CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc

EC Number: 236-671-3

CAS Number: 13463-41-7

Index Number: -

Contact details for dossier submitter:

Swedish Chemicals Agency
P.O. Box 2, SE-172 13 Sundbyberg, Sweden
kemi@kemi.se
Phone: +46 8 519 41 100

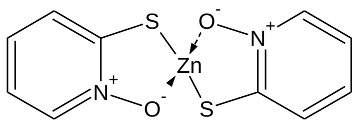
Version number: 5 **Date: 22 May 2017**

CONTENTS

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)	(T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc
Other names (usual name, trade name, abbreviation)	Zinc pyrithione
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	236-671-3
EC name (if available and appropriate)	Pyrithione zinc
CAS number (if available)	13463-41-7
Other identity code (if available)	None
Molecular formula	$C_{10}H_8N_2O_2S_2Zn$
Structural formula	
SMILES notation (if available)	Not applicable (coordination complex)
Molecular weight or molecular weight range	317.69 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable (the substance does not contain any isomers)
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Min: 95%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (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)
Pyrithione zinc CAS no: 13463-41-7	95-100%	None	

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
No impurities present at ≥1%w/w or which contributes to the classification of the substance				

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
No additives					

Table 5 Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information
Pyrithione zinc CAS no: 13463-41-7	Min. 95%	No impurities present that contributes to the classification of the substance	

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal		pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc	236-671-3	13463-41-7	Acute Tox. 3 Acute Tox. 2 Eye Dam. 1 Repr. 1B STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H301 H330 H318 H360D H372 H400H410	GHS05 GHS06 GHS08 GHS09 Dgr	H301 H330 H318 H360D H372 H410	-	M-factor=1000 (acute) M-factor=10 (chronic)	-
RAC opinion		pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc	236-671-3	13463-41-7							
Resulting Annex VI entry if		pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-	236-671-3	13463-41-7							

agreed by RAC and COM		2(1H)-thionato-.kappa.S]zinc									

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

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

Hazardous to the ozone layer	Hazard class not assessed	No

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Zinc pyrithione (ZnPT) has not been previously classified.

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Zinc pyrithione is an active substance in the meaning of Regulation (EU) No 528/2012 repealing Directive 98/8/EC and justification is not required (Article 36 CLP Regulation).

5. IDENTIFIED USES

Zinc pyrithione is used in the context of Regulation (EC) No 528/2012 as an active substance in Product Types 2, 6, 7, 9, 10 and 21, *i.e.*:

- Private area and public health area disinfectants and other biocidal products
- In-can preservatives
- Film preservatives
- Fibre, leather, rubber and polymerised materials preservatives
- Masonry preservatives
- Antifouling products

Zinc pyrithione is also used in rinse-off products (excluding oral hygiene products) and in leave-on hair products which are regulated under the Cosmetics Regulation 1223/2009 (SCCS, 2014).

6. DATA SOURCES

A dossier was received by RMS Sweden from the European Zinc Pyrithione Task Force (EZPTF) consisting of Arch Chemicals Inc. (now Lonza) and Weylchem Gmbh (now Janssen PMP) for review under the Biocidal Directive 98/8/EC (now replaced by the Biocides Regulation (EU) 528/2012). The biocide Competent Authority Report (CAR) based on the dossier is structured as follows:

- Assessment Report
- Doc II Risk Assessment:
- Doc IIA: Effects assessment of active substance
- Doc IIB: Effects and exposure assessment of biocidal product(s)
- Doc IIC: Risk Characterisation for use of active substance in biocidal product(s)
- Doc III: Study Summaries
- Doc IIIA: Active substance
- Doc IIIB: Biocidal product(s)

This report has been prepared based on the data on zinc pyrithione that was submitted in the dossier and evaluated in the CAR. References are made to the study summaries provided in Doc IIIA. The study summaries from Doc IIIA referred to in this report are also provided in confidential appendices to the IUCLID file. Furthermore, information from a dossier on zinc pyrithione submitted by Thor GmbH in June 2015 as part of their BPR (Regulation (EU) 528/2012) Article 95 notification of the substance is also considered in this report. REACH registration dossiers for zinc pyrithione are also available[1] and the relevant data from these is considered in this CLH report.

Zinc pyrithione has also been evaluated by the Scientific Committee for Consumer Products (SCCP) in connection to its use in anti-dandruff shampoo. Reference is made to the SCCP (2014) report.

The Dossier Submitter (DS) acknowledges that zinc pyrithione shows some structural similarity to sodium pyrithione (EC 223-296-5) and copper pyrithione (238-984-0), in that they share the common organic moiety i.e. pyrithione. The DS has assessed a position paper provided by the zinc pyrithione task force in May 2016 wherein a category read-across for sodium-, copper- and zinc pyrithiones was proposed based on the Read-Across Assessment Framework (RAAF) by ECHA[2]. However, for zinc pyrithione there is reliable and adequate substance-specific information (i.e. a complete dataset[3]) precluding the necessity to consider a grouping and/or read-across (see sections 1.1.1.1 and 1.1.1.3 of Annex I to the CLP Regulation). Therefore, the DS submitted a CLH report on zinc pyrithione without including data from the other pyrithiones. In other words, the DS does not use grouping and/or read-across in the CLH proposal for zinc pyrithione.

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	Solid	Figura, 1997a (A3.3/01)	Visual inspection
Melting/freezing point	267°C	Figura, 1997a (A3.1.1/01)	Purified grade a.i. (>95%)
	Decomposition before melting starting at 240°C	Wenighofer, 2002 (A3.1.1/02)	Technical grade a.i. (>95%)
	Self heating of a bulk sample (30 g) starts at 175°C, rapid decomposition at 210-220°C, with max rate at 270°C. Decomposition adducts O2, N2, CO, CO2, COS, CS2 and SO2. ZnPT should not be allowed to reach 150°C to provide a reasonable margin of safety	Cruice, 1976 (A3.10/02) and Polson, 1991 (A3.10/01)	Technical grade a.i. (purity not stated)

[1] https://echa.europa.eu/information-on-chemicals/registered-substances

[2] https://echa.europa.eu/documents/10162/13628/raaf_en.pdf. The DS is aware that the zinc pyrithione task force intends to submit a non-confidential version of the read-across position paper during the Public Consultation. Therefore, this should be available on ECHA webpages later.

[3] Except for the carcinogenicity endpoint (see section 10.9); and for the rapid degradability of zinc pyrithione, information from copper pyrithione dossier was used as supportive evidence for aquatic degradation of a common degradation product (PSA, see section 11.4).

Boiling point	-	Figura, 1997a (A3.1.1/01) Wenighofer, 2002 (A3.1.2/01)	Not relevant as the melting point is high (purified a.i.)/decomposition occurs upon melting (technical grade)
Relative density	1.76 g/cm^3 at 20.1ºC	Figura, 1997a (A3.1.3/01)	Technical grade a.i. (>95%)
	1.81 g/cm^3 at 22.4-22.5ºC	Wenighofer, 2002 (A3.1.3/01)	Technical grade a.i. (>95%)
Vapour pressure	<1 x 10^{-6} Pa at 25°C	Figura, 1997a (A3.2/01)	Based on LOQ of the HPLC-method used for quantification
Surface tension	63.8 mN/m at 20.1°C for 90% saturated aqueous solution	Wenighofer, 2002 (A3.13/02)	Technical grade a.i. (>95%)
Water solubility	7.15 mg/L at 20°C and pH 6.4-8.0 (non-buffered distilled water)	Figura, 1997a (A3.5/01)	Technical grade a.i. (>95%)
	<u>20°C</u> 4.93 mg/L at pH 7.3-7.6 <u>30°C</u> 6.11 mg/L at pH 7.2-7.4	Wenighofer, 2002 (A3.5/02)	Technical grade a.i. (>95%)
	<u>At 25°C</u> pH 4: 15.5 mg/L pH 5: 9.03 mg/L pH 7: 6.48 mg/L pH 8.3: 6.32 mg/L pH 10: 17.0 mg/L	Quin, 2001 (A3.5/03)	The shown difference in solubility at the different pH is neither considered significant nor to be attributed to a dissociation behaviour of ZnPT under the conditions of the study.
Partition coefficient n-octanol/water	At 20°C: Log P_{ow}= 0.88 (in distilled water at pH 6.4-6.5)	Wenighofer, 2002 (A3.9/02)	Given that the solubility in water was 2.4 and 2.6 times higher at pH 4 and 10 respectively than at pH 7 a log Pow of ~0.5 is anticipated at pH 4 and 10.
Flash point	-	Document III-A3.12	Not applicable as the melting point is >40°C
Flammability	Not highly flammable	Russel, 1996 (A3.11/01) Wenighofer, 2002 (A3.11/02)	Technical grade a.i. (>95%)
Explosive properties	Zinc pyrithione is not explosive	Russel, 1996 (A3.15/01) Wenighofer, 2002 (A3.15/02)	Technical grade a.i. (>95%)
Self-ignition temperature	Self-heating starting at 215°C. Self-ignition according to the definition in guideline at 254°C	Wenighofer, 2002 (A3.11/02)	Technical grade a.i. (>95%)

	Dust ignites at 200-205°C	Cruice, 1976 (A3.10/02)	Technical grade a.i. (purity not stated)
	Self heating of a bulk sample (30 g) starts at 175°C, rapid decomposition at 210-220°C, with max rate at 270°C. Decomposition adducts O2, N2, CO, CO2, COS, CS2 and SO2. ZnPT should not be allowed to reach 150°C to provide a reasonable margin of safety	Polson, 1991 (A3.10/01)	Technical grade a.i. (purity not stated)
Oxidising properties	Zinc pyrithione is not oxidising	Russel, 1996 (A3.16/01)	Technical grade a.i. (>95%)
Granulometry	No data available	-	-
Stability in organic solvents and identity of relevant degradation products	Zinc pyrithione is stable within antifouling formulations	DeMatteo, 2009a (A3.8/01) DeMatteo, 2009b (A3.8/02)	Specific formulations tested at 14 days at 54 °C (solvent not reported)
Dissociation constant	Formation constant for the metal complex ZnPT: log K1 = 5.3	Sun *et al*, 1964 & Song *et al*, 1990 (A3.5/01-02) and document III-A3.6	The published articles indicate that the equilibrium is strongly shifted towards the formation of the metal complex ZnPT. Due to the high formation constant no dissociation (breakage) of the metal complex is anticipated during the conditions of e.g. the water solubility study. Nevertheless at environmentally relevant concentrations (i.e. very dilute), ZnPT is suspected to dissociate and the equilibrium and speciation for that reaction might be pH dependant
Viscosity	-	Document III-A3.14	Not applicable as zinc pyrithione is a solid

8. EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC A.14	Zinc pyrithione is not explosive	Technical grade a.i. (>95%)	Russel, 1996 (A3.15/01) Wenighofer, 2002 (A3.15/02)

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

Two studies performed in accordance with EEC A.14 were provided. These studies were both negative.

8.1.2 Comparison with the CLP criteria

Zinc pyrithione does not conform to the waiving criteria for explosive properties based on the structure due to the presence of N-O bonds (N-oxide). The oxygen balance is also not less than -200 (i.e. -111). Moreover, it is not evident from the CLP-guidance that a negative test according to method EEC A.14 automatically means that it is to be regarded as a non-explosive under CLP.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed due to the lack of data derived in accordance with the CLP guidance.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable (zinc pyrithione is not a gas).

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant.

8.2.2 Comparison with the CLP criteria

Not relevant.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable.

8.3 Oxidising gases

Hazard class not applicable (zinc pyrithione is not a gas).

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant.

8.3.2 Comparison with the CLP criteria

Not relevant.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable.

8.4 Gases under pressure

Hazard class not applicable (zinc pyrithione is not a gas).

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant.

8.4.2 Comparison with the CLP criteria

Not relevant.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable.

8.5 Flammable liquids

Hazard class not applicable (zinc pyrithione is not a liquid).

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not relevant.

8.5.2 Comparison with the CLP criteria

Not relevant.

8.5.3 Conclusion on classification and labelling for flammable liquids

Hazard class not applicable.

8.6 Flammable solids

Table 10: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10	Not highly flammable	Technical grade a.i. (>95%)	Wenighofer, 2002 (A3.11/02)
EEC A.10	Not highly flammable	Technical grade a.i. (>95%)	Russel, 1996 (A3.11/01)

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Two studies performed in accordance with EEC A.10 were provided. These studies were both negative and zinc pyrithione is to be regarded as not highly flammable in the sense of the test method.

In the first study (Wenighofer, 2002) the no propagation of flame was observed within 4 minutes in the preliminary test of EEC A.10.

In the second study (Russel, 1996), no propagation of flame was observed in the preliminary test of EEC A.10. However, the test material melted very quickly, black smoke and an orange flame was observed. Therefore the full test of EEC A.10 was performed which was negative (i.e. the material melted but did not ignite).

8.6.2 Comparison with the CLP criteria

The first study (Wenighofer, 2002) was negative in the preliminary test of EEC A.10. This means that the test material should also not be classified as a flammable solid under CLP (i.e. the preliminary test of EEC A.10 and the screening test in CLP are principle the same).

In the second study (Russel, 1996), the material did not ignite in the main test of EEC A.10. Even if the set-up of the main test in EEC A.10 is not the same as in the burning rate test recommended in CLP (UN-MTC,33.2.1), the fact that the material did not ignite means that the test material should not be classified as a flammable solid under CLP.

8.6.3 Conclusion on classification and labelling for flammable solids

No classification is proposed. Data is conclusive but not sufficient for classification.

8.7 Self-reactive substances

Data lacking.

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

No data has been provided addressing this property.

8.7.2 Comparison with the CLP criteria

No data has been provided that addresses this property. Zinc pyrithione does not conform to the waiving criteria for self-reactive substances as there chemical groups associated with explosive properties (i.e. due to the presence of the N-oxides).

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed based on the lack of data.

8.8 Pyrophoric liquids

Hazard class not applicable (zinc pyrithione is not a liquid).

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

Not relevant.

8.8.2 Comparison with the CLP criteria

Not relevant.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Hazard class not applicable.

8.9 Pyrophoric solids

Data lacking.

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No specific data derived in accordance with the recommended test method in CLP has been provided. However, zinc pyrithione has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections).

8.9.2 Comparison with the CLP criteria

Based on experience in handling of zinc pyrithione, it is not a pyrophoric solid (compare with example in CLP guidance section 2.10.7.2).

8.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

8.10 Self-heating substances

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC A.16	Self-heating starting at 215°C. Self-ignition according to the definition in guideline at 254°C	Technical grade a.i. (>95%)	Wenighofer, 2002 (A3.11/02)
Godbert-Greenwald Furnace (minimum ignition temperature of dust layers)	Dust ignites at 200-205°C	Technical grade a.i. (purity not stated)	Cruice, 1976 (A3.10/02)
Heating in a bomb	Self-heating of a bulk sample (30 g) starts at 175°C, rapid decomposition at 210-220°C, with max rate at 270°C. Decomposition adducts O_2, N_2, CO, CO_2, COS, CS_2 and SO_2. ZnPT should not be allowed to reach 150°C to provide a reasonable margin of safety.	Technical grade a.i. (purity not stated)	Polson, 1991 (A3.10/01)

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

The data available indicate that self-heating (in the sense of the test method used) starts at 175°C for bulk samples and around 200°C for smaller samples. Zinc pyrithione dust ignites at ~200°C.

