9 %) of background control data from the conducting laboratory. No treatment-related differences including the mean number of corpora lutea, implantations, losses, and the number of foetal resorptions, and the number of live or dead foetuses were observed. In a preliminary range-finding study (Anonymous 5, 1989a) the NOAEL for maternal toxicity was 1.0 mg/kg bw/day as at 2 mg/kg bw/d one female and at 2.5 mg/kg bw/d two females were euthanized in extremis because of weight loss. The incidence of small pups (less than 32 g) (24.3 %) in this study was above the mean (11.39 %) and range (2.7 - 21.9 %) of background control data (7 studies, 606 foetuses) at 2 mg/kg bw/d, but without dose-response. No further information (e.g. time frame, single study data) on the background data is available except that it comes from the conducting laboratory and refers to the same rabbit strain. Relevance of this HCD is therefore considered limited. An increase of the variation incomplete ossification of hyoid body above the median (29.62 %) and range (9.6–50.7 %) of the background control (15 studies, 1079 foetuses) was reported at 2.5 mg/kg bw/d with an incidence of 61.3 %. The finding of small pups is not attributed to the reported maternal toxicity as body weights in surviving dams was only slightly reduced at 2 mg/kg bw/d and also individual animal data showed that dams having small pups were not more affected than other dams. The number of small pups was moreover also increased in the other rabbit study without co-occurring maternal toxicity. . In both studies the treatment period was not in line with current test guidelines as fosthiazate was only given from day 6 to 19 of presumed gestation. Hence, the sensitivity of these studies might be impaired and the studies were considered to be supplementary.

In summary, in the rat developmental toxicity study no adverse effects on foetuses were observed. However, it should be emphasised, that the treatment period was too short according to current requirements (OECD TG 414) and the high dose level revealed only very slight maternal toxic effects. The study in rabbits (Anonymous 6, 1989b), did not reveal any evidence of maternal toxicity, but adverse effects on development such as increased number of small pups outside historical control data and slightly reduced foetal body weight at highest dose level. The adverse effect on development in terms of small pups is supported by the results of a range-finder study in rabbits. The treatment period in rabbits was also too short according to current guidelines. Due to deviations to current standards both studies were considered only supplementary to examine the developmental toxicity of fosthiazate. However, further adverse developmental effects were observed in the multigeneration studies in rats:

In a range finding study (Anonymous 25, 1990) rats were dosed up to levels of 300 ppm (22.64 mg/kg bw/day in males, 28.19 mg/kg bw/day in females). At the highest dose post implantation survival was only about 58 %

(vs 89 % in ctrl) and live birth index was also lower than in the control group (84 % vs 96 %). Number of live born litters as well as size of litters on day 1 were reduced: 6 litters with a mean litter size of 6.2 vs. 9 litters with a mean size of 12.8 in the control group. On day 4 all litters were dead in the highest dose group and at 100 ppm the viability index was also extremely reduced, only 13 % (vs. 90 % in ctrl) survived. Also at the dose of 30 ppm pup viability at day 4 was already reduced about 17 % in comparison to control. The lactation index was reduced about 43 % on day 7 at 100 ppm, while at this time point no effect was seen in the 30 ppm dose group. Necroscopy of females revealed inactive mammary tissue in 9 out of 10 animals at 100 ppm and in 3 animals from the top dose group (300 ppm). In two animals of the top dose group no milk was present in the mammary tissue and mammary tissue appears light in colour. Observation at necroscopy of offspring dying or sent to necroscopy before weaning revealed no milk in stomach in all dose groups, the highest incidence was observed at 30 ppm.

Under the conditions of the multigeneration study by Anonymous 34, 1990, similar effects of fosthiazate on litter size, live birth index and pup viability as in the range-finding study (Anonymous 25, 1990) were observed and decrease of these parameters was reported for the highest dose group (100 ppm): The litter size at day 1 post-partum was reduced about 18 % (9.8 vs 12 in ctrl) and at day 25 about 41 % (4.6 vs 7.8 in ctrl), live birth index was significantly reduced (-7 %) (92 vs 99 % in ctrl), the viability index was significantly reduced about 50 % (44 vs 94 % in ctrl). Furthermore birth weight was reduced (-11 %) at this dose level and weight at weaning was also reduced (-32 %). The lactation index (from day 7 on) was reduced at 100 ppm (76 vs 99 in ctrl), while day 4-viability of the pups was already reduced at 30 ppm (-12 %). A further decrease of the lactation index (-54 %) was obvious on day 25 post-partum Absence of milk in the stomach was reported for pups of all groups (including control) which died before terminal kill. In pups killed at culling the highest incidence of absence of milk was reported for the top dose group (100 ppm). Mammary tissue was not routinely evaluated, only in (presumptive) 13 F0 females with total litter loss. No adverse findings concerning the mammary glands were reported for these animals. Cholinesterase activity was not measured in this study, hence it remains open whether cholinesterase inhibition was causal for the pup mortality. Data from an age-sensitivity study in rats (Anonymous 7, 2006) showed no increased sensitivity of developing rats to cholinesterase inhibition (exposure during day 6-20 of gestation, euthanized on day 20): significant inhibition (≥ 20 %) of plasma and RBC cholinesterase in the dams was already observed at a 0.7 mg/kg bw. At 5 mg/kg bw also brain cholinesterase was inhibited in females (≥ 20 %) and plasma, RBC and brain cholinesterase were significantly inhibited (≥ 20 %) in the foetuses. The extent of inhibition was greater in the dams (-99.5–89 %) than in the foetuses (-33-22 %). The incidence of small pups (no definite criterion in the report) was increased in the two top dose groups (at 2.62 and 9.34 mg/kg bw/d) for pups examined after culling on day 4 and at terminal kill (culled on day 4: 19.4 and 25 vs 2.7 in ctrl; terminal kill: 5 and 6.3 vs 0 in ctrl). No information on historical control data regarding small pups is available for this study. Onset of eye opening was delayed in F1 (day 14.2 vs 13.4 in ctrl). Also onset of tooth eruption was delayed in F1 (day 10.6 vs 9.3 in ctrl).

In summary, litter size, live-birth index, viability index, lactation index, body weight at birth until weaning, onset of eye opening and tooth eruption and the incidence of small pups were affected in generational studies in rats by fosthiazate and cannot attributed to parental toxicity. The relevance of significantly affected postnatal development and survival until weaning for effects on or via lactation is discussed below.

10.10.6 Comparison with the CLP criteria

Table 27: Results of developmental toxicity studies in comparison with CLP criteria

Toxicological result	CLP criteria
Preliminary teratology study, rat, (Anonymous 24, 1989): Maternal toxicity: 10 mg/kg bw/day body weight gain↓ Developmental effects: No effects **Teratogenicity, Wistar rat,** (Anonymous 41, 1990):	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies

Toxicological result	CLP criteria
Maternal toxicity: 10 mg/kg bw/d body weight gain↓ Developmental effects: No effects **Dose range-finding generation study, rat,** (Anonymous 25, 1990): Maternal toxicity: ≥ 30 ppm (2.03/2.48 mg/kg bw/d, male/female): isolated clinical signs of toxicity (tremors, hunched posture, hair loss) ≥ 100 ppm (7.09/8.93 mg/kg bw/d, male/female): maternal bw gain↓ during gestation , inactive mammary tissue Developmental effects: ≥ 30 ppm (2.03/2.48 mg/kg bw/d, male/female): viability index↓ ≥ 100 ppm (7.09/8.93 mg/kg bw/d, male/female): litter size day 4↓, lactation index (day 7, 14, 21)↓ 300 ppm (22.64/28.19 mg/kg bw/d, male/female): post implantation survival↓, live birth index↓ **2-generation reproduction study in rats, fosthiazate administered via diet** (Anonymous 34, 1990): Maternal toxicity: 100 ppm (7.21/9.34 mg/kg bw/d, male/female): absolute adrenal weight↑ and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams, gestation length prolonged Developmental effects: ≥30 ppm (2.09/2.62 mg/kg bw/d for male/females): viability index↓, litter size at weaning↓, small pups↑ 100 ppm (9.34 mg/kg bw/d for females): live birth index ↓, birth weight and weight at weaning of F1↓, lactation index ↓, delayed onset of eye opening and tooth eruption	- clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
Tolerance study in the rabbit, (Anonymous 36, 1989): Maternal toxicity: 8 mg/kg bw/d: body weight↓, evidence of gastrointestinal tract disturbance and ataxia Developmental effects: Not analysed **Preliminary teratology study, rabbit,** (Anonymous 5, 1989a): Maternal toxicity: ≥2.0 mg/kg body weight/d: bodyweight↓; euthanized in extremis 5.0 mg/kg body weight/d: mortality↑, respiration rate↑, ataxia and loss of muscular coordination Developmental effects: 2 mg/kg bw/day: number of small foetuses↑ **Teratogenicity, Chinchilla rabbit,** (Anonymous 6, 1989b): Maternal toxicity: No effects	

Toxicological result	CLP criteria
Developmental effects: 2 mg/kg bw/day: foetal birth weights↓, number of small foetuses↑ (outside HCD)	

There are no appropriate epidemiological studies available on developmental effects of fosthiazate in humans. Hence, classification with Category 1A according CLP regulation is not necessary.

The prenatal developmental toxicity was investigated in rats and rabbits complying with international test guidelines and GLP, but with deviations in duration of treatment and parameters (AGD, thyroid hormones). In all studies the duration was shorter as recommended by current guidelines: day 6-15 in rats and day 6-19 in rabbits. Therefore the study design may have been less sensitive than required according to current test guidelines. Therefore the studies were considered supplementary.

In rats, no treatment-related findings in foetuses after external, visceral and skeletal examinations were reported in the developmental toxicity studies (Anonymous 24, 1989; Anonymous 41, 1990). However, in the rat multigeneration study and its dose range finding study (Anonymous 34, 1990, Anonymous 25, 1990) developmental toxicity was observed. In the main study by Anonymous 34, 1990) at 9.34 mg/kg bw/d a decrease in litter size on PND 1 (-18 %) and 25 (-41 %), reduced indices for live birth (-7 %), viability (-50 %) and lactation (up to -54% on day 25) were observed as well as a reduced weight at birth (-11%) and at weaning (-32 %) and a delay in onset of eye opening and tooth eruption. The incidence of small pups (no definite criterion in the report) was increased at 2.62 and 9.34 mg/kg bw/d for pups examined after culling on day 4 and at terminal kill. In the range-finder study (Anonymous 25, 1990) similar effects regarding litter size and pup mortality have been observed, supporting the developmental toxicity of fosthiazate. Observed adversity cannot be attributed to maternal toxicity, which was not severe in the main study.

In a first tolerance study in rabbits (Anonymous 36, 1989) only effects on maternal animals were analysed and no data on potential developmental toxicity of fosthiazate was provided.

In a next preliminary teratology study in rabbits (Anonymous 5, 1989a) no treatment-related differences in reproductive parameters in females including the number of corpora lutea, pre- and post-implantation losses, and the number of foetal resorptions, and the number of live or dead foetuses were observed. One female of the 2 mg/kg bw/d group and two animals of the 2.5 mg/bw/d group were euthanized in extremis due to body weight loss. The incidence of small pups (24.3 %) was above the mean (11.39 %) and range (2.7-21.9 %) of background control data at 2 mg/kg bw/d, but without dose-response. An increase of the variation incomplete ossification of hyoid body above the median (29.62 %) and range (9.6–50.7 %) of the background control was reported at 2.5 mg/kg bw/d with an incidence of 61.3 %. There were no further treatment-related effects in foetuses after external, visceral and skeletal examinations (Anonymous 5, 1989a).

In a teratology study in rabbits (Anonymous 6, 1989b) no treatment-related differences in reproductive parameters in females including the mean number of corpora lutea, implantations, losses, and the number of foetal resorptions, and the number of live or dead foetuses were observed. At the highest dose of 2 mg/kg bw/d foetal birth weights were reduced about 5.4 % and the value of 38.6 g was beneath the mean (41 g) but within the range (38.5–45 g) of background control data for this parameter. An increased incidence of small foetuses (27.7 %) was noted at 2 mg/kg bw/day, which was above the mean (11.39 %) and range (2.7 – 21.9 %) of background control data from the laboratory. Maternal toxicity was not observed in this study. No further treatment-related external, visceral or skeletal abnormalities were observed.

In summary, due to deviations from current guidelines the developmental toxicity studies in rats and rabbits are considered supplementary to examine prenatal induced adverse effects of fosthiazate. However, the study in rabbits revealed adverse developmental effects such as increased number of small pups outside historical control data and slightly reduced foetal body weight at highest dose level without any evidence of maternal toxicity. Further adverse developmental effects were observed in the multigeneration studies in F1 animals: litter size, live-birth index, viability index, lactation index, body weight at birth and weaning, onset of eye opening and tooth eruption and the incidence of small pups were affected and cannot attributed to parental toxicity.

Based on the available data a potential for developmental effects cannot be excluded. As there are some

uncertainties related to the data base, classification with Category 1B is not applicable. However, evidence is given for classification with Category 2 (Repr. 2, H361d) as developmental adverse effects in rabbits and in F1 rats were observed in the developmental toxicity study and multigeneration study, respectively.

10.10.7 Adverse effects on or via lactation

Effects of fosthiazate on or via lactation were investigated in a multi-generation study (Anonymous 34, 1990) conducted in rats and a corresponding dose range-finding study (Anonymous 25, 1990). Results of both studies in terms of parental toxicity, effects on sexual function and fertility and on development are summarised in Table 24 (c.f. 10.10.1 Adverse effects on development 10.10.4.). Effects associated with disturbances on or via lactation are summarised below.

No human data on effects on or via lactation are available.

Table 28: Summary table of effects on or via lactation

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Dose range-finding generation study
(5 month)

Range-finding study, no guideline
Oral (dietary)
Rat, CD, groups of 10 M and 10 F
GLP
Supplementary</td>
<td>Fosthiazate (93.3 % purity)
Doses: 0, 10, 30, 100 or 300 ppm.
Equivalent to: Males: 0, 0.68, 2.03, 7.09 and 22.64 mg/kg bw/day; females: 0, 0.81, 2.48, 8.93 and 28.19 mg/kg bw/day
90 days of treatment prior to mating.
Observations on offspring from day 1 after birth. On day 4 post-partum, litter size was reduced to 8 (4 males and 4 females) where possible. Offspring culled at day 4 were killed and grossly examined for</td>
<td>Parental toxicity:
≥ 30 ppm: isolated clinical signs of toxicity in F0 (tremor, hunched posture, hair loss),
≥ 100 ppm: maternal bw gain↓ during gestation, inactive mammary tissue

Effects on development:
≥ 30 ppm: viability index↓
≥ 100 ppm: litter size on day 4↓, lactation (day 7, 14, 21)-index ↓
300 ppm: litter size on day 1↓, post implantation survival↓, live birth index ↓

Effects on or via lactation
<table>
<tr><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><th></th><th>0</th><th>10</th><th>30</th><th>100</th><th>300</th></tr>
<tr><td colspan="6">Parental gross necropsy findings</td></tr>
<tr><td>Number of animals</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Number pregnant</td><td>9</td><td>10</td><td>6</td><td>10</td><td>7</td></tr>
<tr><td>Mammary tissue slightly pale, milk present</td><td>-</td><td>-</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td>Mammary tissue appears light in colour, no milk present</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2</td></tr>
<tr><td>Mammary tissue appears inactive</td><td>-</td><td>-</td><td>-</td><td>9</td><td>3</td></tr>
<tr><td colspan="6">Observations at necroscopy of offspring (F1) dying or sent to necroscopy before weaning [A]</td></tr>
<tr><td>Number of offspring (litters) examined</td><td>13 (7)</td><td>19 (5)</td><td>25 (4)</td><td>91 (9)</td><td>21 (5)</td></tr>
<tr><td>No milk in stomach - % incidence, (litters)</td><td>69.2 (5)</td><td>84.2 (3)</td><td>100 (4)</td><td>94.5 (9)</td><td>85.7 (5)</td></tr>
</table>
[A] excludes missing or severely cannibalised offspring</td>
<td>Anonymous 25, 1990</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>abnormalities. Litters surviving to day 21 were also killed and necropsied.</td><td></td><td></td></tr>
<tr><td>Multigeneration study
Oral (dietary)
Rats, CD, groups of 25 male and 25 female
Method is comparable to OECD Test Guidelines 416 (26 May 1983; 22 January 2001 and satisfies the essential requirements of EC guidelines for a multigeneration study (87/302/EEC Part B)
Stability in the vehicle was not performed in this study. Analysis of the batch performed in another study at the same laboratory</td><td>Fosthiazate (93.3% purity)
0, 3, 10, 30 or 100 ppm. Equivalent to: Males: 0, 0.21, 0.69, 2.09 and 7.21 mg/kg bw/day, females: 0, 0.26, 0.86, 2.62 and 9.34 mg/kg bw/day
Six-week-old F0 rats received fosthiazate for at least 99 days before mating, throughout mating, gestation and the lactation periods. Four days after birth the F1 litters were randomly culled to 8 pups/litter (4 males and 4</td><td>Parental toxicity:
100 ppm: absolute adrenal weight↑ and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams,
Effects on sexual function and fertility:
≥ 10 ppm: altered oestrus cycle, 100 ppm: gestation length prolonged

Effects on development:
≥30 ppm: litter size at weaning ↓, viability index ↓
100 ppm: live birth index ↓, birth weight and weight at weaning of F1↓, lactation index ↓, delay in onset of eye opening and tooth eruption

Findings associated with effects on or via lactation
<table>
<tr><th></th><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><th>Parameter</th><th>Sex</th><th>0</th><th>3</th><th>10</th><th>30</th><th>100</th></tr>
<tr><td>No milk in stomach, % – offspring dying before terminal kill (F1)</td><td>M/F</td><td>90</td><td>55.6</td><td>100</td><td>83.9</td><td>95.2</td></tr>
<tr><td>No milk in stomach, % – offspring culled on day 4 post-partum (F1)</td><td>M/F</td><td>2.7</td><td>2.5</td><td>0</td><td>5.6</td><td>25</td></tr>
</table></td><td>Anonymous 34, 1990</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>indicated that the test material in rodent diet was stable when stored a 4°C for at least 7 days and at room temperature for up to 4 days.
No sperm parameters were analyzed. Mammary glands not evaluated.
GLP
Acceptable</td><td>females) where possible. The F0 dams were allowed to rear the F1 pups before weaning on day 25 post-partum. At 4 weeks old, 25 weanlings/sex were randomly selected from the 0, 3, 10 and 30 ppm dose groups to constitute the F1 parents. The F1 parents were treated for 14 weeks and mated to produce the F2 pups.</td><td></td><td></td></tr>
<tr><td>Nature of the residue of 14C-Fosthiazate (IKI-1145)
using lactating goats - Part I: In-life phase

US EPA Pesticide Assessment Guidelines,</td><td>Two lactating goats received either ^{14}C-B-IKI-1145 or ^{14}C-R-IKI-1145 at an oral dose of 10 ppm, once daily for 4 consecutive days and expired air, excreta and milk was</td><td>Distribution of radioactivity in milk from goats dosed with [^{14}C]-fosthiazate
<table>
<tr><th rowspan="2">Matrix</th><th colspan="2">B-label</th><th colspan="2">R-label</th></tr>
<tr><th>% dose</th><th>mg/kg</th><th>% dose</th><th>mg/kg</th></tr>
<tr><td>Predose</td><td>--</td><td>BDL</td><td>--</td><td>BDL</td></tr>
<tr><td>Milk 0-3 hrs</td><td>0.2</td><td>0.16</td><td>0.1</td><td>0.15</td></tr>
<tr><td>Milk 3-17 hrs</td><td>0.4</td><td>0.28</td><td>0.4</td><td>0.59</td></tr>
<tr><td>Milk 17-27 hrs</td><td>0.2</td><td>0.19</td><td>0.2</td><td>0.40</td></tr>
<tr><td>Milk 27-41 hrs</td><td>0.3</td><td>0.21</td><td>0.6</td><td>0.84</td></tr>
</table></td><td>Anonymus 42, 1995</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Subdivision 0, Residue
Chemistry Section 171-4.

GLP
Acceptable</td><td>collected for the 4 days.
Target dose ^{14}C-B-IKI-1145: 16.02 mg, Target dose ^{14}C-R-IKI-1145: 19.25 mg,</td><td>
<table>
<tr><td>Milk 41-65 hrs</td><td>0.3</td><td>0.20</td><td>0.9</td><td>0.76</td></tr>
<tr><td>Milk 65-92 hrs</td><td>0.5</td><td>0.21</td><td>1.1</td><td>0.81</td></tr>
<tr><td>Total</td><td>1.9</td><td>-</td><td>3.3</td><td>-</td></tr>
</table>
BDL: Below Detection Limit
</td><td></td></tr>
</table>

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

In the range finding study (Anonymous 25, 1990) rats were dosed up to levels of 300 ppm (22.64 mg/kg bw/day in males, 28.19 mg/kg bw/day in females). At the highest dose levels maternal toxicity was evident in terms of tremors, hunched posture, hair loss and reduced body weight gain during gestation. On day 4 all litters were dead in the high dose group (300 ppm) and pup viability was also reduced at 30 and 100 ppm 73 % and 13 %, respectively vs 90 % in ctrl). Lactation index was reduced at a dose level of 100 ppm (day 7/14/21: 55/45/45 vs 98/87/97 in ctrl). Necroscopy of females revealed inactive mammary tissue in 9 out of 10 animals at 100 ppm and in 3 animals from the top dose group (300 ppm). In two animals of the top dose group no milk was present in the mammary tissue and mammary tissue appears light in colour. Observation at necroscopy of offspring dying or sent to necroscopy before weaning revealed no milk in stomach in all dose groups, the highest incidence was observed at 30 ppm.

Under the conditions of the multigeneration study by Anonymous 34, 1990 the survival of the pups was impaired in the dose groups 30 and 100 ppm: the viability index was significantly reduced about 8 % and 50 % respectively at day 4. The lactation index was significantly reduced only at the top dose of 100 ppm from day 7 on (-23 %) and further impaired up to -54 % on day 25 post-partum. Body weight of the pups at weaning was reduced about 32 % at 100 ppm. Absence of milk in stomach was highest (incidence of 25 %) in offspring culled on day 4 in the top dose group. For pups dying before terminal kill the absence of milk was reported for all groups (incidence > 55 %, including control). Mammary glands were only examined for F0 females with total litter loss and no adverse effects were reported. The incidence of small pups was increased in the two top dose groups for pups examined after culling on day 4 and at terminal kill. Onset of eye opening and tooth eruption was significantly delayed at 100 ppm.

From the toxicokinetic studies on fosthiazate no information about transfer of fosthiazate in milk is available. Studies on metabolism on livestock revealed only very limited transfer of fosthiazate in milk in lactating goats. In the respective chapter of the RAR (Vol. 1, Chapter 2.7.3) the following is stated: “Similar results were found in the lactating goat dosed for four consecutive days with either [14C]-B or [14C]-R labelled fosthiazate at 10.7 (87N) and 17.7 mg/kg diet (140 N). The majority of the administered radioactivity was excreted. 2-3 % TRR (total radioactive residue) was found in milk. The plateau level in milk was reached within two days. Compared to eggs, where no plateau was reached, this can be explained by rather low log Kow of 1.7.” Based on the results of the range-finding generation study (Anonymous 25, 1990), there is evidence for impaired milk production by fosthiazate at 100 ppm (8.93 mg/kg bw/day) and above.

Cholinesterase activity was not measured in the studies, hence it remains open whether cholinesterase inhibition was causal for the pup mortality. A developmental neurotoxicity study is not available. Data from an age-sensitivity study in rats (Anonymous 7, 2006) showed no increased sensitivity of developing rats to cholinesterase inhibition (exposure during day 6-20 of gestation, euthanized on day 20): significant inhibition (≥ 20 %) of plasma and RBC cholinesterase in the dams was already observed at a 0.7 mg/kg bw. At 5 mg/kg bw also brain cholinesterase was inhibited in females (≥ 20 %) and plasma, RBC and brain cholinesterase were significantly inhibited (≥ 20 %) in the foetuses. The extent of inhibition was greater in the dams (-99.5–89 %) than in the foetuses (-33-22 %).

On balance, in two studies observed developmental effects such as reduced litter size, live-birth index and body weight are considered as a result of prenatal exposure to fosthiazate. Whereas the significant postnatal effects on offspring viability and development are considered to be 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 range-finding generational study. Developmental toxic effects of fosthiazate via self-feeding of the pups can be ruled out, since already 12/13 complete litter losses died on day 4-4 postnatal Therefore, a lactational effect, rather because of an impairment of milk production in the dams than due to exposure via the milk (as the transfer to the milk in high amounts has not been demonstrated is likely and should result in a respective classification and labelling.

10.10.9Comparison with the CLP criteria

Table 29: CLP criteria – hazard category for effects on or via lactation

CLP criteria
Effects on or via lactation Effects on or via lactation are allocated to a separate single category. It is recognized that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

Thus, classification is proposed that fosthiazate may cause harm due to its effects on lactation/milk production (H362: May cause harm to breast-fed children) in accordance with CLP regulation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Fosthiazate should be classified Repr. 2, H361fd “Suspected of damaging fertility. Suspected of damaging the unborn child” and in addition classification for effects on or via lactation (Lact., H362, May cause harm to breast-fed children) is considered warranted.

10.11 Specific target organ toxicity-single exposure

Several studies relevant for specific target organ toxicity after single exposure of fosthiazate are available and are summarised in Table 30. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in the text below (also found in the Volume 3 B.6 of the Renewal Assessment Report (RAR)) .

Table 30: Summary table of animal studies relevant for STOT SE

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Acute neurotoxicity screening study,
rat, Sprague-Dawley
10 animals of each sex per dose group: five males/group were dosed on the first day; 5 females/group were dosed on the second day; the remaining 5 males/group on the third day; and the remaining 5 females/group on the fourth day

The study satisfies the</td>
<td>Fosthiazate (93.8 purity)

Oral (gavage)
Dose: 0, 0.4, 10 and 40 mg/kg bw/day (males) and 0, 0.4, 10 and 20 mg/kg bw/day (females)
Exposure: 4 days</td>
<td>20/40 mg/kg bw (females/ males):
Anogenital staining, decreased faeces and dried red material on the forepaws, mouth and nose on the first and second day following dosing;
FOB open field evaluations of mobility, posture, gait, arousal, urination and righting reflex different from control on Day 0
Mean motor activity values for high dose males and females were statistically significantly different from the control on Day 0
Male Mean Motor Activity Data
<table>
<tr><th>Dose level (mg/kg bw)</th><th>Distance Traveled</th><th>Resting Time</th><th>Ambulatory Time</th><th>Stereotypic Time</th><th>Bursts of Stereotypic</th><th>Horizontal Counts</th><th>Total Counts</th><th>Vertical Counts</th><th>Vertical Breaks</th></tr>
<tr><td>Baseline</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0</td><td>2553.24</td><td>456.19</td><td>121.51</td><td>322.3</td><td>1002.1</td><td>2205.8</td><td>1361</td><td>146.2</td><td>35.2</td></tr>
<tr><td>0.4</td><td>2261.41</td><td>470.59</td><td>109.92</td><td>319.49</td><td>961</td><td>1978.6</td><td>1140.2</td><td>97</td><td>26.8</td></tr>
<tr><td>10</td><td>2423.97</td><td>483.35</td><td>117.56</td><td>299.09</td><td>960.6</td><td>2007</td><td>1252.2</td><td>110</td><td>30.7</td></tr>
<tr><td>40</td><td>2140.24</td><td>468.11</td><td>105.07</td><td>326.82</td><td>951.3</td><td>2019.6</td><td>1139</td><td>83.6</td><td>23.3</td></tr>
<tr><td>Day 0</td><td>v^{++}</td><td>v</td><td>v^{++}</td><td>++</td><td>v</td><td>v^{++}</td><td>v^{++}</td><td>v^{++}</td><td>v^{++}</td></tr>
<tr><td>0</td><td>1756.58</td><td>574.97</td><td>83.67</td><td>241.36</td><td>740.7</td><td>1545.1</td><td>976.3</td><td>178.4</td><td>31.5</td></tr>
<tr><td>0.4</td><td>1834.35</td><td>556.78</td><td>87.84</td><td>255.38</td><td>789</td><td>1560</td><td>976.2</td><td>184.9</td><td>31</td></tr>
<tr><td>10</td><td>2045.26</td><td>539.53</td><td>98.62</td><td>261.85</td><td>865.3</td><td>1644.4</td><td>1052.4</td><td>171.5</td><td>34.5</td></tr>
<tr><td>40</td><td>278.92**</td><td>784.76**</td><td>16.34**</td><td>98.9**</td><td>261.4**</td><td>323.8**</td><td>137**</td><td>2.4**</td><td>0.4**</td></tr>
<tr><td>Day 7</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0</td><td>2362.7</td><td>513.34</td><td>109.91</td><td>276.75</td><td>893</td><td>1840.6</td><td>1261.3</td><td>285.2</td><td>45.3</td></tr>
</table>
</td>
<td>Anonymous 38, 1997</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

essential requirements of EC guidelines for a neurotoxicity study in rodents (B.43.) or OECD TG 424 (1997)

GLP

Acceptable

0.4	2130.09	541.11	97.84	261.05	812	1684	1090.7	243.7	41.9
10	2052.32	555.21	97.43	247.36	811.5	1570.1	1048.7	272.6	41
40	1725.27	561.91	85.31	252.78	780.4	1382.8	834.8	156.1	28.6
Day 14				v					
0	2418.51	531.73	112.15	256.12	888.7	1770.8	1264.8	349.7	49.9
0.4	2554.38	482.76	119.39	297.85	987.2	1981.4	1352.4	382.4	55.6
10	2473.67	530.93	115.31	253.76	903.9	1762.2	1268.8	346.5	54.1
40	2197.41	538.17	105.5	256.33	881.7	1608.3	1123.1	317.6	48.6

Day 0 = 3 hours postdosing, v Bartlett's test significant, ++ Trend significant at the 0.01 level, **Statistical significant at the 0.01 level

Female Mean Motor Activity Data

Dose level (mg/kg bw)	Distance Traveled	Resting Time	Ambulatory Time	Stereotypic Time	Bursts of Stereotypic	Horizontal Counts	Total Counts	Vertical Counts	Vertical Breaks
Baseline									
0	2853.55	446.68	131.06	322.26	980.3	2407.9	1523.2	163.4	42.9
0.4	2807.74	441.34	128.43	330.23	997.2	2309.6	1470.0	111	36
10	2948.67	410.03	139.4	350.57	1091.6	2478.5	1594.8	200.7	51.3
20	3004.73	413.57	139.38	347.05	1077.5	2496.7	1602.8	146.9	41.7
Day 0								v^{++}	v^{++}
0	2766.18	492.67	121.39	285.94	905.5	2330.1	1587.9	267.5	51.6

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

0.4	2932.28	449.65	128.42	321.93	985.1	2577.6	1727.8	302.1	54.9
10	3417.81	443.65	147.27	309.08	1017.4	2636.9	1905.9	307.9	56.3
20	793.5**	722.02**	38.16**	139.82**	402.5**	699.1**	402.9**	24.1**	6.1**
Day 7									
0	3615.16	432.14	152.93	314.93	1059.5	2936.5	2148.2	534.8	76.6
0.4	3605.38	438.9	150.55	310.55	1042.8	2926.8	2154.5	534.5	74.1
10	3080.69	473.61	134.12	292.27	962.6	2474.1	1790.9	429	68.7
20	2691.2	492.07	120.21	287.72	918.7	2224.0	1514.4	371.3	56.9
Day 14	++	V	++				++	++	
0	3801.79	439.98	155.78	304.24	1042.3	3054.7	2336.4	632.1	81.7
0.4	3580.98	453.21	146.88	299.91	991.3	2953.7	2244.3	576.1	73.5
10	3287.33	455.09	142.32	302.59	1016.1	2581.9	1890.5	473.4	70.1
20	2382.86**	537.62*	105.92**	256.46	815.7*	1934.1**	1355.6**	339.5**	58.1

Day 0 = 3 hours postdosing, v Bartlett's test significant, ++ Trend significant at the 0.01 level, *Statistical significant at the 0.05 level, **Statistical significant at the 0.01 level

≥10 mg/kg: Erythrocyte and brain cholinesterase values↓

Cholinesterase activity in males (percent control)							
Group	**Dose mg/kg bw**	**Day**	**Plasma**	**RBC**	**Brain CC**	**Brain C**	**Brain BS**
2	0.4	0[1]	97	87**	101	100	96
		7	95	89	103	100	100
		14	97	97	97	100	102
3	10	0[1]	26**	72**	79**	76*	72**
		7	87	79**	92	107	92
		14	103	86**	93*	97	96
4	40	0[1]	7**	67**	23**	33**	22**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

		7	89	73**	49**	76**	67**
		14	101	82**	63**	92*	80**

RBC = erythrocyte
CC = cerebral cortex
C = cerebellum
BS = brain stem
[1] = Day 0, 3 hours postdosing
* = statistically significant difference at the 0.05 level
** = statistically significant difference at the 0.01 level

Cholinesterase activity in females (percent control)

Group	Dose mg/kg bw	Day	Plasma	RBC	Brain CC	Brain C	Brain BS
2	0.4	0[1]	96	90	101	100	99
		7	95	101	100	104	110
		14	105	101	102	111	101
3	10	0[1]	14**	75**	70**	69*	65**
		7	89	81**	77**	96	96
		14	100	91**	91*	108	92
4	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 the 0.05 level
** = statistically significant difference at the 0.01 level

NOAEL:

FOB and motor activity: 10 mg/kg bw

Neuropathology: 20 mg/kg bw (females) and 40 mg/kg bw (males)

Cholinesterase inhibition (RBC and brain): 0.4 mg/kg bw

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>NOAEL overall: 0.4 mg/kg bw</td><td></td></tr>
<tr><td>Oral (gavage) age-sensitivity study of fosthiazate in rats
Rat, Crl:CD®(SD)

Determination of the potential of fosthiazate to induce effects on maternal, fetal, neonatal and young adult cholinesterase activity in the blood components and brain after maternal gestational exposure or after acute or short-</td><td>Fosthiazate (94.7% purity)
Oral (gastric intubation)
Dose: 0, 0.1, 0.7, 5 mg/kg bw/d
Number of animals in different test phases: Phase I (Gestational exposure, day 6-20): 12 dams and litters, Phase II (Time of peak effect determination, single dose in PND11 or PND21): PND 11: 60 animals/sex, PND 21: 30 animals/sex</td><td>Phase I (daily dosing from GD 6 - 20): no clinical signs observed during days 6-18. On GD 19 and/or 20 at 5 mg/kg bw: tremors, unkempt appearance, prostration and red and yellow material on various body surfaces, additional in 1 or 2 animals during the twice-weekly examinations repetitive jaw movement, piloerection, gasping and lacrimation; 3 hours following dosing on gestation day 19 lacrimation (1 female), tremors and/or repetitive jaw movement (3 females);
Plasma cholinesterase activities of juveniles and young adults ↓ (≥25 %); erythrocyte cholinesterase activities of juveniles and young adults ↓ (≥25 %);
brain cholinesterase activities of juveniles adults ↓ (≥25 %)
<table>
<tr><th colspan="8">Mean plasma, RBC and brain cholinesterase values in dams and foetuses on gestation day 20 - Phase I: Gestation Exposure</th></tr>
<tr><th></th><th>Control</th><th colspan="2">0.1 mg/kg bw/day</th><th colspan="2">0.7 mg/kg bw/day</th><th colspan="2">5 mg/kg bw/day</th></tr>
<tr><td>Plasma[1]
Maternal
Pooled Foetal</td><td>3147 ± 410.1

491 ± 40.8</td><td>3008 ± 467.3

506 ± 38.8</td><td>96%

103%</td><td>852** ± 139.1

495 ± 50.9</td><td>27%

101%</td><td>142** ± 24.7

329** ± 59.3</td><td>4.5%

67%</td></tr>
<tr><td>RBC[1]
Maternal
Pooled Foetal</td><td>3931 ± 1474.5

2644 ± 644.1</td><td>3831 ± 757.3

3283 ± 992.4</td><td>97%

124%</td><td>2193** ± 712.2

2893 ± 738.3</td><td>56%

109%</td><td>20[2]** ± 0.0

1851* ± 593.4</td><td>0.5%[2]

70%</td></tr>
<tr><td>Brain[1]
Maternal
Pooled Foetal</td><td>49446 ± 2189.8

6612 ± 679.5</td><td>48974 ± 1364.5

6328 ± 476.3</td><td>99%

96%</td><td>47135** ± 1510.0

6251 ± 649.5</td><td>95%

95%</td><td>5152** ± 1718.9

5182** ± 684.5</td><td>10.4%

78%</td></tr>
</table>
[1] = For the control group, the mean (U/L) is shown. For the test article-treated groups, the mean (U/L) and the % of the control activity is shown.
[2] = RBC cholinesterase values for all females were under the range of detection; therefore, the LLOQ (20 U/L) was used for statistical analysis.
* = Significantly different from the control group at 0.05.
** = Significantly different from the control group at 0.01.
Phase II (peak determination) and phase III (acute exposure): no clinical signs post-dosing.</td><td>Anonymous 7, 2006</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>term repeated exposure to rats of various ages. Phase I (Gestational exposure), Phase II (Time of peak effect determination), Phase III (Acute exposure) and Phase IV (Repeated exposure). For Phases II-IV, experimentally naive bred female Crl:CD®(SD) rats were used only to obtain pups and were not administered the test article.</td>
<td>, Phase III (Acute exposure): PND 11 20 animals/sex, PND 21: 10 animals/sex, young adult: 10 and Phase IV (Repeated exposure): PND 21: 10 animals/sex, young adult: 10
Duration of exposure: Phase I maternal animals: once daily, gestation day 6-20. Phase II and Phase III pups were administered a single dose on PND 11 or</td>
<td>
<table>
<tr><th></th><th colspan="3">Mean plasma cholinesterase activity[1] – Phase III – Acute exposure</th></tr>
<tr><th></th><th colspan="2">Males</th><th>Females</th><th colspan="2"></th></tr>
<tr><th>Age</th><th>Control</th><th colspan="2">5 mg/kg bw</th><th>Control</th><th colspan="2">5 mg/kg bw</th></tr>
<tr><td>PND 11[2]</td><td>1536 ± 150.8</td><td>633** ± 62.2</td><td>41%</td><td>1482 ± 64.3</td><td>641** ± 59.7</td><td>43%</td></tr>
<tr><td>PND21[2]</td><td>1094 ± 148.7</td><td>432** ± 46.3</td><td>39%</td><td>1123 ± 157.4</td><td>422** ± 65.2</td><td>38%</td></tr>
<tr><td>Young Adult[3]</td><td>888 ± 63.2</td><td>519** ± 98.7</td><td>58%</td><td>1423 ± 399.3</td><td>472** ± 96.1</td><td>33%</td></tr>
</table>
<table>
<tr><th></th><th colspan="3">Mean RBC cholinesterase activity[1] – Phase III – Acute exposure</th></tr>
<tr><th></th><th colspan="2">Males</th><th>Females</th><th colspan="2"></th></tr>
<tr><th>Age</th><th>Control</th><th colspan="2">5 mg/kg bw</th><th>Control</th><th colspan="2">5 mg/kg bw</th></tr>
<tr><td>PND 11[2]</td><td>4701 ± 1008.2</td><td>3866 ± 3074.8</td><td>82%</td><td>5558 ± 2940.3</td><td>3110* ± 832.2</td><td>56%</td></tr>
<tr><td>PND21[2]</td><td>4929 ± 2165.2</td><td>3085 ± 1263.6</td><td>63%</td><td>4058 ± 1576.4</td><td>2777 ± 879.3</td><td>68%</td></tr>
<tr><td>Young Adult</td><td>3351 ± 635.6</td><td>2582 ± 478.4</td><td>77%</td><td>4469 ± 2964.6</td><td>2387* ± 475.4</td><td>53%</td></tr>
</table>
<table>
<tr><th></th><th colspan="4">Mean Brain Cholinesterase Activity[1] – Phase III – Acute exposure</th></tr>
<tr><th></th><th colspan="2">Males</th><th>Females</th><th colspan="2"></th></tr>
<tr><th>Age</th><th>Control</th><th colspan="2">5 mg/kg bw</th><th>Control</th><th colspan="2">5 mg/kg bw</th></tr>
<tr><td>PND 11[2]</td><td>24360 ± 1177.1</td><td>20730** ± 1386.1</td><td>85%</td><td>24800 ± 932.8</td><td>21286** ± 1277.4</td><td>86%</td></tr>
<tr><td>PND21[2]</td><td>36661 ± 1971.4</td><td>31019 ± 1087.5</td><td>85%</td><td>36797 ± 2081.1</td><td>30784** ± 1843.1</td><td>84%</td></tr>
<tr><td>Young Adult[3]</td><td>47611 ± 2803.8</td><td>47783 ± 2016.4</td><td>100%</td><td>48377 ± 2814.7</td><td>47798 ± 3074.9</td><td>99%</td></tr>
</table>
1 = For the control group, the mean (U/L) ± the standard deviation is shown. For the 5 mg/kg bw group, the mean (U/L) ± the standard deviation and the % of the control group activity is shown.
2 = Analyses conducted 4 hours following dosing.
3 = Analyses conducted 3 hours following dosing.
* = Significantly different from the control group at 0.05.
** = Significantly different from the control group at 0.01.

Phase IV (repeated exposure): no clinical findings post-dosing in young adult or juvenile rats, except for one young adult female that was found dead on the 10th day of dose administration
</td>
<td></td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>No Guideline
GLP

Acceptable</td>
<td>PND 21, Phase IV pups were dosed once daily during PND 11-21, Phase IV young adults were dosed for 11 consecutive days form PND 43-46 on.</td>
<td>
<table>
<tr><th colspan="9">Mean plasma, RBC and brain cholinesterase activities for PND 21 animals – Phase IV- Repeated exposure)</th></tr>
<tr><th colspan="9">PND 21 males</th></tr>
<tr><th></th><th>Control</th><th colspan="2">0.1 mg/kg bw/day</th><th colspan="2">0.7 mg/kg bw/day</th><th colspan="2">5 mg/kg bw/day</th></tr>
<tr><td>Plasma Activity[1]</td><td>1085 ± 109.8</td><td>1055 ± 100.1</td><td>97%</td><td>849** ± 136.9</td><td>78%</td><td>439** ± 180.3</td><td>40%</td></tr>
<tr><td>RBC Activity[1]</td><td>8725 ± 3386.9</td><td>7522 ± 2622.6</td><td>86%</td><td>9528 ± 10383.3</td><td>109%</td><td>1107* ± 1942.2</td><td>13%</td></tr>
<tr><td>Brain Activity[1]</td><td>39177 ± 1109.0</td><td>40093 ± 2031.0</td><td>102%</td><td>37563 ± 825.1</td><td>96%</td><td>19692** ± 11643.7</td><td>50%</td></tr>
<tr><th colspan="9">PND 21 females</th></tr>
<tr><td>Plasma Activity[1]</td><td>1019 ± 253.8</td><td>1061 ± 151.3</td><td>104%</td><td>832 ± 193.0</td><td>82%</td><td>346** ± 104.2</td><td>33%</td></tr>
<tr><td>RBC Activity[1]</td><td>7595 ± 3820.6</td><td>7595 ± 3820.6</td><td>101%</td><td>6523 ± 1381.8</td><td>86%</td><td>508** ± 510.2</td><td>7%</td></tr>
<tr><td>Brain Activity[1]</td><td>37284 ± 8312.6</td><td>40936 ± 1081.5</td><td>110%</td><td>34444 ± 6956.5</td><td>92%</td><td>13125** ± 1810.5</td><td>35%</td></tr>
</table>
1 = For the control group, the mean (U/L) is shown. For the test article-treated groups, the mean (U/L) and the % of the control activity is shown.* = Significantly different from the control group at 0.05.
** =Significantly different from the control group at 0.01
<table>
<tr><th colspan="9">Mean plasma, RBC and brain cholinesterase activities of young animals – Phase IV - Repeated exposure)</th></tr>
<tr><th colspan="9">Young adult males</th></tr>
<tr><th></th><th>Control</th><th colspan="2">0.1 mg/kg bw/day</th><th colspan="2">0.7 mg/kg bw/day</th><th colspan="2">5 mg/kg bw/day</th></tr>
<tr><td>Plasma Activity[1]</td><td>773 ± 155.5</td><td>828 ± 76.9</td><td>107%</td><td>756 ± 152.3</td><td>98%</td><td>325** ± 38.3</td><td>42%</td></tr>
<tr><td>RBC Activity[1]</td><td>2941 ± 792.8</td><td>2915 ± 950.4</td><td>99%</td><td>2821 ± 643.6</td><td>96%</td><td>392** ± 306.0</td><td>13%</td></tr>
<tr><td>Brain Activity[1]</td><td>49176 ± 1372.3</td><td>51198 ± 1783.8</td><td>104%</td><td>49595 ± 907.2</td><td>101%</td><td>38237** ± 4346.9</td><td>78%</td></tr>
<tr><th colspan="9">Young adult females</th></tr>
<tr><td>Plasma Activity[1]</td><td>1991 ± 433.3</td><td>2006 ± 754.3</td><td>101%</td><td>1152** ± 170.3</td><td>58%</td><td>253** ± 27.1</td><td>13%</td></tr>
<tr><td>RBC Activity[1]</td><td>2548 ± 846.7</td><td>3178 ± 1248.9</td><td>125%</td><td>2196 ± 298.2</td><td>86%</td><td>20**[2] ± 0.0</td><td>1%[2]</td></tr>
<tr><td>Brain Activity[1]</td><td>50227 ± 2570.2</td><td>50726 ± 1031.3</td><td>101%</td><td>48882 ± 1780.8</td><td>97%</td><td>21476** ± 4545.2</td><td>43%</td></tr>
</table>
1 = For the control group, the mean (U/L) is shown. For the test article-treated groups, the mean (U/L) and the % of the control activity is shown.
2 = RBC cholinesterase values were under the range of detection; therefore, the LLOQ (20 U/L) was used for statistical analysis.
** = Significantly different from the control group at 0.01.
<u>NOAEL:</u>
Plasma:
</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Dams: 0.1 mg/kg bw/day, Fetuses: 0.7 mg/kg bw/day, Juveniles and young Adults (acute exposure): 0.7 mg/kg bw/day, Juveniles and young adults (repeated exposure): 0.1 mg/kg bw/day; RBC: Dams: 0.1 mg/kg bw/day, Fetuses: 0.7 mg/kg bw/day, Juveniles and young adults (acute or repeated exposure): 0.7 mg/kg bw/day Brain: Dams, Fetuses: 0.7 mg/kg bw/day, Juveniles and young adults (acute and repeated exposure): 0.7 mg/kg bw/day	
Acute oral toxicity, Oral (gavage), Rat, CD (remote Sprague-Dawley), 5M & 5 F Meets the essential criteria of 92/69/EEC Method B1. Body weights of 2 animals from the 64 or 81 mg/kg bw dose groups deviated significantly from their group mean	Fosthiazate (93.4% purity) 41, 51, 64, 81, 128 mg/kg bw in maize oil, single dose	≥64 mg/kg bw: Mortality ↑ (2/5 males, 5/5 females) Clinical signs in both sexes at all dose levels (decreased motor activity, prone or hunched posture, muscle tremor, irregular breathing and piloerection; less frequent signs: 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)	Anonymous 14, 1989b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
body weight. The achieved concentrations of the tested dose formulations were 90% and 77% of the nominal values, 128 and 41 mg/kg bw respectively. GLP Acceptable			
Acute oral toxicity, Oral (gavage), Mouse, CD-1 5M & 5 F Meets the essential criteria of 92/69/EEC Method B1 but uncertainty in	Fosthiazate (93.4% purity) 51, 81, 102, 128, 161 mg/kg bw in maize oil, single dose	≥102 mg/kg bw fosthiazate mortality ↑within 4 days of treatment all animals died except 3/5 males at 128 mg/kg bw Clinical signs of toxicity (≥102 mg/kg bw fosthiazate): decreased motor activity, hunched posture, ataxia and muscle tremor	Anonymous 13, 1989a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
concentration dosing solutions. GLP Supplementary			
Acute dermal toxicity, Rat, CD, 5M & 5 F Meets the essential criteria of 92/69/EEC Method B3. GLP Acceptable	Fosthiazate (93.4% purity) M: 1965, 2472, 3115, 3918 mg/kg bw, F: 309, 494, 779, 1236, 1557, 1965, 2472 mg/kg bw	≥1965 mg/kg bw mortality in males ≥779 mg/kg bw mortality in females within 8 days Clinical signs of toxicity (≥1965 mg/kg bw in males, ≥779 mg/kg bw in females) 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.	Anonymous 15, 1989c
Acute toxicity, inhalation (nose-only) Rat, Sprague-Dawley, 8M & 8 F In accordance	Fosthiazate (92 % purity) 0, 0.53, 0.8, 0.9 and 1.23 mg/L; mass median aerodynamic diameter (MMAD) ranged from	LC50: males: 0.83 mg/L, females: 0.56 mg/L at all dose levels: mortalities occurred within 2 days (males) or 4 days (females), signs of toxicity: reduction in spontaneous motor activity, decrease in respiratory rate, salivation, limb paralysis, adoption of prone position nasal bleedings, red tears and lacrimation. Necropsy findings (rats which died): revealed hyperaemia and/or haemorrhage in the lung, stomach filled with gas, haemorrhage of the mucous membrane in the glandular stomach and cloudy eyeball. Survivors were asymptomatic within 4 days.	Anonymous 21, 1989

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>with US EPA FIFRA Pesticide Assessment guidelines and satisfies the essential criteria of 92/96/EEC.

GLP
Acceptable</td><td>2.04-2.29 µm
4 hours exposure to an aerosol of fosthiazate</td><td>
<table>
<tr><th></th><th colspan="2">Mortality</th><th colspan="2">Decrease of spontaneous motor activity</th><th colspan="2">Limb paralysis</th><th colspan="2">Decrease of respiratory rate</th><th colspan="2">Hyperemia, lung (of died rats)</th><th colspan="2">Haemorrhage, lung (of died rats)</th></tr>
<tr><th>Analytical concentration (mg/L)</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th><th>M</th><th>F</th></tr>
<tr><td>0 (air control)</td><td>0/8</td><td>0/8</td><td>8/8</td><td>8/8</td><td>0/8</td><td>0/8</td><td>0/8</td><td>0/8</td><td>n. a.</td><td>n. a.</td><td>n. a.</td><td>n. a.</td></tr>
<tr><td>0.53</td><td>1/8</td><td>4/8</td><td>8/8</td><td>8/8</td><td>7/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>1/1</td><td>4/4</td><td>1/1</td><td>4/4</td></tr>
<tr><td>0.80</td><td>3/8</td><td>5/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>3/3</td><td>5/5</td><td>2/3</td><td>4/5</td></tr>
<tr><td>0.90</td><td>5/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>7/8</td><td>8/8</td><td>7/8</td><td>5/5</td><td>7/8</td><td>2/5</td><td>5/8</td></tr>
<tr><td>1.23</td><td>7/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>8/8</td><td>7/8</td><td>8/8</td><td>7/8</td><td>7/7</td><td>8/8</td><td>3/7</td><td>3/8</td></tr>
</table>
</td><td></td></tr>
<tr><td>Acute Delayed Neurotoxicity Study, hen
Oral (gavage)
Hens (Gallus gallus domesticus), groups of 6 animals
The study satisfies the essential requirements of OECD</td><td>Fosthiazate (93.7 % purity)
20 mg/kg bw in maize oil
Dosing at day 1 and 23.
Observation for 24 days.</td><td>20 mg/kg bw: mortality↑, marked cholinergic responses and motor impairment; no acute delayed neurotoxicity</td><td>Anonymous 19, 1989g</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Guideline 418
GLP
Acceptable</td><td></td><td></td><td></td></tr>
<tr><td>Determination of a no effect level for cholinesterase inhibition in rats with fosthiazate
Oral (gavage)
Rat, CD, Sprague Dawley
20 M & 20 F
No guideline
GLP
Supplementary</td><td>Fosthiazate (92.1% purity)
Single dose of 0, 0.04, 0.4,
4 mg/kg bw</td><td>
<table>
<tr><th colspan="6">Mean acetylcholinesterase activity (U/L)</th></tr>
<tr><th colspan="6">Males</th></tr>
<tr><th>Dose (mg/kg bw)</th><th>Plasma</th><th>Erythrocyte</th><th>Brain Cerebral cortex</th><th>Brain Cerebellum</th><th>Brain stem</th></tr>
<tr><td>0</td><td>422.8</td><td>139.0</td><td>238.4</td><td>205.8</td><td>514.6</td></tr>
<tr><td>4</td><td>272.0* (64%)</td><td>129.2</td><td>228.4</td><td>202.4</td><td>538.2</td></tr>
<tr><td>0.4</td><td>405.8</td><td>152.4</td><td>188.8</td><td>206.0</td><td>503.2</td></tr>
<tr><td>0.04</td><td>429.4</td><td>171.6</td><td>255.6</td><td>207.4</td><td>594.2</td></tr>
<tr><th colspan="6">Females</th></tr>
<tr><th>Dose (mg/kg bw)</th><th>Plasma</th><th>Erythrocyte</th><th>Brain Cerebral cortex</th><th>Brain Cerebellum</th><th>Brain stem</th></tr>
<tr><td>0</td><td>1145.2</td><td>137.2</td><td>230.0</td><td>186.3</td><td>458.8</td></tr>
<tr><td>4</td><td>218.6* (19%)</td><td>122.6</td><td>225.8</td><td>188.8</td><td>476.2</td></tr>
<tr><td>0.4</td><td>1016.0</td><td>126.4</td><td>252.8</td><td>211.4</td><td>554.6</td></tr>
<tr><td>0.04</td><td>1253.2</td><td>141.8</td><td>247.4</td><td>220.2</td><td>572.2</td></tr>
</table>
* Significantly different from control, p<0.01
(%) = Percent of control
NOEL for inhibition of plasma acetylcholinesterase: 0.4 mg/kg bw; at 4 mg/kg bw inhibition of plasma acetylcholinesterase: ~ 36 % in males, ~81% in females.
NOEL for inhibition of erythrocyte and brain acetylcholinesterase: >4.0 mg/kg bw
</td><td>Anonymous 37, 1994</td></tr>
</table>

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

In an acute neurotoxicity screening study in rats (Anonymous 38, 1997) anogenital staining, decreased faeces and dried red material on the forepaws, mouth and nose were observed in high dose animals on the first and second day following dosing. Similar mean body weights for male and female animals for all groups for pre-test, prefast, and Days 0, 7 and 14 were reported. Significant differences were observed in the functional observational battery (FOB) open field evaluations of mobility, posture, gait, arousal, urination and righting reflex in high dose males (40 mg/kg bw) and posture and gait in high dose females (20 mg/kg bw) on Day 0 (3 hours post dosing): mobility was slightly to moderately impaired in all males (ten animals per group); five of the males showed hunched posture, one splayed hind limbs and one a dragging body; gait abnormalities were severe in two males, considerable in one male and slight in seven males; arousal was low in nine males; four males had one pool of urine, one male two pools, and two males each three and four pools of urine (in control group four males showed one pool of urine); the rightning reflex was slightly impaired in six males, six females showed hunched posture; 7 females showed slightly impaired gait. Mean motor activity values for high dose males and females were statistically reduced in comparison to the control group on Day 0 (3 hours post dosing). Mean plasma cholinesterase values were statistically significantly lower on Day 0 (3 hours postdosing) for both sexes at 10 mg/kg bw and 20 respectively 40 mg/kg. At day 7 and 14 plasma cholinesterase values were comparable to control values in males and females at all dose levels. At 10 and 40 mg/kg bw erythrocyte cholinesterase values were lower (at least 20% lower than control) than control on Day 0 (3 hours postdosing) for males and females, but recovered by Day 14. Also brain cholinesterase was statistically significantly lower (at least 10 %) in males and females on Day 0 (3 hours postdosing) at 10 mg/kg bw and 40 or 20 mg/kg bw, respectively, but was reversed at the mid dose level (10 mg/kg bw) by Day 14. At the high dose a reduced brain cholinesterase activity was still observable at Day 14 in both sexes (20 or 40 mg/kg bw, respectively). No histological findings in evaluated nerve tissues were reported up to the highest tested dose. The study is considered acceptable.

In an oral age sensitivity study in rats (Anonymous 7, 2006) cholinesterase inhibition was, in general, similar in juvenile and young adult males and females for all compartments and no age–related effects of fosthiazate were observed after maternal gestational exposure or after acute or short-term exposure to rats of various ages. Nevertheless, maternal females were much more sensitive to cholinesterase inhibition following direct test article administration than were their foetuses following in utero exposure: significant inhibition (≥ 20 %) of plasma and RBC cholinesterase was already observed at a 0.7 mg/kg bw. At 5 mg/kg bw also brain cholinesterase was inhibited in females and plasma, RBC and brain cholinesterase were significantly inhibited (≥ 20 %) in the foetuses. The extent of inhibition was greater in the dams (-99.5 – 89 %) than in the foetuses (-33-22 %). Following acute exposure, cholinesterase activity was inhibited only at 5 mg/kg bw in juveniles and young adults. After repeated dosing, inhibition occurred at 0.7 and 5 mg/kg bw. RBC inhibition following repeated dosing was severe; all maternal females and several juvenile and young adult animals had values that were below the lower limit of quantitation. The inhibition in the maternal animals at 5 mg/kg bw/day (-99.5 %) was accompanied by functional deficits, including tremors, repetitive jaw movement and piloerection. There were no neurobehavioral findings in the juveniles or young adults following repeated exposure at 5 mg/kg bw/day. Slightly reduced mean body weight gains were observed in the juvenile males and, to a lesser extent, in the females Plasma cholinesterase was determined to be the most sensitive compartment because inhibition occurred at lower dosage levels (0.7 mg/kg bw) and occurred more quickly following test article administration. The study is considered acceptable. No guideline is applicable for this type of study.

In an acute oral toxicity study in rats (Anonymous 14, 1989b) deaths occurred at dose levels of ≥ 64 mg/kg bw within 3 days after treatment. 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. Clinical signs resolved within 3 days in the surviving animals. The study is considered acceptable. Deviations in test concentration were reported and the achieved concentrations of the tested dose formulations were 90 % and 77 % of the nominal values, 128 and 41 mg/kg bw respectively. Another deviation was a significant difference of the body weights of 2 animals from the 64 or 81 mg/kg bw dose in comparison to the group mean body weight. Nevertheless, the results of the study were

considered to be valid, as the deviations didn't influenced the scope of the study.

Also in mice decreased motor activity, hunched posture, ataxia and muscle tremor were observed at ≥ 102 mg/kg bw in an acute oral toxicity study (Anonymous 13, 1989a). Less frequent observations were lethargy, irregular breathing, prone posture and closed eyes. Animals given dose of ≥ 102 mg/kg bw fosthiazate died within 4 days of treatment except 3/5 males at 128 mg/kg bw. Due to uncertainty of dosing solutions the study was considered as supplementary information.

Similar clinical signs of toxicity as after oral exposure were also observed in an acute dermal toxicity study in rats (Anonymous 15, 1989c). 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. Mortality occurred within 8 days at dose levels of ≥1965 mg/kg bw in males and ≥779 mg/kg bw in females. At dose levels of ≥ 3115 mg/kg bw in males and ≥1236 mg/kg bw in females all animals died. Body weight loss was observed in most surviving animals during the first week. Post-mortem examination of the animals which died showed general fur staining, altered gastrointestinal contents, isolated cases of uterine distension, darkened lungs, darkened glandular stomach mucosa and dark encrustation at the treatment site. The study is considered acceptable.

In an acute toxicity study via inhalation route in rats (Anonymous 21, 1989) mortality occurred at all dose levels (0.53 – 1.23 mg/L) within 2 days (males) or 4 days (females). Signs of toxicity included decrease in respiratory rate, salivation, limb paralysis, adoption of prone position nasal bleedings, red tears and lacrimation and were also observed from the lowest dose on. Necropsy of the rats which died showed hyperaemia and/or haemorrhage in the lung, stomach filled with gas, haemorrhage of the mucous membrane in the glandular stomach and cloudy eyeball. Observed decrease in spontaneous motor activity was not treatment-related, as this was also reported for all animals of the control group. Survivors were asymptomatic within 4 days.

In hens, acute delayed neurotoxicity study was investigated (Anonymous 19, 1989g). Seven out of 18 birds treated with 20 mg/kg bw fosthiazate died during the study. Two birds from the treatment-group and 5 of the positive control (TOCP: tri-ortho-cresyl-phosphate) were killed in a moribund condition. Marked cholinergic responses and associated motor impairment was observed in animals treated with fosthiazate. Signs of toxicity consisted of reduced activity, peripheral vasodilation, unsteadiness, drooped wings, resting on hocks and occasional clonic convulsions. No abnormalities were obvious after macroscopic and microscopic examination of organs and tissues including the nervous tissue. Animals from the positive control group showed disturbed balance and or partial paralysis which were consistent with delayed neurotoxicity as well as histopathology findings of these animals: vacuolation of the myelin sheaths, focal gliosis of the spinal cord, eosinophilic hyaline accumulation and swollen axons were observed. The study is considered acceptable.

A no effect level for cholinesterase inhibition on plasma, erythrocyte and brain following a single oral administration of fosthiazate in water to male and female rats was determined in a study by Anonymous 37, 1994. At a dose level of 4 mg/kg bw an inhibition of plasma cholinesterase of about ~ 36 % in males and ~81 % in females was observed. No inhibition of erythrocyte and brain acetylcholinesterase was observed under the conditions of the study up to the top dose level of 4.0 mg/kg bw. No guideline is applicable for this type of study. The study is considered supplementary.

No observations on occupationally and accidentally exposed humans are available.

10.11.2Comparison with the CLP criteria

Table 31: Comparison of results from selected studies with single exposure to classification criteria for Categories 1 and 2 and 3 of specific target organ toxicity – single exposure (C: guidance value)

Toxicological data	CLP criteria	
Acute neurotoxicity screening study, rat (Anonymous 38, 1997): 20/40 mg/kg bw (females/ males):	Category 1 (H370) Oral (rat): C ≤ 300 mg/kg bw	Substances that have produced significant toxicity in humans or that, on the basis of evidence from

Toxicological data	CLP criteria	
Anogenital staining, decreased faeces and dried red material on the forepaws, mouth and nose on the first and second day following dosing; FOB open field evaluations of mobility, posture, gait, arousal, urination and righting reflex, motor activity values different to control; ≥10 mg/kg: Erythrocyte and brain cholinesterase activities in both sexes ↓ (≥20%) **Oral (gavage) age-sensitivity study of technical fosthiazate in rats, acute exposure (Anonymous 7, 2006):** 5 mg/kg bw: on gestation days 19 and/or 20 tremors, unkempt appearance, prostration and red and yellow material on various body surfaces, additional in 1 or 2 animals during the twice-weekly examinations repetitive jaw movement, piloerection, gasping and lacrimation; 3 hours following dosing on gestation day 19 lacrimation (1 female), tremors and/or repetitive jaw movement (3 females); Plasma cholinesterase activities of juveniles and young adults (≥20%) ↓; erythrocyte cholinesterase activities of juveniles and young adults (≥20%) ↓; brain cholinesterase activities of juveniles and young adults (≥20%)↓; Determination of a no effect level for cholinesterase inhibition in rats with fosthiazate, single oral dose, rat (Anonymous 37, 1994): 4.0 mg/kg bw: Plasma acetylcholinesterase activity (≥20%) ↓ **Acute Oral Toxicity Study in the Rat (Anonymous 14, 1989b):** ≥64 mg/kg bw: Mortality ↑ (2/5 males, 5/5 females) Clinical signs in both sexes at all dose levels (decreased motor activity, prone or hunched posture, muscle tremor, irregular breathing and piloerection; less frequent signs: ataxia, muscle spasms, pigmented orbital secretions, irritability, blanching, salivation, pigmented staining of the snout and	Dermal (rat or rabbit): C ≤ 1000 mg/kg bw Inhalative (rat, dust/mist/fume): ≤ 1 mg/L/4 h	studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure - reliable and good quality evidence from human cases or epidemiological studies; or - observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.
	Category 2 (H371) Oral (rat): 2000 ≥ C > 300 mg/kg bw Dermal (rat or rabbit): 2000 ≥ C > 1000 mg/kg bw Inhalative (rat, dust/mist/fume): 5 ≥ C > 1 mg/L/4 h	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure - observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.
	Category 3 (H335/H336) Guidance values do not apply (primarily based on human data).	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.

Toxicological data	CLP criteria	
diarrhoea; clinical signs resolved within 3 days in the surviving animals) **Acute Oral Toxicity Study in the Mice (Anonymous 13, 1989a):** ≥102 mg/kg bw: mortality ↑ Clinical signs of toxicity: decreased motor activity, hunched posture, ataxia and muscle tremor **Acute Dermal Toxicity Study in the Rat (Anonymous 15, 1989c):** ≥1965 mg/kg bw mortality ↑ in males ≥779 mg/kg bw mortality ↑ in females within 8 days Clinical signs of toxicity: 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. **Acute Toxicity Study – inhalation route, rat (Anonymous 21, 1989):** ≥ 0.53 mg/L: Mortality↑, respiratory rate ↓, salivation, prone position, nasal bleeding, limb paralysis, red tears, lacrimation, hyperaemia & haemorrhage in the lung ↑. Survivors were asymptomatic within 4 days. **Acute delayed neurotoxicity study, hen (Anonymous 19, 1989g):** 20 mg/kg bw: Mortality↑, marked cholinergic responses and motor impairment (activity ↓, peripheral vasodilation, unsteadiness, drooped wings, resting on hocks and occasional clonic convulsions, controlled by injections of atropine sulphate and pralidoxime)		

According to the CLP criteria (Guidance on the Application of the CLP Criteria (2017)), chapter 3.8.2.3, "STOT SE Category 3" currently covers only transient effects of `respiratory tract irritation´ and 'narcotic effects´ and is primarily based on human data. No data from occupationally and accidentally exposed humans is available for fosthiazate. In animals studies acute toxicity was tested via oral, dermal and inhalation routes. In these studies no narcotic effects were observed. A decrease in respiratory rate and hyperaemia in the lung at necroscopy of died animals were described in the acute inhalation study in rats. Mortality occurred in all dose levels. Based on data from only the acute toxicity study via inhalation, the weight of evidence for a transient effect of irritation of the respiratory tract induced by fosthiazate is low. Overall, no classification for STOT SE Category 3 is proposed.

According to the CLP guidance (Guidance on the Application of the CLP Criteria (2017)), chapter 3.8.2.2, "STOT SE Category 1 and 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement". Classification after single dose exposure covers all significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed effects are included. "Studies with single administration were conducted with mice, rats and hens. Depending on the findings and their onset, also repeated-dose studies (conducted with mice, rats, dogs and rabbits) can contribute to the assessment of the need for classification for STOT SE. Generally, such studies were conducted according to relevant (OECD) TGs and GLP principles.

Fosthiazate is a neurotoxic substance acting via cholinesterase inhibition. Cholinesterase inhibition (≥20%) and related effects were observed already after single exposure in acute oral toxicity studies in rats including an acute neurotoxicity study (Anonymous 38, 1997), a cholinesterase inhibition study (Anonymous 37, 1994) and an age-sensitivity study (Anonymous 7, 2006). Results of these studies demonstrate acute neurotoxicity after a single dose of fosthiazate. Acute neurotoxicity is indicated especially as inhibition of erythrocyte and brain cholinesterase activities at ≥5 mg/kg bw. Clinical neurotoxic symptoms of cholinergic responses were observed within the CLP guidance value range for Category 1 of specific target organ toxicity-single exposure (STOT SE1, oral rat: C ≤ 300 mg/kg bw) in the acute neurotoxicity screening study (Anonymous 38, 1997), the acute oral toxicity study in rats (Anonymous 14, 1989b), the acute oral toxicity study in mice (Anonymous 13, 1989a) and the acute delayed neurotoxicity study in hens (Anonymous 19, 1989g). Neurotoxic effects of cholinergic responses in an acute inhalation toxicity study in rats (Anonymous 21, 1989) were observed from the lowest dose on (0.53 mg/L), corresponding to guidance value range for Category 1 of specific target organ toxicity-single exposure (STOT SE1, inhalation rat: C ≤ 1 mg/l/4h). Neurotoxic effects in an acute dermal toxicity study in rats (Anonymous 15, 1989c) were observed from 1965 mg/kg bw on in males and 779 mg/kg bw in females. While the effect level in females corresponds to the guidance value range for Category 1 of specific target organ toxicity-single exposure, effects in males were observed at a dose corresponding to Category 2 of specific target organ toxicity-single exposure (STOT SE1, dermal rat: C ≤ 1000 mk/kg bw, STOT SE2, dermal rat: 2000 ≥ C > 1000 mk/kg bw).

Further, cholinesterase inhibition was assessed in repeated-dose toxicity studies in rats and dogs using dermal or oral administration. Inhibition of cholinesterase activity was observed in rats after oral and dermal exposure of fosthiazate (plasma, erythrocyte, brain) and in dogs after oral exposure of fosthiazate (plasma cholinesterase). Studies where effects were seen included: 28-day oral study in rats (Anonymous 11, 1989d), 28-day oral study in dogs (Anonymous 12, 1989e), oral age-sensitivity study of technical fosthiazate in rats (Anonymous 7, 2006), 90-day oral studies in rats (Anonymous 9, 1989b), 90-day oral study in dogs (Anonymous 8, 1989a; Anonymous 22, 1995), 1-year oral study in dogs (Anonymous 39, 1991; Anonymous 23, 1995, 2-year study in rats (Anonymous 4, 1990b) and the 28-day dermal study in rats (Anonymous 11, 1989d), for further details please refer to Table 32.

Comparing the effect dose levels reported in single-dose and repeated-dose studies, little progression towards lower effect dose levels with longer administration was reported. In summary, the observed neurotoxic findings in repeated-dose studies are considered more as repeated acute effects then as genuine repeated-dose effects.

Fosthiazate did not result in pathological or histopathological changes of the nervous system. The inhibition of cholinesterase activity coming along with clinical neurotoxic symptoms are considered as effects that clearly indicate functional impairment and being of considerably adverse nature with significant impact on health.

According to CLP regulation, "STOT SE and acute toxicity are independent of each other and both may be assigned to a substance if the respective criteria are met." (CLP-GD, chapter 3.8.2.5). However, "Care must be taken not to classify for STOT SE for effects which are not yet lethal at a certain dose, but would lead to lethality within the numeric classification criteria. In other words, if lethality would occur at relevant doses then a classification for acute toxicity would take precedence and STOT SE would not be assigned." (CLP-GD, chapter 3.8.2.1.2). Mortalities were observed in acute toxicity studies with oral administration at dose levels of 64 mg/kg bw and above, with dermal administration at dose levels of 779 mg/kg bw and above and

with inhalation administration at 0.53 mg/L. In summary, neurotoxicity was observed at lower dose levels (starting at oral doses of approx. 5 mg/kg bw(/d)) than those associated with mortalities. Hence, for the findings reported for fosthiazate, classification for acute toxicity does not take precedence over STOT-SE classification.

Inhibition of cholinesterase and the related clinical signs of toxicity are mechanisms and effects that can be observed in humans. Hence human relevance of these neurotoxic findings is given. Critical effects of significant inhibition of brain and erythrocyte cholinesterase associated with clinical signs of neurotoxicity are considered to be adverse and toxicologically relevant effects and were reported at dose levels within the guidance value range for STOT SE 1. In summary, classification for specific target organ toxicity after single exposure (STOT SE 1, H370) is required for fosthiazate based on neurotoxicity.

Considering, that neurotoxic effects were observed after administration via several routes (oral, dermal, inhalation) the route of exposure shall not be specified in the hazard statement.

It is noted, that this proposal for STOT SE is in line with recent ECHA/RAC opinions for other neurotoxic compounds (e.g., epsilon-metofluthrin, phosmet or momfluorothrin).

10.11.2.1 Setting of specific concentration limits for STOT SE

CLP Guidance (chapter 3.8.2.6) recommends to set a SCL in case the substance induces relevant effects at dose levels clearly (more than one magnitude) below the guidance values (i.e., 300 mg/kg bw for oral studies). Generally, for fosthiazate neurotoxicity was seen at dose levels of 4-5 mg/kg bw and above. Hence, it is necessary to set a SCL.