8.10.2 Comparison with the CLP criteria

The data available indicate that the onset temperature for self-heating of zinc pyrithione (of bulk samples) is >140 °C and that no classification is thus warranted. However, the data has not been generated in accordance with the recommended test method in CLP and a full assessment can thus not be made.

8.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed due to lack of data.

8.11 Substances which in contact with water emit flammable gases

Data lacking.

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No specific data derived in accordance with the recommended test method in CLP has been provided. However, zinc pyrithione has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas. Moreover, zinc pyrithione is produced commercially in water solutions (for example in dandruff shampoos).

8.11.2 Comparison with the CLP criteria

Based on experience in handling of zinc pyrithione, it is not a substance which in contact with water emit flammable gases (compare with CLP guidance section 2.12.3.2).

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

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

8.12 Oxidising liquids

Hazard class not applicable (zinc pyrithione is not a liquid).

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant.

8.12.2 Comparison with the CLP criteria

Not relevant.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Hazard class not applicable.

8.13 Oxidising solids

Table 12: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC A.17	Zinc pyrithione is not oxidising	Technical grade a.i. (>95%)	Russel, 1996 (A3.16/01)

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

A test performed in accordance with EEC A.17 was provided. The study was negative in the sense of the test method. The sample did not burn in a 4:1 mixture with cellulose but did so in the 1:1 mixture.

8.13.2 Comparison with the CLP criteria

In the decision logic in CLP it is checked whether the sample in the 4:1 or 1:1 mixture with cellulose ignite or burn. In the case of the study provided zinc pyrithione burned in the 1:1 mixture with cellulose (a distance of 19 mm). Since the first step is not passed it should be checked whether the burning rate (for the 1:1 mixture) was less than or equal to that of a 3:7 mixture of potassium bromate. In the case of EEC A.17 ammonium nitrate is used instead of potassium bromate as a reference oxidiser. A conclusion on the need for a classification under CLP can thus not been made even though it is noted that the burning rate for zinc pyrithione:cellulose 1:1 is less than that of ammonium nitrate:cellulose 3:7 which indicates that no classification is warranted under CLP.

8.13.3 Conclusion on classification and labelling for oxidising solids

No classification is proposed due to the lack of data derived in accordance with the CLP guidance.

8.14 Organic peroxides

Hazard class not applicable (zinc pyrithione is not an organic peroxide).

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not relevant.

8.14.2 Comparison with the CLP criteria

Not relevant.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable (zinc pyrithione is not an organic peroxide).

8.15 Corrosive to metals

Data lacking.

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data has been provided addressing this property.

8.15.2 Comparison with the CLP criteria

No data has been provided that addresses this property. Zinc pyrithione is a substance that should be considered for this hazard class (CLP guidance section 2.15.3.1) as the pyrithione part is able to form complexes with metals. However, zinc pyrithione itself is a very stable metal complex; only copper forms more stable pyrithione complexes.In addition to this zinc pyrithione lacks acidic or basic functional groups. In conclusion thus, this indicate that zinc pyrithione should not be corrosive towards copper free steel and aluminium. Nevertheless, a thorough evaluation cannot be done due to the lack of data derived using the recommended test method in CLP.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed due to the lack of data.

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 13: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
ADME No specific guideline, no GLP Published study Oral gavage Rat, Sprague-Dawley (males): 4 Rabbit, New Zealand (females): 4 Monkey, Rhesus (Macaca mulatta) (females): 2 (disposition of radio activity), 3 (urinary metabolite analysis) Dog, Beagle (male): 4 Dose levels: 1 mg/kg bw 2 additional dogs were dosed with 6 mg/kg bw	Absorption: Oral abs >80%. Distribution: Tissue distribution not investigated. Metabolism: The same terminal metabolite, 2-pyridinethiol-1-oxide-S-glucuronide, was found in all species investigated. Excretion: Excretion was rapid (>95% in 72 h), principally via the urine as metabolites (75-94%), faecal excretion being a minor route of excretion (2.6 (rat) - 20% (rabbit)).	ZnPT Reliability: 2 Not GLP No repeated dose group No high-dose group Only male rats 4 rats only No tissue distribution (except carcass) No metabolite analysis in faeces	ZnPT CAR Doc IIIA A6.2/04 Year: 1980
ADME No specific guideline GLP Oral gavage Rat, Sprague-Dawley (Crt:CD®(SD)IGS BR) 5 females/group Dose levels: ZnPT - 5 mg/kg/day for 6 days using non-radiolabelled dose followed by 2 days using radiolabelled dose CuPT - 4 mg/kg/day for 6 days using non-radiolabelled dose followed by 2 days using radiolabelled dose	Absorption: Oral abs >80%. Distribution: Day 8: 49% of the radioactivity remained in the carcass and 1% in blood. Tissue distribution not investigated. Metabolism: Major plasma metabolite: 2-methylsulfonylpyridine. Excretion: Mostly in urine, 63.5% at 24 h. Faeces: 1% at 24 h. Comparison between ZnPT and CuPT: Similar ADME. Differences in effects on body weight, muscle mass and muscle tone.	CuPT and ZnPT Reliability: 2	ZnPT CAR Doc IIIA A6.2/01 Year: 2002
Percutaneous absorption OECD 427 GLP Rat, Sprague-Dawley Dose levels: 100 µl/10 cm^2 or 10 µl/cm^2 2 hour and 8 hours exposure time, 4, 24, 48 and 96 hours sampling time.	A dermal absorption rate of approximately 1-3% was observed, however, no exact value for dermal absorption could be determined. The study can be used as supportive evidence of low dermal absorption.	ZnPT Reliability: 3, since four rats only were used per dose and time point, there were large variations in the data and the test substance seems to have been orally ingested.	ZnPT CAR Doc IIIA A6.2/03 Year: 2005

A toxicokinetics study (OECD 417) and an in vitro dermal absorption study (OECD 428) are available from a dossier on zinc pyrithione submitted by Thor GmbH as part of their Article 95 notification of the substance just before the CLH report was finalised by the DS. These are summarised in the table below.

Table 14: Summary table of toxicokinetic studies from Thor GmbH Art. 95 dossier

Study	Guideline	Species, strain, sex / test system Dose/conc. levels	Dose descriptor/results
Absorption, distribution, metabolism and excretion of zinc pyrithione in the Wistar rats after single and repeated oral administration Year: 2015 Reliability: 1	OECD 417 GLP	Rat, Wistar Han, male and females 2 or 10 mg/kg bw	Highly absorbed, readily distributed into all organs, extensively metabolised and mainly excreted via the urine. Metabolites: 2-mercaptopyridine N-oxide S-glucuronide, 2-mercaptopyridine S-glucuronide, 2-mercaptopyridine S-cysteine, N-acetylcysteine of 2-mercaptopyridine, 2-methylthiopyridine-N-oxide, 2-methylsulphonylpyridine.
Determination of the dermal absorption of zinc pyrithione through human skin *in vitro* Year: 2014 Reliability: 1	OECD 428 GLP	Human skin 297 g/L or 0.56 g/L	Dermal penetration values concentrate: 0.1 ± 0.04 % dilution: 0.6 ± 0.4 %

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

The metabolism and disposition of zinc pyrithione in rabbit, rat, monkey and dog after oral exposure was investigated in a published non-guideline, non-GLP study (ZnPT CAR Doc IIIA A6.2/04). Oral absorption was 73% in rabbits, 81% in rats, 86% in monkeys and 94% in dogs, calculated as the amount of radioactivity found in urine and carcass (including tissues). The rabbit values are unreliable however as the faeces were contaminated with urine and thus oral absorption was probably higher. The metabolic profiles confirmed the presence of the same terminal metabolite in all the species investigated, 2-pyridinethiol-1-oxide-S-glucuronide, which is the glucuronic acid conjugate of free pyrithione. Excretion of zinc pyrithione was found to be rapid (>95% in 72 h), principally via the urine as metabolites (75-94%) with faecal excretion being a minor route of excretion (2.6-20%). There was no observable trend for bioaccumulation. The carcass of the rat contained an average of 5.9% of the dose after 72 hours.

A study with zinc pyrithione and copper pyrithione (ZnPT CAR Doc IIIA A6.2/01) demonstrated that zinc pyrithione was efficiently absorbed as there was a low recovery of radioactivity in the faeces (ZnPT: 1.3% within 24 hours after dosing). Oral absorption calculated as total amount found in urine, blood and carcass (including tissues) was >80% for zinc pyrithione. Four hours after the second radio-labelled ZnPT dose on day 8, 49% of the radioactivity remained in the carcass. Radioactivity in blood was 1% of the total administered radioactivity at the same time of measuring. The radioactivity was distributed equally between the RBC and the plasma. Zinc pyrithione and copper pyrithione were metabolised in the same way as shown by the almost identical radiochromatograms obtained with the two substances, respectively. However the urine metabolites were not identified. No pyrithione was detected in the plasma from any animal and the major plasma metabolite in both dose groups was identified as 2-methylsulfonylpyridine. There were no significant differences between the two dose groups in the distribution of radioactivity in the urine, faeces, blood, plasma, red blood cells or carcass. However, actual tissue distribution was not investigated.

The same study also looked at similarities and differences in toxicological effects after treatment with the two substances, respectively. Female rats were dosed with 4, 6, 9 or 12 mg CuPT /kg bw/day or 3, 5, 8 or 11 mg ZnPT /kg bw/day for 9 days in a range-finding study. Three animals per group were used. Irregular gait, lethargy and hind limb paralysis were the most notable observations, with CuPT-dosed animals more affected than ZnPT-dosed animals. All animals dosed with CuPT except two in the lowest dose group had greatly reduced hind limb muscle mass and very low muscle tone while the ZnPT-dosed animals had normal to slightly reduced muscle mass. The functional assessments in animals dosed with ZnPT were somewhat intermediate between vehicle- and CuPT-dosed groups. Body weights were more severely affected after treatment with CuPT than with ZnPT and the difference was statistically significant.

Another study (ZnPT CAR Doc IIIA A6.2/03) was performed according to OECD 427 to determine the extent of absorption, distribution and elimination of zinc pyrithione following topical application of two paint formulations (architectural paint and antifouling paint) to rats *in vivo*. The first test paint (antifouling paint) contained Zn[^{14}C]PT at ca 5 % (w/w). The second test paint (architectural paint) was water based and contained Zinc [^{14}C]-pyrithione at ca 0.5 % (w/w). The results indicated that the dermal absorption of zinc pyrithione is low as levels of radioactivity measured in whole blood, plasma or subcutaneous fat were, in most samples, below the reliable limit of detection. In addition, when levels of radioactivity were observed to be significantly higher than the limit of detection, these were associated with the GI tract which was indicative of oral ingestion. A dermal absorption rate of approximately 1-3 % was observed, however no exact value could be determined due to a large variation in the data, few data points (four rats only were used per group) and the fact that the test substance seems to have been orally ingested. The study was thus considered to be of low reliability.

In the toxicokinetics study (Thor GmbH Art. 95 dossier, 2015) performed according to OECD 417 and with GLP compliance, Wistar Han rats were given single and repeated oral doses of 2 or 10 mg/kg bw. Zinc pyrithione was highly absorbed, readily distributed into all organs, extensively metabolised and mainly excreted via the urine in this study. The following metabolites were identified in the study: 2-mercaptopyridine N-oxide S-glucuronide, 2-mercaptopyridine S-glucuronide, 2-mercaptopyridine S-cysteine, N-acetylcysteine of 2-mercaptopyridine, 2-methylthiopyridine-N-oxide, 2-methylsulphonylpyridine.

In the study (Thor GmbH Art. 95 dossier, 2014) for determination of dermal absorption using human skin performed according to OECD 428 and with GLP compliance, the dermal penetration values for zinc pyrithione at 297 g/L and 0.56 g/L are 0.1±0.04% and 0.6±0.4%, respectively.

10. EVALUATION OF HEALTH HAZARDS

Several new studies, relevant for evaluation of the health hazards, performed during 2013 and 2014 are available from a dossier on zinc pyrithione submitted by Thor GmbH as part of their Article 95 notification of the substance just before the CLH report was finalised by the dossier submitter (DS). All these new studies are evaluated by the DS and briefly summarised in the tables 15a and 15b below. Among these, the studies that affected the conclusions on classification and labelling that were based on studies from ZnPT CAR Doc IIIA, amongst other, are described under the respective endpoint sections of this report. The studies that did not affect the conclusions on classification and labelling are briefly reported under the respective endpoints.

The reliability scores 1 to 4 assigned to the studies in this CLH report correspond to Klimisch scores 1 to 4.

In all of the studies presented in the tables 15a and 15b, the purity of zinc pyrithione was >95% and came from the same batch.

Table 15a: Summary of *in vitro* studies from Thor GmbH Art. 95 dossier

Study	Guideline	Test system Conc. levels	Results	Described under the respective endpoint sections in this report (Yes/No)
In vitro skin irritation test with zinc pyrithione using a human skin model Reliability: 1 Year: 2013	OECD 439 GLP	EPISKIN Small Model™ 10.2 to 12.4 mg	Equivocal under the experimental conditions	No
Screening for the eye irritancy potential of zinc pyrithione using the bovine corneal opacity and permeability test Reliability: 1 Year: 2013	OECD 437 GLP	Bovine cornea in an isolated system 311 to 320 mg	Not irritating	No
Evaluation of the mutagenic activity of zinc pyrithione in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay Reliability: 1 Year: 2014	OECD 471 GLP	*S. typhimurium:* TA 1535, TA 1537, TA 98 and TA 100 *E. coli:* WP2uvrA First experiment: 0.3 to 100 µg/plate Second experiment: 1 to 100 µg/plate	+ S9-mix: Negative - S9-mix: Negative	No
Evaluation of the ability of zinc pyrithione to induce chromosome aberrations in cultured peripheral human lymphocytes Reliability: 1 Year: 2014	OECD 473 GLP	Cultured peripheral human lymphocytes First experiment, - S9-mix: 0.3, 10 and 15 µg/ml (3 h exp. and 24 h fix.) + S9-mix: 0.3, 15 and 20 µg/ml (3 h exp. and 24 h fix.) Second experiment, - S9-mix: 0.1, 0.66 and 1.5 µg/ml (24 h exp. and 24 h fix.); 0.66, 1.5 and 2 µg/ml (48 h exp. and 48 h fix.) + S9-mix: 3, 15 and 30 µg/ml (3 h exp. and 48 h	+ S9-mix: Positive - S9-mix: Positive	No

Study	Guideline	Test system Conc. levels	Results	Described under the respective endpoint sections in this report (Yes/No)
		fix.)		
Evaluation of the mutagenic activity of zinc pyrithione in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells Reliability: 1 Year: 2014	OECD 476 GLP	L5178Y mouse lymphoma cells Concentrations tested: -S9: 0, 0.01, 0.03, 0.065, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/mL +S9: 0, 0.4, 0.6, 1, 1.5, 2, 2.5, 3 and 3.5 µg/mL Positive controls: -S9: Methyl methanesulfonate +S9: Cyclophosphamide	+ S9-mix: Positive - S9-mix: Positive	Yes

Table 15b: Summary of *in vivo* studies from Thor GmbH Art. 95 dossier

Study	Guideline	Species, strain, sex Dose/conc. levels	Dose descriptor/results	Described under the respective endpoint sections in this report (Yes/No)
Assessment of acute oral toxicity with zinc pyrithione in the rat Reliability: 1 Year: 2014	OECD 423 GLP	Rat, Wistar Han, females Stepwise procedure: 2000, 300, 50 mg/kg bw	LD50: 300 mg/kg bw	No
Assessment of acute dermal toxicity with zinc pyrithione in the rat Reliability: 1 Year: 2014	OECD 402 GLP	Rat, Wistar Han, males and females 2000 mg/kg bw	LD50: > 2000 mg/kg bw	No
Assessment of acute inhalation toxicity with zinc pyrithione in the rat Reliability: 1 Year: 2014	OECD 403 GLP	Rat, Wistar Han, males and females 0.5, 0.05 mg/L	LC50: within the range 0.05 – 0.5 mg/L	Yes
Primary skin irritation/corrosion study with zinc pyrithione in the rabbit	OECD 404 GLP	Albino rabbit, New Zealand White, males 0.5 g	Not irritating	No

Study	Guideline	Species, strain, sex Dose/conc. levels	Dose descriptor/results	Described under the respective endpoint sections in this report (Yes/No)
Reliability: 1 Year: 2014				
Acute eye irritation/corrosion study with zinc pyrithione in the rabbit Reliability: 1 Year: 2014	OECD 405 GLP	Albino rabbit, New Zealand White, males Ca. 42 mg	Irreversible damage	No
Assessment of contact hypersensitivity to zinc pyrithione in the mouse Reliability: 1 Year: 2014	OECD 429 GLP	Mice, CBA/J, females 10, 25 or 50 % w/w	EC3: > 50 %	No
Mammalian Erythrocyte Micronucleus Test (combined with the Comet assay) Reliability: 1 Year: 2014	OECD 474 GLP	Rat, Wistar Han, males 25, 50 and 100 mg/kg bw <u>Positive control:</u> Cyclophosphamide	Negative	No
In vivo Comet assay (combined with the Micronucleus test) Reliability: 1 Year: 2014	ICH S2 (R1), 2012; Tice et al., 2000; Smith et al., 2008; Bowen et al., 2011 GLP	Rat, Wistar Han, males and females 0, 25, 50 and 100 mg/kg bw Positive control: Ethyl methanesulfonate	Negative	Yes
Two-generation reproductive toxicity study in rats by daily gavage Reliability: 1 Year: 2015	OECD 416 GLP	Rat, Wistar Han, males and females 0, 0.2, 0.5, 2.5 mg/kg bw	NOAEL for systemic effects (parental and F1): 0.5 mg/kg bw based on the following effects at the next dose level: toxicologically relevant effects on the skeletal muscle NOAEL for reproductive and developmental toxicity: 2.5 mg/kg bw, the highest dose tested	Yes
Prenatal developmental toxicity study in rats by	OECD 414	Rat, Wistar Han, females	NOAEL for maternal toxicity: 1.18 mg/kg bw	Yes

Study	Guideline	Species, strain, sex Dose/conc. levels	Dose descriptor/results	Described under the respective endpoint sections in this report (Yes/No)
dietary administration Reliability: 1 Year: 2015	GLP	0, 5, 15, 25 ppm (0, 0.4, 1.18, 1.68 mg/kg bw)	based on following effects at the next dose level: clinical signs including abnormal gait (among others), lower body weights and body weight gains, and lower food consumption. NOAEL for developmental toxicity: 1.18 mg/kg bw based on following effects at the next dose level: lower foetal body weights.	
Prenatal developmental toxicity study in rabbits by oral gavage Reliability: 1 Year: 2015	OECD 414 GLP	Rabbit, New Zealand White, females 0, 0.5, 1.5, 4 mg/kg bw	NOAEL for maternal toxicity: 1.5 mg/kg bw based on following effects at the next dose level: red/orange discolouration of the urine, reduced body weight gains and reduced food consumption NOAEL for developmental toxicity: 0.5 mg/kg bw based on increased incidence of early resorptions and malformations at 1.5 and 4 mg/kg bw, and reduced number of litters and decreased foetal body weights at 4 mg/kg bw.	Yes
90-day oral toxicity study combined with a neurotoxicity study with zinc pyrithione by daily gavage in the rat followed by a 14-day recovery period Reliability: 1 Year: 2014	OECD 408 and 424 GLP	Rat, Wistar Han, males and females 0, 0.2, 0.5 and 2.5 mg/kg bw	NOAEL: 0.5 mg/kg bw Based on the following effects observed at the next dose level: clinical signs, lower body weight/weight gains and effects on the hindlimb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration.	No

10.1 Acute toxicity - oral route

Table 16: Summary table of animal studies on acute oral toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 GLP Reliability: 2, since purity of the test substance was not specified.	Rat, Wistar Albino 5/sex/dose	48% dispersion of Zinc pyrithione Batch: specified Purity: not specified	125, 158, 200, 254 and 321 mg/kg bw 14 days post exposure period	221 mg/kg bw	ZnPT CAR Doc IIIA A6.1.1/01 Year: 1986
OECD 401 GLP Reliability: 2, since purity of the test substance was not specified.	Rat Sprague-Dawley CD 5 females /dose	Zinc pyrithione powder in arachis oil Batch: specified Purity: not specified	500, 707 and 1000 mg/kg bw 14 days post exposure period	774 mg/kg bw	ZnPT CAR Doc IIIA A6.1.1/02 Year: 1997
Not specified	Rat Strain not specified	Zinc pyrithione Batch/purity: not specified	Not specified	92-266 mg/kg	SCCS opinion on zinc pyrithione Year: 2014
Acute neurotoxicity OECD 424 Reliability: 1 for acute toxicity	Rat Crl:CD®(SD)IGS BR VAF/Plus®	Zinc pyrithione Batch: specified Purity: >95%	25, 75, 150 mg/kg bw	4/10 females died at 150 mg/kg bw	ZnPT CAR Doc IIIA A6.9/01 Year: 2005

Table 17: Summary table of human data on acute oral toxicity

No data is available.

Table 18: Summary table of other studies relevant for acute oral toxicity

No data is available.

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

Two studies performed with rats and according to GLP and OECD 401 are available on the acute oral toxicity of zinc pyrithione. The purity of the test substance was not stated in either study, making the results unreliable. In the first study (Doc IIIA A6.1.1/01), mortality was noted between 1 and 4 days after dosing. The deaths were preceded by signs of ptosis, diarrhoea, lethargy, piloerection, chromomdacryorrhea, chromorhinorrhea, emaciation, soiling of the body surfaces, and wetness and brown staining of the anogenital area. Necropsy of the dead animals revealed abnormalities of the lungs, liver, spleen and gastrointestinal tract. Reductions in body weight were seen in the mid and high-dose groups but generally returned to normal by day 14. LD_{50} was found to be 221 mg/kg.

In the second study (Doc IIIA A6.1.1/02), according to GLP and OECD 401, mortality was noted between 1 and 7 days after dosing. Signs of systemic toxicity were noted in all dosed groups and included ataxia, diuresis, hunched posture, lethargy and decreased respiratory rate. Red/brown stains around the eyes or snout, piloerection, ptosis and splayed or tiptoe gait were also seen in females of the mid and high-dose groups. The animals that died lost weight while surviving animals gained weight during the post-exposure period. Necropsy of the decedents revealed haemorrhagic or abnormally red lungs, dark liver and kidneys sloughing of the non-glandular epithelium of the stomach and haemorrhage and sloughing of the gastric mucosa. LD_{50} was found to be 774 mg/kg in this study.

In the study (Thor GmbH Art. 95 dossier, 2014) for assessment of acute oral toxicity with zinc pyrithione in the rat, performed according to OECD 423 and with GLP compliance, the LD_{50} was found to be 300 mg/kg bw.

The Scientific Committee on Consumer Safety (SCCS) Opinion on Zinc Pyrithione states that "*LD_{50} values for zinc pyrithione have been determined in various species after oral administration. The values in the rat ranged from 92 to 266 mg/kg and in the mouse from 160 to 1000 mg/kg. Six hundred mg/kg was found to be the LD_{50} when administered orally to dogs*". These studies are not available for evaluation by the DS but it is noted that the studies in rats gave similar results as the evaluated studies and supports classification in the same category. They have therefore not been further evaluated.

An acute neurotoxicity study performed in rats according to GLP and OECD 424 is also mentioned here. Although the aim of the study was to investigate neurotoxicity and establish a NOAEL, it is noted that 4/10 females died at the highest dose level of 150 mg/kg bw. This indicates that the LD_{50} in this study was slightly above 150 mg/kg bw for females in this study.

10.1.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) a substance should be classified as Acute Tox. 3 if the LD_{50} is within the limits $50 < ATE \leq 300$ (oral, mg/kg bw). In all rat studies on zinc pyrithione except one the LD_{50} values obtained were within these limits.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification in Acute Tox. 3 (hazard statement H301 - Toxic if swallowed) is proposed for zinc pyrithione.

10.2 Acute toxicity - dermal route

Table 19: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Value LD_{50}	Reference
US EPA 81- 2 GLP Reliability: 2, since purity of the test substance was not specified.	Rat Sprague-Dawley CD 5 per sex	Zinc pyrithione Batch: specified, Purity: not specified	2000 mg a.i./kg bw (limit test) 14 days post exposure period	>2000 mg/kg bw	ZnPT CAR Doc IIIA A6.1.2/01 Year: 1997

Table 20: Summary table of human data on acute dermal toxicity

No data is available.

Table 21: Summary table of other studies relevant for acute dermal toxicity

No data is available.

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

No signs of toxic effects were seen in a limit study according to GLP and EPA guideline 81-2 where acute dermal toxicity of zinc pyrithione was investigated in rats at a dose of 2000 mg/kg.

In the study (Thor GmbH Art. 95 dossier, 2014) for assessment of acute dermal toxicity with zinc pyrithione in the rat, performed according to OECD 402 and with GLP compliance, the LD_{50} was found to > 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

Not relevant as no effects were seen.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed for zinc pyrithione.

10.3 Acute toxicity - inhalation route

Table 22: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LC_{50}	Reference
Nose-only OECD 403 GLP Reliability: 2, since purity of the test substance was not reported.	Rat Sprague-Dawley CD albino 5/sex/dose	Zinc pyrithione Batch: Tox 1000 Purity: not specified	1.82, 0.95 and 0.53 mg/L MMADs 3.8, 3.5 and 3.3 µm 4 hours exposure; 14 days post exposure period	Males: 0.84 mg/L Females: 1.34 mg/L Males + females: 1.03 mg/L	ZnPT CAR Doc IIIA A6.1.3/01 Year: 1996
Nose-only US EPA 81-3, which complied with OECD 403. GLP Reliability: 1	Rat Sprague-Dawley CD 5/sex/dose	Zinc pyrithione Batch: specified Purity: ≥95 %	0.24 and 0.61 mg/L MMADs 1.9 and 2.3 µm 4 hours exposure; 14 days post exposure period	>0.61mg/L	ZnPT CAR Doc IIIA A6.1.3/03 Year: 1991
Nose-only OECD 403 Reliability: 1	Rat Wistar Han 5/sex/dose	Zinc pyrithione Batch: specified Purity: >95%	0.05 and 0.5 mg/L MMADs 2.7 – 4.4 µm 4 hours exposure; 14 days post exposure period	Within the range of 0.05 – 0.5 mg/L	Thor GmbH Art. 95 dossier Year: 2014
Whole-body US EPA 81-3, which complied with OECD 403. GLP Reliability: 3, since purity of the test substance was not reported and it is likely that the test substance was orally ingested by preening.	Rat Sprague-Dawley CD albino 5/sex/dose	48% (nominal) aqueous suspension of zinc pyrithione Batch/purity: not specified	0.054, 0.14, 0.16, 0.82, 1.4 and 1.5 mg/L MMADs 2.8-5.3 µm 4 hours exposure; 14 days post exposure period	0.14 mg/L	ZnPT CAR Doc IIIA A6.1.3/02 Year: 1991
OPPTS 870.1300 Not evaluated by DS	Rat Sprague-Dawley 5/sex/dose	Zinc pyrithione 48% dispersion Batch/purity: not specified	0.68, 1.19 and 2.25 mg/L 4 hours	5.08 mg/L	SCCS opinion on zinc pyrithione Year: 2014

Table 23: Summary table of human data on acute inhalation toxicity

No data is available.

Table 24: Summary table of other studies relevant for acute inhalation toxicity

No data is available.

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

Four studies which comply with GLP and OECD 403 are available on the acute inhalation toxicity of zinc pyrithione in rats. Three of the studies were performed with nose-only exposure and the fourth with whole body exposure. LC_{50} was found to be 0.84 mg/L in the first nose-only study; however purity of the test substance was not given. Common abnormalities were wet fur, hunched posture, piloerection, decreased respiratory rate, pallor of the extremities, ptosis, incidents of lethargy, ataxia, laboured gasping and noisy respiration and red/brown staining around the eyes, snout and mouth. Occasional or isolated incidents of increased respiratory rate, sneezing, dehydration, increased salivation and an apparent stiffness in the hind legs were also noted. The histopathological examination revealed lung abnormalities, excessive fluid in the thoracic cavity, liver changes, pale kidneys, incidents of congestion and reddening and gaseous distension in the gastro-intestinal tract. One female exposed to 0.53 mg/L showed dark foci on the lungs.

In the second nose-only exposure study 1/5 males died at 0.24 mg/L and 1/5 males and 2/5 females died at 0.61 mg/L. All deaths occurred at day 1 post-exposure. As only two dose levels were investigated in this study, no true LC_{50} value could be calculated but it was found to be more than 0.61 mg/L. Clinical signs of toxicity were increased salivation, laboured breathing, decreased activity and tremors were noted at both exposure levels on day of exposure. Gasping was also noted at the high exposure level. Congested or discoloured (red) lungs were noted at necropsy for all animals dying on study. Necropsy observations for all animals surviving to study end appeared normal.

In the third nose-only exposure study (5/sex/dose), at 0.5 mg/L, one male and one female were sacrificed on day 1 due to ethical reasons, and on day 2, two males and two females were found dead and the remaining animals were sacrificed due to ethical reasons. No mortalities occurred at the other dose level (0.05 mg/L) in this study. At 0.5 mg/L group, on days 1 and/or 2, all animals had lethargy, hunched posture, laboured respiration, gasping, bleeding of the nose, pale appearance, ptosis and/or hypothermia. Macroscopic examination of the dead animals showed dark red discolouration of the mandibular lymph nodes, gelatinous salivary glands, and yellowish content in jejunum and in ileum. At 0.05 mg/L, all animals showed lethargy, hunched posture, laboured respiration, rales and ptosis between days 1 and/or 5. No animals in this dose group showed any abnormalities at macroscopic examination. The LC_{50} value in this study was considered to be within the range of 0.05 – 0.5 mg/L.