The SCL is determined in line with the recommended approach of the CLP-GD:

$$\text{SCL(cat.1)} = \frac{\text{effect dose}}{\text{guidance value 1}} * 100\% = \frac{4 \text{ to } 5 \text{ mg/kg bw}}{300 \text{ mg/kg bw}} * 100\% = 1.3 \text{ to } 1.6\%$$

$$\text{SCL(cat.2)} = \frac{\text{effect dose}}{\text{guidance value 2}} * 100\% = \frac{4 \text{ to } 5 \text{ mg/kg bw}}{2000 \text{ mg/kg bw}} * 100\% = 0.2 \text{ to } 0.25\%$$

The “preferred value approach” leads to a SCL (Cat. 1) of C ≥ 1 % and a SCL (Cat. 2) of 0.2 % ≤ C < 1 %.

10.11.3 Conclusion on classification and labelling for STOT SE

Based on the CLP criteria and guidance given in the Guidance on the Application of the CLP Criteria, Version 5.0 (2017), there is sufficient evidence of specific target organ toxicity after single exposure of fosthiazate in animals. Therefore, classification for specific target organ toxicity after single exposure STOT SE 1 with the nervous system as the target organ is considered appropriate. Also a SCL (Cat. 1) of C ≥ 1 % and a SCL (Cat. 2) of 0.2 % ≤ C < 1 % are proposed.

10.12 Specific target organ toxicity-repeated exposure

Several studies relevant for specific target organ toxicity after repeated exposure of fosthiazate are available and are summarised in Table 67. Further details regarding study design, guideline (and deviations, if any) and information on incidences and severities of findings and extent of changes relative to controls are given in the text below ((also found in the Volume 3 B.6 of the Renewal Assessment Report (RAR)).

Table 32: Summary table of selected animal studies on STOT RE

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Oral 28-day (diet), CD rats, preliminary study (range-finding)

Rat, CD

10 M & 10 F / group

Meets the essential criteria of 92/69/EEC Method B7. Deficiencies: Testes were not weighed, limited number of tissues were analysed microscopically.

GLP

Supplementary</td><td>Fosthiazate (92.6 % purity)

Male: 0, 0.05, 0.10, 0.48, 0.97, 9.69 and 40.87 mg/kg bw/day; female: 0.05, 0.10, 0.5, 1.0, 10.7 and 43.5 mg/kg bw/day (0, 0.5, 1, 5, 10, 100 or 400 ppm)

Exposure for 28 days</td><td>Haematology – Hb ↓, PCV ↓, MCHC ↓, MCV↓. Alkaline phosphatase ↑, alanine aminotransferase ↑

Fur loss, occasional muscle tremors, reduction in cholinesterase activities,

increase in adrenal weight at 400 ppm; ≥100 ppm (M: 9.69 mg/kg bw, F: 10.7 mg/kg bw): changes in the adrenal cortex (cell enlargement and pallor) in females (2 animals affected at 100 ppm, all affected at 400 ppm (M: 40.87 mg/kg bw, F: 43.5 mg/kg bw))

<table>
<tr><th colspan="7">Haematology</th></tr>
<tr><th></th><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><th>Parameter</th><th>Sex</th><th>0</th><th>5</th><th>10</th><th>100</th><th>400</th></tr>
<tr><td>PCV %</td><td>M
F</td><td>46
46</td><td>46
46</td><td>46
47</td><td>46
45</td><td>44** (95.7%)
45</td></tr>
<tr><td>Hb g%</td><td>M
F</td><td>15.6
15.2</td><td>15.5
15.2</td><td>15.6
15.3</td><td>15.7
14.9 (98%)</td><td>14.9 ** (95.5%)
14.9 (98%)</td></tr>
<tr><td>MCHC pg</td><td>M
F</td><td>22
21</td><td>22
21</td><td>22
21</td><td>21 (95.5%)
20[a] (95.2%)</td><td>21 (95.5%)
20** (95.2%)</td></tr>
<tr><td>MCV cμ</td><td>M
F</td><td>64
63</td><td>65
64</td><td>64
64</td><td>63
63</td><td>62* (96.9%)
61* (96.8%)</td></tr>
</table>
* Significantly different from controls, p< 0.05
** Significantly different from controls, p< 0.01
Haemoglobin = Hb
Packed cell volume = PCV
Mean cell volume = MCV
MCHC = Mean cell haemoglobin concentration (calculated as Hb * 100 / PCV)

<table>
<tr><th colspan="5">Clinical chemistry data</th></tr>
<tr><th>Sex</th><th>Dose level mg/kg bw</th><th>Alkaline phosphatase IU/L</th><th>Alanine amino-transferase IU/L</th><th>Plasma protein concentration g%</th></tr>
<tr><td>Male</td><td>0</td><td>166</td><td>35</td><td>6.5</td></tr>
<tr><td></td><td>9.69</td><td>171</td><td>33</td><td>6.6</td></tr>
<tr><td></td><td>40.87</td><td>216***(130%)</td><td>42**(120)</td><td>6.6</td></tr>
<tr><td>Female</td><td>0</td><td>89</td><td>30</td><td>6.9</td></tr>
<tr><td></td><td>10.7</td><td>106</td><td>30</td><td>6.7</td></tr>
</table></td><td>Anonymous 11, 1989d</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

	43.5	121**(136%)	40**(133%)	6.4***(93%)

* Significantly different from controls, p<0.05, ** Significantly different from controls, p<0.01, *** Significantly different from controls, p<0.001, # Achieved dose levels in ppm, (%) Percent of control

Male mean cholinesterase data				
Dose level (mg/kg bw)	**Brain acetyl-cholinesterase (IU/kg)**	**Plasma butyryl-cholinesterase (IU/L)**	**Plasma acetyl-cholinesterase (IU/L)**	**Cell acetyl-cholinesterase (IU/L)**
0	11900	895	662	2333
0.05	12500	936	675	2314
0.1	11900	997*	739	2298
0.48	11200	861	637	2099** (90%)
0.97	11900	907	698	2034*** (87.2%)
9.7	6700*** (56.3%)	630*** (70.4%)	378*** (57.1%)	535*** (22.9%)
40.87	1900*** (16%)	585*** (65.4%)	344*** (52%)	215*** (9.2%)

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

Female mean cholinesterase data				
Dose level (ppm)	**Brain acetyl-cholinesterase (IU/kg)**	**Plasma butyryl-cholinesterase (IU/L)**	**Plasma acetyl-cholinesterase (IU/L)**	**Cell acetyl-cholinesterase (IU/L)**
0	10500	3296	2460	2054
0.05	10600	3499	2558	1944
1	11500**	3273	2458	2149
5	9600* (91.4 %)	2573** (78.1 %)	1688*** (68.6 %)	2085
10	9500** (90.5 %)	2000*** (57.2 %)	1344*** (54.6 %)	1515*** (73.8 %)
100	2800*** (26.4 %)	936*** (26.8 %)	589*** (23.9 %)	445*** (21.7 %)
400	1500*** (14.2 %)	619*** (18.8 %)	341*** (13.9 %)	204*** (9.9 %)

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

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><th colspan="5">Summary of organ weight relative to body weight data</th></tr>
<tr><th>Dose level (ppm) (number in group)</th><th>Terminal body weight (g)</th><th>Adrenals (%)</th><th>Kidneys (%)</th><th>Liver (%)</th></tr>
<tr><td colspan="5">Males</td></tr>
<tr><td>0 (10)</td><td>330.7</td><td>0.0144</td><td>0.902</td><td>4.60</td></tr>
<tr><td>100 (10)</td><td>324.6</td><td>0.0152</td><td>0.885</td><td>4.78</td></tr>
<tr><td>400 (9)</td><td>291.0** (88 %)</td><td>0.0183** (127 %)</td><td>0.915</td><td>5.08</td></tr>
<tr><td colspan="5">Females</td></tr>
<tr><td>0 (10)</td><td>211.7</td><td>0.0306</td><td>0.905</td><td>4.51</td></tr>
<tr><td>100 (10)</td><td>206.0</td><td>0.0335</td><td>0.908</td><td>4.83</td></tr>
<tr><td>400 (10)</td><td>191.1* (90 %)</td><td>0.0429** (141 %)</td><td>0.940</td><td>4.97** (110 %)</td></tr>
</table>
* different from controls, p < 0.05, ** Significantly different from controls, p < 0.01, (%) Percent of control
NOAEL: 10 ppm (0.97 or 1.0 mg/kg bw/day)
</td><td></td></tr>
<tr><td>Oral 28-day (diet), CD-1 mouse, preliminary study
CD-1 mice
12 M & 12 F / group

Meets the essential criteria of 92/69/EEC Method B7. Deviations: no clinical chemistry tests were conducted, testes were not</td><td>Fosthiazate (92.6 % purity)
Male: 0, 0.9, 3.5, 17.6, and 69.0 mg/kg bw/day; female: 0, 0.97, 4.1, 21.4 and 82.4 mg/kg bw/day (0, 5, 20, 100 or 400 ppm)

Exposure for 28 days</td><td>Haematological effects, Hb ↓, PCV ↓, leukocyte count↑, lymphocyte count ↑
Increase in organ weight in the adrenal glands and liver
histopathological changes in the kidneys at 69.0 mg/kg bw/day in males (9/12animals) and 82.4 mg/kg bw/day (8/12 animals): increased incidence of focal tubular basophilia
<table>
<tr><th colspan="6">Haematology</th></tr>
<tr><th>Sex</th><th>Dose level</th><th>Packed cell volume (%)</th><th>Haemoglobin concentration (g %)</th><th>Total leukocyte count</th><th>Lymphocyte count</th></tr>
<tr><td rowspan="3">Male</td><td>Control</td><td>45</td><td>15.3</td><td>10.8</td><td>9.2</td></tr>
<tr><td>100 ppm</td><td>45</td><td>15.2</td><td>9.2</td><td>8.0</td></tr>
<tr><td>400 ppm</td><td>42** (93.3 %)</td><td>14.5** (94.8 %)</td><td>10.4</td><td>8.5</td></tr>
<tr><td rowspan="3">Female</td><td>Control</td><td>46</td><td>15.3</td><td>6.4</td><td>5.3</td></tr>
<tr><td>100 ppm</td><td>46</td><td>15.6</td><td>7.3</td><td>6.3</td></tr>
<tr><td>400 ppm</td><td>44</td><td>15</td><td>9.8*** (153 %)</td><td>8.3** (157 %)</td></tr>
</table>
** Significantly different from controls, p<0.01, *** Significantly different from controls, p<0.001, (%) Percent of control</td><td>Anonymous 1, 1989a</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>weighed and a limited number of tissues were analysed microscopically
GLP
supplementary</td><td></td><td>
<table>
<tr><th colspan="5">Organ weights</th></tr>
<tr><th>Sex</th><th>Dose level</th><th>Adrenals absolute (g)</th><th>Adrenal relative to body weight (g)</th><th>Liver relative to body weight (g)</th></tr>
<tr><td rowspan="3">Male</td><td>Control</td><td>0.005</td><td>0.0152</td><td>5.62</td></tr>
<tr><td>100 ppm</td><td>0.004</td><td>0.0114</td><td>5.57</td></tr>
<tr><td>400 ppm</td><td>0.004</td><td>0.0149</td><td>6.41** (114%)</td></tr>
<tr><td rowspan="3">Female</td><td>Control</td><td>0.006</td><td>0.0258</td><td>6.23</td></tr>
<tr><td>100 ppm</td><td>0.008</td><td>0.0325</td><td>5.85</td></tr>
<tr><td>400 ppm</td><td>0.009* (150%)</td><td>0.0384** (149%)</td><td>6.67</td></tr>
</table>
* Significantly different from controls, p<0.05, ** Significantly different from controls, p<0.01, (%) Percent of control

NOAEL: 100 ppm (17.6 or 21.4 mg/kg bw/day)
</td><td></td></tr>
<tr><td>28-day oral (dietary) immunotoxicity study
Mouse, Crl:CD-1, females, 10 F each in treatment groups and vehicle control group 1, 7 animals in positive control group, 3 animals in vehicle control 2 group
Comparable to EPA Health Effects Test Guideline OCSPP</td><td>Fosthiazate (97.4 % purity)
0, 50, 100, 400 and 200 ppm (0, 10, 22, 61 and 44 mg/kg bw/d fosthiazate, female CD-1 mice.
Duration of exposure: 35 days for Vehicle control 1, positive control and 50 and 100 ppm treatment groups , 28 days for 200 ppm group
Positive control: cyclophosphamide</td><td>400 ppm for 5 consecutive days: not tolerated (mortality);

50, 100, and 200 ppm for a minimum of 28 consecutive days:
no suppression of the functional humoral immune response (T-cell dependent antibody response to sheep red blood cells);

Higher absolute and relative adrenal gland weights in all dose groups; Slightly higher mean absolute and relative liver weights at 200 ppm
<table>
<tr><th colspan="5">Organ weights (mean)</th></tr>
<tr><th>Dose level</th><th>Adrenals absolute (g)</th><th>Adrenals relative to body weight</th><th>Liver absolute (g)</th><th>Liver relative to bodyweight</th></tr>
<tr><td>Vehicle control *</td><td>0.0099</td><td>0.036</td><td>1.3272</td><td>4.772</td></tr>
<tr><td>50 ppm</td><td>0.0109 (110%)</td><td>0.038 (118%)</td><td>1.4058 (106%)</td><td>4.969 (104%)</td></tr>
<tr><td>100 ppm</td><td>0.0113 (114%)</td><td>0.040 (113%)</td><td>1.3846 (104%)</td><td>4.958 (104%)</td></tr>
<tr><td>200 ppm</td><td>0.0116 (117%)</td><td>0.044 (124%)</td><td>1.4212 (107%)</td><td>5.361 (112%)</td></tr>
<tr><td>CPS</td><td>0.0091 (92%)</td><td>0.034 (96%)</td><td>1.4702 (110%)</td><td>5.413 (113%)</td></tr>
<tr><td>ANOVA p-value</td><td>0.297</td><td>0.053</td><td>0.681</td><td>0.099</td></tr>
</table>
*Combination of Vehicle group 1 (n=10) and Vehicle group 2 (n=3), (%) Percent of control</td><td>Anonymous 40, 2011</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
870.7800 Immunotoxicity (1998). Due to overt toxicity the highest dose was offered for only 5 days. Surviving animals of the highest dose group were euthanized and the treatment regime was altered at day 7: a 200 ppm treatment group was established at day 7. GLP Acceptable	monohydrate. (CPS), 50 mg/kg/day. IP injection once daily on study days 31-34.		
Oral 28-day (gelatine capsule), beagle dog, preliminary study, range-finding Oral (gelatine capsule) Beagle dogs, 3M & 3 F /group Similar to	Fosthiazate (92.5-93.5 % purity) in corn oil 0, 0.021, 0.11, 0.54, 5.4 or 26.8 mg/kg bw/day Exposure for 28 days	Signs of toxicity: depressed tactile placement (1F at 5.4 mg/kg bw/day, 2M at 26.8 mg/kg bw/day), rotation of the head backward (1 M & 1F at 26.8 mg/kg bw/day), salivation (2 M) and nasal dryness (both sexes) at 26.8 mg/kg bw/day Increased weight of adrenals at top dose in males and females. Histopathological changes in the adrenals of both sexes: cell enlargement and pallor in the zona fasciculata of the adrenal cortex: 0.54 mg/kg bw/day: 2 M, 0 F 5.4 mg/kg bw/day: 3 M, 1 F, 26.8 mg/kg bw/day: 3 M, 3 F. Toxicologically relevant depression in erythrocyte and brain cholinesterase activities at ≥5.4 mg/kg bw/day.	Anonymous 11, 1989d

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
92/69/EEC Method B7, but limited number of tissues were examined microscopically. GLP Supplementary			

Cholinesterase activity – males group mean values expressed as percentages

Dose level (mg/kg bw/day)	Plasma Butyryl			Plasma Acetyl			Erythrocyte acetyl			Brain acetyl
	Pre	Week 4		Pre	Week 4		Pre	Week 4		Week 5
	A	A	B	A	A	B	A	A	B	A
0	100	100	101	100	100	90	100	100	108	100
0.021	108	118	110	103	120	105	112	126	122	83
0.11	101	98	99	97	104	97	94	100	114	92
0.54	91	41*	46	87	48*	50	101	94	101	92
5.4	85	34*	41	78	40*	46	78	22*	31	67*
26.8	120	56*	47	113	72*	57	108	8*	8	52*

A Percentage of contemporary control group mean
B Percentage of pretreatment group mean
* Mean values were statistically different from controls

Cholinesterase activity – females group mean values expressed as percentages

Dose level (mg/kg bw/day)	Plasma Butyryl			Plasma Acetyl			Erythrocyte acetyl			Brain acetyl
	Pre	Week 4		Pre	Week 4		Pre	Week 4		Week 5
	A	A	B	A	A	B	A	A	B	A
0	100	100	99	100	100	90	100	100	123	100
0.021	115	117	100	114	114	90	115	104	111	100
0.11	79	82	102	83	85	93	115	113	121	100
0.54	95	48*	50	95	56*	53	101	84	103	98
5.4	89	28*	31	91	38*	37	101	23*	28	51*
26.8	88	36*	40	89	54*	55	126	7*	7	56*

A Percentage of contemporary control group mean
B Percentage of pretreatment group mean
* Mean values were statistically different from controls

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><th colspan="4">Organ weight – adrenal gland</th></tr>
<tr><th>Sex</th><th>Dose level (mg/kg bw/day)</th><th>Adrenals absolute (g)</th><th>Adrenals relative to body weight (%)</th></tr>
<tr><td rowspan="3">Males</td><td>0</td><td>0.91</td><td>0.0086</td></tr>
<tr><td>5.4</td><td>1.03</td><td>0.0097</td></tr>
<tr><td>26.8</td><td>1.3* (142 %)</td><td>0.0133* (155 %)</td></tr>
<tr><td rowspan="3">Females</td><td>0</td><td>1.04</td><td>0.0095</td></tr>
<tr><td>5.4</td><td>1.16</td><td>0.011</td></tr>
<tr><td>26.8</td><td>1.22</td><td>0.0126* (133 %)</td></tr>
</table>
NOAEL: 0.11 mg/kg bw/day. Because adverse adrenal effects at 0.54 mg/kg bw/d were not confirmed in the 90-day and 1-year studies in dogs, this value was considered the NOAEL in the RAR, but should revised accordingly,</td><td></td></tr>
<tr><td>Oral 90-day (diet), CD rat
Rats, CD, 10 M & 10 F / group
In accordance with EPA/FIRA guidelines; satisfies the essential requirements of EC guidelines for a 90-day repeat dose toxicity study in rodents except for the duration of the recovery period.
GLP
Acceptable</td><td>Fosthiazate (93.3 % purity)
Male: 0, 0.08, 0.77, 4.12 and 36.4 mg/kg bw/day; female: 0, 0.09, 0.9, 4.7 and 41.0 mg/kg bw/day (0, 1.07, 10.7, 53.6 or 429 ppm)
13 weeks treatment, Satellite group (10 M & 10 F) for dose level 0 and 429 ppm was kept on a fosthiazate-free diet for 10 weeks</td><td>Hair loss during first nine weeks of treatment at 429 ppm, both sexes. Emaciation in five males during first three weeks of treatment, trachypnoea in nine males and one female of the 429 ppm group.
Histopathological changes in the adrenals.
Reduction in cholinesterase activity in particular brain cholinesterase.
Haematological changes (Hb ↓, PCV ↓, RBC ↓ at ≥4.1 or ≥4.7 mg/kg bw/day (53.6 ppm).
<table>
<tr><th></th><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><th>Parameter</th><th>Sex</th><th>0</th><th>1.07</th><th>10.7</th><th>53.6</th><th>429</th></tr>
<tr><td>Feed consumption week 1-13 (% of control)</td><td>M
F</td><td>100
100</td><td>101
98</td><td>99
102</td><td>102
103</td><td>100
107</td></tr>
<tr><td>Body weight gain week 1 - 13 (g)</td><td>M
F</td><td>431
180</td><td>445
189</td><td>471*
194</td><td>434
175</td><td>365*** (84.7 %)
157**</td></tr>
<tr><td>PCV (%) week 12</td><td>M
F</td><td>52
48</td><td>50
45</td><td>50
42</td><td>52
43</td><td>47*** (90 %)
62</td></tr>
<tr><td>Hb (g %) week12</td><td>M
F</td><td>16.2
15.9</td><td>16
16</td><td>16
15.8</td><td>16.4
14.9** (93.7 %)</td><td>15.2*** (93.8 %)
14.6*** (91.8 %)</td></tr>
<tr><td>RBC (mil/cmm) week12</td><td>M
F</td><td>8.12
7.56</td><td>7.95
7.72</td><td>7.96
7.39</td><td>7.98
7.00*** (92.6 %)</td><td>7.86* (96.8%)
7.15*** (94.6 %)</td></tr>
<tr><td>MCV (cμ) week12</td><td>M
F</td><td>64
64</td><td>63
65</td><td>64
66*</td><td>65*
66*</td><td>60*** (93.8 %)
62*** (96.9 %)</td></tr>
</table></td><td>Anonymous 9, 1989b</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
*** significantly different from controls, p<0.001
(%) Percent of control

		Dose (ppm)				
Parameter	**Sex**	**0**	**1.07**	**10.7**	**53.6**	**429**
ALT (iu/l)	M	32	33	32	31	39***
	F	26	32** (123%)	32* (123%)	33** (127%)	34*** (131%)
AST (iu/l)	M	74	77	76	65* (87.8%)	86** (116%)
	F	74	85**	81	79	89*** (120%)
Urea	M	30	29	30	32	32* (107%)
	F	40	49**	46	40	57*** (143%)
Protein	M	7.2	7.4	7.2	7.2	7.1*
	F	7.4	8.3***	7.8**	7.4	6.9***
Cell acetyl	M	2052	1810** (88.2%)	1867	888*** (43.3%)	214*** (10.4%)
cholinesterase (iu/mL)	F	1946	2129**	1625*** (83.5%)	572*** (29.4%)	144*** (7.4%)
Brain acetyl	M	10000	9900	10400	7700*** (77%)	1900*** (19%)
Cholinesterase (iu/kg)	F	10000	9600* (96%)	9600* (96%)	3400*** (34%)	1000*** (10%)
Plasma acetyl	M	831	888	663*** (79.8%)	461*** (55.5%)	322*** (38.7)
cholinesterase (iu/mL)	F	3796	4136	1909*** (50.3%)	834*** (22%)	450*** (11.9%)
Plasma butyryl	M	1232	1188	858*** (69.6%)	736*** (59.7%)	642*** (52.1%)
cholinesterase (iu/mL)	F	6278	6442	2838*** (45.2%)	1372*** (21.9%)	811*** (12.9%)
Histopathology						
Vacuolation of the zona	M	2	4	5	8	10
fasciculata	F	0	0	0	2	10
Vacuolation of the zona	M	2	0	2	2	10
glomerulosa	F	3	3	3	5	10

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
*** significantly different from controls, p<0.001
(%) Percent of control

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><th colspan="5">Organ weights</th></tr>
<tr><th>Dose level (ppm)</th><th>Adrenals (g)</th><th>Adrenals vs body weight (%)</th><th>Liver (g)</th><th>Liver vs. body weight (%)</th></tr>
<tr><th colspan="5">Males</th></tr>
<tr><td>0</td><td>0.050</td><td>0.0097</td><td>18.7</td><td>3.52</td></tr>
<tr><td>53.6</td><td>0.058</td><td>0.0104</td><td>20.9</td><td>3.77</td></tr>
<tr><td>429</td><td>0.065**</td><td>0.0134** (138%)</td><td>19.6</td><td>4.01</td></tr>
<tr><th colspan="5">Females</th></tr>
<tr><td>0</td><td>0.075</td><td>0.0246</td><td>11.1</td><td>3.63</td></tr>
<tr><td>53.6</td><td>0.071</td><td>0.0251</td><td>10.7</td><td>3.75</td></tr>
<tr><td>429</td><td>0.085</td><td>0.0319* (130%)</td><td>11.1</td><td>4.14</td></tr>
</table>
* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
(%) Percent of control

NOAEL: 10.7 ppm (0.8 or 0.9 mg/kg bw/day)
</td><td></td></tr>
<tr><td>Oral 90-day (gelatine capsule), beagle dog

4M & 4 F / group

In accordance with EPA/FIRA guidelines; satisfies the essential requirements of EC guidelines for a subchronic oral toxicity study</td><td>Fosthiazate (93.3% purity) in corn oil

0, 0.054, 0.11, 0.54, or 5.4 mg/kg bw/day

Exposure: 13 weeks</td><td>
Haematological changes in females at 5.4 mg/kg bw/d: Hb ↓, PCV ↓, RBC ↓

Reduction in brain acetylcholinesterase, plasma butyrylcholinesterase, plasma acetylcholinesterase and erythrocyte acetylcholinesterase

Increase in adrenal weight and in histopathological changes in the adrenal cortex at 5.4 mg/kg bw/day
<table>
<tr><th></th><th></th><th colspan="5">Dose (mg/kg bw/day)</th></tr>
<tr><th>Parameter</th><th>Sex</th><th>0</th><th>0.054</th><th>0.11</th><th>0.54</th><th>5.4</th></tr>
<tr><th>Haematology</th><th></th><th></th><th></th><th></th><th></th><th></th></tr>
<tr><td>PCV (%)
wk 13</td><td>M
F</td><td>39
46</td><td>43*
45</td><td>44** (113 %)
46</td><td>43* (110 %)
43</td><td>39
41* (89 %)</td></tr>
<tr><td>Hb (g%)
wk13</td><td>M
F</td><td>13.4
15.7</td><td>14.9*
15.6</td><td>15.0*
15.9</td><td>14.7
15.0</td><td>13.5
14.1* (89.8 %)</td></tr>
<tr><td>RBC (mil/cmm)
wk13</td><td>M
F</td><td>5.66
6.73</td><td>6.30*
6.63</td><td>6.31
6.58</td><td>6.26*
6.21</td><td>5.53
5.78* (85.9 %)</td></tr>
<tr><th>Cholinesterase</th><th></th><th></th><th></th><th></th><th></th><th></th></tr>
<tr><td>Brain acetyl
Cholinesterase (iu/kg)</td><td>M
F</td><td>3100
3800</td><td>3800*
3900</td><td>3500
3900</td><td>3400
3700</td><td>2400* (77.4 %)
2600*** (68.4 %)</td></tr>
</table>
</td><td>Anonymous 8, 1989a

Anonymous 23, 1995</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

GLP

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

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
*** significantly different from controls, p<0.001
(%) Percent of control

Dose (mg/kg bw/day)	Adrenals (g)	Adrenals vs body weight (%)
Males		
0	1.12	0.0084
0.054	1.01	0.0083
0.11	1.06	0.008
0.54	1.1	0.0086
5.4	1.41**(126 %)	0.0108**(129 %)
Females		
0	1.11	0.0107
0.054	1.00	0.0089
0.11	1.07	0.0101
0.54	1.09	0.0101
5.4	1.25	0.0114

*** significantly different from controls, p<0.001, (%) Percent of control

Incidence and severity of microscopic changes in the adrenal cortex										
	Males					Females				
Dose level (mg/kg bw/day)	**0**	**0.054**	**0.11**	**0.54**	**5.4**	**0**	**0.054**	**0.11**	**0.54**	**5.4**
No. of animals	4	4	4	4	4	4	4	4	4	4
Adrenal cortex, zona fasciculate										
Hypertrophy										
Not remarkable		1				2	3	1	1	
Minimal	4	3	4	4		2	1	3	3	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Slight</td><td></td><td></td><td></td><td></td><td>2</td><td></td><td></td><td></td><td></td><td>3</td></tr>
<tr><td>Moderate</td><td></td><td></td><td></td><td></td><td>2</td><td></td><td></td><td></td><td></td><td>1</td></tr>
<tr><td>Moderately severe</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="11">Increase in palor</td></tr>
<tr><td>Not remarkable</td><td></td><td></td><td></td><td></td><td></td><td></td><td>1</td><td></td><td></td><td></td></tr>
<tr><td>Minimal</td><td>3</td><td>4</td><td>4</td><td>3</td><td></td><td>3</td><td>3</td><td>4</td><td>4</td><td></td></tr>
<tr><td>Slight</td><td>1</td><td></td><td></td><td>1</td><td>1</td><td>1</td><td></td><td></td><td></td><td>3</td></tr>
<tr><td>Moderate</td><td></td><td></td><td></td><td></td><td>3</td><td></td><td></td><td></td><td></td><td>1</td></tr>
<tr><td>Moderately severe</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="11">Adrenal cortex, zona glomerulosa</td></tr>
<tr><td colspan="11">Hypertrophy</td></tr>
<tr><td>Not remarkable</td><td>1</td><td></td><td></td><td></td><td></td><td></td><td>1</td><td>1</td><td>1</td><td>1</td></tr>
<tr><td>Minimal</td><td>3</td><td>4</td><td>4</td><td>4</td><td></td><td>4</td><td>3</td><td>2</td><td>2</td><td>1</td></tr>
<tr><td>Slight</td><td></td><td></td><td></td><td></td><td>2</td><td></td><td></td><td></td><td></td><td>1</td></tr>
<tr><td>Moderate</td><td></td><td></td><td></td><td></td><td>2</td><td></td><td></td><td></td><td></td><td>1</td></tr>
<tr><td colspan="11">Increase in pallor</td></tr>
<tr><td>Not remarkable</td><td></td><td></td><td></td><td></td><td></td><td></td><td>1</td><td></td><td></td><td></td></tr>
<tr><td>Minimal</td><td>4</td><td>4</td><td>4</td><td>4</td><td></td><td>4</td><td>3</td><td>3</td><td>2</td><td>1</td></tr>
<tr><td>Slight</td><td></td><td></td><td></td><td></td><td>1</td><td></td><td></td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>Moderate</td><td></td><td></td><td></td><td></td><td>3</td><td></td><td></td><td></td><td></td><td>1</td></tr>
<tr><td>Moderately severe</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
NOAEL: 0.54 mg/kg bw/day
</td><td></td></tr>
<tr><td>Oral 1 year (capsule), beagle dog
4M & 4 F / group
In accordance with US EPA FIFRA guidelines and satisfies the essential criteria</td><td>Fosthiazate (93.8% purity) in corn oil
0, 0.05, 0.1, 0.5, or 5 mg/kg bw/day
Exposure: 12 month</td><td>5.0 mg/kg bw/d: haematological changes (erythrocyte counts (16.4 - 11.6 %, 3 - 12 month) ↓ and haemoglobin concentration (10.9 - 15.7%) ↓ in males; haematocrit concentration ↓ (transient) and MCH ↑ and reticulocyte ratios (≥ 300 %) ↑ in females); adrenal glands (pallor of the zona glomerulosa and fasciculata and hypertrophy of the zona fasciculata, in males and females)
≥ 0.5 mg/kg bw/d: plasma cholinesterase ↓ in both sexes (≥ 25 %)
<table>
<tr><th colspan="4">Male haematology values</th></tr>
<tr><th rowspan="2">Parameter</th><th rowspan="2">Interval(month)</th><th colspan="3">Dose level (mg/kg bw/d)</th></tr>
<tr><th>0</th><th>0.5</th><th>5.0</th></tr>
<tr><td>Erythrozyte count (10^4/mm^3)</td><td>3</td><td>755</td><td>740</td><td>634** (84 %)</td></tr>
<tr><td></td><td>6</td><td>804</td><td>770</td><td>672* (83.6 %)</td></tr>
</table>
</td><td>Anonymous 39, 1991
Anonymous 23, 1995</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

of EU guidelines for a subchronic oral toxicity study.

GLP

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	9	794	770	680** (85.6 %)
	12	773	755	683** (88.4 %)
Haematocrit concentration (Vol%)	3	51.3	50.5	45.8* (89.3 %)
	6	51.6	48.9	44.3* (85.5 %)
	9	53.6	52.2	48.0* (89.6 %)
	12	49.8	49.2	45.5
Hemoglobin concentration (g/dL)	3	16.5	16.4	14.7* (89.1 %)
	6	18.5	17.2	15.6* (84.3 %)
	9	18.5	17.4	16.6* (89.7 %)
	12	17.9	16.9	16.1* (89.9 %)

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
(%) Percent of control

Female haematology values

Parameter	Interval (month)	Dose level (mg/kg bw/d)		
		0	**0.5**	**5.0**
Reticulocyte ratio (%)	3	0.4	0.5	1.2** (300 %)
	6	0.4	0.5	0.9
	9	0.3	0.6	1.1** (366 %)
	12	0.3	0.5	0.9** (300 %)
Haematocrit concentration (Vol%)	3	50.5	46.8	44.1* (87.3 %)
	6	48.6	49.1	46.7
	9	50.1	47.0	49.0
	12	48.4	46.4	47.3
Mean Corpuscular haemoglobin (pg)	3	21.6	21.9	23.5** (109 %)
	6	22.4	22.3	22.6
	9	23.2	22.5	23.7
	12	21.9	22.6	23.4

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
(%) Percent of control

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Male cholinesterase activities

Type	Interval (month)	Dose level (mg/kg bw/d)			
		0	0.1	0.5	5.0
True plasma (IU/L)	3	3008	2653	1677** (55 %)	1764** (58.6 %)
	6	3925	3383	2395** (61 %)	2475** (63 %)
	9	3166	2870	2075* (65.5 %)	2397* (75.7 %)
	12	3802	3453	2534** (66.6 %)	2845** (74.8 %)
Pseudo Plasma (IU/L)	3	4027	3227	1414	1014* (25.2 %)
	6	5162	4611	1895** (36.7 %)	1385** (26.8 %)
	9	4545	3596	1597* (35.1 %)	1507* (33.2 %)
	12	5332	4216	2046** (38.4 %)	1785** (33.5 %)
Erythrocyte (IU/L)	3	17923	17755	17893	17938
	6	20078	20458	20975	17007
	9	22826	23031	22861	21516
	12	22854	23367	23164	21816
Brain (IU/L)	12	3057	2972	3003	2886

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
(%) Percent of control

Female cholinesterase activities

Type	Interval (month)	Dose level (mg/kg bw/d)			
		0	0.1	0.5	5.0
True plasma (IU/L)	3	2729	2723	2095	1688
	6	2929	2916	1861** (63.5 %)	1900** (64.9 %)
	9	3645	3243* (89 %)	2595* (71.2 %)	2527* (69.3 %)
	12	2729	2651	1995* (73.1 %)	2041* (74.8 %)
Pseudo Plasma (IU/L)	3	4464	3585	1964	1094* (24.5 %)
	6	4487	47736	1748** (39 %)	1616** (36 %)
	9	4059	3670	2114** (52.1 %)	1660** (40.9 %)
	12	4496	4558	2177** (48.4 %)	1914** (42.6 %)
Erythrocyte (IU/L)	3	25854	26862	25961	25393
	6	24763	26124	25992	25865
	9	21775	23258	22025	21741

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

	12	20301	19337	20237	19405
Brain (IU/L)	12	3139	3093	3091	3138

* significantly different from controls, p<0.05
** significantly different from controls, p<0.01
(%) Percent of control

Incidence and severity of microscopic changes in the adrenal cortex

	Males					Females				
Dose level (mg/kg bw/day)	**0**	**0.05**	**0.1**	**0.5**	**5.0**	**0**	**0.05**	**0.1**	**0.5**	**5.0**
No. of animals	5	5	5	5	5	5	5	5	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 palor										
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
Increase in palor										
Not remarkable						1				
Minimal	4	3	1	1		3		1	3	
Slight	1	1	3	3		1	5	4	2	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Moderate</td><td></td><td>1</td><td>1</td><td>1</td><td>2</td><td></td><td></td><td></td><td></td><td>3</td></tr>
<tr><td>Moderately severe</td><td></td><td></td><td></td><td></td><td>3</td><td></td><td></td><td></td><td></td><td>2</td></tr>
</table>
NOAEL: 0.5 mg/kg bw/day Anonymous 39, 1991
Anonymous 23, 1995</td><td></td></tr>
<tr><td>Combined long-term and carcinogenicity study in the rat
Rats, CD
50 M & 50 F / group, + 10 M & F /group as interim kill
Oral (dietary)
In accordance with US EPA/FIFRA guidelines and meets the essential criteria of current EC guidelines for a chronic toxicity and carcinogenicity study in rodents

GLP
Acceptable</td><td>Fosthiazate (93.3% purity)
Male: 0, 0.042, 0.41, 2.08 and 8.94 mg/kg bw/day; female: 0, 0.055, 0.54, 2.63 and 12.53 mg/kg bw/day (0, 1.07, 10.7, 53.6 or 214 ppm)
Exposure: 24 month & 12 month for interim kill</td><td>Histopathological changes (adrenals, eyes, ovaries, pituitary) at dose levels of ≥ 53.6 ppm (≥2.08 or ≥2.63 mg/kg bw/day for males and females, respectively).
Reduction in brain cholinesterase activity in males and females and haematological changes (lower packed cell volumes, lower haemoglobin concentration, erythrocyte counts) in females at dose levels of ≥53.6 ppm (≥2.08 or ≥2.63 mg/kg bw/day for males and females, respectively).
<table>
<tr><th colspan="6">Haematology summary, males</th></tr>
<tr><th rowspan="2">Weeks of change</th><th colspan="5">Dose level (ppm)</th></tr>
<tr><th>0</th><th>1.07</th><th>10.7</th><th>53.6</th><th>214</th></tr>
<tr><td>Hb (g %)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>12 week</td><td>15.8</td><td>16.2</td><td>15.9</td><td>16.1</td><td>15.4</td></tr>
<tr><td>51 week</td><td>15.9</td><td>15.7</td><td>15.6</td><td>15.3* (96.2 %)</td><td>15.2* (95.6 %)</td></tr>
<tr><td>102 week</td><td>12.8</td><td>12.7</td><td>12.0</td><td>12.4</td><td>13.3</td></tr>
<tr><td>RBC (mil/cmm)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>12 week</td><td>8.84</td><td>8.72</td><td>8.27</td><td>8.43</td><td>8.41</td></tr>
<tr><td>51 week</td><td>8.51</td><td>8.48</td><td>8.22</td><td>8.10* (95.2 %)</td><td>8.32</td></tr>
<tr><td>102 week</td><td>6.94</td><td>6.96</td><td>6.56</td><td>6.60</td><td>7.27</td></tr>
<tr><td>PCV (%)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>12 week</td><td>49</td><td>50</td><td>48</td><td>40</td><td>47</td></tr>
<tr><td>51 week</td><td>48</td><td>48</td><td>48</td><td>47* (97.9 %)</td><td>46** (95.8 %)</td></tr>
<tr><td>102 week</td><td>38</td><td>38</td><td>35</td><td>36</td><td>40</td></tr>
</table>
* Significantly different from controls, p<0.05
** Significantly different from controls, p<0.01
*** Significantly different from controls, p<0.001
<table>
<tr><th colspan="6">Haematology summary, females</th></tr>
<tr><th rowspan="2">Weeks of change</th><th colspan="5">Dose level (ppm)</th></tr>
<tr><th>0</th><th>1.07</th><th>10.7</th><th>53.6</th><th>214</th></tr>
<tr><td>Hb (g %)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>12 week</td><td>15.9</td><td>15.8</td><td>15.7</td><td>14.8*** (93.1 %)</td><td>14.4*** (90.6 %)</td></tr>
</table></td><td>Anonymous 4, 1990b</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results					Reference
		51 week	15.2	15.4	15.3	14.8	14.1*** (92.8 %)	
		102 week	13.8	14.3	13.8	12.8* (92.8 %)	12.6** (91.3 %)	
		RBC (mil/cmm)						
		12 week	8.19	8.05	8.07	7.41*** (90.5 %)	7.57*** (92.4 %)	
		51 week	7.38	7.34	7.31	6.91** (93.6 %)	6.91** (93.6 %)	
		102 week	6.96	7.35	7.08	6.19** (88.9 %)	6.32* (90.8 %)	
		PCV (%)						
		12 week	48	48	47	44*** (91.7 %)	44*** (91.7 %)	
		51 week	46	46	46	44* (95.7 %)	42*** (91.3 %)	
		102 week	43	43	42	39* (90.7 %)	38** (88.4 %)	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Cholinesterase activity - group mean values (% of the control activity)

Sex	Dose level (ppm)	Week 12	Week 24	Week 51	Week 76	Week 102
Plasma butyryl cholinesterase						
Male	1.07	97	94	104	66	105
	10.7	77	76	69*	71	55**
	53.6	51**	64**	44**	31**	37**
	214	45**	48**	37**	28**	27**
Females	1.07	68	78	72	113	92
	10.7	32**	35	35**	57**	41**
	53.6	19**	18**	19**	26**	23**
	214	12**	12**	15**	21**	18**
Plasma acetyl cholinesterase						
Males	1.07	99	105	101	73	105
	10.7	85	83	76	74	61
	53.6	60**	57**	43**	35**	33**
	214	45**	44**	32**	24**	22**
Females	1.07	67	74	73	113	95
	10.7	35**	32	34**	56**	47**
	53.6	20**	19**	19**	26**	25**
	214	13**	12**	13**	20**	18**
Cell acetyl cholinesterase						
Males	1.07	100	109*	103	101	101
	10.7	81**	90	84*	81*	89*
	53.6	35**	36**	33**	36**	48**
	214	17**	14**	15**	12**	14**
Females	1.07	98	91*	96	99	104
	10.7	68**	75**	64**	77**	71**
	53.6	28**	24**	25**	36**	33**
	214	12**	7**	11**	8**	14**

* Absolute activities significantly different from controls, $p<0.05$

** Absolute activities significantly different from controls, $p<0.01$

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Brain acetylcholinesterase activity - group mean values (% of the control activity)			
Sex	**Dose level (ppm)**	**Week 53**	**Week 104**
Male	1.07	105	101
	10.7	102	99
	53.6	51***	51***
	214	20***	15***
Females	1.07	98	104
	10.7	97	116***
	53.6	36***	38***
	214	12***	10***

*** Significantly different from controls, p<0.001

Dose (mg/kg bw/day)	Adrenals (g)	Adrenals vs body weight (%)
Males		
0	0.113	0.0074
1.07	0.1	0.0074
10.7	0.29	0.0077
53.6	0.12	0.0081
214	0.123	0.0088
Females		
0	0.091	0.0186
1.07	0.092	0.0184
10.7	0.076	0.0192
53.6	0.1	0.0153
214	0.12* (132 %)	0.0219

* Significantly different from controls, p<0.05, (%) Percent of control

Micropathological findings for animals at terminal sacrifice or those dying during the study period					
Finding	**Dose level (ppm)**				
	0	**1.07**	**10.7**	**53.6**	**214**
Males					
Adrenal-vacuolation of zona fasciculate	7	7	6	4	27***
Eyes- cataract	6	1	2	4	3

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Eyes-retinal atrophy</td><td>1</td><td>1</td><td>1</td><td>0</td><td>11**</td></tr>
<tr><td>Pituitary-pars nervosa vacuolation</td><td>8</td><td>3</td><td>8</td><td>7</td><td>24**</td></tr>
<tr><td>Females</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Adrenal-vacuolation of zona fasciculate</td><td>2</td><td>1</td><td>2</td><td>5</td><td>12**</td></tr>
<tr><td>Eyes- cataract</td><td>0</td><td>1</td><td>0</td><td>3</td><td>8**</td></tr>
<tr><td>Eyes-retinal atrophy</td><td>3</td><td>0</td><td>4</td><td>1</td><td>27***</td></tr>
<tr><td>Ovary-foamy interstitial cells</td><td>9</td><td>8</td><td>7</td><td>30***</td><td>41***</td></tr>
<tr><td>Pituitary-pars nervosa vacuolation</td><td>7</td><td>10</td><td>9</td><td>10</td><td>26***</td></tr>
</table>
* Significantly different from controls, p<0.05, ** Significantly different from controls, p<0.01, *** Significantly different from controls, p<0.001 (Fisher’s Exact Test)
NOAEL: 10.7 ppm (0.42 mg/kg bw/day)
</td><td></td></tr>
<tr><td>Carcinogenicity study in the
Mouse, CD-1
60 M & 60 F/group

Oral (dietary) administration to CD-1 Mice for up to 104 Weeks
Conform to the current guideline requirements for oncogenicity studies except that the study was allowed to continue until survival was low.
GLP</td><td>Fosthiazate (93.3% purity)
Male: 0, 1.02, 3.1, 10.3 and 30.5 mg/kg bw/day; female: 0, 1.11, 3.2, 10.4 and 39.17 mg/kg bw/day (0, 0, 10, 30, 100 or 300 ppm)
Exposure for 102 weeks</td><td>
Increased adrenal weights at 300 ppm in both sexes (30.5 mg/kg bw/day (males) and 39.17 mg/kg bw/day (females)), reduced weights of kidney and liver.
Histopathological changes in the adrenal cortex (corticomedullary pigmentation of the adrenal cortex) in females at 100 ppm (10.4 mg/kg bw/day) and in males at 300 ppm (30.5 mg/kg bw/day). Histopathological changes in kidney (papillary mineralization) and pituitary (vacuolation of pars nervosa) in males and females at the top dose.
<table>
<tr><th colspan="5">Absolute organ weights (g)</th></tr>
<tr><th>Organ</th><th>0 ppm</th><th>0 ppm</th><th>107 ppm</th><th>322 ppm</th></tr>
<tr><td>Males</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Adrenal</td><td>0.004</td><td>0.005</td><td>0.004</td><td>0.005</td></tr>
<tr><td>Kidney</td><td>0.877</td><td>0.846</td><td>0.850</td><td>0.705** (80 %)</td></tr>
<tr><td>Liver</td><td>2.87</td><td>3.03</td><td>2.91</td><td>2.07** (72 %)</td></tr>
<tr><td>Females</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Adrenal</td><td>0.007</td><td>0.007</td><td>0.005</td><td>0.010* (143 %)</td></tr>
<tr><td>Kidney</td><td>0.535</td><td>0.535</td><td>0.534</td><td>0.439* (82 %)</td></tr>
<tr><td>Liver</td><td>2.07</td><td>1.89</td><td>2.11</td><td>1.64** (79%)</td></tr>
</table>
* Significantly different from combined controls, p<0.05, ** Significantly different from combined controls, p<0.01
(%) Percent of control
<table>
<tr><th colspan="5">Organ weights relative to body weights (g)</th></tr>
<tr><th>Organ</th><th>0 ppm</th><th>0 ppm</th><th>107 ppm</th><th>322 ppm</th></tr>
<tr><td>Males</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Adrenal</td><td>0.0094</td><td>0.0106</td><td>0.0088</td><td>0.0125 (133 %)</td></tr>
</table>
</td><td>Anonymous 3, 1990a</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Acceptable

Kidney	1.8470	1.8535	1.9143	1.9281 (104 %)
Liver	2.87	3.03	2.91	2.07** (72 %)
Females				
Adrenal	0.0157	0.0158	0.0132	0.0330* (212 %)
Kidney	1.2910	1.2621	1.2859	1.4862* (115 %)
Liver	4.657	4.421	4.997*	5.535* (119 %)

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

(%) Percent of control

Histopathological changes (after 102 weeks treatment)						
	Dose (ppm)					
Finding	**0**	**0**	**10.7**	**32.2**	**107**	**322**
Males						
Adrenal cortex, cortico-medullary pigment (number examined)	24 (59)	24 (60)	19 (59)	14* (58)	33 (59)	48*** (59)
Adrenal cortex, mineralization of pigmented cells (number examined)	0 (59)	0 (60)	0 (59)	1 (58)	2 (59)	6** (59)
Kidney, papillary mineralization (number examined)	1 (60)	0 (60)	0 (60)	0 (60)	0 (60)	50*** (60)
Pituitary, vacuolation of the pars nervosa (number examined)	3 (27)	5 (25)	6 (36)	3 (27)	5 (34)	31*** (38)
Lungs – pulmonary adenoma	3 (24)	3 (21)	5 (18)	4 (22)	3 (22)	2 (13)
Lungs – pulmonary carcinoma	5 (24)	5 (21)	2 (18)	4 (22)	2 (22)	3 (13)
Females						
Adrenal cortex, cortico-medullary pigment (number examined)	2 (60)	4 (58)	6 (59)	8 (60)	20*** (60)	56*** (60)
Adrenal cortex, mineralization of pigmented cells (number examined)	1 (60)	0 (58)	0 (59)	0 (60)	1 (60)	13*** (60)
Kidney, papillary mineralization (number examined)	0 (60)	1 (60)	1 (60)	0 (60)	0 (60)	45*** (60)
Pituitary, vacuolation of the pars nervosa (number examined)	3 (16)	2 (15)	2 (36)	8 (38)	4 (31)	40*** (41)
Lungs – pulmonary adenoma	2 (26)	5 (29)	8 (32)	0 (14)	1 (36)	0 (17)
Lungs – pulmonary carcinoma	1 (26)	1 (29)	3 (32)	2 (14)	7 (36)*	1 (17)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>* Significantly different from combined controls, p<0.05
** Significantly different from combined controls, p<0.01
*** Significantly different from combined controls, p<0.001
NOAEL: 32.2 ppm (3.2 mg/kg bw/day)</td><td></td></tr>
<tr><td>Supplementary histopathological assessment of the adrenal glands of selected females from the carcinogenicity study by Anonymous 3, 1990a:
Mouse, CD-1
No guideline applicable
no GLP
Supplementary</td><td>Fosthiazate (93.3% purity)

0 and 39.17 mg/kg bw/day (0 and 300 ppm)
Eight adrenals from four high dose females that were reported with the corticomedullary pigment and up to nine adrenals from six control females; where possible, three reported with the corticomedullary pigment and three reported without the pigment.</td><td>Cortico-medullary pigment reported in the study of Anonymous 3, 1990a was PAS (periodic acid/Schiff staining) positive, LZN (Long Zeihl Neelson stain) positive, Schmorl's negative and Perls' negative indicating that the pigment is likely to be ceroid, which is a spontaneous age-related finding in CD-I mice. The result suggests that fosthiazate exacerbated this background change
<table>
<tr><th colspan="4">Findings in PAS stained adrenal glands of selected female mice</th></tr>
<tr><th>Group/sex
Level (ppm)</th><th>1F
0</th><th>2F
0</th><th>6F
300</th></tr>
<tr><td colspan="4">Left adrenal
PAS positive staining</td></tr>
<tr><td>Minimal</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>Slight</td><td>1</td><td>0</td><td>1</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Marked</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Total</td><td>2</td><td>1</td><td>4</td></tr>
<tr><td>Number of tissues examined</td><td>2</td><td>1</td><td>4</td></tr>
<tr><td colspan="4">Right adrenal
PAS positive staining</td></tr>
<tr><td>Minimal</td><td>2</td><td>1</td><td>0</td></tr>
<tr><td>Slight</td><td>1</td><td>2</td><td>0</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Marked</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Total</td><td>3</td><td>3</td><td>4</td></tr>
<tr><td>Number of tissues examined</td><td>3</td><td>3</td><td>4</td></tr>
</table></td><td>Anonymous 35, 2013</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><th colspan="4">Findings in LZN astained adrenal glands of selected females</th></tr>
<tr><td>Group/sex
Level (ppm)</td><td>1F
0</td><td>2F
0</td><td>6F
300</td></tr>
<tr><td colspan="4">Left adrenal
LZN positive staining</td></tr>
<tr><td>Minimal</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>Slight</td><td>1</td><td>0</td><td>2</td></tr>
<tr><td>Moderate</td><td>2</td><td>0</td><td>2</td></tr>
<tr><td>Total</td><td>2</td><td>1</td><td>4</td></tr>
<tr><td>Number of tissues examined</td><td>2</td><td>1</td><td>4</td></tr>
<tr><td colspan="4">Right adrenal
LZN positive staining</td></tr>
<tr><td>Minimal</td><td>1</td><td>-</td><td>0</td></tr>
<tr><td>Slight</td><td>1</td><td>-</td><td>1</td></tr>
<tr><td>Moderate</td><td>0</td><td>-</td><td>1</td></tr>
<tr><td>Marked</td><td>0</td><td>-</td><td>2</td></tr>
<tr><td>Total</td><td>2</td><td>-</td><td>4</td></tr>
<tr><td>Number of tissues examined</td><td>2</td><td>0</td><td>4</td></tr>
</table>
</td><td></td></tr>
<tr><td>Multigeneration study
Oral (dietary)
Rats, CD, groups of 25 male and 25 female
Method is comparable to OECD Test Guidelines 416 (26 May 1983; 22 January 2001 and satisfies the</td><td>Fosthiazate (93.3% purity)
0, 3, 10, 30 or 100 ppm. Equivalent to: Males: 0, 0.21, 0.69, 2.09 and 7.21 mg/kg bw/day, females: 0, 0.26, 0.86, 2.62 and 9.34 mg/kg bw/day
Six-week-old F0 rats received fosthiazate for at</td><td>100 ppm: absolute adrenal weight↑ and hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams;
≥30 ppm: pup viability↓ during lactation during the first generation (for detailed results please see Chapter 10.10.1)
<table>
<tr><th></th><th colspan="5">Dose (ppm)</th></tr>
<tr><td></td><td>0</td><td>3</td><td>10</td><td>30</td><td>100</td></tr>
<tr><td>FO generation</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Terminal body weight (g)</td><td>335</td><td>337</td><td>348</td><td>361</td><td>367*</td></tr>
<tr><td>Absolute adrenal weight in F_0, g</td><td>0.062</td><td>0.070</td><td>0.071* (115 %)</td><td>0.073** (118 %)</td><td>0.077** (124 %)</td></tr>
<tr><td>Relative adrenal wt (%)</td><td>0.0188</td><td>0.0208</td><td>0.0206</td><td>0.0207</td><td>0.0212</td></tr>
<tr><td>Adrenal Cortex – hypertrophy of zona glomerulosa</td><td>0</td><td>0</td><td>0</td><td>3</td><td>22***</td></tr>
</table>
* Significantly different from controls at p<0.05</td><td>Anonymous 34, 1990</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
essential requirements of EC guidelines for a multigeneration study (87/302/EEC Part B) Stability in the vehicle was not performed in this study. Analysis of the batch performed in another study at the same laboratory indicated that the test material in rodent diet was stable when stored a 4°C for at least 7 days and at room temperature for up to 4 days. No sperm parameters were analyzed. Mammary glands not evaluated. GLP Acceptable	least 99 days before mating, throughout mating, gestation and the lactation periods. Four days after birth the F1 litters were randomly culled to 8 pups/litter (4 males and 4 females) where possible. The F0 dams were allowed to rear the F1 pups before weaning on day 25 post-partum. At 4 weeks old, 25 weanlings/sex were randomly selected from the 0, 3, 10 and 30 ppm dose groups to constitute the F1 parents. The F1 parents were treated for 14 weeks and mated to produce the F2 pups.	** Significantly different from controls at p<0.01. *** Significantly different from controls at p<0.00	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Oral (gavage) age-sensitivity study of fosthiazate in rats

Rat, Crl:CD®(SD)

Determination of the potential of fosthiazate to induce effects on maternal, fetal, neonatal and young adult cholinesterase activity in the blood components and brain after maternal gestational exposure or after acute or short-term repeated exposure to rats of various ages. Phase I (Gestational exposure), Phase II (Time of peak effect determination),</td>
<td>Fosthiazate (94.7 % purity)
Oral (gastric intubation)
Dose: 0, 0.1, 0.7, 5 mg/kg bw/d
Number of animals in different test phases: Phase I (Gestational exposure): 12 dams and litters, Phase II (Time of peak effect determination): PND 11: 60 animals/sex, PND 21: 30 animals/sex, Phase III (Acute exposure): PND 11 20 animals/sex, PND 21: 10 animals/sex, young adult: 10 and Phase IV (Repeated exposure): PND 21: 10 animals/sex, young adult: 10
Duration of exposure: Phase I</td>
<td>5 mg/kg bw: on gestation days 19 and/or 20 tremors, unkempt appearance, prostration and red and yellow material on various body surfaces, additional in 1 or 2 animals during the twice-weekly examinations repetitive jaw movement, piloerection, gasping and lacrimation; 3 hours following dosing on gestation day 19 lacrimation (1 female), tremors and/or repetitive jaw movement (3 females);
Plasma cholinesterase activities of juveniles and young adults ↓ (≥ 25 %); erythrocyte cholinesterase activities of juveniles and young adults ↓ (≥ 25 %);
brain cholinesterase activities of juveniles adults ↓ (≥ 25 %)
<table>
<tr><th colspan="8">Mean plasma, RBC and brain cholinesterase values in dams and foetuses on gestation day 20 - Phase I - Gestation Exposure</th></tr>
<tr><th></th><th>Control</th><th colspan="2">0.1 mg/kg bw/day</th><th colspan="2">0.7 mg/kg bw/day</th><th colspan="2">5 mg/kg bw/day</th></tr>
<tr><td>Plasma[1]
Maternal
Pooled Foetal</td><td>3147 ± 410.1

491 ± 40.8</td><td>3008 ± 467.3

506 ± 38.8</td><td>96 %

103 %</td><td>852** ± 139.1

495 ± 50.9</td><td>27%

101 %</td><td>142** ± 24.7

329** ± 59.3</td><td>4.5 %

67 %</td></tr>
<tr><td>RBC[1]
Maternal
Pooled Foetal</td><td>3931 ± 1474.5

2644 ± 644.1</td><td>3831 ± 757.3

3283 ± 992.4</td><td>97 %

124 %</td><td>2193** ± 712.2

2893 ± 738.3</td><td>56 %

109 %</td><td>20[2]** ± 0.0

1851* ± 593.4</td><td>0.5 %[2]

70 %</td></tr>
<tr><td>Brain[1]
Maternal
Pooled Foetal</td><td>49446 ± 2189.8

6612 ± 679.5</td><td>48974 ± 1364.5

6328 ± 476.3</td><td>99 %

96 %</td><td>47135** ± 1510.0

6251 ± 649.5</td><td>95 %

95 %</td><td>5152** ± 1718.9

5182** ± 684.5</td><td>10.4 %

78%</td></tr>
</table>
[1] For the control group, the mean (U/L) is shown. For the test article-treated groups, the mean (U/L) and the % of the control activity is shown.
[2] RBC cholinesterase values for all females were under the range of detection; therefore, the LLOQ (20 U/L) was used for statistical analysis.
* Significantly different from the control group at 0.05.
** Significantly different from the control group at 0.01.</td>
<td>Anonymous 7, 2006</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Phase III (Acute exposure) and Phase IV (Repeated exposure). For Phases II-IV, experimentally naive bred female Crl:CD®(SD) rats were used only to obtain pups and were not administered the test article.

No Guideline
GLP
Acceptable</td><td>maternal animals: once daily, gestation day 6-20,. Phase II and Phase III pups were administered a single dose on PND 11 or PND 21</td><td>
<table>
<tr><th></th><th colspan="6">Mean plasma cholinesterase activity[1] – Phase III – Acute exposure</th></tr>
<tr><th></th><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><th>Age</th><th>Control</th><th colspan="2">5 mg/kg bw</th><th>Control</th><th colspan="2">5 mg/kg bw</th></tr>
<tr><td>PND 11[2]</td><td>1536 ± 150.8</td><td>633** ± 62.2</td><td>41 %</td><td>1482 ± 64.3</td><td>641** ± 59.7</td><td>43 %</td></tr>
<tr><td>PND21[2]</td><td>1094 ± 148.7</td><td>432** ± 46.3</td><td>39 %</td><td>1123 ± 157.4</td><td>422** ± 65.2</td><td>38 %</td></tr>
<tr><td>Young Adult[3]</td><td>888 ± 63.2</td><td>519** ± 98.7</td><td>58 %</td><td>1423 ± 399.3</td><td>472** ± 96.1</td><td>33 %</td></tr>
</table>
<table>
<tr><th></th><th colspan="6">Mean RBC cholinesterase activity[1] – Phase III – Acute exposure</th></tr>
<tr><th></th><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><th>Age</th><th>Control</th><th colspan="2">5 mg/kg bw</th><th>Control</th><th colspan="2">5 mg/kg bw</th></tr>
<tr><td>PND 11[2]</td><td>4701 ± 1008.2</td><td>3866 ± 3074.8</td><td>82 %</td><td>5558 ± 2940.3</td><td>3110* ± 832.2</td><td>56 %</td></tr>
<tr><td>PND21[2]</td><td>4929 ± 2165.2</td><td>3085 ± 1263.6</td><td>63 %</td><td>4058 ± 1576.4</td><td>2777 ± 879.3</td><td>68 %</td></tr>
<tr><td>Young Adult</td><td>3351 ± 635.6</td><td>2582 ± 478.4</td><td>77 %</td><td>4469 ± 2964.6</td><td>2387* ± 475.4</td><td>53 %</td></tr>
</table>
<table>
<tr><th></th><th colspan="6">Mean Brain Cholinesterase Activity1 – Phase III – Acute exposure</th></tr>
<tr><th></th><th colspan="3">Males</th><th colspan="3">Females</th></tr>
<tr><th>Age</th><th>Control</th><th colspan="2">5 mg/kg bw</th><th>Control</th><th colspan="2">5 mg/kg bw</th></tr>
<tr><td>PND 11[2]</td><td>24360 ± 1177.1</td><td>20730** ± 1386.1</td><td>85 %</td><td>24800 ± 932.8</td><td>21286** ± 1277.4</td><td>86 %</td></tr>
<tr><td>PND21[2]</td><td>36661 ± 1971.4</td><td>31019 ± 1087.5</td><td>85 %</td><td>36797 ± 2081.1</td><td>30784** ± 1843.1</td><td>84 %</td></tr>
<tr><td>Young Adult[3]</td><td>47611 ± 2803.8</td><td>47783 ± 2016.4</td><td>100 %</td><td>48377 ± 2814.7</td><td>47798 ± 3074.9</td><td>99 %</td></tr>
</table>
1 For the control group, the mean (U/L) ± the standard deviation is shown. For the 5 mg/kg bw group, the mean (U/L) ± the standard deviation and the % of the control group activity is shown.
2 Analyses conducted 4 hours following dosing.
3 Analyses conducted 3 hours following dosing.
* Significantly different from the control group at 0.05.
** Significantly different from the control group at 0.01.
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Mean plasma, RBC and brain cholinesterase activities for PND 21 animals – Phase IV- Repeated exposure)

PND 21 males							
	Control	**0.1 mg/kg bw/day**		**0.7 mg/kg bw/day**		**5 mg/kg bw/day**	
Plasma Activity[1]	1085 ± 109.8	1055 ± 100.1	97 %	849** ± 136.9	78 %	439** ± 180.3	40 %
RBC Activity[1]	8725 ± 3386.9	7522 ± 2622.6	86 %	9528 ± 10383.3	109 %	1107* ± 1942.2	13 %
Brain Activity[1]	39177 ± 1109.0	40093 ± 2031.0	102 %	37563 ± 825.1	96 %	19692** ± 11643.7	50 %
PND 21 females							
Plasma Activity[1]	1019 ± 253.8	1061 ± 151.3	104 %	832 ± 193.0	82 %	346** ± 104.2	33 %
RBC Activity[1]	7595 ± 3820.6	7595 ± 3820.6	101 %	6523 ± 1381.8	86 %	508** ± 510.2	7 %
Brain Activity[1]	37284 ± 8312.6	40936 ± 1081.5	110 %	34444 ± 6956.5	92 %	13125** ± 1810.5	35 %

1 = For the control group, the mean (U/L) is shown. For the test article-treated groups, the mean (U/L) and the % of the control activity is shown.

* = Significantly different from the control group at 0.05.

** =Significantly different from the control group at 0.01

Mean plasma, RBC and brain cholinesterase activities of young animals – Phase IV - Repeated exposure)

Young adult males							
	Control	**0.1 mg/kg bw/day**		**0.7 mg/kg bw/day**		**5 mg/kg bw/day**	
Plasma Activity[1]	773 ± 155.5	828 ± 76.9	107 %	756 ± 152.3	98 %	325** ± 38.3	42 %
RBC Activity[1]	2941 ± 792.8	2915 ± 950.4	99 %	2821 ± 643.6	96 %	392** ± 306.0	13 %
Brain Activity[1]	49176 ± 1372.3	51198 ± 1783.8	104 %	49595 ± 907.2	101 %	38237** ± 4346.9	78 %
Young adult females							
Plasma Activity[1]	1991 ± 433.3	2006 ± 754.3	101 %	1152** ± 170.3	58 %	253** ± 27.1	13 %
RBC	2548 ± 846.7	3178 ± 1248.9	125 %	2196 ± 298.2	86 %	20**[2] ± 0.0	1 %[2]

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Activity[1]</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Brain Activity[1]</td><td>50227 ± 2570.2</td><td>50726 ± 1031.3</td><td>101 %</td><td>48882 ± 1780.8</td><td>97 %</td><td>21476** ± 4545.2</td><td>43 %</td></tr>
</table>
1 = For the control group, the mean (U/L) is shown. For the test article-treated groups, the mean (U/L) and the % of the control activity is shown.
2 = RBC cholinesterase values were under the range of detection; therefore, the LLOQ (20 U/L) was used for statistical analysis.
** = Significantly different from the control group at 0.01.
</td><td></td></tr>
<tr><td>Dermal 21-day,
CD rat
5M & 5F / group
In accordance with US EPA FIFRA Pesticide assessment guidelines and satisfies the essential criteria EC guidelines for subacute dermal toxicity testing except that importantly the animals were treated for 21 days rather than 28 days.

GLP
Acceptable</td><td>Fosthiazate (95.5 % purity) in corn oil
0, 0.5, 2.5, 25 or 250 mg/kg bw/day
Exposure: 6-8 hours per day, 3 weeks</td><td>
Reduced feed consumption in males, increase in urea concentrations in the blood in males, reduction in brain cholinesterase in both sexes at ≥25 mg/kg bw/day;
reduction in erythrocyte cholinesterase in females at ≥2.5 mg/kg bw/day
250 mg/kg bw/day: increased adrenal weights in both sexes and vacuolation of zona fasciculata in the adrenals of males (4/5) and females (2/5)
<table>
<tr><th></th><th></th><th colspan="5">Dose (mg/kg bw/day)</th></tr>
<tr><th>Parameter</th><th>Sex</th><th>0</th><th>0.5</th><th>2.5</th><th>25</th><th>250</th></tr>
<tr><td>Feed consumption week 1-3 (g/rat/week)</td><td>M
F</td><td>544
414</td><td>547
418</td><td>525
423</td><td>505
438</td><td>502
388</td></tr>
<tr><td>Body weight gain wk 1 – 3 (g)</td><td>M
F</td><td>62
7</td><td>70
5</td><td>55
5</td><td>49
10</td><td>15**
A</td></tr>
<tr><td>Urea Concentration mg (%)</td><td>M
F</td><td>43
48</td><td>42
45</td><td>41
42</td><td>51
43</td><td>53
62</td></tr>
<tr><td>Cell acetyl cholinesterase (iu/mL)</td><td>M
F</td><td>3728
3484</td><td>3606
3415</td><td>3384
2758* (79.2 %)</td><td>1681*** (45.1 %)
830*** (23.8 %)</td><td>205*** (5.5 %)
361a</td></tr>
<tr><td>Brain acetyl Cholinesterase (iu/kg)</td><td>M
F</td><td>9600
10000</td><td>9700
11100*</td><td>9500
10700*</td><td>7100*** (74 %)
3300*** (33 %)</td><td>1200*** (12.5 %)
800a</td></tr>
<tr><td>Plasma acetyl cholinesterase (iu/mL)</td><td>M
F</td><td>743
2458</td><td>795
2362</td><td>817
1568* (63.8 %)</td><td>662
559*** (22.7 %)</td><td>344*** (46.3 %)
368a</td></tr>
<tr><td>Plasma butyryl cholinesterase (iu/mL)</td><td>M
F</td><td>1325
3705</td><td>1408
3685</td><td>1408
2484* (67 %)</td><td>1118* (84.4 %)
994*** (26.8 %)</td><td>863*** (65.1 %)
828a</td></tr>
</table>
A not determined
a no statistics conducted since only one animal survived
</td><td>Anonymous 10, 1989c</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>** significantly different from controls, P<0.01
*** significantly different from controls, P<0.001
<table>
<tr><th>Dose (mg/kg bw/day)</th><th>Adrenals (g)</th><th>Adrenals vs body weight (%)</th><th>Vacuolation Zona fasciculata (animals examined: 5)</th></tr>
<tr><td>Males</td><td></td><td></td><td></td></tr>
<tr><td>0</td><td>0.054</td><td>0.0196</td><td>0</td></tr>
<tr><td>0.5</td><td>0.053</td><td>0.0184</td><td>0</td></tr>
<tr><td>2.5</td><td>0.049</td><td>0.0183</td><td>0</td></tr>
<tr><td>25</td><td>0.046</td><td>0.0175</td><td>0</td></tr>
<tr><td>250</td><td>0.057</td><td>0.0247</td><td>4</td></tr>
<tr><td>Females</td><td></td><td></td><td></td></tr>
<tr><td>0</td><td>0.072</td><td>0.0322</td><td>0</td></tr>
<tr><td>0.5</td><td>0.071</td><td>0.0323</td><td>0</td></tr>
<tr><td>2.5</td><td>0.074</td><td>0.0328</td><td>0</td></tr>
<tr><td>25</td><td>0.082</td><td>0.0361</td><td>0</td></tr>
<tr><td>250</td><td>0.095</td><td>0.0516*</td><td>2</td></tr>
</table>
* Significantly different from control s, p<0.05.</td><td></td></tr>
</table>

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In an oral 28-day study in rats (Anonymous 11, 1989d) signs of toxicity were occasional muscle tremors in 4 females at the top dose of 43.5 mg/kg bw/d and in one male (40.87 mg/kg bw/d) only which died on day 21. Fur loss, predominantly on the dorsal body surface and head was observed in all males and in one female at this dose level. Haematology at day 25 (males) or 26 (females) showed statistically significant but slight reductions (~2-4.5 %) in haemoglobin and packed cell volume only in males at 400 ppm (43.5 / 40.87 mg/kg bw/d). Mean cell volume was reduced in both sexes at the top dose about ~ 3%. It is also considered that reductions in Hb (2 %) and MCHC (4.8 %) in females at ≥100 ppm (10.7 mg/kg bw/d) is also possibly treatment-related. At dose level of ≥1.0 mg/kg bw/d erythrocyte cholinesterase was reduced in females (≥ 26.2 %). At dose level of ≥ 9.7 mg/kg bw/d erythrocyte cholinesterase was reduced in males (≥ 77 %) and brain cholinesterase activity was reduced in males (≥ 43.7 %) and females (≥73.4 %). There was cell enlargement and pallor in the adrenal cortex in 2 females at 10.7 mg/kg bw/d and in all females at 43.5 mg/kg bw/d. At the top dose level of 400 ppm (43.5 / 40.87 mg/kg bw/d) organ weights showed an increase in the absolute and relative adrenal weights in females (27 % and 41 %, respectively) and in the relative adrenal weights in males (27 %). Relative liver weight was also increased in females at this dose and an increase in alkaline phosphate in males (30 %) and females (36 %) and an increase in alanine aminotransferase in males (20 %) and females (33 %) was observed.

As the study design exhibited some deficiencies in comparison to the essential criteria of 92/69/EEC Method B7, testes were not weighed and a limited number of tissues were analysed microscopically, the study was considered to be of supplementary information.

While in a mice a 28-day range-finding study (Anonymous 1, 1989a) no cholinesterase investigations were reported, the study provides information on haematological effects as well as effects on adrenals, liver and kidney (weight and histopathology): at a dose level of 400 ppm (69.0 or 82.4 mg/kg bw/day) there were reductions in levels of haemoglobin (5.2 %) and packed cell volume (6.7 %) in males during week 4, an increase in leucocyte and lymphocyte counts in females, an increase in the absolute (+50 %) and relative (+49 %) adrenal weight in females and in the relative liver weight (+14 %) in males and an increase in the incidence of focal tubular hyperplasia of the kidneys in males (9/12) and females (8/12). The study is considered supplementary, as no clinical chemistry tests were conducted, testes were not weighed and a limited number of tissues was analysed microscopically.

Anonymous 40 (2011) conducted a 28-day dietary study in female CD-1 mice to evaluate a potential immunotoxicity of fosthiazate. The T-cell dependent antibody response to sheep red blood cells was assessed via a splenic antibody-forming cell assay. As test-substance related overt toxicity occurred and mortality was observed in the highest dose group (400 ppm / 61 mg/kg bw/d) from day 4 on, the treatment regime was altered and a 200 ppm group (44 mg/kg bw/d) was included in the study on day 7. This group received fosthiazate for 28 days, the other groups for 35 days. Observed clinical signs in the 400 ppm group were hypoactivity, tremors (intermittent and/or continuous), labored respiration, extremities cool to touch, bodycool to touch, decreased defecation, and/or feces smaller than normal. Immune response, measured by T-cell dependent antibody response to sheep red blood cells, was not suppressed by fosthiazate treatment. Absolute and relative weight of adrenal glands was increased in all treatment groups (up to 17 and 24 % at the highest dose, respectively). Relative liver weight was increased in in the 44 mg/kg bw/d group (+12 %).