The fourth study, performed with whole-body exposure, gave a LC_{50} value at 0.14 mg/L. Clinical signs of toxicity included prostration, gasping, laboured breathing, rales, trembling, urine-stained abdomen, lacrimation, hunched posture and red material around the nose/eyes/mouth. Gasping and laboured breathing were noted even at the lowest dose of 0.054 mg/L. Whole body exposure is considered a less accurate exposure method since an unknown amount of test substance is likely to be ingested by preening. The result of this study is therefore likely an overestimation of the inhalation toxicity of zinc pyrithione and the three nose-only exposure studies are considered for the purpose of classification of zinc pyrithione. In the first nose-only exposure study, the purity of zinc pyrithione was not specified.

The fifth study is not available to the DS and has not been evaluated in this context. LC_{50} is stated to be 5.08 mg/L which is considerably higher than the results obtained in the other studies.

10.3.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) a substance should be classified as Acute Tox. 2 if the LC_{50} is within the limits 0.05 < ATE ≤ 0.5 (inhalation of dust/mists, mg/l). The LC_{50} value in an acute inhalation toxicity study with high reliability and high purity of zinc pyrithione was within these limits.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification in Acute Tox. 2 (hazard statement H330: Fatal if inhaled) is proposed for zinc pyrithione.

10.4 Skin corrosion/irritation

Table 25: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404 GLP Reliability factor: 1	Rabbit, New Zealand Albino 3 females	Zinc pyrithione Batch no: specified Purity: >95 %	0.5 g (dry weight) 4 h	Observations made at 1, 24, 48 and 72 hours. Erythema: 0 Oedema: 0 Reversibility: Not applicable	ZnPT CAR Doc IIIA A6.1.4/01 Year: 2001

Table 26: Summary table of human data on skin corrosion/irritation

No data.

Table 27: Summary table of other studies relevant for skin corrosion/irritation

No data.

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The acute skin irritation potential of zinc pyrithione was investigated in a study on rabbit in accordance with GLP and OECD 404. General signs of toxicity were investigated daily and skin examinations were performed after 1, 24, 48 and 72 hours of patch removal. All treated areas were normal at each observations time. Zinc pyrithione was thus found not to be irritating to the skin.

In the study (Thor GmbH Art. 95 dossier, 2014) for assessment of skin irritation/corrosion in the rabbit, performed according to OECD 404 and with GLP compliance, zinc pyrithione was found to be not irritating.

In an *in vitro* skin irritation test using human skin (EPISKIN Small Model^{TM}), performed according to OECD 439 and with GLP compliance, zinc pyrithione gave equivocal results (Thor GmbH Art. 95 dossier, 2013).

10.4.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.2.2 a substance should be classified for skin irritation Category 2 in the case where

(1) Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Zinc pyrithione does not fulfil the criteria for skin irritation as the scores for erythema and oedema were below 2.3 in all animals at all time points and no signs of inflammation were observed.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed for zinc pyrithione.

10.5 Serious eye damage/eye irritation

Table 28: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 405 GLP Reliability factor: 1	Rabbit New Zealand White 1 female	Zinc pyrithione Batch no: specified Purity: >95%	84 mg 24h Examinations: 1 and 2 h.	Corneal opacity: 24 h: 4 Iritis: 24 h: no ophthalmological examination possible Reversibility: No Animal sacrificed at 24 hours due to the severity of the lesions.	ZnPT dossier Doc IIIA A6.1.4/02 Year: 2001

Table 29: Summary table of human data on serious eye damage/eye irritation

No data.

Table 30: Summary table of other studies relevant for serious eye damage/eye irritation

No data.

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

The acute eye irritation potential of zinc pyrithione was investigated in a study in rabbits in accordance with GLP and OECD 405. Severe and irreversible cornea lesions, redness and swelling were noted 24 hours post application. No observation of the iris was possible because of the severity of chemosis and of corneal alteration. Due to the severity of lesions the animal was euthanized and no additional animal were exposed to the test substance.

In the study (Thor GmbH Art. 95 dossier, 2014) for assessment of acute eye irritation/corrosion in the rabbit, performed according to OECD 405 and with GLP compliance, zinc pyrithione caused irreversible eye damage.

In an *in vitro* screening test for eye irritancy potential using bovine cornea in an isolated system, performed according to OECD 437 and with GLP compliance, zinc pyrithione was found to be not irritating (Thor GmbH Art. 95 dossier, 2013).

10.5.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Section 3.3.2.2 a substance should be classified in Category 1 (serious eye damage) if at least in one animal effects on the cornea, iris or conjunctiva are not expected to reverse in 21 days and/or if in at least 2 of 3 tested animals, a score for corneal opacity of ≥3 and/or iritis >1.5 is observed, calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

In the study (ZnPT dossier Doc IIIA A6.1.4/02, 2001), zinc pyrithione gave irreversible damage to the eye and the lesions were considered so severe that the animal was sacrificed at 24 hours post-administration. The score for corneal opacity at 24 hours was 4 while the score for iritis could not be determined due to the severity of chemosis and of corneal alteration.

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

Classification in Eye Dam. 1 (hazard statement H318 – Causes serious eye damage) is proposed for zinc pyrithione.

10.6 Respiratory sensitisation

No data.

10.7 Skin sensitisation

Table 31: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
OECD 406 Maximization test modified according to Maurer and Hess GLP Reliability factor: 1	Guinea pig, Dunkin Hartley albino Females 20 (test group) 10 (control group)	Zinc pyrithione Batch no: specified Purity: >95%	Way of induction: epicutaneous Concentrations: 25% (w/w) in white petrolatum (induction) 10% (w/w) in white petrolatum (challenge) Removal of the test substance: 24 h	24 h: 2/20 48 h: 0/20 Negative	ZnPT CAR Doc IIIA A6.1.5/01 Year: 2002

Table 32: Summary table of human data on skin sensitisation

No data.

Table 33: Summary table of other studies relevant for skin sensitisation

No data.

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The sensitisation potential of zinc pyrithione was investigated in a Maximisation study in accordance with GLP and OECD 406. As the test substance was insoluble the protocol according to Maurer & Hess was followed. The study was performed in two consecutive steps. In each step 10 females were used for the test substance group and another 5 females for the negative control group. Immediately after the injection of Freund`s complete adjuvant the test substance was administered epicutaneously on the same area. One week later a second epicutaneous induction exposure followed and 2 weeks afterwards the epicutaneous challenge exposure.

The results of both steps were combined for the final conclusion. All animals survived until the end of the study. Intradermal injections of Freund’s adjuvant caused severe local reactions in all animals. No other adverse effects were noted. After the challenge exposure, 2/20 animals of the test substance group had positive skin reactions 24 h after the end of the exposures. No adverse skin reactions were observed in the control animals. Therefore 2/20 animals of the test substance group (10 %) were regarded as sensitised.

In the study (Thor GmbH Art. 95 dossier, 2014) for assessment of contact hypersensitivity to zinc pyrithione in the mouse, performed according to OECD 429 and with GLP compliance, the EC3 value was found to be > 50%.

10.7.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.4.3 an incidence of ≥ 30 % in a Guinea Pig Maximisation Test (GPMT) is considered a positive response triggering classification. The incidence observed with zinc pyrithione was 10% and zinc pyrithione thus did not fulfil the classification criteria under the conditions of the study.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification is proposed for zinc pyrithione.

10.8 Germ cell mutagenicity

Table 34: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance, reference to table 5**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
In vitro gene mutation in bacteria OECD 471 (1997), EEC Council Directive 2000/32, Annex 4D; ICH S2A, Step 5. GLP Reliability: 1	Zinc pyrithione Batch: specified Purity: >95%	Organism/strain: *S. typhimurium:* TA 1535, TA 1537, TA 98, TA 100, TA 102 Concentrations tested: Expt. 1, strains TA 1535, TA 1537, TA 98, TA 100: 0; 6.25; 12.5; 25.0; 50.0; 100 µg/plate Expt. 1, strain TA 102: 0; 3.13; 6.25; 12.5; 25.0; 50 µg/plate Expt. 2, strains TA 1535, TA 1537, TA 98, TA 100: 0; 1.56; 3.13; 6.25; 12.5; 25.0; 50.0 µg/plate Expt. 2, strain TA 102: 0; 1.56; 3.13; 6.25; 12.5; 25.0; 35.0 µg a.s./plate Positive controls: Sodium azide; 9-Aminoacridine; 2-Nitrofluorene; 2-Aminoanthracene; Cumene hydroperoxide; Dimethylsulfoxide	+ S9: Negative - S9: Negative Cytotoxicity: Experiment 1: TA 1535, TA 1537, TA 98, TA 100, TA 102 (in absence and presence of S9 metabolic activation): 50 and/or 100 µg/plate Experiment 2: TA 1535, TA 1537, TA 98, TA 100 (in absence and presence of S9 metabolic activation): 50 µg/plate; TA 102 (in absence and presence of S9 metabolic activation): 35 µg/plate	ZnPT CAR Doc IIIA A6.6.1/01 Year: 2002
ZnPT *In vitro* chromosomal aberration assay in mammalian cells. OECD 473 GLP Reliability: 1	Zinc pyrithione Batch: specified Purity: >95 %	Organism/strain: Chinese hamster lung fibroblasts (V79 cell line) Concentrations tested: Expt.1 +/- S9: 0, 0.0488, 0.0977, 0.195, 0.395, 0.781, 1.56, 3.13 and 6.25 µg/mL Expt. 2 -S9: 0, 0.12, 0.023, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5 and 3.0 µg/mL Expt. 2 +S9: 0, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5, 3.0, 6.0 and 12.0 µg/mL Positive controls: Mitomycin C; Cyclophosphamide	+ S9: Positive - S9: Positive Cytotoxicity: Parameter: reduction of the number of viable cells (% of negative control value) Experiment 1 (-S9): 6.25 µg/mL: 0 % 0.195 - 3.13 µg/mL: 10 % - 47 %; 0.0488 – 0.0977µg/mL: > 100 %. Experiment 1 (+S9): 6.25 µg/mL: 57 % 0.0488 – 3.13 µg/mL: 89 % - 101 %. Experiment 2 (-S9, 20 h): 0.375 – 3.00 µg/mL: 1 %– 5 %; 0.094 – 0.188 µg/mL: 22 % - 26 %; 0-047 – 0-012 µg/mL: 44 % - 66 %.	ZnPT CAR Doc IIIA A6.6.2/01 Year: 2002

Method, guideline, deviations if any	**Test substance, reference to table 5**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
			Experiment 2 (+S9): 1.50 – 12.0 µg/mL: 2 % - 03 %; 0.047 – 0.750 µg/mL: 32 % - 101 %. Experiment 2 (-S9, 31 h): 0.750 – 3.00 µg/mL: 1 %– 40 %; 0.012 – 0.375 µg/mL: 80 % - 100 %	
In vitro gene mutation in mammalian cells OECD 476; EEC Council Directive 2000/32, Annex 4E GLP Reliability: 1	Zinc pyrithione Batch: specified Purity: >95 %	Organism/strain: Chinese hamster V79 cells. Concentrations tested: Assay 1 -S9: 0.0244, 0.0488, 0.0977, 0.195, 0.293 and 0.391 µg/ml Assay 1 +S9: 0.391, 0.781, 1.56, 3.13, 4.69 and 6.25 µg/mL Assay 2 -S9: 0.0773, 0.116, 0.174, 0.261, 0.391 and 0.587 µg/mL Level 2 +S9: 1.23, 1.85, 2.78, 4.17 and 6.25 µg/mL Positive controls: Ethylmethanesulfonate; 7,12-dimethyl-benz(a)anthracene	Equivocal Cytotoxicity: + S9: 6.25 µg/mL: 22% relative survival - S9: 0.391 µg/mL: 45% relative survival	ZnPT CAR Doc IIIA A6.6.3/01 Year: 2002
In vitro gene mutation in mammalian cells OECD 476 GLP Reliability: 1	Zinc pyrithione Batch: specified Purity: >95%	Organism/strain: L5178Y mouse lymphoma cells Concentrations tested: -S9: 0, 0.01, 0.03, 0.065, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/mL +S9: 0, 0.4, 0.6, , 1, 1.5, 2, 2.5, 3 and 3.5 µg/mL Positive controls: -S9: Methyl methanesulfonate +S9: Cyclophosphamide	+ S9: Positive - S9: Positive Cytotoxicity: -S9: 0.5 µg/mL: 12% relative survival +S9: 3.5 µg/mL: 11% relative survival	Thor GmbH Art 95 dossier Year: 2014
In vitro Comet assay	Zinc pyrithione	Organism/strain:	-S9: Positive	Lamore et al, 2010[4]

[4] Lamore SD, Cabello CM, Wondrak GT (2010). The topical antimicrobial zinc pyrithione is a heat shock response inducer that causes DNA damage and PARP-dependent energy crisis in human skin cells. Cell Stress Chaperones 15:309–322.

Method, guideline, deviations if any	**Test substance, reference to table 5**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Published study No guideline	Batch: not stated Purity: not stated	Human epithelial keratinocytes Doses levels/sampling times: 100 nM: 1, 3, 12 h 500 nM: 1, 3, 12 h Positive control: Hydrogen peroxide	Cytotoxicity: 100 nM: 91% (24h) 500 nM: 92% (1h); 90% (6h); 75% (12 h)	

Table 35: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	**Test substance, reference to table 5**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Mammalian erythrocyte micronucleus test OECD 474 EPA 84-2 GLP Reliability: 2, because the longer sampling time (48 h) was used for the highest dose group and the negative control group only, which prevents any identification of a dose-response relationship.	Zinc pyrithione Batch: specified Purity: >95 %	Animal/strain: Mouse/ Crl:NMRI BR 5/sex/dose Doses levels/sampling times: 800, 1000 and 1300 mg/kg Single dose, gavage 24 and 48 hours Positive control: 2-acetylaminofluorene and dimethylnitrosamine Negative control: Phosphate-buffered saline (PBS)	Negative Cytotoxicity: Mortality: 6/15 males, 2/15 females (spare animals included) in high-dose. 1/5 males, 2/5 females in mid-dose. Sedation, reduced locomotion, exsiccation, generally weak condition in high-dose animals.	ZnPT CAR Doc IIIA A6.6.4/01 Year: 2001
Mammalian erythrocyte micronucleus test EPA OPP 84-2 GLP No reliability factor is given because the study has not been evaluated by the DS. Only a short summary is available with no infor-mation regarding the purity	Zinc pyrithione Batch: not stated Purity: not stated	Animal/strain: Mouse/Sprague-Dawley (Obviously a typo but this is the information given) 5 animals/sex/dose Doses levels/sampling times: 0, 11, 22, 44 mg/kg Single i.p. injection	Negative	Arch registration dossier Year: 1990

Method, guideline, deviations if any	**Test substance, reference to table 5**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
and bioavailability of the test substance.		24, 48 and 72 hours		
In vivo chromosome aberration test Japanese MITI guideline GLP The method is similar to EC method B.10 and OECD 473 *In vitro* Mammalian Chromosome Aberration Test, except that instead of treating cells harvested from untreated animals and then incubating them for testing, the animals were treated with the test substance and then the cells were harvested and incubated for testing. Reliability: 3, since there was no information on the average cell cycle length of the lymphocytes and the cells were cultivated longer than appropriate (for human peripheral blood lympho-cytes, incubation beyond 50 hours is not recommended since this allows a greater proportion of the cells to enter into a second cell cycle, resulting in cells with extensive chromosomal damage dying prior to detection. However this refers only to the last dose since single dosing is recommended for both *in vitro* and *in vivo* studies. It is unclear how the repeated dosing scheme affected the outcome of the study). No positive control was used.	Zinc pyrithione Batch: specified Purity: >95%	Animal/strain: Monkey/Cynomolgus 4/sex/group Doses levels/sampling times: 0, 5.5, 11 and 22 mg/kg bw/day Oral (capsule), once daily for 28 days. On the day after 28 days dosing period.	Negative Cytotoxicity: One female in the 22.0 mg/kg dose group died on day 10 of the dosing period. Clinical signs: vomiting, diarrhoea or soft stool, decreased appetite and spontaneous activity and reduced body weight. One female showed no test related effects.	ZnPT CAR Doc IIIA A6.6.5/01 Year: 1992
In vivo Comet assay ICH S2 (R1), 2012; Tice et al., 2000; Smith et al., 2008; Bowen et al., 2011 GLP Reliability: 1	Zinc pyrithione Batch: specified Purity: >95%	Animal/strain: Rat/Wistar Han 5/sex/group Doses levels/sampling times:	Negative Cytotoxicity: Viability of cells of all dose levels was 94-100%. Doses were chosen based on a range-finding test	Thor GmbH Art 95 dossier Year: 2014

Method, guideline, deviations if any	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
The DNA damage in blood (20.5%) and duodenum cells (42.02%) from vehicle treated animals was higher than the acceptance criteria (<15%). However, the positive control clearly induced DNA damage according to the acceptance criteria.		0, 25, 50 and 100 mg/kg bw/day Oral (gavage), once daily for 3 days. Positive control: Ethyl methanesulfonate Tissues investigated: liver, blood, and duodenum	where 3/6 animals died at 200 mg/kg bw/day.	

Table 36: Summary table of human data relevant for germ cell mutagenicity

No data is available.

Table 37: Summary table of other studies relevant for germ cell mutagenicity

No data is available.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

10.8.1.1 *In vitro* data

Zinc pyrithione was found to be negative for mutagenicity in an *in vitro* gene mutation test in the *Salmonella typhimurium* performed according to GLP and OECD 471 (ZnPT CAR Doc IIIA A6.6.1/01).

Zinc pyrithione was found to be negative for mutagenicity also in the *in vitro* gene mutation test in the *Salmonella typhimurium* and the *Escherichia coli,* performed according to OECD 471 and with GLP compliance (Thor GmbH Art. 95 dossier, 2014).

Zinc pyrithione was found to be positive (both in the presence and absence of S9-mix) in the chromosome aberrations study in the cultured peripheral human lymphocytes, performed according to OECD 473 and with GLP compliance (Thor GmbH Art. 95 dossier, 2014).

In the chromosome aberration study in Chinese hamster V79 cells (ZnPT CAR Doc IIIA A6.6.2/01) statistically significant increases in the number of cells bearing aberrations (including and excluding gaps) were observed both in the absence and presence of S9 metabolism at the 20 hour sampling time and in the absence of S9 metabolism at the dose level selected for scoring at the 31 hour sampling time. The incidences in aberrations exceeded the historical values for background controls of the laboratory. Zinc pyrithione was thus found to be clastogenic under the conditions of the study.

Table 38: Table for Cytogenetic *In Vitro* Test ZnPT CAR Doc IIIA A6.6.2/01: Chromosomal Analysis: without metabolic activation, experiment 1, treatment 3 h, sampling time 20 h

		Control/ solvent	**Low-dose 0.0977 µg/mL**	**Mid-dose 0.195 µg/mL**	**High-dose 0.391 µg/mL**
cytotoxicity (reduction of the number of viable cells to xx % of negative control value)		n.a./n.a.	103 %	47 %	38 %
Aberrations per 100 cells					
gaps		0.5 / 0.5	0.5	1.0	2.5
chromatid aberrations	breaks	0 / 1.5	1.0	1.5	3.5
	interchanges	0 / 0.5	0	0	6.5
isochromatid aberrations	breaks	0 / 0	0	0	0
	interchanges	0 / 1.5	0	0.5	0
others	heavily damaged cells/100 cells (>5 aberrations/cell)	0.5 / 0	0	0	0.5
mitotic index		n.a.	n.a.	n.a.	n.a.
polyploidy		0.5 / 0	1.0	1.5	4.5
endo reduplication		0 / 0	0	0	0.5

Table 39: Table for Cytogenetic *In Vitro* Test ZnPT CAR Doc IIIA A6.6.2/01: Chromosomal Analysis: with metabolic activation, experiment 1, treatment 3 h, sampling time 20 h

		Control/ solvent	Low-dose 1.56 µg/mL	Mid-dose 3.13 µg/mL	High-dose 6.25 µg/mL
cytotoxicity (reduction of the number of viable cells to xx % of negative control value)		n.a./n.a.	96 %	89 %	57 %
Aberrations per 100 cells					
gaps		0.5 / 0	0.5	7.5	2.0
chromatid aberrations	breaks	0.5 / 2.5	1.0	3.5	6.0
	interchanges	0 / 0.5	2.0	3.0	1.5
isochromatid aberrations	breaks	0 / 0	0	0	0
	interchanges	0 / 1.5	0	1.0	0
others	heavily damaged cells/100 cells (>5 aberrations/cell)	0 / 0	0	0	0
mitotic index		n.a.	n.a.	n.a.	n.a.
polyploidy		1.0 / 0.5	2.5	4.0	0.5
endo reduplication		0 / 0	0	0	0

Table 40: Table for Cytogenetic *In Vitro* Test ZnPT CAR Doc IIIA A6.6.2/01: Chromosomal Analysis: without metabolic activation, experiment 2, treatment 20 h, sampling time 20 h

		Control/ solvent	Low-dose 0.012 µg/mL	Mid-dose 0.023 µg/mL	High-dose 0.047 µg/mL
cytotoxicity (reduction of the number of viable cells to xx % of negative control value)		n.a./n.a.	66 %	47 %	44 %
Aberrations per 100 cells					
gaps		0.5 / 0.5	2.5	0.5	0.5
chromatid aberrations	breaks	1.5 / 0	0	1.0	3.0
	interchanges	0 / 0	0.5	0	0
isochromatid aberrations	breaks	0 / 0	0	0	0
	interchanges	0 / 0	0	0.5	0
others	heavily damaged cells/100 cells (>5 aberrations/cell)	0 / 0	0	0	0
mitotic index		n.a.	n.a.	n.a.	n.a.
polyploidy		0 / 0	0	0	4.5
endo reduplication		0 / 0	0	0	0.5

Table 41: Table for Cytogenetic *In Vitro* Test ZnPT CAR Doc IIIA A6.6.2/01: Chromosomal Analysis: with metabolic activation, experiment 2, treatment 3 h, sampling time 20 h

		Control/ solvent	Low-dose 0.188 µg/mL	Mid-dose 0.375 µg/mL	High-dose 0.750 µg/mL
cytotoxicity (reduction of the number of viable cells to xx % of negative control value)		n.a./n.a.	52 %	26 %	32 %
Aberrations per 100 cells					
gaps		0.5 / 0.5	0	1.0	2.5
chromatid aberrations	breaks	0.5 / 1.0	0.5	2.5	1.0
	interchanges	0 / 0.5	0	0	1.5
isochromatid aberrations	breaks	0 / 0	0	0	0
	interchanges	0 / 0	0	0	0.5
others	heavily damaged cells/100 cells (>5 aberrations/cell)	0 / 0	0	0.5	1.5
mitotic index		n.a.	n.a.	n.a.	n.a.
polyploidy		1.0 / 0.5	1.5	0.5	9.5
endo reduplication		0 / 0	0	0	0

Table 42: Table for Cytogenetic *In Vitro* Test ZnPT CAR Doc IIIA A6.6.2/01: Chromosomal Analysis: without metabolic activation, experiment 2, treatment 31 h, sampling time 31 h

		Control/ solvent	High-dose 0.750 µg/mL	-	-
cytotoxicity (reduction of the number of viable cells to xx % of negative control value)		n.a./n.a.	40 %	-	-
Aberrations per 100 cells				-	-
gaps		0.5 / 0	1.0	-	-
chromatid aberrations	breaks	0 / 0	2.5	-	-
	interchanges	0 / 0	2.0	-	-
isochromatid aberrations	breaks	0 / 0.5	1.0	-	-
	interchanges	0 / 0	0	-	-
others	heavily damaged cells/100 cells (>5 aberrations/cell)	0 / 0	0	-	-
mitotic index		n.a.	n.a.	-	-
polyploidy		0 / 0	0	-	-
endo reduplication		0 / 0	0	-	-

Table 43: Table for Cytogenetic *In Vitro* Test ZnPT CAR Doc IIIA A6.6.2/01: Chromosomal Analysis: with metabolic activation, experiment 2, treatment 3 h, sampling time 31 h

		Control/ solvent	**High-dose 3.00 µg/mL**	-	-
cytotoxicity (reduction of the number of viable cells to xx % of negative control value)		n.a./n.a.	73 %	-	-
Aberrations per 100 cells				-	-
gaps		1.0 / 1.0	1.0	-	-
chromatid aberrations	breaks	1.0 / 1.0	0.5	-	-
	interchanges	0 / 0	2.0	-	-
isochromatid aberrations	breaks	0.5 / 0	0	-	-
	interchanges	0 / 0	0	-	-
others	heavily damaged cells/100 cells (>5 aberrations/cell)	0 / 0	0	-	-
mitotic index		n.a.	n.a.	-	-
polyploidy		0 / 0	0	-	-
endo reduplication		0 / 0	0	-	-

Zinc pyrithione was also tested in an *in vitro* mammalian cell gene mutation test (ZnPT CAR Doc IIIA A6.6.3/01) according to GLP and OECD 476. In the presence of S9 metabolic activation a statistically significant effect of dose level was observed in the ANOVA analysis performed by the laboratory ($p<0.001$ in Assay 1 and $p<0.01$ in Assay 2); this was considered by the study author not to be of biological relevance since the increase was less than five-fold which is the cut-off value for interpretation of a positive result established by the performing laboratory due to variation in historical negative control data. No dose-response relationship was observed, but the mutation frequency recorded for the highest dose with >50% relative survival in Assay 2 (day 6) was approximately three times higher than the control (69.94 compared to 23.81) and well outside the historical negative control range. In the absence of S9 metabolic activation the average mutation frequencies were also approximately two to three times higher in the highest dose levels (not taking into account the dose level giving <50 % relative survival) compared to controls. In Assay 1 (days 6 and 9) the mutation frequency was approximately twice the historical control mean value (8.80) but still within the historical control range. In Assay 2 the value was higher than the recorded historical control range. The result of the study is therefore considered to be positive. The ratio of small versus large colonies was not measured so no conclusion can be drawn as to whether the results would indicate a possible mutagenic or clastogenic effect.

Table 44: Table for *in vitro* mammalian cell gene mutation test in mammalian cells (ZnPT CAR Doc IIIA A6.6.3/01): Summarised results from mutation assay 1

Without metabolic activation				With metabolic activation			
Dose level (µg a.s./mL)	**%RS**	**MF day 6**	**MF day 9**	**Dose level (µg a.s./mL)**	**%RS**	**MF day 6**	**MF day 9**
0.00	100	5.74	4.59	**0.00**	100	7.78	9.84
0.0244	96	9.50	6.11	**0.391**	97	4.60	5.54
0.0488	85	7.29	6.40	**0.781**	88	4.69	3.61
0.0977	85	5.12	6.82	**1.56**	86	11.17	14.94
0.195	73	5.12	14.86	**3.13**	75	7.68	15.67
0.293	63	15.65	18.10	**4.69**	73	10.36	9.32
0.391	45	5.36	8.10	**6.25**	22	22.82	29.70
EMS 10.0 mM	74	1137.37	1155.46	**DMBA 10.0 mM**	81	694.87	701.65
Historical mean negative control (n=62)		8.80	10.6	**Historical mean negative control (n=62)**		9.10	11.8
Historical negative control range		1.01-39.3	2.22 – 43.3	**Historical negative control range**		2.25-47.7	2.22 – 56.1
%RS = Percentage relative survival MF = Average mutation frequencies per million surviving cells							

Table 45: Table for *in vitro* mammalian cell gene mutation test in mammalian cells (ZnPT CAR Doc IIIA A6.6.3/01): Summarised results from mutation assay 2

Without metabolic activation				With metabolic activation			
Dose level (µg a.s./mL)	**%RS**	**MF day 6**	**MF day 9**	**Dose level (µg a.s./mL)**	**%RS**	**MF day 6**	**MF day 9**
0.00	100	25.68	24.68	**0.00**	100	23.81	22.82
0.0773	78	10.19	15.25	**1.23**	127	37.45	35.92
0.116	95	39.16	58.78	**1.85**	114	24.62	29.44
0.174	55	44.92	46.24	**2.78**	123	39.15	42.45
0.261	45	49.29	26.19	**4.17**	75	69.94	26.21
0.391	18	4.29	1.24*	**6.25**	34	53.86	47.87
EMS 10.0 mM	104	1467.49	1246.27	**DMBA 10.0 mM**	102	1169.68	1192.79
Historical mean negative control (n=62)		8.80	10.6	**Historical mean negative control (n=62)**		9.10	11.8
Historical negative control range		1.01-39.3	2.22 – 43.3	**Historical negative control range**		2.25-47.7	2.22 – 56.1
%RS = Percentage relative survival MF = Average mutation frequencies per million surviving cells * = Mutation frequency, assuming 1 colony on mutation plates							

A second *in vitro* mutagenicity study is available from a dossier on zinc pyrithione submitted by Thor GmbH as part of their Article 95 notification of the substance. The study was performed in 2013 (with the final report dated 2014) in accordance with GLP and OECD 476 and investigated mutagenicity at the TK locus in L5178Y mouse lymphoma cells. Zinc pyrithione was tested at concentrations up to 0.5 µg/mL in the absence of S9-mix and up to 3.5 µg/mL in the presence of S9-mix. The negative (solvent) and positive (-S9: methyl methanesulfonate, +S9: cyclophosphamide) controls gave appropriate results. In the absence of metabolic activation, zinc pyrithione induced an up to 6.7-fold dose-related increase in the mutation frequency (477 per 10^6 survivors), which was well above the historical control range and the GEF + $MF_{(controls)}$ (*i.e.* 126 + 71 = 197 per 10^6 survivors). Both small and large colonies were increased. The relative total growth at the highest dose was reduced by 88% which is acceptable by the guideline. In the presence of metabolic activation, zinc pyrithione induced an up to 8.3-fold dose-related increase in the mutation frequency (748 per 10^6 survivors), which was also outside the historical control range and the GEF + $MF_{(controls)}$ (*i.e.* 126 + 90 = 216 per 10^6 survivors). Both small and large colonies were increased. The relative total growth at the highest dose was reduced by 89% which is acceptable by the guideline. Both small and large colonies were increased. Zinc pyrithione was therefore considered to be mutagenic in both the absence and presence of metabolic activation under the conditions of the test. Please see table 46 for summarised results.