A 28-day oral study in dogs (Anonymous 12, 1989e) revealed an significant increase in absolute weight of adrenals the top dose level, 26.8 mg/kg bw/d, in males (+ 42 %) and also relative weight of adrenals was increased at this dose level in males and females (+55 % and +33 % respectively). Histopathological examination of the adrenal glands revealed cell enlargement and pallor in the zona fasciculata of the adrenal cortices in 2 males already at 0.54 mg/kg bw/day, in all males and one female at 5.4 mg/kg bw/day and in all dogs at 26.8 mg/kg bw/day. Erythrocyte cholinesterase was markedly reduced in both sexes at dose levels of ≥ 5.4 mg/kg bw/day (> 77 %) compared with controls. Brain cholinesterase was reduced at dose levels of ≥ 5.4 mg/kg bw/day (> 33 % in males and 49 % in females) in both sexes compared with controls. As the study was a range-finding study with a limited number of organs which were examined microscopically (in comparison to the requirements of 92/69/EEC Method B7 guidelines for a subchronic oral toxicity study) it is

considered supplementary.

During an oral 90-day study in rats (Anonymous 9, 1989b) signs of toxicity including hair loss, emaciation, tachypnoea were observed at the highest dose level of 429 ppm (36.4 and 41 mg/kg bw/d in males and females, respectively). At dose levels of ≥ 4.12 mg/kg bw/d in males and 41 mg/kg bw/d in females there was vacuolation of the adrenal cortex. At dose levels of ≥ 4.12 mg/kg bw/d in males (≥ 23 %) and ≥ 4.7 mg/kg bw/d in females (≥ 66 %) there was a significant reduction in brain cholinesterase. Erythrocyte (cell acetyl) cholinesterase was also significantly reduced in both sexes at ≥53.6 ppm (4.12 and 4.7 mg/kg bw/d, respectively) in both sexes. Significant changes of lower packed cell volume, haemoglobin concentration, and erythrocyte counts in males and females receiving 429 ppm and in females receiving 53.6 ppm were observed (< 10 %). All these changes were reversible after a three week period. The study satisfies the essential requirements of current EC guidelines for a 90-day repeat dose toxicity study in rodents except for the duration of the recovery period. It is considered that the differences in methodology do not negatively impact the scientific interpretation of the results. Therefor the study is considered acceptable.

Similar to the 90- day rat study also in dogs effects on haematology, cholinesterase and adrenals were reported (Anonymous 8, 1989a, Anonymous 22, 1995): a decrease of haematologic parameters was observed in females at a dose level of 5.4 mg/kg bw/day: packed cell volume (11 %), haemoglobin (10 %), red blood cell count (14 %). At 0.11 mg/kg bw/day plasma butyryl and acetyl-cholinesterase activities were reduced in females (21-23 %). At ≥0.54 mg/kg bw/day plasma butyryl and acetyl-cholinesterase activities were reduced in both sexes. At the highest dose level of 5.4 mg/kg bw/day erythrocyte and brain cholinesterase were markedly reduced in both sexes (22-68 %). At this dose level an increase in the absolute and relative adrenal weights in males (+29 and +26 %) and an increase in the severity of cytoplasmic hypertrophy and/or increased pallor of cells in the zona glomerulosa and zona fasciculata of the adrenal cortex was observed in males and females. The study is considered acceptable.

In a 1-year oral study in dogs (Anonymous 39, 1991, Anonymous 23, 1995) haematological changes were observed in males including reduction in erythrocyte counts (16.4-11.6 %) and reduced haemoglobin concentration (10.9-15.7 %) at a dose level of 5.0 mg/kg bw/day. There was also a transient reduction in haematocrit concentration and an increase in MCH in females. Increased reticulocyte ratios were observed in females. Furthermore, there were histopathological findings in the adrenals, pallor of the zona glomerulosa and fasciculata and hypertrophy of the zona fasciculata, in males and females at the high dose of 5.0 mg/kg bw/day. At 0.5 mg/kg bw/day and above an inhibition of plasma cholinesterase activity was observed in both sexes (≥ 20 %). The study is considered acceptable.

The combined chronic toxicity and carcinogenicity study in CD rats (Anonymous 4, 1990b) is considered acceptable. 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 and EU test guidelines (OECD Guideline for testing of chemicals, "Combined Chronic Toxicity/Carcinogenicity Studies", 453, 12 May 1981; ASB2015-11698; Carcinogenicity Studies, 451, 7 September 2009; ASB2015-11697). However, the survival was higher than 50 % till week 92. An increase in mortality above 50 % began at week 93 (male animals at 53.6 ppm group only). At the end of the study survival was far above 25% in all groups. According to the OECD Guidance Document N° 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting TG 451, 452, 453, 2ND Edition (13-Apr-2012; ASB2015-8445) “For a negative result to be acceptable in a rat carcinogenicity bioassay, survival in the study should ideally be no less than 50 % in all groups at 24 months, while for “life span studies” survival at study termination should not be less than 25 %.” According to the US EPA Health Effects Test Guidelines OPPTS 870.4300 and 870.4200 survival in any group should not fall below 50 % at 18 months (72 week) in the case of rats, or below 25% at 24 months (ASB2015-11685). While this study may not meet the OECD and EU guideline requirements for long term/carcinogenicity testing concerning survival, it is concluded however that the results are useful without prejudice to the assessment of carcinogenicity or long-term toxicity.

At the top dose level of 214 ppm (8.94 in males or 12.53 mg/kg bw/d in females) body weight gain was reduced in both sexes. Overall body weight gain was statistically significantly reduced in females (24 %) only at 214 ppm compared with controls. Lower packed cell volumes, lower haemoglobin concentration, erythrocyte counts up to ~ 11 % was observed in haematology tests after 12, 51 and 102 weeks in females at dose levels of 2.63 and 12.53 mg/kg bw/d. Organ weights at 104 weeks showed increases in the absolute (+ 32 %) and

relative adrenal weights in females at the top dose (12.53 mg/kg bw/d). Gross examination at necropsy revealed an increased incidence of pallor and areas of change in the adrenals of females. Histopathological examination revealed changes in both sexes including vacuolation of the zona fasciculata of the adrenal gland, retinal atrophy and vacuolation of the pars nervosa of the pituitary at 8.94 in males or 12.53 mg/kg bw/d in females. An increase in the incidence of degenerative myopathy in the skeletal muscles in both sexes was also noted in males and females at this dose level. The latter change was apparent only in animals killed after 52 weeks. Foamy interstitial cells in the ovaries were observed in females at dose levels of ≥ 2.63 mg/kg bw/d. Plasma acetyl and butyryl cholinesterase and erythrocyte cholinesterase activities were reduced in both sexes at dose levels of ≥ 0.41 mg/kg bw/d compared with controls. Brain acetylcholinesterase was reduced in both sexes at dose levels of ≥ 2.63 mg/kg bw/day more than 60 %. Haematological observations were not associated with any histopathological changes. They are considered to be of minimal toxicological significance. Under the experimental conditions of this study, there was no evidence of any oncogenic potential for fosthiazate. The study is considered acceptable.

In a carcinogenicity study (Anonymous 3, 1990) in mice feed consumption was very slightly reduced in males (14 %) compared with the combined control mean at the top dose of 322 ppm (30.5 or 39.17 mg/kg bw/d). Overall body weight gain was markedly reduced in males and females (43 %) compared with the combined control mean and the remaining treatment groups. Mortality in male and females was increased. An increase in the absolute (+ 43 %) and relative (+ 112 %) adrenal weights was observed in females. There was a reduction in the absolute kidney weight in males and females (20 or 18 %) at the top dose. An increase in the relative kidney weight was considered to be possibly due to the lower body weights. Histopathological changes included an increase in corticomedullary pigmentation of the adrenal cortex in males, increase in mineralization of pigmented cells of the adrenal cortex, papillary mineralization in the kidney and vacuolation of the pars nervosa of the pituitary in both sexes. At ≥ 107 (10.4 mg/kg bw/d) ppm there was an increase in the relative liver weight (7 % at 10.4 mg/kg bw/d, 19 % at 39.17 mg/kg bw/d) and an increase in corticomedullary pigmentation of the adrenal cortex in females.

The higher incidence of pulmonary carcinoma in females at 107 ppm was not considered to be treatment-related due to its absence at the higher treatment level and from the males at 100 ppm.

There was no indication of a carcinogenic potential of fosthiazate in the mouse. The carcinogenicity study in mice is considered acceptable as the study methodology conformed to the current guideline requirements for oncogenicity studies except that the study was allowed to continue until survival was low. For clarification of the observed corticomedullary pigmentation of the adrenal cortex in males and females of the carcinogenicity study a follow-up study was conducted by (Anonymous 35, 2013). As the design of the study does not meet any requirements of a specific guideline it is considered as supplementary information. Eight adrenals from four high-dose female mice were selected for histopathologic examination in comparison to selected adrenals from control animals. It was demonstrated that the pigmentation was PAS positive, LZN positive, Schmorl's negative and Perl's negative. This finding indicates that the pigmentation is likely to be ceroid, a spontaneous age-related finding in CD-I mice. Fosthiazate may have accelerated this background finding.

Effects of fosthiazate on weight of adrenal glands were also reported in a two generation study in rats (Anonymous 34, 1990): a statistically significant increase in absolute adrenal weight was observed from a dose level of 0.86 mg/kg bw/d on (+15%) in females. At the highest dose (9.34 mg/kg bw/d) the absolute adrenal weight in F0 dams was increased about 24% in comparison to control animals and a statistically significant increase in the incidence of hypertrophy of the zona glomerulosa of the adrenal cortex in F0 dams was observed. Further information on effects of fosthiazate on pup viability are described in Chapter 10.10.1. The study is considered acceptable.

In an oral age sensitivity study in rats (Anonymous 7, 2006) cholinesterase inhibition was, in general, similar in juvenile and young adult males and females for all compartments and no age–related effects of fosthiazate were observed after maternal gestational exposure or after acute or short-term exposure to rats of various ages. Nevertheless, maternal females were much more sensitive to cholinesterase inhibition following direct test substance administration than were their foetuses following in utero exposure: significant inhibition (≥ 20 %) of plasma and RBC cholinesterase was already observed at a 0.7 mg/kg bw. At 5 mg/kg bw also brain cholinesterase was inhibited in females and plasma, RBC and brain cholinesterase were significantly inhibited (≥ 20 %) in the foetuses. The extent of inhibition was greater in the dams (-99.5–89 %) than in the foetuses

(-33-22 %). Following acute exposure, cholinesterase activity was inhibited only at 5 mg/kg bw in juveniles and young adults. After repeated dosing, inhibition occurred at 0.7 and 5 mg/kg bw. RBC inhibition following repeated dosing was severe; all maternal females and several juvenile and young adult animals had values that were below the lower limit of quantitation. The inhibition in the maternal animals at 5 mg/kg bw/day (-99.5%) was accompanied by functional deficits, including tremors, repetitive jaw movement and piloerection. There were no neurobehavioral findings in the juveniles or young adults following repeated exposure at 5 mg/kg bw/day but no specific neurobehavioral tests were performed. Slightly reduced mean body weight gains were observed in the juvenile males and, to a lesser extent, in the females. Plasma cholinesterase was determined to be the most sensitive compartment because inhibition occurred at lower dosage levels (0.7 mg/kg bw) and occurred more quickly following test substance administration. The study is considered acceptable. No guideline is applicable for this type of study.

Effects of fosthiazate on adrenals and cholinesterase were also reported after dermal exposure. In a 21-day study in rats (Anonymous 10, 1989c) clinical signs of toxicity including emaciation, hunched posture, torpor and tremor in both sexes, additional hypothermia, gasping, hypersensitivity to noise, pallor, trachypnoea and piloerection in females were observed at 250 mg/kg bw/day. Feed consumption was slightly reduced in females. Statistically significant overall lower body weight gain was observed in both sexes at this dose level. Leukocyte and platelet numbers were reduced in males at 250 mg/kg bw/day. Clinical chemistry showed a significant increase in urea in the surviving female at 250 mg/kg bw/day. Pathology revealed increased adrenal weights in both and vacuolation of the zona fasciculata of the adrenals in males (4/5) and females (2/5) at 250 mg/kg bw/day. At dose levels of ≥ 25 mg/kg bw/day feed consumption was slightly reduced in males (7-8 %). Clinical chemistry showed a significant increase in urea in males at ≥ 25 mg/kg bw/day. At ≥ 25 mg/kg bw/day erythrocyte cholinesterase was markedly reduced in males (≥ 55 %). Brain cholinesterase showed treatment-related reduction in both sexes at dose levels of ≥ 25 mg/kg bw/day. At ≥ 2.5 mg/kg bw/day erythrocyte cholinesterase was markedly reduced in females (≥ 21 %). The study is considered acceptable, albeit the animals were treated for 21 days rather than 28 days.

10.12.2 Comparison with the CLP criteria

Table 33: Toxicological results (at dose levels below the guidance values) of repeated dose studies in comparison with criteria of specific target organ toxicity – repeated exposure

Toxicological result within the guidance value range for STOT RE 1 and STOT RE 2	CLP criteria
28-day oral study in rats (Anonymous 11, 1989d): STOT RE 1 (≤30 mg/kg bw/d): at ≥1.0 mg/kg bw/d reduced erythrocyte cholinesterase in females (≥ 26.2 %), at 100 ppm (9.69 mg/kg bw/d males; 10.7 mg/kg bw/day females): Reductions in Hb (2 %) and MCHC (4.8 %) in females, reduced erythrocyte cholinesterase (≥ 77 %) and brain cholinesterase activity (≥ 43.7 %) in males (≥ 43.7 %) and in females (≥73.4 %). Cell enlargement and pallor in the adrenal cortex in 2/10 females. Histopathology supported by increased absolute and relative adrenal weights in females and relative adrenal weights in males. STOT RE 2 (30 < C ≤ 300 mg/kg bw/d): at 40.87 mg/kg bw/d (m) and 43.5 mg/kg bw/d (f): fur loss, occasional muscle tremors in both sexes (4 females and in one male (died on day 21)), reductions in haemoglobin (~2-4.5 %) in males and females, in packed cell volume in males, and in mean cell volume (~ 3%) in both sexes.	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations: Oral, rat: 28-day: ≤30 mg/kg bw/d

absolute and relative (41%) adrenal weights in females ↑, relative adrenal weights in males (27%) ↑, cell enlargement and pallor in the adrenal cortex in all females. relative body weight in females ↓; relative liver weight in females↑, alkaline phosphate in males (30%) ↑ and females (36%) ↑, alkaline phosphate in males (30 %) and females (36 %) ↑, alanine aminotransferase in males (20%) ↑ and females (33%) ↑ after 25 or 26 days; **28-day oral study in mice** (Anonymous 1, 1989a): STOT RE 2 (30 < C ≤ 300 mg/kg bw/d): 69.0 or 82.4 mg/kg bw/d, male/female: absolute (50 %) and relative (49 %) adrenal weight in females increased, haemoglobin (5.2 %) ↓ and packed cell volume (6.7 %) ↓ in males during week 4; leucocyte and lymphocyte counts in females (> 50 %) ↑, relative liver weight in males (14 %)↑; incidence of focal tubular hyperplasia of the kidneys in males (9/12) ↑ and females (8/12) ↑ **28-day oral immunotoxicity study** (Anonymous 40, 2011): STOT RE 1 (≤30 mg/kg bw/d): ≥10 mg/kg bw/d: absolute (18-24%) and relative (10-17%) adrenal glands weights ↑ STOT RE 2 (30 < C ≤ 300 mg/kg bw/d): 44 mg/kg bw/d: mean absolute (7%) and relative (12%) liver weights ↑ 61 mg/kg bw/d for 5 consecutive days: mortality ↑; **28-day oral study in dogs** (Anonymous 12, 1989e): STOT RE 1 (≤30 mg/kg bw/d): ≥0.54 mg/kg bw/d: adrenal glands (cell enlargement and pallor in the zona fasciculata of the adrenal cortices) ≥5.4 mg/kg bw/d: depressed tactile placement (1 female); erythrocyte cholinesterase in both sexes ↓ (>77%); brain cholinesterase in males ↓ (>33%) and in females↓ (49%); 26.8 mg/kg bw/d: salivation (2 males) and nasal dryness (both sexes), depressed tactile placement (2 males), listlessness (1 male), rotation of the head backward, absolute adrenal weight (42%) ↑ in males, relative adrenal weight ↑ in males (55%) and females (33%) **90-day oral study in rats** (Anonymous 9, 1989b): STOT RE 1 (≤10 mg/kg bw/d): ≥4.12/4.7 mg/kg bw/d in males/females: adrenal glands (vacuolation of the adrenal cortex) in males, brain cholinesterase in males ↓ (≥23%) and females ↓ (≥66%); erythrocyte (cell acetyl) cholinesterase in both sexes ↓ (≥50%); reversible changes of lower packed cell volume, haemoglobin concentration, and erythrocyte counts in females STOT RE 2 (10 < C ≤ 100 mg/kg bw/d):	90-day: ≤10 mg/kg bw/d Adjusted guidance values 1 year ≤2.5 mg/kg bw/d 2 year ≤1.25 mg/kg bw/d Dermal: 28-day: ≤ 60 mg/kg bw/d 90-day: ≤ 20 mg/kg bw/d Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for different study durations: Oral, rat: 28-day: 30 < C ≤ 300 mg/kg bw/d 90-day: 10 < C ≤ 100 mg/kg bw/d Adjusted guidance values 1 year 2.5 < C ≤ 25 mg/kg bw/d 2 year 1.25 < C ≤ 12.5 mg/kg bw/d Dermal: 28-day: 60 < C ≤ 600 mg/kg bw/d 90-day: 20 < C ≤ 200 mg/kg bw/d

36.4/41.0 mg/kg bw/d in males/females): adrenal glands (vacuolation of the adrenal cortex) in females emaciation (5 males) and tachypnoea (9 males, 1 female) for up to 4 weeks, hair loss during the first 9 weeks of the study in both sexes; adrenal glands (vacuolation of the adrenal cortex) in females; reversible changes of lower packed cell volume, haemoglobin concentration, and erythrocyte counts in both sexes; **90-day oral study in dogs** (Anonymous 8, 1989a; Anonymous 22. 1995): STOT RE 1 (≤10 mg/kg bw/d): ≥0.54 mg/kg bw/d: plasma butryl and acetyl-cholinesterase in both sexes ↓; 0.11 mg/kg bw/d: plasma butryl and acetyl-cholinesterase in females ↓ (21 - 23%); 5.4 mg/kg bw/d: erythrocyte (≥32.1%) and brain cholinesterase (≥68.4%) in both sexes ↓; absolute and relative adrenal weights in males ↑; adrenal cortex (severity of cytoplasmic hypertrophy ↑ and/or pallor of cells ↑ in the zona glomerulosa and zona fasciculata) in males and females **1-year oral study in dogs** (Anonymous 39, 1991; Anonymous 23, 1995): STOT RE 1 (≤2.5 mg/kg bw/d): ≥0.5 mg/kg bw/d: plasma cholinesterase ↓ in both sexes (≥25%) STOT RE 2 (2.5 < C ≤ 25 mg/kg bw/d): 5.0 mg/kg bw/d: haematological changes (erythrocyte counts (16.4 - 11.6%, 3 - 12 month) ↓ and haemoglobin concentration (10.9 - 15.7%) ↓ in males; haematocrit concentration ↓ (transient) and MCH ↑ and reticulocyte ratios (≥300%) ↑ in females); adrenal glands (pallor of the zona glomerulosa and fasciculata and hypertrophy of the zona fasciculata, in males and females) **2-year study in rats** (Anonymous 4, 1990b): STOT RE 1 (≤1.25 mg/kg bw/d): ≥ 0.41/0.54 mg/kg bw/d, males/females: reticulocytes counts ↑; plasma acetyl and butryl cholinesterase (≤69%) and erythrocyte cholinesterase (≤89%) activities were reduced in both sexes ↓ STOT RE 2 (1.25 < C ≤ 12.5 mg/kg bw/d): ≥ 2.08/2.63 mg/kg bw/d, males/females: after 12, 20, 28 and 51, 76 and 102 weeks packed cell volumes, haemoglobin concentration, erythrocyte counts in females ↓ (≤11.6%); brain cholinesterase at 53 and 104 weeks in males and females (≤51%)↓; 8.94/12.53 mg/kg bw/d, males/females: body weight gain in both sexes ↓; packed cell volumes, haemoglobin	

concentration, erythrocyte counts after 12, 20, 28 and 51, 76 and 102 weeks in males ↓ (≤4.4%); alkaline phosphatase in males and females ↑, total plasma proteins during the first year in females ↓, specific urine gravities predominantly in females ↓, urinary output in females ↓; vacuolation of the zona fasciculata and glomerulosa of the adrenal gland in both sexes ↑, retinal atrophy in both sexes ↑, vacuolation of the pars nervosa of the pituitary in both sexes ↑, degenerative myopathy in the skeletal muscles in both sexes ↑; ovary-foamy interstitial cells in females ↑; **104 week study in mice** (Anonymous 3, 1990; Anonymous 35, 2013): STOT RE 2 (1.25 < C ≤ 12.5 mg/kg bw/d): ≥100 ppm (10.4 mg/kg bw/d): relative liver weight in females ↑; adrenal glands (corticomedullary pigmentation of the adrenal cortex in females) **2-generation reproduction study in rats, fosthiazate administered via diet** (Anonymous 34, 1990): 9.34 mg/kg bw/d: hypertrophy of the zona glomerulosa of the adrenal cortex supported by absolute adrenal weight increase in F0 dams ≥ 2.09/2.62 mg/kg bw/d, male/female): pup viability↓ during lactation during the first generation **Oral (gavage) age-sensitivity study of technical fosthiazate in rats, repeated exposure** (Anonymous 7): 5 mg/kg bw: on gestation days 19 and/or 20 tremors, unkempt appearance, prostration and red and yellow material on various body surfaces, additional in 1 or 2 animals during the twice-weekly examinations repetitive jaw movement, piloerection, gasping and lacrimation; 3 hours following dosing on gestation day 19 lacrimation (1 female), tremors and/or repetitive jaw movement (3 females); Plasma cholinesterase activities of juveniles and young adults ↓ (≥25%); erythrocyte cholinesterase activities of juveniles and young adults ↓ (≥25%); brain cholinesterase activities of juveniles adults ↓ (≥25%) **21-day dermal studies in rats** (Anonymous 10)**:** STOT RE 1 (≤60 mg/kg bw/d): ≥2.5 mg/kg bw/d: erythrocyte cholinesterase in females (≥21%) ↓ ≥25 mg/kg bw/d: feed consumption in males ↓; urea in male ↑; erythrocyte cholinesterase in males ↓ (≥55%), brain cholinesterase in both sexes ↓ (≥25%);	

STOT RE 2 (60 < C ≤ 600 mg/kg bw/d): 250 mg/kg bw/d: clinical signs of toxicity (emaciation, hunched posture, torpor and tremor in both sexes, additional hypothermia, gasping, hypersensitivity to noise, pallor, tachypnoea and piloerection in females); feed consumption in females ↓; body weight gain in both sexes ↓; urea in female ↑; adrenal glands (organ weight in both sexes ↑, vacuolation of the *zona fasciculata* in males (4/5) and females (2/5)	

10.12.2.1 Nervous system:

Fosthiazate is a neurotoxic organophosphorus compound due to inhibiting cholinesterase. Considering cholinesterase inhibition after repeated exposure relevant information is obtained from repeated oral and dermal toxicity studies in rats and dogs.

Inhibition of cholinesterase activity was observed in rats after oral and dermal exposure of fosthiazate (plasma, erythrocyte, brain) and in dogs after oral exposure of fosthiazate (plasma cholinesterase). Cholinesterase inhibition occurred below cut-off values for classification as STOT RE 1. However, inhibition of cholinesterase activity was already obvious after single oral exposure of fosthiazate, identified as an acute toxic effect. Hence, the observed neurotoxic findings in repeated-dose studies are considered more as repeated acute effects. In summary, the neurotoxic findings in repeated-dose studies are covered by the proposed classification with STOT-SE.

10.12.2.2 Adrenal glands:

Adrenal glands were affected in rats, mice and dogs in all repeated dose studies. Organ weights were increased (>20%) in all species. Histopathology revealed vacuolation of the zona fasciculata and glomerulosa of the adrenal gland in rats being probably storage lesions (Anonymous 8, 1989a; Anonymous 11, 1989d; Anonymous 4, 1990b), an increase in the incidence of hypertrophy of the zona glomerulosa of the adrenal cortex in female rats (Anonymous 34, 1990), corticomedullary pigmentation of the adrenal cortex and mineralization of pigmented cells of the adrenal cortex in mice (Anonymous 3, 1990a; Anonymous 35, 2013) and an increase in the severity of cytoplasmic hypertrophy and/or pallor of cells in the zona glomerulosa and zona fasciculata in dogs (Anonymous 8, 1989a; Anonymous 22, 1995; Anonymous 39, 1991; Anonymous 23, 1995).

In the subchronic toxicity study in rats, recovery from adrenal changes during the subsequent reversibility period was reported (Anonymous 9, 1989b).

In the mice carcinogenicity study the application of special stains to additional sections of adrenal from selected control and high dose female mice indicated that the cortico-medullary pigment is likely to be ceroid, which is a spontaneous age-related finding in CD-I mice. Nevertheless fosthiazate exacerbated this background change (Anonymous 3, 1990a).

In summary, adrenal changes are found as consistent and identifiable toxic effects in experimental animals, where dogs are the most sensitive species. According to the CLP Criteria and guidance (Guidance on the Application of the CLP Criteria, chapter 3.9.1.3) such changes have to be considered as adverse health effects and are supporting classification for specific target organ toxicity after repeated exposure; changes in adrenals were seen in dogs (28-/90-day studies) at dose levels leading to category 1 (STOT RE 1, H372). The adrenal effects observed in the 28-/90-day studies were not markedly increased in terms of severity in the 1-year dog study at comparable dosages. However, the effect levels in the 1-year studylead to category 2 (STOT RE, H373). In the CLP guidance (Guidance on the Application of the CLP Criteria (2017) 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)”. Overall, category 2 (STOT RE 2, H373) is thus considered more appropriate.

10.12.2.3 Haematology:

Haematological changes were observed in oral subacute, subchronic and long-term studies in all species.

In the 28-day studies in rats and mice the reduction of the haemoglobin concentration (Hb) and changes of other haematological parameter values were below 10% in comparison to controls (Anonymous 11, 1989d; Anonymous 1, 1989a). In the 28-day oral study in dogs (Anonymous 9, 1989b) haematological examinations did not reveal any treatment-related changes either.

In the subchronic studies in rats and dogs changes of haematological parameter values are below 10% in comparison to controls. These changes were reversible (rat) (Anonymous 9, 1989b) or within the normal range (dog) (Anonymous 12, 1989e). In the 1-year oral study in dogs 5.0 mg/kg bw/d revealed anaemic changes. The reduction of red blood cell parameters was above 10%. However, anaemic changes did not increase with the progress of the study and no associating clinical signs were detected in these animals. Therefore the anaemic changes in these animals were considered to be minimal and reversible effects of fosthiazate.

In the 2-year study in rats (Anonymous 4, 1990b) haematology tests showed lower packed cell volumes, lower haemoglobin concentration, erythrocyte counts in females at dose levels of ≥2.63 mg/kg bw/d (≤11.6%) and in males at 8.94 mg/kg bw/d (≤4.4%). At ≥0.41 mg/kg bw/d there was a minimal increase in the numbers of reticulocytes in animals. These observations were not associated with any histopathological changes. Therefore, the haematological changes are considered to be of minimal toxicological significance.

In the 104 week study in mice (Anonymous 3, 1990a, 1990; Anonymous 35, 2013) haematology at 51, 76 and 101 weeks did not reveal any treatment-related changes.

In summary, it can be concluded that haematological changes were small. They were not associated with any histopathological or clinical changes. Therefore, they are of doubtful or minimal toxicological importance. According to the CLP Criteria and guidance (Guidance on the Application of the CLP Criteria, chapter 3.9.2.8.1) small effects on haematology are considered not to support classification for specific target organ toxicity following repeated exposure when such effects are of doubtful or minimal toxicological importance.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the CLP criteria and guidance given in the Guidance on the Application of the CLP Criteria, Version 5.0 (2017), there is sufficient evidence of specific target organ toxicity after repeated exposure of fosthiazate in animals. Classification of fosthiazate with STOT RE 2 (H373), is proposed with the adrenals as the target organ.

10.13 Aspiration hazard

This endpoint is not addressed in this CLH report and is outside the scope of the consultation.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

All the information on ready biodegradability are taken from the RAR and list of endpoints for fosthiazate, September 2020.

Table 34: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD 301 F	*Ready biodegradability:* 0% degradation after 28 days Fosthiazate is not readily biodegradable.	The study is considered acceptable.	Anonymous (2012)
US EPA 161-1	*Hydrolytic degradation of the active substance and metabolites>10%:* DT_{50} at 25°C 177, 104 and 3.2 days at pH 5, 7 and 9 Maximal amounts of Metabolites: pH 5: DETO 10 % at day 30 pH 7: DETO 14 % at day 30 pH 9: DBTO: 45 % at day 14 TZO: 40 % at day 14 DTTO: 44 % at day 14 .	The study is considered acceptable. Non- GLP study	Anonymous (1989)
OECD 111	*Hydrolytic degradation of the active substance and metabolites>10%:* DT_{50} pH 4, 7 and 9 buffer at 20°C = 312 d, 255 d and 9 d. Maxiamal amounts of Metabolites: DETO : 65.0 % at 50°C, pH 7 TZO: 20% at 50°C, pH 7 DTTO : 34.8% at 50°C, pH 7 DBTO: 10% at 50°C, pH 7 volatile degradate: sec-butyl mercaptan	The study is considered acceptable. GLP study	Anonymous (2014)
Not indicated	*Direct photochemical degradation in water* DT50 : negligible, (Xenon lamp, 12h on/ 12h off cycle) Met DETO: 8 % AR (30d)	The study is considered acceptable.	Anonymous (1993)

Method	Results	Remarks	Reference
OECD TG No 309	*Aerobic mineralisation in surface water:* System: Pond Schoonrewoerdse Wiel, pH 7.3, 20°C: SFO - DT_{50} /DT_{90} values: [^{14}C–R]-fosthiazate: 10 µg/L: 15.2/50.5 days 100 µg/L: 15.5/51.7 days [^{14}C–B]-fosthiazate: 10µg/L: 14.7/48.7 days 100 µg/L: 15.9/52.7 days Metabolite TZO: Max in total system: *36,1* % after *31* days, 10 µg/L Metabolite *DBTO-Li:* Max. in total system: *80.3 %* after 56 days, 100 µg/L Metabolite BSA: Max. in total system: 29.9 % after 56 days, 100 µg/L Mineralisation (for parent dosed experiments): [^{14}C–R]-fosthiazate: 10 µg/L: 21% after 56 d 100 µg/L: 4 % after 56 d [^{14}C–B]-fosthiazate: 10 µg/L: 6% after 56 d 100 µg/L: 3% after 56 d	The study is considered acceptable.	Anonymous (2014)
BBA Part IV, US EPA 162-4	*Degradation in water/sed system:* System: Grand River, [^{14}C-B]-lab.: pH water/sediment: 7.4 / 7.0 SFO - DT_{50}/DT_{90} , whole system: 70.2/233 d No major metabolites were detected.	The study is in regard to the river system considered acceptable.	Anonymous (1996)

Method	Results	Remarks	Reference
BBA Guideline Part IV, US EPA 162-4	*Degradation in water/sed system:* System: Homestead Road Pond, silt loam, [^{14}C-T]-lab. pH water/sediment: 6.5 / 4.9 SFO - DT_{50}/DT_{90} , whole system: 43.7 / 145 d Mineralisation: 28.2% after 100 days System: Grand River [^{14}C-T]-lab. pH water/sediment: 7.9 / 7.3 SFO - DT_{50}/DT_{90} , whole system: 30.1/100 d Mineralisation: 36.2% after 100 days Metabolite TZO : 32.9 % AR water phase and 35.7 % whole system after 30 d Total system DT_{50} TZO: 11.4 and 19.7 days in the pond and river system,	The study is considered acceptable.	Anonymous (1998)
OECD No. 308	*Degradation in water/sed system:* System: Swiss Lake (fresh) sand, [^{14}C-B]-lab pH water/sediment: not given / 5.5 SFO - DT_{50}/DT_{90} , whole system: 72.1/239 d Metabolite DTTO: 16.2% AR whole system day 7 DT_{50} /DT_{90} DTTO : 36 /120 days Mineralisation: 18.8% after 100 days	The study is considered acceptable only for the Swiss Lake system.	Anonymous(2013)
No test guideline	*Aerobic degradation of stereoisomers of active substance in water:* Four isomers of fosthiazate Calwich Abbey Lake: No significant differences between the DT_{50} values of the stereoisomers pH 9 buffer solution: No significant differences between the hydrolytic DT50 values of the stereoisomers	Acceptable	Anonymous (2014)

11.1.1 Ready biodegradability

Reference:	**Anonymous (2012), IKI-1145 (FOSTHIAZATE) - ASSESSMENT OF READY BIODEGRADABILITY BY RESPIROMETRY**
Report No.	JSM0297
Published:	No
Test guideline used:	EC 440/2008, C.4-D, OECD No. 301F
Deviations:	None
GLP:	Yes; laboratory certified by the Department of Health of the Government of the United Kingdom

The study is acceptable. The biodegradation of fosthiazate was 0 % on each of the 28 days of the test.

Fosthiazate was considered to be not readily biodegradable under the conditions of this test.

The validity criteria for this test were fulfilled, as the biodegradation of the reference substance sodium benzoate was 65 % of its ThOD after 4 days and the degradation in the toxicity test was more than 25 % of the total ThOD (32 % in relation to the THOD of sodium benzoate and fosthiazate).

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Reference:	**Anonymous: HYDROLYSIS IN BUFFER SOLUTIONS AT PH 5, 7 AND 9.**
Report No:	1145-89-16101-1/I-221, DP 29431
Guidelines:	US EPA 161-1
GLP	No
Previous evaluation:	In DAR December 1997

Anonymous (1989) conducted a hydrolysis study where both [14C-thiazolidine]-fosthiazate and [14C-sec-butyl]-fosthiazate was added to sterile buffer solutions at pH 5, 7 and 9 (4 mg/l) at 25°C. DT50 values for fosthiazate were 177, 104 and 3.2 days at pH 5, 7 and 9 respectively. At pH 5 and 7 the major metabolite was DETO (10 % respectively 14 % at day 30). At pH 9 DBTO reached 45 % and TZO reached 40 % at day 14 from the thiazolidine-labelled compound and DTTO reached 44 % and volatile ^{14}C reached 30 % from the sec-butyl labelled compound.

In order to provide a GLP compliant study (OECD 111), a new study was provided.

Anonymous (2014)

Reference:	**Anonymous: Hydrolysis in water**
Document No:	JSM0298, Huntingdon Life Science Ltd.
Guidelines:	OECD 111
GLP	Yes
Materials and Methods:	
Test substance:	IKI-1145 (Fosthiazate)
Chemical name:	*(RS)*-*S*-*sec*-butyl *O*-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphonothionate (IUPAC)
CAS number:	98886-44-3
Molecular formula:	$C_9H_{18}NO_3PS_2$
Molecular weight:	283.34 g/mol
Appearance:	Liquid
Water solubility (25°C)	pH 5: 9.88 g/L pH 7: 9.00 g/L pH 9: 9.46 g/L

Results

DT_{50} values for the hydrolysis of fosthiazate in pH 4 buffer were 312, 90 and 10 days at 20, 30 and 50ºC, respectively. In pH 7 buffer the DT_{50} values were 255, 68 and 7 days at 20, 30 and 50ºC, respectively. In pH 9 buffer the DT_{50} values (based on the R-label values only) were 35, 9 and 5 days at 10, 20 and 25ºC, respectively.