Table 46: Table for *in vitro* mammalian cell gene mutation test in mammalian cells (Thor GmbH, 2014): Summarised results of cytotoxicity and mutagenicity assays.

Dose (µg/mL)	Relative survival (%)	Relative total growth (%)	Mutation frequency per 10^6 survivors		
			total	small colonies	large colonies
Without metabolic activation					
0	100	100	73	41	29
0	100	100	69	35	31
0.01	95	80	72	39	31
0.03	76	75	94	57	34
0.065	106	93	86	53	29
0.1	87	78	123	68	48
0.2	82	65	140	77	55
0.3	85	45	140	71	61
0.4	101	34	250	142	76
0.5	69	12	477	286	117
MMS	42	28	1195	718	331
With metabolic activation					
0	100	100	91	52	35
0	100	100	89	47	38
0.4	116	116	56	18	36
0.6	89	85	81	45	33
1	80	67	118	61	51
1.5	86	68	331	174	109

2	71	32	601	243	226
2.5	81	25	519	215	191
3	70	16	748	299	251
3.5	68	11	650	273	230
CP	30	14	1662	833	617

A published study is available (Lamore et al, 2010) which among other tests included an *in vitro* alkaline single cell gel electrophoresis (Comet assay). In one part of the study, primary human epidermal keratinocytes were treated with 100 or 500 nM zinc pyrithione for 1, 3 or 12 hours. Cells treated with hydrogen peroxide served as positive controls and untreated cells as negative controls. After treatment, cells were harvested and analysed for comets with a fluorescence microscope and CASP software. At least 100 tail moments for each group were analysed in order to calculate the mean ±SD for each group. Cytotoxicity was investigated for 100 nM at 24 hours and 500 nM at 1, 6, 12 and 24 hours. The results showed a statistically significant and dose-dependent increase in tail moments at both dose levels that increased with increased exposure time. Tail moments were increased approximately 3-fold within 1 hour of exposure and approximately 5-fold within 12 hours of exposure. It should be noted that loss of genomic integrity occurred at doses that did not impair viability of the cells. In a second part of the study, primary human epidermal melanocytes were exposed to 500 nM zinc pyrithione for 1 hour. An approximately 20-fold increase in tail moment was observed. The cytotoxicity assessment showed almost complete inhibition of proliferation at 100 nM, however loss of viability was only observed upon much higher exposure concentrations (2 µM). Based on these results zinc pyrithione was considered positive for induction of comet assays under the conditions of the study.

10.8.1.2 In vivo data

Zinc pyrithione was investigated in a micronucleus study (ZnPT CAR Doc IIIA A6.6.4/01) performed according to OECD guideline 474 and GLP. Doses of 800, 1000 and 1300 mg/kg were administered to 5 mice/sex/dose via oral gavage. The animals were sacrificed at 24 and 48 hours post-administration. There was no statistically significant increase in the numbers of micronucleated polychromatic erythrocytes at any dose level tested at any time point for males. The results were within the range of historical negative controls. In females of the high-dose group sacrificed at 48 h p.a., the number of micronucleated polychromatic erythrocytes was statistically significantly higher (2.10) than in the corresponding negative control group (0.9), but still within historical negative control data (range: 0.0-3.0) and was therefore considered by the study author to be without biological relevance. It should also be noted that it was below the negative control value at the 24 h sampling time was (2.90) and the DS therefore agrees that the study was negative. Bioavailability of the test substance was proven by mortality and cytotoxicity at the high and mid-dose levels.

Table 47: Table for micronucleus study in mice (ZnPT CAR Doc IIIA A6.6.4/01): Summarised results for males

Parameter/dose level (mg/kg bw)		**Neg control**		**800**	**1000**	**1300**		**Pos control**
Number of cells (polychromatic erythrocytes) evaluated		2000		2000	2000	2000		2000
Sampling time (h)		24	48	24	24	24	48	24
Percentage of all cells	**Nucleated cells**	56.8	62.9	45.9	32.2*	29.3*	32.1*	43.0
Ratio of erythrocytes	**Normochromatic (%)**	46.2	44.8	54.4	60.6*	67.8*	66.5*	52.8
	Polychromatic (%)	53.8	55.2	45.6	39.4*	32.2*	33.5*	47.2
	Polychromatic / normochromatic (%)	1.19	1.24	0.87	0.66*	0.48*	0.53*	0.91
	Polychromatic with micronuclei (‰)	1.70	1.30	1.50	1.88	2.40	1.50	9.80*
	Normochromatic with micronuclei (‰)	2.23	1.25	1.43	3.72	1.19	1.96	1.48
** Statistically significant (level not indicated)*								

Table 48: Table for micronucleus study in mice (ZnPT CAR Doc IIIA A6.6.4/01): Summarised results for females

Parameter/dose level (mg/kg bw)		**Neg control**		**800**	**1000**	**1300**		**Pos control**
Number of cells (polychromatic erythrocytes) evaluated		2000		2000	2000	2000		2000
Sampling time (h)		24	48	24	24	24	48	24
Percentage of all cells	**Nucleated cells**	63.2	65.2	41.0*	57.9	38.4*	41.1*	45.0*
Ratio of erythrocytes	**Normochromatic (%)**	41.6	41.9	56.1	43.1	59.0	65.5*	53.2*
	Polychromatic (%)	58.4	58.1	43.9	56.9	41.0	34.5*	46.8*
	Polychromatic / normochromatic (%)	1.42	1.42	0.86	1.33	0.77	0.56*	0.88*
	Polychromatic with micronuclei (‰)	2.90	0.90	2.40	2.83	1.60	2.10*	13.00*
	Normochromatic with micronuclei (‰)	2.85	0.46	2.23	3.87	1.37	1.16	1.83
** Statistically significant (level not indicated)*								

A second micronucleus study with zinc pyrithione is available from the registration dossier and has not been evaluated by the DS. The reporting is very limited and shows deficiencies, e.g. the purity of the test substance was not given, the species was specified as "mouse Sprague-Dawley" and no information regarding cytotoxicity was given to prove the bioavailability of the test substance. Therefore, it is only included as supporting information. The study was performed according to EPA OPP 84-2 guideline. Zinc pyrithione was administered by one intraperitoneal injection in doses of 0, 11, 22 or 44 mg/kg to 5 animals/dose group. The result of the study is reported to be negative.

In a mammalian erythrocyte micronucleus test in the rat, performed according to OECD 474 and with GLP compliance, zinc pyrithione was found to be negative (Thor GmbH Art. 95 dossier, 2014).

Zinc pyrithione was also investigated in a chromosomal aberration study (ZnPT CAR Doc IIIA A6.6.5) performed according to GLP but not following any OECD guideline. The study was performed in connection with a 28 day oral toxicity study in monkeys where two animals per sex were given an oral dose of 5.5, 11.0 or 22.0 mg/kg bw for 28 consecutive days and blood was drawn approximately 24 hours following the last dose. The peripheral lymphocytes were then cultivated for 66 hours after which time they were treated with Colcemid and incubated for an additional 6 hours. The cells were then analysed for chromosomal aberrations. There were no significant differences in the average frequencies of structural aberrations or polyploidy between the zinc pyrithione treated groups and the negative control group and zinc pyrithione was considered negative for clastogenicity in this test. The study was found to be of limited quality since there was no information on the average cell cycle length of the lymphocytes and the cells were cultivated longer than appropriate; for human peripheral blood lymphocytes, incubation beyond 50 hours is not recommended since this allows a greater proportion of the cells to enter into a second cell cycle, resulting in cells with extensive chromosomal damage dying prior to detection. However this refers only to the last dose since single dosing is recommended for both *in vitro* and *in vivo* studies. It is unclear how the repeated dosing scheme affected the outcome of the study. Moreover, no positive control was used.

An *in vivo* Comet assay is available from a dossier on zinc pyrithione submitted by Thor GmbH as part of their Article 95 notification of the substance. The study was performed in mid-2014 in accordance with the GLP and the guidelines/recommendations in ICH S2(R1), 2012; Tice et al., 2000; Smith et al., 2008; Bowen et al., 2011. Five male Wistar Han rats per treatment group received zinc pyrithione (purity: >95%) by oral gavage at 0 (negative control/vehicle-treated), 25, 50 and 100 mg/kg for three consecutive days. In a positive control group, the rats received ethyl methanesulfonate twice at 200 mg/kg. Liver, blood and duodenum were collected after approx. 3-4 hours and single cell suspensions were prepared followed by Comet slides. The mean tail intensity in liver, blood, and duodenum cells of the negative controls was 9.72%, 20.50% and 42.02%, respectively. The positive control induced a tail intensity of 9.5-, 4.7-, and 2.2-fold in liver, blood and duodenum, respectively. The DNA damage in blood and duodenum cells from negative controls was higher than the acceptance criteria (<15%). However, the positive control clearly induced DNA damage according to the acceptance criteria. A statistically significant increase in the mean tail intensity (15%; 1.5-fold increase compared to negative controls) was observed in the low dose group. As no effects were observed at mid- or high-dose groups, the effects at low-dose group were considered not biologically relevant. Under these experimental conditions of the Comet assay, it was concluded that zinc pyrithione does not cause biologically relevant DNA damage.

10.8.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.5.1, classification in Category 2 mutagen is based on:

> - *Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*

- Somatic cell mutagenicity tests in vivo, in mammals; or

- Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."

Zinc pyrithione tested positive for clastogenicity *in vitro* but an *in vivo* micronucleus study of high reliability gave negative results which were supported by two studies of low reliability (a second micronucleus study and a 28-day chromosomal aberration study in monkeys).

Zinc pyrithione tested positive for gene mutations *in vitro* but was negative in an *in vivo* comet assay.

It is concluded that zinc pyrithione does not fulfil the classification criteria for germ cell mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification is proposed for zinc pyrithione.

10.9 Carcinogenicity

Table 49: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
No specific guideline No GLP Reliability: 3	Rat (Strain not stated) 10/sex/dose	Zinc pyrithione Batch: Not specified Purity: Not specified	0, 2, 5, 10, 25, 50 ppm (food consumption per day not specified) Daily treatment 10 ppm corresponds to approx. 1.3 mg/kg bw/day for males and females. 2 year Oral, in diet	No increase in tumour formation. 2, 5 and 10 ppm: No adverse effects 25 ppm ~2 mg/kg bw/day: ↑ mortality (f) ↑ hind limb paralysis (f) ↓ body weight gain (f) 50 ppm: ↑ mortality (10 f, 6 m) ↑ hind limb paralysis (m, f) ↓ body weight gain NOAEL = 10 ppm based on death of animals and decreased weight gain.	ZnPT CAR Doc IIIA 6.5/03 Year: 1958

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
US EPA 83-2, which complies with OECD 453. GLP Reliability: 2	Rat Crl: CD (SD) (VAF Plus) 50/sex/dose	Sodium pyrithione (NaPT)	0, 0.5, 1.5, 5 (decreased to 3.5 after 12 weeks) mg/kg bw/day 2 year Oral, gavage	No evident NaPT-induced tumour increase NOAEL < 0.5 mg/kg bw based on increased incidences of hind leg wasting and spinal chord degradation. Low survival in some groups and therefore the study cannot be regarded as truly negative cancer study.	Doc IIIA A6.5.1/01 and A6.7/02 (1991, unpublished)
Conclusion of the same study in REACH registration dossier	[NaPT] did not affect tumour formation adversely. Decreases in body weight gain, hind limb muscle atrophy and histopathological changes in skeletal muscle, spinal cord and in the eyes were observed in the high dose group. Some, but not all of these effects were observed to a lesser degree in the mid dose group. LO(A)EL: 1.5 mg/kg bw/day; NO(A)EL: 0.5 mg/kg bw/day Reliability – 1				
US EPA 83-2, which complies with OECD 453 Reliability: 2	Rat Hsd: Sprague Dawley SD Control, low and medium dose: 12/sex/dose; high dose: 20/sex/dose; veterinary control: 16/sex/dose	NaPT	0, 0.5, 1.4, 4 mg/kg bw/day (reduced to 2.8 after 7 weeks and decreased to 2.1 for female after 9 months) 2 year Oral, gavage	No evident NaPT-induced tumour increase NOAEL < 0.5 based on sciatic nerve degeneration in 1/12 males Low survival in some groups reducing the reliability of the negative result.	Doc IIIA A6.5.1/02 and A6.7/03 (2004, unpublished)
Conclusion of the same study in REACH registration dossier	Signs of toxicity, such as, ataxia, decreased muscle tone and emaciation were seen in animals of both sexes. In addition, a lower body weight was noted in low and high-dose males and in mid- and high- dose females when compared to controls. There were no incidences of neoplasia with sodium pyrithione up to 2.8 mg/kg/day. NO(A)EL: 0.5 mg/kg bw/day Reliability: 1				
US EPA 83-2, which complies with OECD 453. GLP Reliability: 1	Mouse Crl: CD-1 (ICR) BR (VAF Plus) 50/sex/dose	NaPT	0, 5, 15, 40 mg/kg bw/ day 80 weeks Dermal	No NaPT-induced tumour increase 2 year this administration route circumvents the first-pass effect of the liver	Doc IIIA A6.7.1/01 (1991, unpublished)
Conclusion of the same study in REACH registration dossier	[NaPT] did not affect tumour formation adversely. The only observed lesion, which appeared to be related to the treatment, was epidermal hyperplasia at the application sites of high and mid dose animals. LO(A)EL: 15 mg/kg bw/day NO(A)EL: 5 mg/kg bw/day Reliability: 1				

Table 50: Summary table of human data on carcinogenicity

No data is available.

Table 51: Summary table of other studies relevant for carcinogenicity

No data is available.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

There is no robust substance-specific data available to assess the carcinogenic potential of zinc pyrithione. Information of some relevance for this endpoint is available in a chronic toxicity study in which histopathological examinations were included (the chronic part is presented in section 10.12). However, this study was performed in 1958, before GLP or any guidelines were established and the study is poorly reported lacking information on purity, unclear dose levels and several deviations from OECD 451 (e.g. 10 animals per sex instead of 50; lack of analyses of urine or clinical chemistry; no weekly recording of body weight during the first 13 weeks of the test period and no recording “at least once every four weeks” thereafter and no measurements of food consumption). Although the study provides some information on the carcinogenic potential of zinc pyrithione (see below), the results of the study are not considered sufficiently reliable to serve as a stand-alone key data.