Table 35: Hydrolysis DT_{50} values at different temperatures at pH values

Buffer 4	20°C: 312 d	30°C: 90 d	50°C: 10 d
Buffer 7	20°C: 255 d	30°C: 68 d	50°C: 7 d
Buffer 9	10°C: 35 d	20°C: 9 d	25°C: 5 d

Fosthiazate was degraded to DETO, TZO, DTTO and DBTO and a volatile degradate, sec-butyl mercaptan as shown in the figure below.

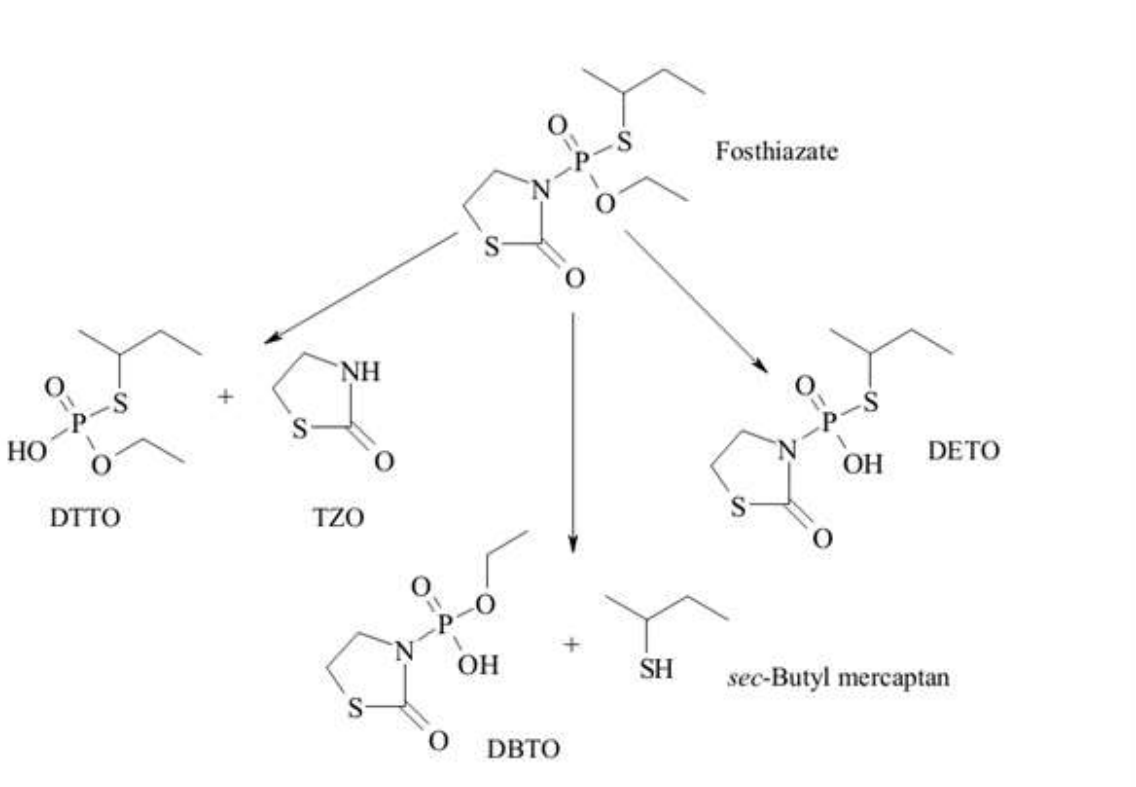


Figure 2: Proposed pathway for the hydrolysis of fosthiazate

At pH 4, the major hydrolysis product was DETO accounting for a maximum of 5.5% AR (20°C), 20.4% AR (30°C) and 85.5% AR (50°C). TZO and DTTO were also detected, accounting for no greater than 4.0% and 1.2% AR, respectively.

At pH 7, the major hydrolysis product was DETO accounting for a maximum of 65.0% AR (50°C) after 20 days incubation before declining slightly (in the B-label only) to a maximum of 62.1% AR after 30 days. Other significant hydrolysis products were DTTO which accounted for a maximum of 34.8% AR after 30 days at 50°C; TZO which accounted for a maximum of 20.0% AR on day 24 at 50°C and DBTO which accounted for 10.0 % AR on day 30 at 50°C.

Table 36: Maximal amounts of metabolites in aqueous solutions at pH 7

Metabolite	20°C	30°C	50°C
TZO	2.8%	7.5%	20%
DETO	5.2%	14.7%	65.0%
DBTO	0.9%	1.9%	10.0%
DTTO	3.1%	12.6%	34.8%

At pH 9, the major hydrolysis products were TZO (maximum of 47.6% AR at 25°C), DTTO (44.2% AR) and DBTO (33.8% AR). DETO was a minor hydrolysis product at this pH and accounted for no greater than 3.8% AR. One volatile degradate (predominantly associated with the toluene traps), and considered to be associated with sec-butyl mercaptan, was detected at each temperature and accounted for a maximum of 21.8% AR (25°C).

Conclusion

The study is acceptable. Both hydrolysis studies, the non GLP study and the new one according to OECD No 111, show similar results: relative hydrolytic stability of fosthiazate was in the order pH4 > pH 7 > pH 9.

At pH 4 and 5, respectively, in both studies the main metabolite was DETO, and at pH 9 the main metabolites were DTTO, DBTO and TZO. At pH 7 the metabolites TZO, DETO, DBTO, and DTTO with amounts > 10 % were detected at 30°C and 50°C. The detection of the volatile sec-butyl mercaptan could be an explanation of losses of radioactivity in aquatic systems of other studies.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant for C & L.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

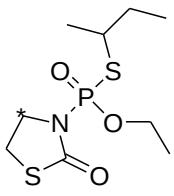
11.1.4.3.1 Aerobic mineralization in surface water

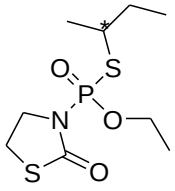
Reference:	**Anonymous (2015), FINAL REPORT - AEROBIC MINERALISATION OF FOSTHIAZATE IN SURFACE WATER**
Report No :	714-003
Laboratory report No.	503279
Testing facility:	WIL Research Europe B.V., 's-Hertogenbosch, The Netherlands
Published:	No
Test guideline used:	OECD No. 309
Deviations:	None
GLP:	Yes; certified by Staatstoezicht op de Volksgezondheid - Health Care Inspectorate, Ministry of Health, Welfare and Sport, Den Haag

Materials and Methods:

Name:	**Fosthiazate**
Chemical name:	*(RS)-S-sec*-butyl *O*-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphonothionate (IUPAC)
Molecular formula:	$C_9H_{18}NO_3PS_2$
Molecular weight:	283.35 (unlabelled Fosthiazate)

Radiolabelled test substances:

Name:	**[^{14}C-R]-Fosthiazate (Substance 204978/A)**
Label position:	 * Denotes ^{14}C-label

Name:	**[^{14}C-B]-Fosthiazate (Substance 205175/B)**
Label position:	 * Denotes ^{14}C-label

Reference substances of possible metabolites:

The reference substances in the tables below are possible metabolites formed in this study and were compared based on retention time and m/z values.

Name:	**DBTO-Li**
Chemical name:	Lithium *O*-ethyl hydrogen 2-oxo-1,3-thiazolidin-3-ylphosphonothioate
Batch:	120417
Molecular formula	$C_5H_9LiNO_4PS$
Molecular weight	217.11

Name:	**TZO**
Chemical name:	1,3-thiazolidin-2-one
Batch:	110609
Molecular formula	C_3H_5NOS
Molecular weight	103.14

Name:	**DTTO**
Chemical name:	*(RS)-S-sec*-butyl *O*-ethyl hydrogen phosphorothioate
Batch:	20121015
Molecular formula	$C_6H_{15}O_3PS$
Molecular weight	198.22

Name:	**DETO-Li**
Chemical name:	Lithium *(RS)-S-sec*-butyl hydrogen 2-oxo-1,3-thiazolidin-3-ylphosphonothioate
Batch:	120516
Molecular formula	$C_7H_{13}LiNO_3PS_2$
Molecular weight	261.23

Name:	**2-Butanethiol**
Chemical name:	2-Buthanethiol
Batch:	01396EJV
Molecular formula	$C_4H_{10}S$
Molecular weight	90.19

Name:	**MBSo**
Chemical name:	*Sec*-butyl methylsulfone
Batch:	20130109
Molecular formula	$C_5H_{12}O_2S$ (determined by WIL Research Europe B.V.)
Molecular weight	136.2

Name:	**BSA**
Chemical name:	2-butanesulfonic acid
Batch:	20120904

Table 37: Characteristics of surface water

Parameter	Value
pH (at sampling)	7.3
Temperature (at sampling)	13.4°C
Oxygen (at sampling)	6.3 mg/L (59%)
Total Organic Carbon	10.9 mg/L
Dissolved Organic Carbon	0.6 mg/L
Total Nitrogen as N	1.7 mg/L
Nitrate	< 2.2 mg/L
Nitrite	< 1.6 mg/L
Ammonium	0.6 mg/L
Total hardness	142 mg/L as CaCO3
Phosphate/orthophosphates	0.4 mg/L
Total Phosphorus as P	<0.1 mg/L

Results:

Table 38: Distribution of radioactivity in test systems after application of [^{14}C-R]-Fosthiazate (10, 100 µl), [^{14}C-B]-Fosthiazate (10, 100 µl) as well as for sterile and reference control

Time (days)	PUF	EGEE	Total NaOH	Total water	Total water stripped of CO_2	Mass balance
	[% AR]					
	[^{14}C-R]-Fosthiazate, Application of 10 µg/L					
0	n.a.	n.a.	n.a.	84.9	84.5	84.9
1	n.a.	0.0	0.0	98.5	98.8	98.5
3	n.a.	0.0	0.1	99.0	97.8	99.0
7	n.a.	0.1	0.2	97.1	98.6	97.4
14	n.a.	0.0	2.3	98.1	92.4	100.5
31	n.a.	0.0	7.7	89.6	67.9	97.3
45	n.a.	0.0	4.4	74.4	65.0	78.8
56	0.0	0.0	20.9	71.9	56.7	92.8
56[1)]	0.0	0.0	11.7	76.3	64.9	88.0
	[^{14}C-R]-Fosthiazate, Application of 100 µg/L					
0	n.a.	n.a.	n.a.	103.5	100.5	103.5
1	n.a.	0.1	0.0	104.5	103.9	104.6
3	n.a.	0.1	0.1	102.1	101.3	102.2
7	n.a.	0.0	0.3	101.4	101.4	101.8
14	n.a.	0.0	2.0	100.9	93.8	102.9
31	n.a.	0.0	5.0	96.9	81.8	101.9
45	n.a.	0.0	1.4	93.4	78.3	94.8

Time (days)	PUF	EGEE	Total NaOH	Total water	Total water stripped of CO_2	Mass balance
56	0.0	0.0	4.4	91.1	72.0	95.5
56[1)]	0.0	0.0	3.9	90.5	84.9	94.5
	[14C-B]-Fosthiazate, Application of 10 µg/L					
0	n.a.	n.a.	n.a.	98.3	94.9	98.3
1	n.a.	1.1	0.2	96.0	96.0	97.3
3	n.a.	2.0	0.9	93.0	95.3	95.9
7	n.a.	3.6	1.9	88.2	87.8	93.7
14	n.a.	6.8	2.8	82.0	80.8	91.6
31	n.a.	8.1	4.8	76.0	66.3	88.9
45	n.a.	8.3	5.3	69.3	65.8	83.0
56	0.5	8.6	6.3	65.4	61.3	80.7
	[^{14}C-B]-Fosthiazate, Application of 100 µg/L					
0	n.a.	n.a.	n.a.	109.0	107.4	109.0
1	n.a.	0.7	0.1	107.8	106.9	108.5
3	n.a.	1.8	0.7	103.6	102.1	106.1
7	n.a.	2.1	2.0	97.6	96.8	101.7
14	n.a.	2.4	4.6	93.2	95.9	100.2
31	n.a.	5.1	9.3	71.3	63.6	85.7
45	n.a.	5.5	7.1	64.7	59.7	77.3
56	1.2	5.6	12.3	62.3	54.6	81.3
	Sterile control, Application of 10 /100 µg/L [^{14}C-R]-Fosthiazate					
0	n.a./n.a.	n.a./n.a.	n.a./n.a.	74.5/101.4	73.2/98.7	74.5/101.4
1	n.a./n.a.	0.0/0.0	0.0/0.0	95.0/102.8	95.5/102.8	95.0/102.8
3	n.a./n.a.	0.0/0.1	0.0/0.0	101.2/101.8	94.4/103.0	101.3/101.8
7	n.a./n.a.	0.1/0.1	0.0/0.0	98.2/102.8	94.8/99.5	98.3/103.0
14	n.a./n.a.	0.0/0.0	0.8/1.1	96.7/101.5	84.4/93.1	97.6/102.6
31	n.a./n.a.	0.0/0.0	2.1/6.0	93.1/88.5	83.5/82.0	95.3/94.5
45	n.a./n.a.	0.0/0.0	2.0/2.6	88.8/82.1	78.4/76.0	90.7/84.7
56	0.1/0.0	0.0/0.0	8.2/7.5	87.5/81.2	78.7/64.2	95.7/88.8
	Reference control, Application of Benzoic Acid					
0	n.a.	n.a.	n.a.	94.0	-	94.0
1	n.a.	0.0	3.4	69.8	-	73.2
3	n.a.	0.0	7.1	45.8	-	52.9
7	n.a.	0.0	19.0	23.6	-	42.7
14	n.a.	0.0	88.8	9.2	-	98.0
31	n.a.	0.0	94.8	4.5	-	99.4
45	n.a.	0.0	89.0	5.5	-	94.5

Time (days)	PUF	EGEE	Total NaOH	Total water	Total water stripped of CO_2	Mass balance
56	0.0	0.0	91.3	4.2	-	95.4
n.a. = not applicable 1) flasks prepared for establishment of mass balance						

In the surface water supplemented with 10 µg/L [^{14}C-R]-fosthiazate, the amount of radioactivity decreased to 71.9 % AR (56.7 % after stripping of CO_2) and in surface water supplemented with 100 µg/L [^{14}C-R]-fosthiazate it decreased to 91.1 % AR (72.0 % after stripping of CO_2) after 56 days of incubation.

After addition of [^{14}C-B]-fosthiazate, radioactivity decreased to 65.4 and 62.3 % AR after application of 10 and 100 µl, respectively (61.3 and 54.6 % AR after stripping of CO_2).

In the sterile controls approximately 84 % of applied (72 % after stripping of CO_2) was recovered at the end of the incubation period (56 days).

Identification of metabolites:

Table 39: Parent and metabolites in test systems after application of [^{14}C-R]-Fosthiazate

Time (days)	[% AR]		
	Parent	TZO	DBTO-Li
Application of 10 µl/L			
1	98.5	0.0	0.0
3	82.7	5.7	10.5
7	69.1	12.3	15.7
14	56.4	18.4	23.3
31	25.8	36.1	27.7
45	11.5	0.0	55.3
56	4.7	0.0	67.2
Application of 100 µl/L			
1	96.7	3.7	4.1
3	82.7	6.7	8.1
7	72.8	11.2	17.3
14	56.1	16.7	25.5
31	23.7	26.7	44.7
45	13.2	0.0	77.5
56	6.6	0.0	80.3

Table 40: Parent and metabolites in test systems after application of [^{14}C-B]-Fosthiazate

Time (days)	[% AR]				
	Parent	MB-1	MB-2 (BSA)	MB-4	MB-5
	Application of 10 µg/L				
1	96.0	0.0	0.0	0.0	0.0
3	80.8	0.0	11.1	0.0	0.0

7	72.5	0.0	15.7	0.0	0.0
14	53.6	1.5	0.0	0.0	0.0
31	25.1	3.8	0.0	47.1	0.0
45	8.1	0.0	0.0	0.0	61.2
56	0.0	0.0	0.0	0.0	66.4
	Application of 100 µg/L				
	Parent	**MB-1**	**MB-2 (BSA)**	**MB-3**	**MB-4**
1	102.3	1.5	0.0	0.0	0.0
3	92.1	1.5	4.4	0.0	0.0
7	78.3	2.1	16.0	0.0	0.0
14	61.4	4.3	0.0	0.0	26.2
31	29.3	19.7	0.0	0.0	15.5
45	12.7	27.8	0.0	12.2	4.9
56	5.2	1.2	29.9	19.8	1.2

Several metabolites were formed in the surface water in the course of the study. Concentrations of (R)-fosthiazate and detected metabolites are shown in Table 39 while amounts of (B)-Fosthiazate and formed metabolites are presented in Table 40.

In the test systems with [^{14}C-R]-fosthiazate, the metabolites MR-2 and MR-3 were formed. After 31 days of incubation, MR-2 reached a maximum of 36.1 % and 26.7 % AR in the low and high concentrations of (R)-fosthiazate test systems, respectively. The metabolite MR-3 reached a maximum of 67.2 % and 80.3 % AR at the low and high concentration, respectively, at the end of the incubation period (56 days). Metabolites MR-2 and MR-3 were assigned as TZO and DBTO-Li, respectively.

Metabolite MB-1 reached a maximum of 28% after 45 days of incubation at the high concentration (B)-fosthiazate test systems and degraded to 1% after 56 days of incubation. Metabolite MB-1 was not significant in the low concentration test systems. Metabolite MB-2 reached a maximum of 30% after 56 days of incubation in the high concentration (B)-fosthiazate systems and a maximum of 16% after 7 days of incubation in the low concentration (B)-fosthiazate test systems. Metabolite MB-3 reached a maximum of 20% at the end of the incubation period for the high concentration (B)-fosthiazate and was not observed in the low concentration systems. Metabolites MB-4 reached a maximum of 26% after 14 days of incubation in the high concentration (B)-fosthiazate systems and degraded to 5% of applied after 45 days of incubation. Metabolite MB-4 was only observed once at 47% of applied after 31 days of incubation in the low concentration (B)-fosthiazate test systems. Metabolite MB-5 reached a maximum of 66% of applied radioactivity after 56 days in the low (B)-fosthiazate concentration and was not observed in the high (B)-fosthiazate test concentration systems.

Metabolite MB-2 was assigned as BSA (AS1462). Metabolite MB-1, MB-3, MB-4 and MB-5 could not be identified. The metabolites were not stable in the freezer and do not have UV absorbance. It was therefore decided to analyse the metabolites fresh by re-incubation of the study. This was performed twice and the chromatograms of all three incubations did not (always) match. Due to these instabilities it was not possible to conduct additional identification work. Since 67-80% of the metabolites deriving from (R)-fosthiazate could be identified as DBTO-Li, the metabolites deriving from (B)-fosthiazate were small metabolites formed from 2-butanthiol.

Volatilised radioactivity:

For [^{14}C -R]-fosthiazate, 21 % (10 µg/L) and 4 % (100 µg/L) AR was detected as $^{14}CO_2$. For [^{14}C-B]-fosthiazate, 6 % (10 µg/L) and 12 % (100 µg/L) AR was recovered in NaOH traps. Approximately 27 % of the high concentration flask (100 µg/L) was confirmed to be CO_2 after precipitation with barium hydroxide which means a total of 3% CO_2.

For [^{14}C-R]-fosthiazate, no radioactivity (0.0 %) was detected in the PUF plugs and EGEE traps. For [^{14}C-B]-

fosthiazate negligible amounts of radioactivity (<2 %) were found in the PUF plugs. In the EGEE traps 8.6 % (10 µg/L) and 5.6 % AR (100 µg/L) were recovered.

Reference control:

Radioactivity in surface water decreased to <10 % AR within 14 days of incubation. Significant amounts of radioactivity (91.3 % AR) were detected at the study end (56 days) in NaOH traps and confirmed to be $^{14}CO_2$ after precipitation with Barium hydroxide.

These results indicate viable conditions in the surface water.

Table 41: DT_{50} and DT_{90} calculations

Test substance	Initial concentration [µg/L]	Kinetic model	DT_{50} [days]	DT_{90} [days]	Chi^2
[^{14}C-R]-Fosthiazate	10	SFO	15.2	50.5	4.9
	100	SFO	15.5	51.6	3.4
	10	FOMC	13.8	46.0	6.2
	100	FOMC	14.4	47.9	4.5
[^{14}C-B]-Fosthiazate	10	SFO	14.7	48.7	6.4
	100	SFO	15.9	52.7	3.5
	10	FOMC	13.0	43.2	7.7
	100	FOMC	13.4	44.5	6.8

DT_{50} and DT_{90} values for fosthiazate were calculated based on HPLC results. The SFO and FOMC model were fitted to the data. As the Chi^2 values of the SFO kinetics are lower, SFO was chosen. SFO DT_{50} values were in the range from 14.7 to 15.9 days. Calculated SFO DT_{90} values ranged from 48.7 to 52.7 days.

Conclusion:

The mass balance shows deficiencies at all used labels and concentrations of the test substance, but according to the OECD 309 guideline the radiolabelled mass balance should range from 90% to 110%, whereas these ranges should be interpreted as targets and should not be used as criteria for acceptance of the test. The reference substance is degraded within two weeks to <10 % AR.

Metabolite TZO reached a maximum of 36.1 % AR after 31 days at the low concentration. Metabolite DBTO-Li reached a maximum of 80.3 % AR after 56 days at the high concentration. Metabolite BSA reached a maximum of 20.9 % AR after 56 days at the high concentration.

Metabolite MB-1, MB-3, MB-4 and MB-5 were instable and could not be identified. However, the explanation in the study that the unidentified metabolites deriving from (B)-fosthiazate were small metabolites formed from 2-butanthiol, is plausible, since according to pathway given in Figure 2 the formation of DBTO is linked with the formation of Butyl-mercaptan (=$C_4H_{10}S$), and over 80 % of the metabolites deriving from (R)-fosthiazate in the aerobic mineralisation study could be identified as DBTO.

Mainly primary degradation of fosthiazate to the metabolites TZO, DBTO-Li and BSA was observed and only low mineralization with 3 to 21% AR. Therefore based on the study results fosthiazate is considered to be not rapid biodegradable, although DT50 values are < 16 days.

11.1.4.3.2 Water/sediment studies:

There are valid data from four water/sediment systems available.

From the study Anonymous (1996) of the fosthiazate DAR (December 1997) one system was considered as valid and re-assessed according to the FOCUS degradation kinetics guidance (2006, 2011) by Anonymous

(2014). Two water/sediment studies Anonymous (1998) and Anonymous (2013) each with two systems performed with radiolabelled fosthiazate were provided in support of the assessment. In one water/sediment system of the study by Anonymous (2013), the mass balance was below 90 % at more than one sampling day, therefore only one system from this study was accepted.

Mineralisation in the four considered systems was limited to moderate and ranges from 10.4 % do 36.2 % AR after 100 days.

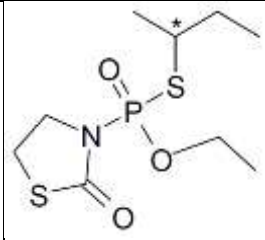
Bound residues were moderate to high ranging from 18.8 % do 36.6 % AR after 100 days, the maximum amount of bound residues during the time of the studies was 36.6 % after 100 days.

DT_{50} values for modelling purposes in the overall water/sediment systems ranged from 30.1 to 72.1 days. Best fit DT_{50}-values for persistence purposes ranged from 43.7 to 79.8 days. Primary degradation in the whole system was slowly.

Main metabolites were DTTO with ≤ 16.2 % and TZO with a maximum amount 32.9 % AR in the water phase and 2.8 % AR in the sediment phase.

Reference:	**Anonymous (1996), [^{14}C-B]IKI-1145: DEGRADATION AND FATE IN WATER/SEDIMENT SYSTEM**
Report No:	6214-94-0186-EF-001, DP 29440
Guidelines:	BBA Part IV, US EPA 162-4
GLP	Yes
Previous evaluation:	In DAR December 1997

Materials and Methods:

Test material:	**[^{14}C]IKI-1145 (= (*RS*)-*S*-*sec*-butyl O-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphorothioate = Fosthiazate)**
Structure:	
Position of radiolabel (*):	B-label
Origin of water /sediment system:	Grand River, Elm Street, Fairpoort, (Lake County, Ohio)
Sediment Class	Loam
% sand % silt % clay	42.0 40.0 18.0
Organic carbon [%]	5.06
pH value	7.0
Cation exchange capacity [meq/ 100 g]	6.59
Water	
Total organic carbon [mg/L]	8.0
Hardness (mg $CaCO_3$/L)	206
pH value	7.41

Results:

The overall recoveries of radioactivity from the Grand River system with butyl-label ranged from 91.3 % to 101.8 % AR.

Table 42: Mass balance for the Grand River water/sediment system treated with [^{14}C-B]-IKI-1145

Sample day	Water phase	Sediment extracts	Bound residues		$^{14}CO_2$	VOC	Total recovery
		[% AR]					
0	96.8	3.0	0.2		-	-	100.1
0.25	95.1	4.7	0.6		-	-	100.4
1	96.7	4.8	0.4		-		101.8
3	96.5	4.7	0.5		-	-	101.7
7	88.7	8.9	1.4		0.1	2.6	101.7
14	77.1	13.1	4.1		0.2	4.1	98.5
30	62.1	14.6	7.0		0.7	6.9	91.3
49	54.1	17.6	10.9		2.0	7.7	92.2
60	49.4	19.2	12.1		3.3	7.7	91.7
100	39.1	17.6	20.2		10.4	7.8	95.0
140	25.7	14.7	23.0		22.3	8.4	94.0

na: not analysed
VOC: volatile organic compounds

Only minor metabolites MBSo, DETO, DTTO and BSA were detected in the Grand River System in concentration below 10 % AR.

Table 43: Percent Distribution of the Applied ^{14}C in the Surface Water of the Grand River System Treated with [^{14}C-B]-IKI-1145

Sample day	MBSo	BSA	DETO/DTTO	IKI-1145	% Applied in Surface Water
	[% AR]				
0	BD	BD	0.3	94.9	96.8
0.25	0.1	0.1	0.6	92.7	95.1
1	0.1	0.2	1.4	93.6	96.7
3	BD	0.3	2.7	92.0	96.5
7	0.2	0.5	2.5	83.4	88.7
14	0.4	0.7	2.4	71.3	77.1
30	0.8	1.4	2.7	54.1	62.1
49	1.6	2.5	3.9	43.0	54.1
60	1.5	2.9	2.4	39.2	49.4
100	3.9	4.5	3.0	25.5	39.1
140	3.1	4.2	2.3	13.5	25.7

Table 44: Percent Distribution of the Applied ^{14}C in the Sediment Extract of the Grand River System Treated with [^{14}C-B]-IKI-1145

Sample Day	MBSo	BSA	DETO/DTTO	IKI-1145	% Applied in Soil Extract
	[% AR]				
0	BD	BD	BD	2.9	3.0
0.25	BD	BD	BD	4.6	4.7
1	BD	BD	BD	4.6	4.8
2	BD	BD	0.1	4.4	4.7
7	0.1	BD	0.1	8.5	8.9
14	0.1	0.1	0.2	12.5	13.1
30	0.2	0.1	0.4	13.4	14.6
49	0.3	0.1	0.1	13.5	17.6
60	2.2%	0.2	0.6	15.4	18.2
100	1.0	0.6	0.5	14.5	17.6
140	1.5	0.6	0.6	11.2	14.7

BD = Below Detection
ND = Not Determined

The DT_{50} values were recalculated according to FOCUS degradation kinetics guidance (2006, 2011) by Anonymous (2014). In the US river system the recalculated DT_{50} values for fosthiazate accounted for 46.0, 214.0 and 70.2 days in the water, sediment and total system, respectively.

Table 45: DT50 and DT90 values of IKI-1145 calculated for water/sediment systems treated with [^{14}C-B]-IKI-1145

	Whole system	Water phase	Sediment phase
DT_{50}	70.2 d	46 d	214 d
DT_{90}	233 d	153 d	710 d
Chi^2 err. [%]	2.3	3.7	3.7
t-test Prob > t Parameter k:	4.5-010	2.1e-004	0.023

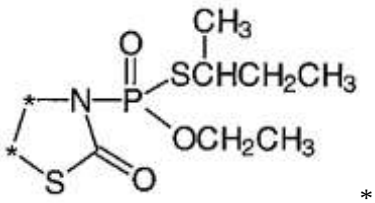
Conclusion:

The study is in regard to the river system acceptable. No major metabolites were detected. The maximal amount of the metabolite BSA is 5.1 % AR in the whole system at one sampling point. DT50 whole system is longer than 16 day, therefore, fosthiazate is not rapid biodegradable.

11.1.4.3.2.1 ANONYMOUS (1998)

Reference:	Anonymous (1998), [14C-R]IKI-1145: DEGRADATION AND FATE IN WATER/SEDIMENT SYSTEM
Report No.:	7247-97-0104-EF-001
Testing facility:	Ricerca, LLC, Painesville, Ohio, USA
Published:	No
Test guideline used:	SETAC-Europe Guidelines (March 1995), BBA Guideline Part IV, US EPA 162-4
Deviations:	None
GLP:	Yes; laboratories in the USA are not certified by any governmental agency, but are subject to official inspections

Materials and Methods:

Test material:	**[^{14}C]IKI-1145 (= (RS)-S-sec-butyl O-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphorothioate = Fosthiazate)**
	 * Denotes ^{14}C-label
Description:	Not stated
Lot/Batch #:	21072-89-06
Specific radioactivity:	51 mCi/mmol, 1.89 GBq/mmole
Radiochemical purity:	100 %
CAS #:	Not stated
Stability of test compound:	Expiry date: not stated Storage at < -5°C in the dark Relatively stable under experimental conditions for 98 days (purity of 94.5 % when stored in the environmental chamber)

The degradation of [^{14}C]IKI-1145 in aquatic systems under aerobic conditions was studied in two water/sediment systems. The water/sediment systems were collected from Lake and Medina Counties in Ohio.

Table 46: Water/Sediment systems used to investigate the degradation of [^{14}C]-IKI 1145

	System Homestead Road Pond	**System Grand River**
Origin	Homestead Road Pond in Medina County, Wadsworth Twp., Ohio	Grand River in Lake County, Fairport, Ohio
	Sediment	
Sediment texture	Silt loam	Sandy loam
% sand (63 µm-2 mm) % silt (2 –63 µm) % clay (< 2 µm)	23.2 56.4 20.4	69.2 22.4 8.4
Organic matter [%]	6.22 (study initiation)	1.35 (study initiation)
pH value (water)	4.9 (study initiation)	7.3 (study initiation)
Cation exchange capacity [meq/ 100 g]	11.19	7.69
Microbial biomass [CFUs/g dry soil]	1.8 x 10^6 (bacterial; beginning of study) 8.7 x 10^4 (fungal; beginning of study) 2.2 x 10^6 (bacterial; end of study) 6.9 x 10^4 (fungal; end of study)	1.1 x 10^7 (bacterial; beginning of study) 2.5 x 10^3 (fungal; beginning of study) 5.1 x 10^6 (bacterial; end of study) 6.0 x 10^3 (fungal; end of study)
	Water	
Total organic carbon [mg/L]	16.3 (study initiation)	5.8 (study initiation)
Hardness (mg $CaCO_3$/L)	98 (study initiation)	1510 (study initiation)
pH value	6.52 (study initiation)	7.95 (study initiation)

Results:

The total mean recoveries for the Homestead Road Pond water/sediment system were between 80.7 and 111.4 %. The mass balance was less than 90 % on day 0.25 and day 100.

The overall recoveries of radioactivity from the Grand River system ranged from 93.3 to 100.4 % AR

Table 47: Mass balance for the Homestead Road Pond water/sediment system treated with [^{14}C-R]-Fosthiazate

Sample day	Water phase	Sediment extracts	Bound residues	$^{14}CO_2$	VOC	Total recovery
	[% AR]					
0	97.2	2.7	0.1	NA	NA	100.0
0.25	85.1	3.5	0.1	NA	NA	88.7
1	89.9	6.4	0.3	NA	NA	96.6
2	93.8	8.0	3.7	0.1	< 0.1	105.5
7	71.6	18.2	3.0	0.3	< 0.1	93.2
14	71.9	20.4	6.4	2.3	< 0.1	100.9
30	68.2	16.7	16.0	10.6	< 0.1	111.4
60	29.0	10.9	29.4	21.2	< 0.1	90.5
100	15.8	9.1	27.7	28.2	< 0.1	80.7
na: not analysed VOC: volatile organic compounds						

Table 48: Mass balance for the Grand River water/sediment system treated with [^{14}C-R]-Fosthiazate

Sample day	Water phase	Sediment extracts	Bound residues	$^{14}CO_2$	VOC	Total recovery
	[% AR]					
0	96.1	3.9	0.0	NA	NA	100.0
0.25	93.8	5.3	0.1	NA	NA	99.1
1	93.8	6.5	0.1	NA	NA	100.4
2	84.0	11.6	0.5	<0.1	<0.1	96.2
7	81.4	14.3	1.1	0.2	<0.1	97.0
15	77.4	14.6	4.0	1.1	<0.1	97.0
30	65.0	12.5	9.9	11.9	<0.1	99.3
60	22.5	10.7	28.0	32.8	<0.1	94.0
100	12.1	8.3	36.6	36.2	<0.1	93.3
na: not analysed VOC: volatile organic compounds						

Water and sediment extract samples were analysed by HPLC to detect IKI-1145 and possible degradation products.

The average amount of IKI-1145 in the water steadily decreased from 97.2 and 96.1 % AR on day 0 to an average of 14.3 and 9.7 % AR by day 100 in the pH 6.3 and pH 7.7 systems, respectively.

The average amount of radioactivity as IKI-1145 in the extractable sediment fraction increased from 2.7 and 3.9 % AR (day 0) to an average maximum of 19.4 % AR (day 14) and 12.4 % AR (day 7) in the pH 6.3 and pH 7.7 systems, respectively. IKI-1145 decreased afterwards to a level of 8.7 and 8.1 % AR at day 100 in the

pH 6.3 and pH 7.7 systems, respectively.

The major metabolite TZO was detected in a maximum amount 32.9 % AR in the water phase and 2.8 % AR in the sediment phase at day 30 in the pH 7.7 system.

Table 49: Distribution of radioactive residues in the Homestead Road Pond water/sediment system treated with [^{14}C-R]-Fosthiazate

Sampling Day	TZO		DETO		IKI-1145		Total	
	Water phase	Sediment extract	Water phase	Sediment extract	Water phase	Sediment extract	Water phase	Sediment extract
	[% AR]		[% AR]		[% AR]		[% AR]	
0	ND	ND	ND	ND	97.2	2.7	97.2	2.7
0.25	ND	ND	ND	ND	85.1	3.5	85.1	3.5
1	ND	ND	ND	ND	89.9	6.4	89.9	6.4
2	ND	ND	ND	ND	93.8	8.0	93.8	8.0
7	4.2	0.4	1.6	ND	65.9	1.8	71.6	18.2
14	8.1	0.8	1.7	0.2	61.2	19.4	71.9	20.4
30	15.0	1.7	1.9	0.2	51.4	14.8	68.2	16.7
60	7.4	1.3	0.4	ND	20.0	9.6	29.0	10.9
100	1.5	0.4	ND	ND	14.3	8.7	15.8	9.1

Table 50: Distribution of radioactive residues in the Grand River water/sediment system treated with [^{14}C-R]-Fosthiazate

Sampling Day	TZO		DETO		IKI-1145		Total	
	Water phase	Sediment extract	Water phase	Sediment extract	Water phase	Sediment extract	Water phase	Sediment extract
	[% AR]		[% AR]		[% AR]		[% AR]	
0	ND	ND	ND	ND	96.1	3.9	96.1	3.9
0.25	ND	ND	ND	ND	93.8	5.3	93.8	5.3
1	1.8	0.2	0.8	ND	90.0	6.3	93.8	6.5
2	6.4	0.6	ND	ND	77.7	11.0	84.0	11.6
7	21.7	1.9	ND	ND	59.7	12.4	81.4	14.3
15	28.9	2.7	ND	ND	48.5	11.9	77.4	14.6
30	32.9	2.8	ND	ND	32.3	9.8	65.0	12.5
60	0.6	ND	0.4	0.1	21.2	10.6	22.5	10.
100	1.2	ND	0.8	0.2	9.7	8.1	12.1	8.3

The data of this study have been re-assessed concerning the DT_{50} values according to the FOCUS degradation kinetics guidance (2006, 2011) by Anonymous (2014). For fosthiazate DT_{50} values for water, sediment and total system were derived accounting for 30.4, 62.0 and 43.7 days in the pond system and 19.8, 172.0 and 79.8 days in the river system, respectively. In addition total system DT_{50} values were derived for TZO with 11.4 and 19.7 days in the pond and river system, respectively.