Rats were exposed to 2, 5, 10, 25 and 50 ppm zinc pyrithione in diet for up to 104 weeks. Food consumption was not measured but the test substance intake can be calculated using default values[5] and a dose of 25 ppm equals approximately 2 mg/kg bw/day in females (see study summary). There were no effects on survival in male rats whereas the survival rate in female rats decreased markedly at 25 and 50 ppm. Only eight and six of the ten females in the 25 and 50 ppm dose groups were alive after 20 weeks. None of the high dose females were alive after 80 weeks and there were only 3 surviving females in the 25 ppm group (compared to 8 in controls). In similarity with results from other studies hind limb paralysis and reduced body weight gain was noted in females administered 25 ppm and in both males and females administered 50 ppm. The tissues examined histopathologically include heart, lung, liver, spleen, kidney, GI tract, bone marrow, brain, spinal cord, muscle, eye, bladder, pancreas, adrenal, thyroid and gonad. The examinations did not reveal any increase in tumour formation. However, it is noted that only five high-dose females were subjected to histopathological examinations and as a consequence of the mortality rate these females were only exposed to the test substance for 20, 62, 62, 78 and 78 weeks, respectively. The ten high-dose males that were subjected to a histopathological examination were exposed for 62 (1 male), 77 (4 males), 78 (1 male) and 104 (4 males) weeks, respectively. Since the exposure duration of females only represent approximately half the life-span of the rat it can be questioned if this time period was sufficient for detecting tumours, taking into account tumour latency.

Data available for sodium pyrithione:

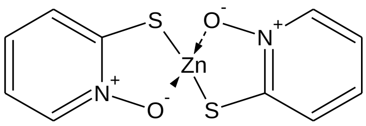
The REACH registration dossier[6] as well as the dossier submitted for the biocides review of zinc pyrithione also include chronic studies performed with a different type of pyrithione, i.e. sodium pyrithione (see table 49).

[5] Technical Guidance Document on Risk Assessment (TGD), 2003. Annex VI, Default reference values for biological parameters. Tables 2 and 3.

[6] https://echa.europa.eu/registration-dossier/-/registered-dossier/14333/7/8

Both substances share the pyrithione moiety, however, while zinc pyrithione is a chelate, sodium pyrithione is a soluble salt (Fig. 10.9.1-1)[7]. This is reflected in different water solubilities of the two substances. Sodium pyrithione is an ionic substance and highly soluble in water thus in aqueous solutions the sodium cations and the counter-anion coexist. In contrast, zinc pyrithione is predominantly a covalent substance and barely soluble in water. Theoretically, the low hydrolysis rate of zinc pyrithione may result in the co-existence of zinc pyrithione in both chelated and dissociated forms which in turn may cause differences in toxicokinetic and toxicological profiles for zinc pyrithione and sodium pyrithione. However, it cannot be excluded that zinc pyrithione would dissociate to a high extent in the low pH of gastric juice following oral administration.

Zinc pyrithione



Sodium pyrithione

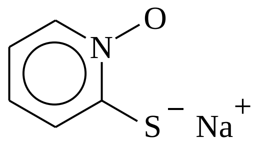


Figure 10.9.1-1 Chemical structures of zinc pyrithione and sodium pyrithione

Although some effects observed among studies most certainly can be linked to the pyrithione moiety, the toxicological significance of the Zn^{2+} in zinc pyrithione, either in isolation or in synergy with the pyrithione, for tumorigenesis is unknown. Information regarding the carcinogenic potential of zinc salts appears to be limited and the final risk assessment reports on zinc sulphate[8] and zinc chloride concludes[9] *"The available data are limited. Zinc deficiency or supplementation may influence carcinogenesis, since promoting and inhibiting actions have been reported. However, there is no clear experimental or epidemiological evidence for a direct carcinogenic action of zinc or its compounds."* Due to the uncertainty with respect to any impact of Zn on carcinogenicity, it is not considered scientifically justified to consider the data obtained with sodium pyrithione in a weight of evidence determination for zinc pyrithione. In addition, the reliability of the results from the two oral chronic toxicity/carcinogenicity studies with sodium pyrithione can be questioned since the survival rate was less than 50% in some groups (see table 49). It is thus not possible to exclude that the mortality rate could have masked tumour formation, taking into account latency. The reliability of the 1991 study is further reduced by the lack of information regarding purity and stability of the test substance.

In conclusion, the data available on sodium pyrithione is not completely relevant for the assessment of the carcinogenic potential of zinc pyrithione as the influence of zinc is not addressed.

10.9.2 Comparison with the CLP criteria

In the absence of robust information on the carcinogenic potential of zinc pyrithione, a meaningful comparison of results with CLP criteria cannot be made.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Due to the lack of data on zinc pyrithione, it is not possible to present a classification proposal for this hazard class.

[7] Picture provided by Arch Chemicals for the biocides review

[8] RISK ASSESSMENT REPORT ZINC SULPHATE, final report May 2008, CAS-No.: 7733-02-0, EINECS-No.: 231-793-3

[9] RISK ASSESSMENT REPORT ZINC CHLORIDE, final report, May 2008, CAS-No.: 7646-85-7, EINECS-No.: 231-592-0

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 52: Summary table of animal studies on adverse effects on sexual function and fertility

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
OECD 416 EPA OPPTS 870.3800 EU B.35	Reliability factor: 1 No major deviations from the guideline that adversely affected the study integrity.	Rat Crl:WI(Han) Male and female 24/sex/dose	Zinc pyrithione Batch: specified Purity: >95%	0, 0.2, 0.5, 2.5 mg/kg bw Oral gavage P animals: Min. 70 days pre-mating and 15 days mating. For females the dosing continued until lactation day 21-23 F_1 animals: After weaning, similar to P animals	High-dose P animals: • two females had total litter loss which the author considered were not treatment related. Macroscopic examination showed one of them with ↓ size of femoral muscle. Uterus of the same female had a fetus and the other female had fluid in the uterus which was considered as an incidental finding by the author. • hunched posture, piloerection and lean appearance were noted for 6 females • ↓ body weight gains in females from days 22-64 (-10 to -20%) • ↓ size of skeletal muscle in 3 females • ↑ relative liver (8%) and spleen (11%) weights in females. The author did not consider these to be adverse as the difference from controls was slight and there were no histological findings. • skeletal muscle histopathological findings in 10 females include: - atrophy in 7 - fat replacement in 6 - axonal degeneration in 4 • statistically significantly lower epididymal sperm concentrations in males compared to controls. The author did not consider it as toxicologically relevant as the values were within the normal ranges for the age and strain. Reproduction/developmental data in all dose groups of P animals/F_1 pups: • There were no adverse effects on any of the reproduction parameters or on pup development that were attributed to the treatment by the author. • one high-dose female had implantation sites only • two mid-dose females were non-pregnant	Thor GmbH Art. 95 dossier Year: 2015

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					• one female at low-dose had implantation sites only and one did not mate • the mean litter size (9.7) of the high-dose females was lower than the control (10.9) and the historical control (11.5) means • in total 9 pups were dead at high-dose at the first litter check, 6 of those pups were of the female with the total litter loss and 3 from an another female • all 4 pups of a litter of a high-dose female were lost from PND 7-14 • one high-dose female pup was euthanized on PND 22 after signs of piloerection, lethargy, swelling of the head, pale appearance, a wound, and skin abnormalities • vaginal patency was delayed by on average two days compared to controls in the females of all treated groups F_1 animals of all dose groups: • one high-dose female was euthanized when she had a total litter loss in which the single pup went missing on PND 5. The author did not consider it to be treatment related. • statistically significant lower absolute body weights (but not body weight gains) for high-dose females on GD 4-20 and LD 1 compared to controls • there was higher incidence of fluid in the uterus in mid and high-dose females which the author considered as not treatment related or toxicologically relevant • skeletal muscle histopathological findings in 10 high-dose females included atrophy in 1 • the mean litter size (10.2) of the high-dose females was lower than the control (11.3) and the historical control (11.5) means • there were no treatment related or toxicologically relevant effects on the developmental parameters of the pups of F_1 animals (F_2 generation)	

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

In a two-generation reproductive toxicity study performed according to the guidelines (OECD 416/EPA OPPTS 870.3800/EU B.35) and with GLP, zinc pyrithione (purity >95%) was given to Wistar Han rats by daily oral gavage at dose levels of 0, 0.2, 0.5, and 2.5 mg/kg bw (Thor GmbH Art. 95 dossier). Treatment related adverse effects in the parental animals was limited to high-dose females with skeletal muscle being the primary target including reduced size of the hindlimb muscle with corresponding histopathological observations of atrophy, fat replacement of myofibres and axonal degeneration. The high-dose parental females only had lower body weight gains during pre-mating treatment days 22-64. The only adverse effects noted in F_1 animals was atrophy of the skeletal muscle in only one high-dose female. There were no treatment related adverse effects on reproductive or developmental parameters for any generation at the dose levels tested.

The selection of dose levels for this two-generation study was based on a 14-day range finding study and the observations during the first weeks of a 90-day study, both with dose levels of 0.2, 0.5, and 2.5 mg/kg bw. The high-dose females of the 14-day study had slightly lower body weight gains from days 8-14 and slightly higher relative liver weights, and the high-dose males had slightly lower relative food consumption at the end of the study. One high-dose female of the 90-d study lost weight from days 15-29 and showed clinical signs including hunched posture, uncoordinated movements, abnormal gait and lean appearance.

10.10.3 Comparison with the CLP criteria

There were no treatment related adverse effects on sexual function and fertility.

10.10.4 Adverse effects on development

In June 2016, the zinc pyrithione task force provided a document to the DS wherein the developmental toxicity studies on zinc pyrithione were reviewed individually and in a weight of evidence according to the CLP criteria. This document is included as a confidential appendix to this CLH report.

Although the unpublished developmental toxicity studies on zinc pyrithione are sufficiently described by the DS in this section, the full study reports of these are attached as confidential appendices to this CLH report in order for the Committee for Risk Assessment (RAC) to have access to all the details.

Table 53: Summary table of animal studies on adverse effects on development

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
EPA 83-3	Reliability factor: 2, as the purity of the test substance prior to suspension in water was not specified	Rat Sprague-Dawley Crl:CD VAF/plus 30 females/ group	Zinc pyrithione Batch: specified 52.2% aq solution. Purity of the active ingredient prior to suspension in water not	0, 0.75, 3.0, 15.0 mg/kg bw Oral gavage day 6-15 post mating 5 days post exposure period	<u>Maternal tox – 3.0 mg/kg bw:</u> ↑ salivation (8) <u>Maternal tox – 15.0 mg/kg bw:</u> ↓ food consumption gestation d6-16 (- 48%, p<0.01) ↓ adjusted body weight at gestation day 20 (-8%, p<0.01) ↓ adjusted body weight gain (-38%, p<0.01) ↑ number of animals with dilated pupils before and after dosing (17 animals) ↓ gravid uterine weight compared	ZnPT CAR Doc IIIA A6.8.1/02 Year: 1993

			specified.		to control (-17%, dose-response but not statistically significant) ↑ salivation (29) <u>Foetal tox – 3.0 mg/kg bw:</u> ↑ total number of examined foetuses with malformations compared to controls (7/1, $p<0.05$) ↑ number of examined foetuses with skeletal malformations: (mid-dose/controls) • fused ribs (3/0) • pelvic malformation (1/0) • tail malformation (1/0) ↑ number of examined foetuses with soft tissue malformations: (mid-dose/control) • diaphragmatic hernia (2/0) • anal atresia (1(the foetus with tail malformation)/0) <u>Foetal tox – 15.0 mg/kg bw:</u> (high-dose/controls) ↓ mean foetal body weights (♀: -15% ,$p<0.01$; ♂:-17%, $p<0.01$) ↑ post-implantation loss (3.7/0.8 $p<0.01$) ↓ mean number of viable foetuses per litter (12.5/14.5, $p<0.05$) ↑ total number of examined foetuses with malformations (168/1, $p<0.01$) ↑ number of examined foetuses with vertebral malformations with or without an associated rib malformation (89% of foetuses examined skeletally) ↑ number of examined foetuses with skeletal malformations: (high-dose/controls) • rib malformation (3/0) • fused ribs (3/0) • fused sternebrae (30/0) • sternal malformation (35/0) • limb malformation (ulna or radus missing) (2/0) • adactyly (1/0) • ectrodactyly (9/0) • syndactyly (1/0) • polydactyly (1/0) ↑ number of examined foetuses with soft tissue malformations: • dimorphism (2/0) • malformed brain (1/0) • renal hypoplasia (1/0)	

					↑ total number of examined foetuses with developmental variations (171/79, p<0.05): • less than 13 pairs of ribs (122 compared to 0 in control) • variations in the number of presacral vertebrae (24, 25, or 27) (in total 126 foetuses compared to 1 in control) • unossified sternebrae 5 or 6 (91 compared to 58 in control) • unossified sternebrae 1, 2, 3 or 4 (23 compared to 1 in control) • misaligned sternebrae (66 compared to 4 in control) ↑ number of examined foetuses with soft tissue developmental variations: • undeveloped renal papillae (12/1)	
EPA 83-3 GLP	Reliability factor: 2, because of the lack of examination of tissue alterations and information on purity <u>Deviations from OECD 414:</u> Maternal body weights were recorded every 6th day instead of every 3rd day of dosing. No heads of foetuses were examined for soft tissue alterations (including eyes, brain, nasal passages and tongue).	Rabbit New Zealand White 20 f/group	Zinc pyrithione Batch: specified 52.2% aq solution. Purity of the active ingredient prior to suspension in water not specified.	0, 0.5, 1.5, 3.0 mg/kg bw Oral gavage day 6-18 post mating	<u>Maternal tox – 1.5 mg/kg bw:</u> ↓ body weight gain d 6-19 (-41%, p<0.01) ↓ mean gravid uterus weights (-32% but not statistically significant) ↓ food consumption compared to controls (-14-23%, p<0.01) <u>Maternal tox – 3.0 mg/kg bw:</u> ↓ food consumption (-13-31%, p<0.01) ↓ body weight gain d 6-19 (-98%, p<0.01) ↓ mean gravid uterus weights (-32% but not statistically significant) <u>Foetal tox – 1.5 mg/kg bw:</u> ↑ resorption (mean 1.6 per pregnant dam compared to mean 0.8 in control group, dose-response but not statistically significant) ↑ post-implantation loss (29% compared to 11% in control) <u>Foetal tox – 3.0 mg/kg bw:</u> ↑ resorption (mean 3.3 per pregnant dam compared to mean 0.8 in control group, dose-response but not statistically significant) ↑ post-implantation loss (65% compared to 11% in control) ↓ mean number of viable foetuses (2 compared to 6.2 in control, p<0.05)	ZnPT CAR Doc IIIA A6.8.1/01 Year: 1993