Table 51: DT_{50} and DT_{90} values of IKI-1145 calculated for water/sediment systems treated with [^{14}C-R]-IKI-1145

	Whole system	Water phase	Sediment phase
Homestead Road Pond, SFO			
DT_{50}	43.7	30.4	62
DT_{90}	145	101	206
Chi^2 error	5.8	7.5	7.9
t-test	2.7e-005	8.6e-005	0.0274
Grand River, SFO			
DT_{50}	30.1	19.8	172.4
DT_{90}	100	65.9	572.9
Chi^2 error	7.4	9.0	5.2
t-test	8.0e-005	2.1e-004	0.0233
Grand River, FOMC			
DT_{50}	20.9 (DT90/ 3.32=79.8)		
DT_{90}	264.7		
Chi^2 error	2.1		
t-test	9.2e-005		

In case of the Grand River system the FOMC fit is better than the SFO fit for the whole system. But as the DT_{90} is not reached during the study, FOMC is not appropriate for modelling. For persistence purposes the from the FOMC DT_{90}-value recalculated DT_{50} is useful.

Table 52: DT_{50} and DT_{90} values of metabolite TZO calculated for water/sediment systems treated with [^{14}C-R]-IKI-1145

	Whole system	Water phase	Sediment phase
Homestead Road Pond (parent SFO, metabolite SFO)			
DT_{50}	11.4 d		
DT_{90}	37.9 d		
Formation fraction	0.76		
Chi^2 error	36.6		
t-test	Passed		
Grand River (parent FOMC, metabolite SFO)			
DT_{50}	19.7 d		
DT_{90}	65.4 d		
Formation fraction	1		
Chi^2 error	23.4		
t-test	Passed		

Conclusions:

The study is acceptable. The mass balance deficiencies on day 0.25 and day 100 in the Homestead Road Pond water/sediment system were noticed. However, the study is accepted, as at the first time with mass balance

below 90% the intervals between the sampling days (day 0, day 0.25, day 1) were very short. The other time with insufficient recovery was at the end of the study.

Instead of day 14 in the re-evaluation by Anonymous (2014) of DT_{50} values day 15 was used as time for measured values. This can only impact the results to be longer and is therefore accepted.

The main metabolite after labelling in the thiazolidine ring is TZO. TZO reaches a maximal amount of 35.7 % in the whole system.

Fosthiazate is not rapidly degradable, as even at the end of the study mineralisation range from 28-32% and is not greater than 70 % after 28 days.

11.1.4.3.2.2 ANONYMOUS (2013)

Reference:	**Anonymous (2013), FOSTHIAZATE: AEROBIC AQUATIC METABOLISM**
Report No.:	JSM0294
Testing facility:	Huntingdon Life Sciences Ltd., Cambridgeshire, United Kingdom
Published:	No
Test guideline used:	OECD No. 308
Deviations:	None
GLP:	Yes; certified by The Department of Health of the Government of the United Kingdom

Materials and Methods:

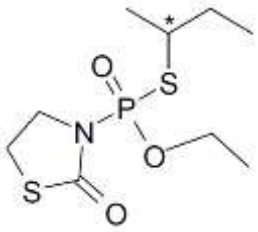
Test material:	**[^{14}C]IKI-1145 (= (*RS*)-*S*-*sec*-butyl O-ethyl 2-oxo-1,3-thiazolidin-3-ylphosphorothioate = Fosthiazate)**
Structure:	
Position of radiolabel (*):	B-label

Table 53: Water/Sediment systems used to investigate the degradation of [^{14}C-B]-Fosthiazate

	Calwich Abbey Lake	**Swiss Lake**
Origin	Derbyshire, UK	Derbyshire, UK
	Sediment	
Sediment texture (USDA)	Silt loam	Sand
% sand (63 µm-2 mm) % silt (2 –63 µm) % clay (< 2 µm)	21 65 14	96 2 2
Organic carbon [%]	5.06	0.46
pH value (in water) pH value (in 0.01 M $CaCl_2$)	7.9 7.2	6.2 5.5
Cation exchange capacity [meq/ 100 g]	16.1	2.4

	Calwich Abbey Lake	Swiss Lake
Microbial biomass [CFUs/g dry soil]	Sediment, beginning of the study: 7.60 x 10^5 (aerobic bacteria) 4.75 x 10^5 (anaerobic bacteria) 4.30 x 10^3 (Actinomycetes) 2.40 x 10^3 (Fungi) Water, beginning of the study: 4.90 x 10^3 (aerobic bacteria) 3.05 x 10^2 (anaerobic bacteria) 10 (Actinomycetes), 40 (Fungi) Sediment, Day 100: 6.75 x 10^5 (aerobic bacteria) 5.90 x 10^4 (anaerobic bacteria) 2.55 x 10^3 (Actinomycetes) 2.40 x 10^3 (Fungi) Water, Day 100: 1.56 x 10^4 (aerobic bacteria) 1.45 x 10^2 (anaerobic bacteria) <10 (Actinomycetes), 20 (Fungi)	Sediment, beginning of the study: 4.05 x 10^5 (aerobic bacteria) 1.22 x 10^4 (anaerobic bacteria) 2.25 x 10^3 (Actinomycetes) 7.95 x 10^2 (Fungi) Water, beginning of the study: 9.40 x 10^3 (aerobic bacteria) <10 (Actinomycetes), <10 (Fungi) Sediment, Day 100: 7.00 x 10^5 (aerobic bacteria) 8.05 x 10^3 (anaerobic bacteria) 2.00 x 10^3 (Actinomycetes) 9.00 x 10^2 (Fungi) Water, Day 100: 4.50 x 10^3 (aerobic bacteria) <10 (Actinomycetes), <10 (Fungi)
	Water	
Total organic carbon [mg/L]	41.8	13.1
Hardness (mg $CaCO_3$/L)	258	27.5
pH value	Not stated	Not stated

Results:

Table 54: Distribution and recovery of radioactivity in Calwich Abbey Lake aquatic sediment after application of [^{14}C-B]-Fosthiazate

Sample day	**0**		**7**		**14**		**30**		**61**		**100**	
Sample ID	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**
	[% AR]											
Water	98.1	101.3	69.0	69.0	56.4	45.8	37.0	38.9	23.8	14.5	5.2	8.4
Sediment: Extractable	6.0	3.1	20.7	21.8	25.4	23.3	26.8	27.8	20.4	16.6	8.5	13.5
Non-extractable	0.8	0.4	4.4	5.0	8.3	8.0	19.7	18.5	26.5	26.1	26.7	26.5
VOC	ns	ns	0.4	0.9	1.4	0.9	2.3	1.3	1.8	1.5	1.3	0.8
$^{14}CO_2$	ns	ns	2.1	3.7	3.8	7.5	7.1	3.2	13.5	20.6	38.6	27.5
Total volatiles	ns	ns	2.5	4.6	5.2	8.4	9.4	4.5	15.3	22.1	39.9	28.3
Total recovery	104.9	104.8	96.6	100.4	95.3	85.5	92.9	89.7	86.0	79.3	80.3	76.7

The total recoveries in the Calwich Abbey Lake aquatic sediment were lower than 90 % AR in one of the

duplicates on day 14 and day 30, and in both replicates on day 61 at day 100. Because of that, the study is in regard to the Calwich Abbey Lake system not valid.

Table 55: Distribution and recovery of radioactivity in Swiss Lake aquatic sediment aquatic sediment after application of [^{14}C-B]-Fosthiazate

Sample day	**0**		**7**		**14**		**30**		**61**		**100**	
Sample ID	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
	[% AR]											
Water	101.8	101.9	76.5	77.0	69.6	66.7	52.5	53.6	40.1	43.5	34.7	27.4
Sediment: Extractable	2.6	2.9	18.4	17.8	20.4	27.6	26.8	22.2	21.8	21.1	17.6	18.9
Non-extractable	nd	nd	1.0	4.4	3.3	2.6	8.4	8.2	14.5	15.6	17.3	20.3
VOC	ns	ns	0.1	0.1	0.3	0.7	0.8	0.2	0.6	2.5	0.3	2.0
$^{14}CO_2$	ns	ns	1.5	2.3	2.6	1.3	5.5	3.6	12.2	9.5	11.7	16.5
Total volatiles	ns	ns	1.6	2.4	2.9	2.0	6.3	3.8	12.8	12.0	12.0	18.5
Total recovery	104.4	104.8	97.5	101.6	96.2	98.8	94.0	87.8	89.2	92.2	81.6	85.1

The overall recoveries of radioactivity from the Swiss Lake aquatic system ranged from 81.6 to 104.8 % AR. The mass balance is lower than 90 % on day 100. The notifier attributed the lower recovery to volatile radioactivity not trapped by the trapping system used in this study.

In the Swiss Lake sediment, maximal 24.5 % (mean of duplicates) bound residues were detected after 30 days, after 100 days the mean value of bound residues were 18.8 % AR.

Characterisation of the bound residues was performed for one of the duplicates of day 100. The highest amount of ^{14}C in the Swiss Lake sediment was associated with the humin and humic acid fractions (4.7 and 6.8 % AR, respectively).

Table 56: Characterisation of non-extractable radioactivity in sediment samples taken 100 days after application of [^{14}C-B]-Fosthiazate

			Non-extractable ^{14}C associated with:			
Sample ID	**Aquatic sediment**	**Total non-extractable ^{14}C**	**Fulvic acid**	**Humic acid**	**Humin**	**Loss[a]**
		[% AR]				
A12	Calwich Abbey Lake	26.5	0.3	1.7	14.1	10.4
B12	Swiss Lake	20.3	0.9	6.8	4.7	7.9

In the Swiss Lake system, volatile radioactivity accounted for 12.0 /18.5 % AR (duplicates) at day 100, which was further characterised as mainly $^{14}CO_2$ (11.7 /16.5 % AR, mean 14.1 %).

Table 57: Distribution of radioactive residues in the water and sediment phase of the Calwich Abbey Lake system following addition with [^{14}C-B]-Fosthiazate

Sample day	**0**		**7**		**14**		**30**		**61**		**100**	
Sample ID	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**	**A12**
	[% AR]											
	Water phase											
Fosthiazate	91.6	98.6	45.0	46.9	37.7	28.7	22.1	24.7	11.9	7.7	2.3	4.8
Sd-A	-	-	1.3	1.2	-	0.6	1.2	1.5	3.7	2.7	0.9	1.0
DTTO	1.6	1.8	13.1	15.8	13.9	10.9	7.5	7.0	1.4	0.6	0.5	0.8
Sd-B1	-	-	-	-	-	-	-	-	-	-	-	-
DETO	-	-	1.1	1.1	2.3	1.6	2.0	1.9	2.0	1.7	0.6	1.0
Sd-D	-	-	2.2	2.8	1.9	1.7	2.2	2.0	3.4	0.2	0.1	0.1
Sd-E	-	-	-	-	-	-	-	-	0.3	0.5	0.2	-
Others (a)	4.9	1.0	6.3	1.2	0.6	2.4	2.0	1.8	1.1	1.0	0.7	0.7
	Sediment phase											
Fosthiazate	5.9	3.1	19.9	21.0	24.2	22.9	22.2	22.1	15.4	12.7	5.0	10.3
Sd-A	-	-	0.1	0.1	0.1	-	0.2	0.2	0.2	0.3	0.5	0.5
DTTO	-	-	-	0.4	0.1	-	1.4	1.2	0.4	0.3	0.3	0.5
Sd-B1	-	-	-	-	-	-	-	-	-	-	-	-
DETO	-	-	0.2	0.2	0.2	0.2	0.6	1.1	1.0	1.0	0.5	0.8
Sd-D	-	-	-	-	-	-	0.2	0.7	1.1	0.4	0.1	-
Sd-E	-	-	-	-	-	-	0.2	0.7	0.4	0.2	0.2	0.1
Others (a)	0.1	-	0.5	0.2	0.7	0.2	1.9	1.8	2.0	1.6	1.9	1.5

Fosthiazate was shown to degrade to the major metabolite DTTO, which a maximal amount of 16.2 % AR in the overall system of the Calwich Abbey Lake system.

DTTO increased rapidly until day 7 in the water phase which (13.1 /15.8 % AR in Calwich Abbey Lake system, 5.0 /9.5 % AR in Swiss Lake system) and decreased thereafter until day 100 (0.5 /0.8 % AR Calwich Abbey Lake system, 0.4 /2.1 % AR in Swiss Lake system).

DTTO accounted for a maximum amount of 1.4 /1. % AR in the sediment phase of the Calwich Abbey Lake system on day 30.

Table 58: Distribution of radioactive residues in the water and sediment phase of the Swiss Lake system following addition with [^{14}C]-Fosthiazate

Sample day	**0**		**7**		**14**		**30**		**61**		**100**	
Sample ID	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
	[% AR]											
	Water phase											
Fosthiazate	101.4	100.1	67.4	61.8	56.3	52.0	39.7	40.5	31.2	36.0	27.5	17.8
Sd-A	-	-	0.7	0.9	1.3	2.4	-	-	0.2	0.3	0.1	0.8
DTTO	-	1.5	5.0	9.5	5.1	5.2	5.4	5.1	2.6	1.8	0.4	2.1

Sample day	0		7		14		30		61		100	
Sample ID	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
Sd-B1	-	-	-	-	-	-	1.3	1.5	2.6	2.4	0.8	0.7
DETO	-	-	-	-	1.6	1.8	1.1	1.2	1.1	1.1	1.6	1.4
Sd-D	-	-	-	-	1.6	1.5	2.1	2.0	1.5	1.2	2.6	1.2
Sd-E	-	-	-	-	-	-	0.6	-	-	-	0.7	0.8
Others (a)	0.4	0.3	3.5	4.8	3.7	3.8	2.4	3.4	1.0	0.7	1.0	2.6
	Sediment phase											
Fosthiazate	2.5	2.8	16.6	16.1	18.1	25.7	24.1	20.3	18.8	18.2	14.4	15.4
Sd-A	-	-	-	-	-	-	0.2	0.2	0.1	0.2	0.4	0.5
DTTO	-	-	0.8	0.9	1.2	1.0	0.4	0.2	0.2	0.2	0.2	0.2
Sd-B1	-	-	-	-	-	-	-	-	-	-	-	-
DETO	-	-	0.3	0.4	0.5	0.7	0.5	0.2	0.4	0.3	0.4	0.3
Sd-D	-	-	0.2	0.3	0.3	-	0.5	0.4	0.6	0.6	0.6	0.5
Sd-E	-	-	-	-	-	-	0.1	-	0.2	0.2	0.3	0.5
Others (a)	0.1	0.1	0.4	0.1	0.3	0.2	1.1	0.9	1.4	1.4	1.3	1.5

As mass balance was not acceptable for the Calwich Abbey Lake system, only the Swiss Lake system was considered for deriving endpoints. The $DissT_{50}$ and $DissT_{90}$ values in the water layer were 37.6 and 125 days. DT_{50} and DT_{90} values in the sediment phase were 56.4 and 187 days. In the total systems, DT_{50} value was 72.1 days, while DT_{90} value was 129 days.

Table 59: DT_{50} and DT_{90} values of IKI-1145 calculated for Swiss Lake system water/sediment systems treated with [^{14}C-B]IKI-1145

	Whole system	Water phase	Sediment phase
Swiss Lake system			
DT_{50}	71.2	37.6	56.4
DT_{90}	239	125	187
Chi^2 error	5.3	15.1	14.7
Visual fit	Acceptable	-	Only three time points

Table 60: DT_{50} and DT_{90} values calculated for the decline of DTTO in aquatic sediment systems treated with [^{14}C-B]-Fosthiazate

	Whole system	Water phase	Sediment phase
Swiss Lake system, metabolite DDTO			
DT_{50}	36.0 d	37.7 d	10.3 d
DT_{90}	120 d	125 d	34.1 d
Chi^2 error	7.0	10.8 d	23.6 d

DT_{50} and DT_{90} values for the decline of the metabolite DTTO were taken from the Swiss Lake system. The $DissT_{50}$ value in the water phase is 37.7 day, the DT_{50} value in the sediment phase is 10.3 days and in the total system the DT_{50} value is 36.0 days. DT_{50} values in the Calwich Abbey Lake system are shorter and cannot be accepted because of the mass balance of less than 90 % AR at several samplings points.

Conclusions:

The degradation of fosthiazate was examined in two water and sediment systems under aerobic aquatic conditions. In one of the systems, the Calwich Abbey Lake system, the mass balance is lower than 90 % on more than one sampling day. Losses due to volatile radioactivity was not demonstrated with another trapping system than used in the study. The study is therefore in regard to the primary degradation of fosthiazate in the Calwich Abbey Lake system not acceptable. Nevertheless, the formation of the metabolite DTTO was in the Calwich Abbey Lake system higher than in the other system, and therefore the maximal amount of 16.2 % DTTO in the whole Calwich Abbey Lake system was used.

The mass balance of the other system, the Swiss Lake system, is only lower 90 % on day 100. RMS decided to accept this system.

Fosthiazate dissipated from the water phase with a DT_{50} value of 37.6 days. DT_{50} value in the sediment was 56.4 days. Decline in the overall system corresponded to a DT_{50} values of 72.1 days.

Fosthiazate was shown to degrade to DTTO ($\leq$ 16.2 % in the overall system). The decline of DTTO in the overall system corresponded to a DT_{50} value of 36.0 days.

As sec-butyl chain labelled fosthiazate was used, metabolites (like TZO) without this part in their structure cannot be detected in this study.

As the DT_{50} values of the whole systems are longer than 16 days, fosthiazate is not rapidly degradable.

11.1.4.3.2.3 ANONYMOUS. (2014)

Reference:	**Anonymous (2014), BEHAVIOR OF STEREOISOMER OF FOSTHIAZATE IN WATER**
Report no.:	711-001
Report No.:	I-271
Testing facility:	Ishihara Sangyo Kaisha, Ltd., Central Research Institute, Shiga-Ken, Japan
Published:	No
Test guideline used:	-
Deviations:	-
GLP:	No

The objective of this study was to investigate the distribution and degradation behaviour of fosthiazate stereoisomers in two natural European water systems from Calwich Abbey Lake and Swiss Lake (both UK) and in three buffer solutions (pH values: 4, 7 and 9). The study was conducted under constant laboratory conditions in the dark, whereas a mixture of thiazolidine-ring-labelled and non-labelled fosthiazate has been applied to give a nominal concentration of 10 mg/L in the respective testing systems.

Results:

No significant decrease of radioactivity was observed for the buffer systems pH 4 and pH 7 as well as for the Swiss lake system.

The radioactivity in the pH 9 buffer system was characterised by a faster decrease with 99.7 % AR at day 0 and 9.6 % AR after 29 days than in the Calwich Abbey Lake system. Here, the observed decline was much slower with 95.6 % AR (day 0) and 41.4 % AR (day 31). Thus, a degradation of fosthiazate can only be stated for the pH 9 buffer system and natural water from Calwich Abbey Lake. Four isomers of Fosthiazate were detected by chiral column HPLC. The results are presented in the tables below.

Table 61: The amount of Fosthiazate and each isomer in pH 4 buffer solution

DAT	Fosthiazate (%AR)	I (%AR)	II (%AR)	III (%AR)	IV (%AR)
0	94.2	22.3	24.3	23.9	23.7
7	100.8	24.3	26.0	25.1	25.4
14	95.5	23.0	24.3	23.8	24.4
29	96.9	22.6	25.3	24.4	24.7

Table 62: The amount of Fosthiazate and each isomer in pH 7 buffer solution

DAT	Fosthiazate (%AR)	I (%AR)	II (%AR)	III (%AR)	IV (%AR)
0	97.3	23.1	26.1	24.3	23.8
7	102.5	24.9	26.7	25.5	26.3
14	101.2	24.7	26.4	24.5	25.6
29	89.3	21.3	23.5	22.3	22.2

Table 63: The amount of Fosthiazate and each isomer in pH 9 buffer solution

DAT	Fosthiazate (%AR)	I (%AR)	II (%AR)	III (%AR)	IV (%AR)
0	99.7	23.9	26.3	24.6	24.9
3	71.3	17.1	18.0	18.2	18.0
7	44.9	11.2	11.4	11.3	11.0
14	26.4	7.0	6.3	6.8	6.3
29	9.6	2.5	2.3	2.7	2.1

Table 64: The amount of Fosthiazate and each isomer in natural water from Calwich Lake

DAT	Fosthiazate (%AR)	I (%AR)	II (%AR)	III (%AR)	IV (%AR)
0	95.6	22.6	24.8	23.9	24.2
3	79.3	18.6	20.6	20.1	20.0
7	72.4	17.3	18.5	18.7	18.0
14	62.2	15.7	15.4	15.5	15.6
31	41.4	10.6	10.3	10.6	9.9

Table 65: The amount of Fosthiazate and each isomer in Swiss water

DAT	Fosthiazate (%AR)	I (%AR)	II (%AR)	III (%AR)	IV (%AR)
0	94.0	22.8	24.5	23.2	23.5
3	91.7	21.2	23.8	23.2	23.4
7	94.9	22.1	24.8	24.1	23.9
14	88.8	21.1	23.0	22.3	22.3
31	90.3	21.8	22.5	22.3	23.8

On average, these isomers were equally distributed in the analysed solutions. Dissipation rates of fosthiazate and their stereoisomers were derived for the two systems, where a decline of radioactivity was observed. The DT_{50} values ranged from 8.4 to 9.2 days for the pH 9 buffer system and from 25.7 to 31.7 days for the natural water from Calwich Abbey Lake. The corresponding averaged DT_{50} values accounted for 8.8 days and 27.7 days, respectively.

Table 66: DT50 -values of Fosthiazate and each isomer

	Fosthiazate	Isomer I	Isomer II	Isomer III	Isomer IV
DT_{50} (d) Buffer solution pH 9	8.8	9.1	8.5	9.2	8.4
DT_{50} (d) Calwich Lake	27.7	31.5	25.7	27.7	25.7

Conclusion:

Four isomers of fosthiazate were identified and quantified in the natural waters and buffer systems, treated with thiazolidine-ring-labelled fosthiazate. It has been evidenced that at the individual sampling days these isomers were equally distributed in the respective waters or buffered solutions. No significant differences between the DT_{50} values of the stereoisomers were observed in the Calwich Abbey Lake. No significant differences were observed between the hydrolytic DT_{50} values among the stereoisomers at pH 9.

In conclusion, the stereoisomeric composition is of no concern for environmental fate risk assessment in water.

11.1.4.4 **Photochemical degradation**

11.1.4.4.1 Anonymous (1993)

Reference:	**Anonymous (1993), A PHOTOLYSIS STUDY OF FOSTHIAZATE (IKI-1145) IN WATER AT PH 5**
Report No:	5287-92-0153-EF-001, DP 29435
Guidelines:	Not indicated
GLP	Yes

Results:

Aqueous photolysis was examined in aqueous buffer solution at pH 5, where fosthiazate was most hydrolytically stable. The samples were exposed to simulated sunlight (Xenon lamp with filters) with a 12 hours dark /12 hours light cycle for 30 days at 25 °C.

After 30 days fosthiazate accounted for 87 % AR in the dark control and 88 % in the light exposed samples. No significant photodegradation occurs and fosthiazate degrades only by hydrolysis to DETO. DETO reaches maximal amounts of 9 % AR after 30 days in the dark control and 8 % AR after 30 days in the light exposed samples.

Conclusion

According to the evaluation during Annex I Inclusion it was agreed that direct photochemical degradation in aquatic systems is considered to be negligible.

11.2 Environmental transformation of metals or inorganic metals compounds

Not applicable.

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

Not applicable, see summary above.

11.4 Bioaccumulation

All the information on Bioaccumulation are taken from the RAR, September 2020.

Table 67: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
shake flask method	At 25 °C log Kow : 1.68	-	Lorence (1993), CHE2000-1139
shake flask method	At 25 °C log Kow : 1.75	-	Kanza (1989), CHE2000-1140
US EPA Guideline 165-4	The steady state total ^{14}C-BCF for whole fish was 3.2.	Only one concentration tested. No growth rate and fish lipid content determined, no information about organic carbon concentration in the test medium, 12% mortality.	Anonymous (1989) 89-11-3157

11.4.1 Bioaccumulation test data

11.4.1.1 Anonymous (1989)

Report:	**CA 8.2.2.3/01, Anonymous (1989)**
Title:	IKI-1145 - Bioconcentration and Elimination of 14C-Residues by Bluegill (*Lepomis macrochirus*)
Document No:	Report Number: 89-11-3157, DP 29463
Guidelines:	US EPA Guideline 165-4
GLP	Yes
Validity	Yes

Materials and Methods:

Test Material:	**Radiolabeled IKI1145 (fosthiazate)**
Lot/Batch #:	C14 (B) IKI 1145, LOT CP816 ; specific activity 1.35 MBq/mg C14 (B) IKI 1145, LOT CP815 ; specific activity 1.35 MBq/mg
Vehicle:	Acetone
Negative control:	Solvent control aquarium with 9.0 µl/L, the concentration of solvent present in the treatment aquarium
Analytical verification of test concentration	Yes
Species:	*Lepomis macrochirus*
Mean length:	49 +/- 4.4 mm
Mean weight:	1.50 +/- 0.36 g
Temperature:	17-20°C
Dissolved oxygen:	62 – 100 %
pH:	6.9-7.2
Hardness:	28-30mg/L
Test design and test procedure	The nominal continuous flow through exposure concentration selected was 1.5 mg/L, equivalent to 1/100 th of the 96 h LC_{50} for bluegilll. The exposure was continuous through the exposure phase of 28 days. 225 bluegill were placed into each of two aquaria, the treatment and the solvent control. Following exposure, 35 fish from the treatment were transferred in untreated control water for 14 days to provide data on the elimination rate of fosthiazate. Five fish were removed for total ^{14}C measurement on days 1, 3, 7, 10, 14, 21 and 28 of the exposure period and on days 1, 3, 7, 10 and 14 of the depuration period. Based on a mean measured concentration of 1.9 (+/- 0.2) mg/L IKI-1145 in the test medium during exposure phase and the mean steady state tissue concentrations, bioconcentration factors were calculated.
Analytical methods	Medium samples were taken on days 0, 1, 3, 7, 10, 14, 21, 28 of exposure and on days 0, 1, 3, 7, 10 and 14 of depuration. A liquid scintillation spectrometer was used. Recovery rates were determined to be 97.4-103%.

Results and Conclusion:

On day 16 a diluter malfunction occurred resulting in the death of 26 treatment fish and 12 solvent control fish between day 16 and 17. The overall percent mortality during the 42 d study was 12 % in the treatment aquarium and 9% in the solvent control aquarium.

The concentration of fosthiazate remained relatively constant throughout the 28 day exposure period (1.9 +/- 0.2). No information about dissolved or total organic carbon is given. No information about growth rate and fish lipid content is given.

In this study using Bluegill sunfish, mean bioconcentration factors in fish tissue were determined to be 2.3, 4.5 and 3.2 for edible, non-edible and whole body tissue, respectively. Steady state in all tissues was reached after 1 day of exposure.

11.5 Acute aquatic hazard

All the information on acute aquatic hazards are taken from the RAR, September 2020. Based on the available acute data degradation products of fosthiazate are less hazardous to the aquatic environment compared to the parent compound. Therefore, degradation products are not relevant for classification purposes.

Table 68: Summary of relevant information on acute aquatic toxicity

Method	**Species**	**Test material**	**Results**	**Remarks**	**Reference**
US EPA 72-1	*Oncorhynchus mykiss*	Fosthiazate (93.5 %)	Acute 96 h (static) LC_{50} = 114 mg a.s./L_{nom}	Reliability 1	Anonymous (1991a) 91/ISK177/0015

US EPA 72-1	*Lepomis macrochirus*	Fosthiazate (93.5 %)	Acute 96 h (static) LC_{50} = 171 mg a.s./L $_{nom}$	Reliability 1	Anonymous (1991b) 91/0016 DP 29462
OECD 202	*Daphnia magna*	Fosthiazate (93.5 %)	Acute 48 h (static) EC_{50} = 0.28 mg a.s./L $_{nom}$	Reliability 1	Anonymous (1991) 91/ISK179/0017
US EPA 850.1035, 72-3	*Americamysis bahia*	Fosthiazate (94.7 %)	Acute 96 h (flow-through) EC_{50} = 0.429 mg a.s./L $_{mm}$	Reliability 1	Anonymous (2001) 012914
US EPA 122-2	*Raphidocelis subcapitata*	Fosthiazate (93.6 %)	5 d (static) E_rC_{50} = > 4.51 mg a.s./L $_{mm}$	Reliability 2 Not all validity criteria were fulfilled, but still considered useful information for classification (see 11.5.3)	Anonymous (1995) 16-01-1

11.5.1 Acute (short-term) toxicity to fish

11.5.1.1 Anonymous (1991a)

Report:	**CA 8.2.1/01, Anonymous, C.A. (1991)**
Title:	IKI-1145 Technical: Acute Toxicity to Rainbow Trout
Document No:	Report Number: 91/ISK177/0015, DP 29460
Guidelines:	US EPA 72-1
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	**IKI 1145 (fosthiazate)**
Description:	Straw coloured liquid
Lot/Batch #:	7391
Content a.s.:	93.5%
Vehicle:	Aqueous stock (800ml) dispersion containing acetone (1.3 ml)
Species:	rainbow trout
Mean length:	4.4 cm
Mean weight:	1 g
Source:	Hauxton Fishery Services Limited, Cambridge
Acclimatisation	14 days period
Diet:	Trout pellets BP Nutrition Ltd., Mainstream Trout Fry o2
Test medium:	Tap water
Test unit:	15 l aquaria
Temperature:	12 ± 2 °C
pH:	7.1 to7.9
Hardness:	200-250 mg/L CaCO3
Dissolved oxygen:	96 – 98%
Test conditions:	15 l aquaria
Test design and test procedure	The acute lethal toxicity of IKI 1145 tech. (fosthiazate) to rainbow trout was assessed under static exposure conditions over a period of 96 h.

	Groups of 10 fish were exposed to IKI 1145 tech. at nom. concentrations of 25.9, 43.2, 72, 120 and 200 mg/L. Each test dilution was made from an appropriate, individually prepared concentrated aqueous stock dispersion containing acetone that had been subjected to ultrasound treatment for 30 minutes. Control groups were placed in dilution water alone or dilution water containing acetone at the same level as in the test dilutions (0.1ml/L). The test was conducted at 12.7 ± 1 in treated tap water of hardness 49-55 mg/L as CACO3 and at pH values in the range of 7.1 to 7.9. At the start the dilutions were clear and colourless with small quantities of material on their surfaces. After 96 h the remaining dilutions da become hazy. Observation of the fish were made at least at 24 h intervals during the test.
Analytical Methods	Exposure levels were monitored by an HPLC method of analysis. Chemical analysis of duplicate mid vessel samples at the beginning and end of the indicated that intended exposure concentrations of IKI tech. were achieved and adequately maintained (between 91-111% of the nominal concentrations).

For the test to be valid the following conditions should be fulfilled:

- Mortality in control group should not exceed 10% (or one fish if less than ten are used) at the end of the test
- Constant exposure conditions to be maintained
- Dissolved oxygen concentration ≥ 60% of air saturation
- Measured concentrations of the test substance should be between 80 and 120% of nominal concentration.

Results:

The highest nominal concentration at which no mortalities occurred and the lowest concentration with 100% mortality after 96 h were 43.2 and 200 mg/L, respectively.

Treatment related effects seen at exposure levels of 72 mg/L and above included lethargic behaviour, oedema and loss of coordination. The effects, which were apparent throughout the test, increased in severity during the test. At 72 mg/L up to four fish were added and at 120 mg/L all fish were affected after 24 hours. At 200 mg/L all fish were affected after two hours and had died after 24 hours.

The NOEC was therefore 43.2 mg/L.

Table 69: Definitive test cumulative mortality

	Nominal IKI 1145 concentrations mg/L					
time	**0***	**25.9**	**43.2**	**72**	**120**	**200**
2h	0	0	0	0	0	0
4h	0	0	0	0	0	0
24h	0	0	0	0	0	10
48h	0	0	0	0	1	10
72h	0	0	0	1	1	10
96h	0	0	0	1	4	10

*Both control groups, dilution water alone and dilution water:acetone, ten fish were exposed at each control and test group

Conclusion:

The present study is considered valid according to OECD guideline 203 and can be used for classification purpose. In this static study with *Oncorhynchus mykiss* and fosthiazate a LC_{50} of 114 mg/L based on nominal concentrations was derived after 96 hours.

11.5.1.2 Anonymous (1991b)

Report:	CA 8.2.1/02, Anonymous, C.A. (1991)
Title:	IKI-1145 Technical: Acute Toxicity to Bluegill sunfish
Document No:	Report Number: 91/0016 DP 29462
Guidelines:	US EPA 72-1
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	Tech. fosthiazate (IKI 1145 tech.)
Description:	Straw coloured liquid
Lot/Batch #:	7391
Content a.s.:	93.5 % fosthiazate
Stability of compound:	Suitably stable in the vehicle for the duration of the study
Vehicle:	Water/acetone
Species:	Bluegill sunfish (*Lepomis macrochirus*), ten fish per concentration
Mean length:	3.9 cm
Mean weight:	1.0 g
Source:	Monksfield Aquatic Centre, Cambridge
Test unit:	glass aquaria having a capacity of 25L
Temperature:	21 - 22.4 °C
Dissolved oxygen:	65 – 100 %
pH:	7.14 - 7-86
Hardness:	35 - 42 mg/L $CaCO_3$
Photoperiod:	16/8h
Test conditions:	Static fresh water conditions with gentle aeration. The test vessels were glass aquaria having a capacity of 25 L. The water was dechlorinated, aged tap water with a total hardness ranging from 40 to 75 mg/L as $CaCO_3$.The pH was not adjusted.
Test design and test procedure	The acute lethal toxicity of IKI 1145 tech. to the bluegill sunfish was assessed under static exposure conditions over a period of 96h. Groups of 10 fish were exposed to IKI 1145 tech. at nominal concentrations of 41.5, 69.1, 115.2, 192 and 320 mg/L. Test media were individually prepared from concentrated aqueous dispersions in which acetone was added directly to the test material before adding the dilution water. To assist in the dispersion of the test material, these concentrated stock dispersions were sonicated for 20 minutes before being further diluted with water in the test vessels. Control groups were placed in dilution water alone or dilutions water containing acetone at the same level as in the test dilutions (0.1 mL/L). The test was conducted at 21.7 ± 0.7 °C in treated tap water of hardness 35 - 42 mg/L $CaCO_3$ and at pH values in the range of 7.1 to7.9. At the start the dilutions were clear and colourless with small quantities of material on their surfaces at a level above 41.5 mg/L. After 96 h the highest remaining test concentrations became hazy (115.2 and 192 mg/L).
Analytical Methods	Exposure levels were monitored by an HPLC method of analysis. Chemical analysis of duplicate mid vessel samples at the beginning and end of the test indicated that intended exposure concentrations of IKI tech. were achieved and adequately maintained (between 91-103% of the nominal concentrations). Observation of the fish were made at least at 24h intervals during the test.

Results

The highest nominal concentrations at which no mortalities occurred and the lowest at which there was 100% mortality after 96 h were 115.2 and 320 mg/L, respectively.

Treatment related effects seen at exposure levels of 115.2 mg/L and above included darkened pigmentation, lethargic behaviour and loss of coordination. At 192 and 320 mg/L all fish were affected after exposure for two hours, at 115.2 mg/L effects were evident after 24 h and were sustained for the duration of the test. The NOEC was therefore 69.1 mg/L. The LC_{50} after 96 h was determined as 171 mg/L.

Table 70: Definitive test cumulative mortality

	Nominal IKI 1145 concentrations mg/L					
time	**0***	**41.5**	**69.1**	**115.2**	**192**	**320**
2h	0	0	0	0	0	0
4h	0	0	0	0	0	0
24h	0	0	0	0	0	10
48h	0	0	0	0	2	10
72h	0	0	0	0	7	10
96h	0	0	0	0	7	10

*Both control groups, dilution water alone and dilution water:acetone, ten fish were exposed at each control and test group

Conclusion

The present study is considered valid according to OECD guideline 203 and can be used for classification purpose. In this static study with *Lepomis machrochirus* and fosthiazate a LC_{50} of 171 mg/L based on nominal concentrations was derived after 96 hours.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

11.5.2.1 Anonymous (1991)

Report:	**CA 8.2.4/01, Anonymous (1991)**
Title:	IKI-1145 Technical: Acute Toxicity to *Daphnia magna*.
Document No:	Report Number: 91/ISK179/0017, DP 29464
Guidelines:	OECD 202
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	**IKI-1145 (fosthiazate)**
Description:	Straw coloured liquid
Content a.s.:	93.5 %
Vehicle:	The test media were made by dilution of appropriate individually prepared acetone stock solution.
Species:	*Daphnia magna*
Source:	University of Sheffield
Acclimatisation period:	Daphnia have been maintained in parthenogenetic culture at the aquatic studies laboratories of Life Science Research.
Diet:	*Chlorella vulgaris* and yeast
Test medium:	Tests conducted in treated tap water: blending of water with a supply of softened water treated by reverse osmosis to reduce hardness to between 40 and 75 mg/L.
Replication:	Five test concentrations, solvent control and negative control were run in quadruplicate, each replicate containing 5 daphnids.
Test unit	Glass jars with 150-ml capacity
Temperature:	20 to 20.4 °C
Dissolved oxygen:	94 – 99 %
pH:	7.4 - 7.7
Hardness:	46 - 48 mg/L as $CaCO_3$
Photoperiod:	16/8 h
Test conditions:	The cultures were kept in a temperature maintained at 20 ± 2 °C.
Test design and test procedure	Groups of 20 Daphnia were exposed to IKI 1145 tech. at nominal concentrations of 39, 65, 108, 180, 300 and 500 µg/L selected following a preliminary rage finding test. The test media were

	made by dilution of appropriate individually prepared acetone stock solution. Control groups of Daphnia were placed in dilution water alone or dilution water containing acetone at the same level as in the test dilutions (0.1 ml/L).
Analytical methods	Results of chemical analysis of duplicate samples at the beginning and the end of the test, although variable at the lower levels generally indicated that intended exposure concentrations of IKI 1145 tech. above 39 µg/L were achieved and adequately maintained (mean measured value were between 93 and 110 % of their nominal concentrations); at 39 µg/l the achieved concentration was below the limit of the assay (10 µg/l). Observations of Daphnia were made after 24 and 48 Hours of exposure.

Results

The acute toxicity of IKI tech. to *Daphnia magna* was assessed under static exposure conditions over a period of 48 h.

The measured concentrations showed high variation at low exposure concentrations (65 µg/L: 58-128 % of nominal; 108 µg/L: 73-146 % of nominal) and were below the limit of detection at the lowest exposure concentration (39 µg/L: ND). However, the measured concentrations at the higher exposure concentration were close to nominal concentrations (180 µg/L: 88-109 % of nominal; 300 µg/L: 89-102 % of nominal; 500 µg/L: 91-107 % of nominal). Since no effects were observed at lower test concentrations, it was considered adequate to base effect concentrations on nominal values.

The highest nominal concentration at which no immobilization occurred after 48 hours was 180 µg/L; immobile Daphnia were found at 65 and 108 µg/L but these differences were not considered to be significant because their numbers were not directly related to exposure concentrations. At the highest exposure concentration (500 µg/L) 95 % of the Daphnia were immobilized. The NOEC based on nominal exposure levels was considered to be 180 µg/L. The LC_{50} based on nominal concentrations was 280 µg/L.

It should be noted that in the range of the NOEC and EC_{50} test concentrations were sufficiently maintained (80 – 120 % of nominal). Therefore, results can be based on nominal concentrations.

Table 71: Definitive test: observation of the number of mobile, immobile and floating *Daphnia magna*

No. Conc. (µg/L)	**Mobile no. of Daphnia**		**Immobile no. of Daphnia**	
	Submerged	floating	submerged	floating
Control (water)	19	0	1	0
Control (acetone)	19	1	0	0
39	19	1	0	0
65	16	1	3	0
108	19	0	1	0
180	19	1	0	0
300	4	0	13	3
500	1	0	17	2

Conclusion

The present study can be used for classification purpose. In this static study with fosthiazate a 48 h EC_{50} of 0.28 mg/L (nominal) was derived for *Daphnia magna*.

11.5.2.2 Anonymous (2001)

Report:	**CA 8.2.4.1/01, Anonymous (2001)**
Title:	Technical IKI-1145: Acute Toxicity to the mysid *Americamysis bahia*
Document No:	012914 2117-SK
Guidelines:	US EPA 850.1035, 72-3
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	**Fosthiazate (technical)**
Lot/Batch #:	21007013
Content a.s.:	94.7 %
CAS #:	98886-44-3
Stability of test compound:	Stored in a refrigerator in the dark
Vehicle:	None
Negative control:	Natural seawater
Species:	Mysid shrimp (*Americamysis bahia*)
Age:	Juveniles, < 24 hours at test initiation
Source:	In house culture (T.R. Wilbury Laboratory, Massachusetts), the original brood stock was received from a commercial supplier (Aquatic BioSystems, Fort Collins, Colorado, December 1998)
Acclimatisation period:	Juvenile mysids produced by parents that were cultured within the laboratory were used. During acclimation to the test conditions (> 14 days, 24.5 to 25.6°C, salinity 13 to 17 ‰), mysids were not treated for disease and were free of apparent disease, injuries and abnormalities.
Diet:	None
Test medium:	Carbon filtered, natural seawater with adjusted salinity, 17 ‰
Test unit:	20 L glass aquaria containing 15 L test solution (water depth approx. 19 cm). Inside the aquaria, mysids were exposed in glass cylinders (8 cm in height and 8 cm diameter) with a Nitrex® screen (mesh size 350 µm) attached to the bottom.
Temperature:	24.7 – 26.0 °C
Dissolved oxygen:	7.5 – 7.9 mg/L
pH:	7.9 - 8.1
Hardness:	No data
Photoperiod:	Light/dark cycle of 14 hours/10 hours
Test conditions:	Flow-through, (renewal rate: approximately 6.2 times per day)
Test design and test procedure	Treatments consisted of a dilution water control and the nominal concentrations of 0.1, 0.18, 0.29, 0.48 and 0.80 mg a.s./L (measured: 0.0978, 0.172, 0.283, 0.481 and 0.802 mg a.s./L). There were 2 replicates per treatment with 10 mysids each. The numbers of surviving mysids and the occurrence of sublethal effects were recorded after 24, 48, 72 and 96 hours.
Analytical Methods	Samples of test solutions taken after 0 and 96 hours were analysed using high performance liquid chromatography (HPLC) analysis equipped with an UV-VIS detector.
Statistics	LC_{50}-values were calculated using Probit analysis.

Results

Referring to nominal concentrations of 0.1, 0.18, 0.29, 0.48 and 0.80 mg a.s./L the measured concentrations were 0.0978, 0.172, 0.283, 0.481 and 0.802 mg a.s./L, which was 96 % to 100 % of the nominal values. All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within acceptable ranges.

Based on mean measured concentrations, the 96 h LC_{50} in *Americamysis bahia* was calculated in the report to be 0.429 mg a.s./L (95 %-CI: 0.345-0.560 mg a.s./L). The 96 h NOEC was 0.0978 mg a.s./L.

Table 72: Summary of mortality and sublethal effects of *Americamysis bahia* exposed to Fosthiazate

Mean measured concentrations of Fosthiazate [mg/L]	Mortality [%] / sublethal effects [%]			
	24 h	48 h	72 h	96 h
Dilution water control	0/0	0/0	0/0	0/0
0.0978	0/0	0/0	0/0	1/0
0.172	0/0	0/0	0/0	3/0
0.283	0/0	0/0	0/1	3/0
0.481	0/0	0/0	4/5	10/5
0.802	0/0	0/0	17/3	18/2

Conclusion

The present study is considered valid according to US EPA guideline 850.1035, 72-3 and can be used for classification purpose. Based on mean measured concentrations, the 96 h LC_{50} of 0.429 mg a.s./L was calculated for *Americamysis bahia*.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

11.5.3.1 Anonymous (1995)

Report:	CA 8.2.6/01, Anonymous (1995)
Title:	The Toxicity of IKI-1145 to *Selenastrum capricornutum*.
Document No:	Report No. 16-01-1, DP 29467
Guidelines:	US EPA 122-2
GLP	Yes
Validity	No (not valid according to OECD 201)

Materials and Methods

Test Material:	Fosthiazate (technical)
Lot/Batch #:	21007013
Content a.s.:	93.6 %
CAS #:	98886-44-3
Stability of test compound:	Stable for two years at 4 °C. Stored refrigerated and protected from light.
Vehicle:	DMF, 0.5 mL/L
Negative control:	AAP medium
Species:	*Selenastrum capricornutum = Raphidocelis subcapitata*
Source:	Laboratory stock culture
Initial cell concentration	3000 cells/mL
Test medium:	Synthetic algal assay procedure medium (AAP medium)
Test unit:	250 mL Erlenmeyer flasks with foam stoppers
Temperature:	22.82 – 23.32 °C
pH:	6.93 – 7.57
Photoperiod:	Continuous, 4306 ± 646 lumen/m² (= 300 µE/m²s)
Test conditions:	Continuous shaken at 100 oscillations per minute
Test design and test procedure	• Limit test with a maximum concentration of 5.1 mg a.s./L (nominal) = 4.51 mg a.s./L (mean measured) • 3 replicates per treatment • daily repositioning to minimise spatial effects • cell counts using Coulter Counter on day 3,4 and 5
Analytical Methods	HPLC + UV (limit of detection = 0.831 mg/L)
Statistics	Based on mean measured concentrations, comparison of treatment concentrations with the combined controls

In this static study with *Raphidocelis subcapitata* (*Selenastrum capricornutum*) only one concentration of 4.51 mg a.s./L (mean measured) was tested. Algal cell counts were performed at 72, 96 and 120 h.

Table 73: Summary mean cell number of *Selenastrum capricornutum* = *Raphidocelis subcapitata* exposed to Fosthiazate

Mean measured concentrations of Fosthiazate [mg/L]	**Mean cell counts (cells x 10^4/mL) during test + standard deviation**		
	72 h	**96 h**	**120 h**
Negative control	28.65 ± 6.26	172.24 ± 52.4	389.35 ± 74.6
Solvent control	27.52 ± 5.63	160.84 ± 33.5	367.98 ± 42.9
4.51	30.67 ± 9.89	179.48 ± 56.2	388.6 ± 66.3

As no effects could be observed after 120 hours, the E_rC_{50} and E_bC_{50} are > 4.51 mg a.s./L (measured). A t-test revealed no significant difference of the pooled control and the tested concentration (NOEC 4.51 mg/L). The test was not conducted according to OECD TG 201 and does not meet all validity criteria of this guideline. Cell counts for 24 and 48 h were not reported. It should be noted that the mean coefficient of variation for section-by-section specific growth rates was 50% (for the test to be valid according to OECD 201 it should not exceed 35%). Furthermore, the solvent concentration (solvent used: DMF) was 0.5 mL/L, but should according to OECD 201 not exceed 0.1 mL/L.

However, the study as a limit test is still regarded useful to provide information about the toxicity of fosthiazate to algae for classification purposes. The test substance was stably maintained in the test system and algal cell numbers increased by a factor of 95.5 (negative control) and 91.7 (solvent control) during the first 72 h. No inhibitory effect on algal growth was observed at the tested concentration of 4.51 mg a.s./L. For invertebrates, the derived effect concentrations were considerably lower (EC50 = 0.28 mg a.s./L). Hence, invertebrates are the most sensitive taxon and most relevant for the classification of aquatic toxicity. It is not expected that another study on algal toxicity would lead to completely different results and derive a lower effect concentration for algae than for invertebrates. Thus, the study on algal toxicity is sufficient to judge that this organism group is of low relevance for the classification of aquatic toxicity of fosthiazate. Therefore, the study in considered for classification purposes despite its shortcomings.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No information available.

11.6 Long-term aquatic hazard

All the information on long-term aquatic hazards are taken from the RAR, September 2020.

Table 74: Summary of relevant information on chronic aquatic toxicity

Method	**Species**	**Test material**	**Results[1]**	**Remarks**	**Reference**
OECD 204	*Oncorhynchus mykiss*	Fosthiazate (95.6 %)	28 d (flow-through) $NOEC_{Mortality}$ = 2.4 mg a.s./L $_{nom}$ $NOEC_{body\ weight}$ = 7.8 mg a.s./L $_{nom}$	Reliability 1, but only as supportive information, since the OECD TG 204 is not considered a chronic test	Anonymous (1999) 729156

OECD 210	*Pimephales promelas*	Fosthiazate (94.6 %)	28 d ELS (flow-through) $NOEC_{body\ weight}$ = 3.64 mg a.s./L $_{nom}$ $NOEC_{length}$ = 1.65 mg a.s./L $_{nom}$ $EC_{10\ body\ weight}$ = 1.91 mg a.s./L $_{nom}$ $EC_{10\ length}$ = 4 mg a.s./L $_{nom}$	Reliability 1	Anonymous (2013) 96207-662-12-E-6207
OECD 211	*Daphnia magna*	Fosthiazate (93.8 %)	21 d (semi-static) $NOEC_{reproduction}$ = 0.06 mg a.s./L $NOEC_{mortality}$ = 0.06 mg a.s./L	Reliability 1	Anonymous (1994) 123468
US EPA 122-2	*Raphidocelis subcapitata*	Fosthiazate	5 d (static) E_rC_{50} = > 4.51 mg a.s./L $_{mm}$ NOEC = 4.51 mg a.s./L $_{mm}$	Reliability 2 Not all validity criteria were fulfilled, but still considered useful information for classification (see 11.5.3)	Anonymous (1995) 16-01-1
OECD 219 Draft (1998)	*Chironomus riparius*	Fosthiazate	24 d (static) $NOEC_{development}$ = 0.1 mg a.s./L $_{nom}$ $NOEC_{emergence}$ = 0.1 mg a.s./L $_{nom}$	Reliability 2	Anonymous (1999) 729191

11.6.1 Chronic toxicity to fish

11.6.1.1 Anonymous (1999)

Report:	**CA 8.2.2.1/01, Anonymous, A. (1999)**
Title:	Sublethal toxic effects of Fosthiazate on rainbow trout (*Oncorhynchus mykiss*) in a fish juvenile growth test over 28 days
Document No:	729156
Guidelines:	OECD No. 204
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	**Fosthiazate technical (95.6% w/w purity)**
Lot/Batch #:	80204013
Content a.s.:	Fosthiazate technical (95.6 % w/w purity)
Stability of test compound:	Stable in water during the application period
Solvent:	None
Negative control:	Tap water

Species:	*Oncorhynchus mykiss*
Mean weight:	2.0 g
Source:	P. Hohler, Zeiningen
Acclimatisation	2 weeks prior to the test start
Diet:	Hokovit 502, 1.2mm
Test medium:	Tap water. The nominal fosthiazate concentrations were 0.24, 0.76, 2.4, 7.8, 25 and 80 mg/L.
Test unit:	48 l test aquaria
Temperature:	at 15 ±1 °C
Dissolved oxygen:	8 mg/l, >60 %
pH:	7.4-7.9
Hardness:	4.1 mmol/L (410mg/L as CaCO3)
Photoperiod:	16h/8h, 200-800lux
Test conditions:	at 15 ±1 °C
Test design and test procedure	Rainbow trout, of mean initial individual weight 2.0 g, were exposed to maintained concentrations of fosthiazate technical (95.6% w/w purity) in a flow-through system for 28 days at 15 ±1°C. The nominal fosthiazate concentrations were 0.24, 0.76, 2.4, 7.8, 25 and 80 mg/l. Test concentrations were based on two range-finding studies and solubility studies. Exchange solutions were renewed every 2 days for the first 10 days, thereafter dosing solutions were renewed every 3 or 4 days. A control group of fish was exposed to dilution water only. Test media volumes in the aquaria were exchanged at a rate of 2 volumes per day. Actual concentrations of fosthiazate were determined by chemical analysis on days 0, 7, 14, 21 and 28. Mortality and symptoms of toxicity were recorded daily (except for weekends) over the 28-day exposure period. Bodyweights of individual fish were recorded at the start, and of surviving fish at the end of the study (day 28).
Analytical Methods	Actual concentrations of fosthiazate were determined by chemical analysis on days 0, 7, 14, 21 and 28.
Statistics	ANOVA

Results

Mean measured concentrations of fosthiazate ranged from 91 to 124 % of the nominal values for all test levels. The results were therefore reported as nominal concentrations. No mortality or symptoms of intoxication were observed during the test in the control and at the nominal test concentrations up to and including 2.4 mg/L. At the nominal test concentration of 7.8 mg/L one of the fish was lying apathetic on the bottom of the aquarium from day 19 until the end of the test. This was by the authors not considered to be a toxic effect, since no signs of intoxication were observed in this aquarium up to that time and all the other fish showed no signs of intoxication until the end of the test. In the next higher concentration (nominal 25 mg a.s./L), one fish showed symptoms of intoxication with increasing intensity from day 16 onwards. These symptoms were considered to be a toxic effect, since other fish in the aquarium showed hesitancy in food uptake which lead to a reduced bodyweight. At the highest concentration (nominal 80 mg a.s./L) all fish showed strong symptoms of intoxication from day 1 on. The first fish died on day 2, on day 8 80 % of fish were dead, by day 9 all fish had died.

In contrast to the evaluation in the study report, the NOEC (highest concentration tested without observed toxic effects) is considered to be 2.4 mg/L. At the nominal test concentration of 7.8 mg/L one of the fish was lying apathetic on the bottom of the aquarium and there is no explanation why this effects should be neglected. At lower concentrations no mortality occurred and the observed effects were also seen in higher concentrations displaying in consequence a concentration dependent effect.

Day 28 mean body weights were 6.0, 5.8, 5.9, 6.1, 5.9 and 2.5 g in the control, 0.24, 0.76, 2.4, 7.8, 25 and 80 mg a.s./L test concentrations, respectively. Reductions in the 25 mg a.s./L concentration were significantly different to the control.

Table 75: Sublethal toxic effects of Fosthiazate on rainbow trout (Oncorhynchus mykiss) in a fish juvenile growth test

	Symptoms of intoxication (symptoms/ dead)		Mean body weight ±SD	
day	0	28	0	28
control	0/0	0/0	2.0±0.12	6.0±1.52
0.24 (mg/L) nom.	0/0	0/0	1.9±0.11	5.8±0.48
0.76(mg/L) nom.	0/0	0/0	2.0±0.14	6.0±1.52
2.4(mg/L) nom.	0/0	0/0	2.0±0.12	6.1±1.29
7.8(mg/L) nom.	0/0	1 apathy, fish lying on the side or back on the bottom /0	2.0±0.17	6.0±1.49
25(mg/L) nom.	0/0	1 apathy, fish lying on the side or back on the bottom/0	1.9±0.12	2.5±0.73
80(mg/L) nom.	10 strongly change body colour/ 0 dead	0/10 all fish dead	1.9±0.0812	-/-

Conclusion

In this flow-through test with *Oncorhynchus mykiss* and fosthiazate a NOEC of 2.4 mg a.s./L is considered. At lower concentrations no mortality occurred and the observed effects were also seen in higher concentrations displaying in consequence a concentration dependent effect.

A NOEC of 7.8 mg a.s./L and a LOEC of 25 mg a.s./L were derived for body weight changes based on nominal concentrations.

However, since the OECD TG 204 study is not considered a chronic value equivalent to the ones indicated in both the CLP and REACH Guidance (e.g. OECD TG 210), the endpoints are only used as supportive information. The available early-life stage toxicity test on fathead minnow is the relevant study for the classification of chronic toxicity to fish.

11.6.1.2 Anonymous (2013)

Report:	**CA 8.2.2.1/01, Anonymous (2013)**
Title:	An Early-life Stage Toxicity Study of Fosthiazate TGAI in Fathead minnow
Document No:	Report Number: 96207-662-12-E-6207
Guidelines:	OECD No. 210, OPPTS 850.1400
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	**Fosthiazate (technical)**
Description:	Light brown liquid
Lot/Batch #:	21007013
Content a.s.:	94.6 %
CAS #:	98886-44-3
Stability of test compound:	DT50: 104 days (in water), 80.3-89.0 days (to sunlight in water), stable to heat. The stability of the test item under the storage condition was confirmed by comparing the IR spectrum of the test item before and after the completion of the experiment
Vehicle:	None
Negative control:	Dechlorinated tap water
Species:	Fathead minnow (*Pimephales promelas*)

Age:	Healthy and normal test organisms by viewing were selected. One male and two female fish pair of sex were paired and the fertilised eggs were used for the study.
Mean length:	not applicable
Mean weight:	not applicable
Source:	National Institute for environmental Studies (NIES)
Acclimatisation period (adults for spawning):	25 ± 1.5°C, > 80 % air saturation, 16 h light and 8 h dark
Diet:	Acclimation: Feed for fry of carp "crumble2C" (Nippon formula Feed), corresponding to 3 % of bw/day Pairing (spawning): newly hatched Artemia nauplii Test: Fish after hatching were fed newly hatched Artemia nauplii at least twice per day
Test medium:	Dechlorinated tap water, aerated and temperature controlled
Test unit:	3 L glass tank (diameter: 16 cm, depth 17 cm). The tank had an overflow tube set at approximately 1.8 L level.
Temperature:	25 ± 1.5 °C
Dissolved oxygen:	7.5 - 8.3 mg/L
pH:	7.5 - 8.0
Hardness:	30 - 44 mg CaCO3/L
Photoperiod:	Light/dark cycle of 18 hours/8 hours
Test conditions:	Flow-through, (renewal rate: approximately 12 times per day)
In life dates:	24.07.2013 - 31.08.2013
Test design and test procedure	The objective of this study is to estimate the LOEC and NOEC or ECx by conducting an early-life stage toxicity study of the fosthiazate in fathead minnow. Fertilized eggs of fathead minnow (*Pimephales promelas*) were exposed to five initial test item concentrations for 28 days post-hatch. The nominal test concentrations were 0.342, 0.751, 1.65, 3.64 and 8.00 mg a.s./L (four replicates per test level, 80 fish per test level, 20 fish per test vessel). The test-item was dissolved into dilution water in order to prepare the stock and the test solutions. The developmental stage of the embryos at the start of exposure was the morula stage. Eggs were incubated in a cylindrical glass cup which was covered with stainless steel mesh at the bottom and moved up and down at a slow pace in the test vessel during the embryo phase. Observations on hatch, development, survival and abnormal appearance of behaviour were made daily. Dead embryos, larvae and juvenile fish were removed as soon as observed. Survival rates, body weights and lengths were determined at the end of exposure.
Analytical Methods	Samples of test solutions were analysed using high performance liquid chromatography (HPLC) analysis equipped with an UV-VIS detector.
Statistics	ECx values were calculated based on non-linear regression model according to OECD guidance for statistical analysis (ENV/JM/MONO (2006)18). Non-linear regression analysis was conducted by using SPSS 10.0.5J for Windows.

Results

The results of the measured concentrations of the test item in the test solutions revealed mean measured test concentrations between 88.4 % and 104 % of the nominal values. All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within acceptable ranges. In one of the dilution water tanks temperature increased from 23°C to 26.1°C. It was confirmed that the temperature monitored in the definitive study was hardly influencing the temperature of the supply water in the flow-through system.

a) No significant differences in hatchability were found between all test concentrations and the control.

b) The mean post hatch survival at the end of the test in the controls was greater than 90 %,

c) oxygen saturation was between 7.5 and 8.3 mg/L,

d) the temperature was 25 ± 1.5°C and chemical analysis was performed,

thus the study is considered valid.

No significant reduction in post hatch survival compared to the control could be detected at all test concentrations. A statistically significant reduction in body weight was observed at the highest tested

concentration (8.00 mg a.s./L). For wet body weight of survival fish at the end of exposure, a significant difference (p<0.05) and a significant difference (p<0.01) were observed at 1.65 mg a.i./L and 8.00 mg a.i./L, respectively. However, as no significant difference (p<0.05) at the upper exposure level of 3.64 mg a.i./L was observed, the significant difference at 1.65 mg a.i./L was not considered for NOEC determination.

A significantly reduced total length appeared at 3.64 and 8.00 mg a.s./L. Therefore, the LOEC and NOEC were 3.64 and 1.65 mg a.s./L, respectively. The EC10 and EC20 for body weight were 1.91 and 4.01 mg a.s./L, respectively. The EC10 and EC20 for total length were 4.00 and 7.89 mg a.s./L, respectively.

A summary of egg hatch, post hatch survival, length and wet weight is presented in the table below.

Table 76: Toxicity of Fosthiazate TGAI in an Early-life Stage Toxicity Study with fathead minnow

Nominal Concentration of Fosthiazate [mg/L]	**Control**	**0.342**	**0.751**	**1.65**	**3.64**	**8**
Measured Concentration (mean) [mg/L]	n.d.	0.330	0.708	1.59	3.46	7.75
Egg no. at test start	80	80	80	80	80	80
[n]						
Egg Survival, Hatching [%]	100 ± 0	100 ± 0	100 ± 0	98.8 ± 2.5	100 ± 0	98.8 ± 2.5
Post Hatch Survival day 28 [%]	86.3 ± 2.5	91.3 ± 11.1	90.0 ± 0	96.3 ± 4.8	87.5 ± 2.9	87.4 ± 6.4
Weight, day 28 of the single replicates [mg]*	38.3 ± 12.4	39.8 ± 14.4	39.8 ± 24.8	38.6 ± 14.2	36.1 ± 16.0	24.3 ± 9.5
	41.8 ± 23.7	44.2 ± 17.8	39.7 ± 16.6	32.7 ± 11.2	34.8 ± 13.6	25.2 ± 11.7
	42.3 ± 19.1	40.1 ± 19.1	41.3 ± 17.7	38.7 ± 17.7	35.4 ± 14.2	26.1 ± 14.4
	41.2 ± 17.5	44.1 ± 18.2	31.7 ± 8.2	29.7 ± 12.2	36.1 ± 16.6	27.0 ± 10.9
Length, day 28 of the single replicates [cm]*	1.79 ± 0.16	1.78 ± 0.19	1.73 ± 0.16	1.72 ± 0.16	1.66 ± 0.19	1.38 ± 0.11
	1.72 ± 0.30	1.79 ± 0.21	1.76 ± 0.19	1.71 ± 0.13	1.64 ± 0.19	1.37 ± 0.13
	1.79 ± 0.21	1.76 ± 0.21	1.81 ± 0.17	1.74 ± 0.20	1.65 ± 0.16	1.40 ± 0.15
	1.79 ± 0.19	1.84 ± 0.21	1.68 ± 0.13	1.61 ± 0.19	1.65 ± 0.17	1.45 ± 0.17

* Arithmetic mean and standard deviation
n.d. = not determined

Table 77: Results of statistical analysis in each endpoint comparing with control and exposure level observed toxic symptoms remarkably were shown below.

Nominal concentration (mg a.i./L)	**Hatchability**	**Average of days to hatch**	**Rate of developmental abnormity**	**Survival rate after hatching**	**Body weight**	**Total length**	**Abnormal response**
0.342	-	-	-	-	-	-	-
0.751	-	-	-	-	-	-	-
1.65	-	-	-	-	(*)	-	-
3.64	-	-	-	-	-	**	-
8.00	-	-	-	-	**	**	+

No significantly different at $p<0.05$ or no observed toxic symptom remarkably

+ Exposure level observed toxic symptoms remarkably

* Significantly different from control at $p<0.05$

(*) It was judged that it was proper that the approved significant difference ($p<0.05$) at 1.65 mg a.i./L was dismissed, because no significant difference ($p<0.05$) at the upper exposure level of 3.64 mg a.i./L was observed.

** Significantly different from control at $p<0.01$

Conclusion

The present study is considered valid according to OECD guideline 210.

No significant reduction in post hatch survival compared to the control could be detected at all test concentrations. A statistically significant reduction in body weight was observed at the highest tested concentration (8.00 mg a.s./L) and a significantly reduced total length at 3.64 and 8.00 mg a.s./L. Therefore, the LOEC and NOEC were 3.64 and 1.65 mg a.s./L, respectively.

The EC_{10} and EC_{20} for body weight were 1.91 and 4.01 mg a.s./L, respectively. And the EC_{10} and EC_{20} for total length were 4.00 and 7.89 mg a.s./L, respectively.

11.6.2 Chronic toxicity to aquatic invertebrates

11.6.2.1 Anonymous (1994)

Report:	**Anonymous (1994)**
Title:	*Daphnia magna* reproduction test with IKI-1145 technical
Document No:	123468
Guidelines:	OECD 202, Part II
GLP	Yes
Validity	Yes

Materials and Methods

Test Material:	**IKI-1145 technical (fosthiazate)**
Description:	Yellowish liquid
Lot/Batch #:	21007013
Content a.s.:	93.8 %
CAS #:	98886-44-3
Stability of test compound:	Stable as stored in a refrigerator in the dark
Vehicle:	None
Negative control:	Dilution medium
Species:	*Daphnia magna* (Straus, 1820)
Age:	< 24 hours
Acclimatisation period:	Young daphnids produced by parents that were cultured within the laboratory were used. The parents of the young daphnids were bred in the same quality of water as used in the test.
Diet:	*Chlorella pyrenoidosa*, 50×10^6 cells per daphnid at each medium renewal
Test medium:	M4 medium (Elendt, 1990)
Test unit:	Glass beakers with a volume of 50 mL test medium.
Temperature:	19 – 21 °C (18.5 °C at day 16 in all test solutions)
Dissolved oxygen:	>6 mg/L
pH:	7.4 – 8.7
Hardness:	250 mg $CaCO_3$/L
Photoperiod:	Light/dark cycle of 16 hours/8 hours, 600 lux
Test conditions:	Semi-static, renewal three times a week
In life dates:	21.09.1994 – 12.10.1994
Test design and test procedure	Treatments consisted of a dilution water control and concentrations of 5.5, 12, 27, 60 and 132 µg a.s./L. Ten daphnids per test concentration and 20 in the controls were exposed individually. Immobility and newborn young were recorded every workday.
Analytical Methods	Samples of test solutions with 27, 60 and 132 µg/L were taken from freshly prepared and altered solutions for analysis. At day 0 all concentrations were sampled and analysed. High performance liquid chromatography (HPLC) analysis equipped with an UV-VIS detector was used for analysis.

Results

Measured concentrations ranged from 91 to 122 % of the nominal values. The measured concentrations were stable during the periods between fresh preparation and renewal of the test media. All chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within acceptable ranges.

No immobility could be observed in the controls at any time. 100 % immobility could be observed at 132 µg/L after 12 days. After 21 days a nominal NOEC of 60 µg a.s./L referring to reproduction and parental mortality was determined for *Daphnia magna*. The results are documented in the following table.

Table 78: Effects of Fosthiazate on immobilisation and reproduction of *Daphnia magna* after 21 days

Nominal concentrations [µg a.s./L]	Cumulative immobilisation	Immobilisation [%]	Cumulative mean reproduction	Reproduction [%]
Control	2	10	180	100
5.5	2	20	183	102
12	1	10	194	108
27	2	20	182	101
60	2	20	178	99
132	10	100	35[1]	42[1]

[1]for reproduction until day 12

Conclusion

The present study is considered valid according to OECD 202 as parental mortalities did not exceed 20 % in the controls and the average cumulative number of young per female in the controls was > 60 after 21 days. The study is acceptable for risk assessment.

Based on nominal concentrations a NOEC of 60 µg a.s./L referring to reproduction and parental mortality was determined for *Daphnia magna* after 21 days.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to section 11.5.3.

11.6.4 Chronic toxicity to other aquatic organisms

11.6.4.1 Anonymous (1999)

Report:	Anonymous (1999)
Title:	Effects of fosthiazate on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system.
Document No:	RCC project 729191, DP 95116
Guidelines:	OECD 219 Draft (1998)
GLP	Yes
Validity	Yes

In this static study the water phase was spiked with Fosthiazate. After 24 days a NOEC of 0.1 mg a.s./L (nominal, highest tested concentration) was derived for emergence and development rates of *Chironomus riparius*. The test design deviated from the guideline OECD as the sediment phase contained 10% peat instead of the suggested 5%.

Partitioning into the sediment is not a major removal process. Maximal 26 % AR Fosthiazate can be found in the sediment after 14 days. As Fosthiazate is not expected to accumulate at organic fractions (low Kfoc), this deviation does not seem to have influenced the test results.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Fosthiazate fulfils the classification criteria for Aquatic Acute 1, since its acute toxicity to aquatic crustaceans is below 1 mg/L (EC_{50} *D. magna*: 0.282 mg/L, EC_{50} *A. bahia*: 0.429 mg a.s./L $_{mm}$). The acute toxicity to fish (LC_{50} *O. mykiss*: 114 mg/L) and algae (E_rC_{50} *R. subcapitata*: > 4.51 mg/L) is above 1 mg/L. Since the lowest

EC_{50} is between 0.1 and 1 mg/L, an acute M-factor of 1 is set for fosthiazate.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

For classification purpose it is applicable to classify fosthiazate as not rapidly degradable: According to the ready biodegradability test OECD 301F (0 % degradation within 28 days) fosthiazate is not readily biodegradable. In the water-sediment studies DT50 whole system range from 30.1 to 72.1 days. Although in an aerobic mineralization in surface water study (OECD 309) a half-life of < 16 days (14.7 – 15.9 d) was observed, fosthiazate is not considered to be rapidly biodegradable, because mainly primary degradation and low mineralization (3-21%) was observed. The abiotic degradation showed that the DT_{50} of fosthiazate due to hydrolysis is above 16 days (312 d, longest half-life within pH 4 – 9).

Based on a log K_{ow} of 1.75, which is below the trigger value of 4, and an experimentally determined BCF of 3.2 in fish, which is below the trigger value of 500, fosthiazate is not considered to possess the potential to bioaccumulate.

Fosthiazate fulfils the criteria for classification as Aquatic Chronic 1 since its chronic toxicity to aquatic crustaceans is below 0.1 mg/L (NOEC *D. magna*: 0.06 mg/L) and combined with that the substance is not rapidly degradable. The toxicity of fosthiazate to fish (NOEC *P. promelas*: 1.65) and Algae (NOEC *R. subcapitata*: > 4.51 mg/L) is above 0.1 mg/L. Since the lowest NOEC is between 0.01 and 0.1 mg/L, a chronic M-factor of 1 is set for fosthiazate.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Fosthiazate can be classified as Aquatic Acute 1 with an M-factor of 1 ($0.1 < L(E)C_{50} <= 1$ mg/L).

Fosthiazate can be classified as Aquatic Chronic 1 with an M-factor of 1 ($0.01 < NOEC < 0.1$).

Hazard statement codes: *Hazardous to the aquatic environment*

Aquatic Acute 1; H400, M-factor 1

Aquatic Chronic 1; H410, M-factor 1

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not assessed in this dossier.

13 ADDITIONAL LABELLING

13.1 Toxic by eye contact

13.1.1 Short summary and overall relevance of the provided information on toxicity by eye contact

Two studies investigating the eye damage/irritating potential of fosthiazate are available (please see Table 22). The second study (Anonymous 18, 1989) was a supplement for the first study (Anonymous 17, 1989) and was conducted with the deviation of washing the eyes already after 2-3 minutes after instillation. Due to this deviation the study is considered as supplementary for classification and labelling. Anonymous 18 (1989)

reported that 3 of 6 treated rabbits showed signs of systemic toxicity and died or were killed in extremis after ocular instillation (see Table 22).

13.1.2 Comparison with the CLP criteria

Table 79 presents the result of the acceptable acute eye toxicity study in comparison with the CLP criteria for the assignment of the supplemental labelling element EUH070.

Table 79: Results of acute toxicity by eye contact in comparison with CLP criteria

Toxicological result	CLP criteria
3 of 6 treated rabbits died/were killed *in extremis* after ocular instillation	**Toxic by eye contact (EUH070)** 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. The statement shall also be applied if there is evidence in humans for systemic toxicity after eye contact.

Based on the available data fosthiazate meets the criteria to have the supplemental labelling element EUH070 assigned for toxicity by eye contact.

13.1.3 Conclusion on classification and labelling for toxicity by eye contact

Supplemental labelling with “Toxic by eye contact” (EUH070) is considered appropriate.

14 REFERENCES

Author(s)	Year	Title source (where different from company) report no. published or not
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Anonymous 3	1990a	IKI-1145 (Fosthiazate): Carcinogenicity study by dietary administration to CD-1 mice for up to 104 weeks, 89/ISK088/0624 ! 89/0624 ! ISK/088/1145. Unpublished study.
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