					↑ number (7/26, 27%) of examined foetuses with malformations (7/105, 0.07%), p<0.05: • anencephaly (1) • hydrocephaly (1) • rigid flexure of shoulders and elbows (1) • cleft palate (1) • microglossia (1) • malformed testis (1) • malformed skull bones (1) • craniorachischisis (1) • vertebral malformation with or without associated rib malformation (2) • ectrodactyly (1) • bent limb bone: tibiofibula (1) • malformed scapulae (1) • humerus and ulna absent (1)	
No specific guideline, but comparable to OECD 414 No GLP	Reliability factor: 3 Reporting was more succinct than the guideline demands. Notable omissions are: clinical observations, organ weights and individual animal data.	Rabbit 20 females/ group	Zinc pyrithione Lot: specified 48% aq suspension. Purity of active ingredient prior to suspension in water not specified	1.0, 2.5 and 5.0 mg/kg bw day 6-18 of gestation Oral gavage	<u>Maternal tox – 2.5 mg/kg bw:</u> ↓ body weight gain (-71%, not statistically significant) <u>Maternal tox – 5.0 mg/kg bw:</u> ↓ food consumption (-17%, p<0.05) ↓ body weight days 6-18 (-136g compared to +175g in controls. No info on total weights.) <u>Foetal tox – 2.5 mg/kg bw:</u> ↑ post-implantation loss (47% compared to 12% in controls, not statistically significant) <u>Foetal tox –5.0 mg/kg bw:</u> ↑ post-implantation loss (83%, p<0.05)	Nolen and Dierckman, 1979[10]
No specific guideline, but comparable to OECD 414 No GLP	Reliability factor: 3 Two instead of three dose levels were used. Reporting was more succinct than the guideline demands. Notable omissions	Rat Sprague-Dawley 10 females/ group	Zinc pyrithione Lot: specified 48% aq suspension. Purity of active ingredient prior to suspension in water not specified	Untreated, vehicle, 7.5 or 15 mg/kg bw day 6-15 post mating Oral gavage	<u>Maternal tox – 7.5 mg/kg bw:</u> ↓ body weight gain days 0-15 (-71%, p<0.05) ↑ hind limb paralysis (5 compared to 0 in controls) <u>Maternal tox – 15 mg/kg bw:</u> ↓ body weight gain days 0-15 (-83%, p<0.05) ↑ hind limb paralysis (5 compared to 0 in controls) <u>Foetal tox – 15 mg/kg bw:</u> ↓ body weights (-23%, p<0.05)	

[10] Nolen, G.A. and Dierckman, T.A. 1979 Reproduction and teratology studies of zinc pyrithione administered orally or topically to rats and rabbits. Food and Cosmetics Toxicology 1979 Dec;17(6):639-49.

	are: clinical observations, food consumption, organ weights and individual animal data.				↑ incidence in skeletal abnormalities (82% compared to 45% in vehicle controls, p<0.05): • forked ribs (11% compared to 0 in controls) • missing ribs (18% compared to 0 in controls) • floating ribs (29% compared to 0 in controls)	
OPPTS 870.3700 GLP	Reliability factor: 1	Rat Crl:CD (SD)IGS BR VAF/Plus 25 females/ group	Zinc pyrithione Batch: specified Purity: >95%	0, 10, 15, 30, 60 mg/kg bw 6 h/day on gestation days 0-21 Dermal	<u>Maternal tox – 30 mg/kg:</u> ↓ adjusted body weight (-12%, p<0.01) <u>Maternal tox – 60 mg/kg:</u> (high-dose/controls) ↓ relative food consumption (-11-21%, p<0.01) ↓ adjusted body weight compared to controls (-31%, p<0.01) ↓ mean gravid uterine weights (-24%, p<0.01) ↓ muscle tone days 12-20 (16-21/ 0, p<0.01) ↓ muscle mass (6-12/0 in controls, p<0.01) ↑ number of rats with clinical observations (p<0.01 in all cases): • flaking grade 1 (14) • limited use of hindlimbs (24) • shuffing gait (22) • dehydration (21) • ungroomed coat (19) • urine-stained abdominal fur (12) • low carriage (11) • chromodacryorrhea (9) • emaciation (7) • chromorhinorrhea (8) • hunched posture (4) <u>Foetal tox – 60 mg/kg:</u> (high-dose/control) ↓ foetal body weights (♂: -21%, p<0.01; ♀: -18%, p<0.01) ↑ number of foetuses with any alteration (malformations or variations) (12%/6%, p<0.01) ↑ number of foetuses with skeletal variations (p<0.01): • wavy ribs (3%) • incomplete ossification of sternal centra (5%) ↓ foetal ossification sites averages compared to controls: • caudal vertebrae (p<0.01) • forelimb phalanges and metacarpals (p<0.05) • hindlimb phalanges (p<0.05) and metatarsals (p<0.01)	ZnPT CAR Doc IIIA A6.8.1/03 Year: 2005

					↑ number of foetuses with foetal gross external alterations: • medial rotation of both hindlimbs (1) • absent tail (1) ↑ number of foetuses with foetal soft tissue variations: • depressed eye bulges and microphtalmia (1)	
OECD 414 EPA OPPTS 870.3700 EU B.31	Reliability factor: 1	Rat Crl:WI(Han) 22 females/group	Zinc pyrithione Batch: specified Purity: >95%	0, 5, 15, 25 ppm (0, 0.4, 1.18, 1.68 mg/kg bw) Oral via diet from GD 6-20	Maternal tox – 1.68 mg/kg bw: • Abnormal gait, piloerection and pale faeces was noted in most animals. One female on a single occasion had hunched posture. • ↓ absolute bw, bw gains (ranging -36 to -69%), and adjusted bw gains from GD 15-20 • ↓ absolute and relative food consumption (ranging -21 to -45%) from GD 14-20 Foetal tox – 1.68 mg/kg bw: Statistically significant ↓ mean foetal body weights (♀: -9%; ♂: -8%) *No treatment related maternal or developmental findings at other dose levels.*	Thor GmbH Art. 95 dossier Year: 2015
OECD 414 EPA OPPTS 870.3700 EU B.31	Reliability factor: 1	Rabbit New Zealand White 22 females/group	Zinc pyrithione Batch: specified Purity: >95%	0, 0.5, 1.5, 4 mg/kg bw Oral gavage from GD 7-28	Maternal tox – 4 mg/kg bw: • red or orange discoloration of urine in 10 animals (which all had early resorptions) • Urinalysis on a single day of four animals showed high levels of blood in the urine. One of these animals also had high levels of glucose in the urine. • five animals had ↓ faeces for 2-6 days • ↓ absolute bw (ranging -8 to -9%) during GD 20-29 and ↓ bw gains (ranging -55 to -100%) during GD 13-29. The author considered that this was caused by early resorptions in the animals which resulted in also ↓ mean uterus weight and ↑ corrected absolute bw gain which were not statistically significant. • ↓ absolute (ranging -15 to -32% during GD 10-23) and relative food consumption (ranging -16 to -28% during GD 10-20) Foetal tox – 4 mg/kg: • statistically significant ↓ mean of viable foetuses (33% compared to 92% in controls) • statistically significant ↑ post-	Thor GmbH Art. 95 dossier Year: 2015

					implantation loss (67% compared to 8% in controls; a mean of 4.5 per litter compared to a mean of 0.5 in both controls and historical controls) • two foetuses (from two litters) had external malformations of omphalocele; tail was absent in one of these foetuses (these findings were not found in controls, were found only in one historical control foetus and also in the 1.5 mg/kg group; thus, the author considered these as treatment related) • Three foetuses (from two litters) had following visceral malformations (none in controls) - Urinary tract malformations/variations in one foetus such as absent right kidney and ureter, dilated left ureter and absent urine bladder (None in controls and only absent urine bladder finding in only one historical control foetus. These urinary tract malformations/variations were seen in the same foetus that showed omphalocele & absent tail. The author considered these as treatment related.) - one foetus showed absent lung lobe and one foetus had right-sided aortic arch (the author considered these as incidental findings) • eleven foetuses (from 5 litters) had following skeletal malformations (statistically significant) compared to 2 foetuses (from 2 litters) in controls - fused sternebrae (15.9%/litter; 7 foetuses from 5 litters) - rib anomaly (6.5%/litter; 2 foetuses from 2 litters) - vertebral anomaly with/without associated with rib anomaly (6%/litter; 2 foetuses from 3 litters) - single findings for the following: fused skull bones, costal cartilage anomaly and bent limb bones (the author considered these as treatment related as the litter incidences were well above historical control data) • Six foetuses (from 5 litters) had following treatment related (according to author) skeletal variations	

					- branched sternebrae (3 foetuses from 2 litters) - vertebral supernumerary sites (3 foetuses from 3 litters) • statistically significant ↑ in litter incidences of 13th full rib and pelvic girdle caudal shift (also found in 1.5 mg/kg group; the author considered these to be not toxicologically relevant) <u>Maternal tox – 1.5 mg/kg bw:</u> • red or orange discoloration of urine in 1 animal (which had early resorption) • there were no statistically significant changes compared to controls in body weights (gain) or (relative) food consumption • four animals had ↓ faeces for 3-6 days <u>Foetal tox – 1.5 mg/kg:</u> • statistically significant ↓ mean of viable foetuses (77% compared to 92% in controls) • statistically significant ↑ post-implantation loss (23% compared to 8% in controls; a mean of 1.7 per litter compared to a mean of 0.5 in both controls and historical controls) • two foetuses (from two litters) had external malformations of omphalocele; tail was absent in one of these foetuses (these findings were not found in controls, were found only in one historical control foetus and also in the 4 mg/kg group; thus, the author considered these as treatment related) • one foetus showed incidental visceral malformation (the author did not consider it as treatment related) consisting of teratology of fallot (i.e. pulmonary stenosis, ventricular septum defect, dextaposed aorta overriding the ventricular septum and thickened right ventricular wall) • statistically significant ↑ in litter incidences of 13th full rib and pelvic girdle caudal shift (also found in 4 mg/kg group; the author considered these to be not toxicologically relevant)	

Table 54: Summary table of human data on adverse effects on development

No data is available.

Table 55: Summary table of other studies relevant for developmental toxicity

No data is available.

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

A developmental toxicity study (ZnPT CAR Doc IIIA A6.8.1/02) was performed with rats exposed to zinc pyrithione by the oral route in accordance with GLP and OECD 414. The dose levels used were 0, 0.75, 3.0 and 15.0 mg/kg bw. Maternal toxicity was observed as decreased adjusted body weight[11] (-8% compared to controls) and adjusted body weight change (-38% compared to controls) in the highest dose group and one high-dose dam was found dead on gestation day 16. Reduced body weight gain during the last days of dosing was also recorded in the intermediate dose group. The only clinical signs observed were increased salivation and dilated pupils; no effects on hind limbs were recorded.

Post-implantation loss (3.7 compared to 0.8 in control group, p<0.01) and consequently a decrease in the mean number of viable foetuses/litter (12.5 compared to 14.5 in control group, p<0.05) was observed in the highest dose group together with a decreased gravid uterine weight (-17% compared to controls, not statistically significant). Increased post-implantation loss and reduced gravid uterus weight were also recorded in the intermediate dose group but were not statistically significant. Whole litter resorptions were recorded in three high-dose dams. A reduction in mean foetal body weights (-15% in females, p<0.01, and -17% in males, p<0.01) was also recorded but is considered likely to have been caused by maternal toxicity.

Foetuses of the high-dose group exhibited a high incidence of malformations (168 of examined foetuses compared to 1 in controls, p<0.01) and developmental variations (171 of examined foetuses compared to 79 in controls, p<0.05). The most common malformation observed was a vertebral malformation with or without an associated rib malformation observed in 89% of the high-dose foetuses examined. A high incidence of fused sternebrae (30 foetuses in 14 litters) and other sternebral malformations (35 foetuses in 13 litters) was observed in the high-dose group. Additional malformations in high-dose foetuses included ulna or radus missing, adactlyly/ectro-dactyly/syndactyly/polydactyly. The incidence of these malformations was 1 or 2 except for ectrodactyly that were recorded in 9 foetuses from 5 litters. In addition, the following soft tissue malformations were observed: dimorphism (2), malformed brain (1) and renal hypoplasia (1). None of these malformations were seen in the control group. Malformations were also seen in the intermediate dose group (7 foetuses), however with the exception of fused ribs none of them were the same as those observed in the high-dose group.

Table 56a: Maternal toxicity and signs of developmental toxicity observed in study ZnPT CAR Doc IIIA A6.8.1/02

Reference	**Maternal toxicity**	**Foetal viability**	**Malformations**
ZnPT CAR Doc IIIA A6.8.1/02	<u>15.0 mg/kg bw/day:</u> ↓ food consumption during gestation days 6-16 (up to 48% less, p<0.01)	<u>15.0 mg/kg bw/day:</u> ↓ mean foetal body weights (-15% in ♀	<u>15.0 mg/kg bw/day:</u> ↑ total number (168) of examined foetuses with malformations compared to controls (1), p<0.01

[11] Calculated as body weight minus gravid uterine weight

Oral rat	↓ adjusted body weight compared to controls at gestation day 20 (- 8%, p<0.01) ↓ adjusted body weight change compared to controls (-38%, p<0.01) ↑ number of animals with dilated pupils before and after dosing (17 animals) ↓ gravid uterine weight compared to control (-17%, dose-response but not statistically significant) ↑ salivation after dosing (29) 3.0 mg/kg bw/day: ↑ salivation after dosing (8)	(p<0.01) and -17% in ♂ (p<0.01) ↑ post-implantation loss (mean 3.7 per pregnant dam compared to mean 0.8 in control group, p<0.01) Whole litter resorption in 3 dams ↓ mean number of viable foetuses per litter (12.5/14.5, p<0.05) 3.0 mg/kg bw/day: ↑ post-implantation loss (mean 1.4 per pregnant dam compared to mean 0.8 in control group, not statistically significant)	↑ number (153) of examined foetuses with vertebral malformations with or without an associated rib malformation (89% of foetuses examined skeletally) ↑ number of examined foetuses with skeletal malformations: (high-dose/controls) • rib malformation (3/0) • fused ribs (3/0) • fused sternebrae (30/0) • sternal malformation (35/0) • limb malformation (ulna or radus missing) (2/0) • adactyly (1/0) • ectrodactyly (9/0) • syndactyly (1/0) • polydactyly (1/0) ↑ number of examined foetuses with soft tissue malformations: (high-dose/controls) • dimorphism (2/0) • malformed brain (1/0) • renal hypoplasia (1/0) ↑ total number (171) of examined foetuses with developmental variations (79), p<0.05 ↑ number of examined foetuses with developmental variations: (high-dose/controls) • less than 13 pairs of ribs (122/0) • variations in the number of presacral vertebrae (24, 25, or 27) (126/1) • unossified sternebrae 5 or 6 (91/58) • unossified sternebrae 1, 2, 3 or 4 (23/1) • misaligned sternebrae (66/4) ↑ number of examined foetuses with soft tissue developmental variations: (high-dose/controls) • distended ureter(s) (16/7) • undeveloped renal papillae (12/1) 3.0 mg/kg bw/day: ↑ total number of foetuses with malformations (7/1 in mid-dose/controls, p<0.05) ↑ number of examined foetuses with skeletal malformations: (mid-dose/controls) • fused ribs (3/0) • pelvic malformation (1/0) • tail malformation (1/0)

			↑ number of examined foetuses with soft tissue malformations: (mid-dose/controls) • a diaphragmatic hernia (2/0) • anal atresia (the foetus with the tail malformation/0)

Table 56b: Maternal effects in the study ZnPT CAR Doc IIIA A6.8.1/02

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	
	historical	study	**(0.75 mg/Kg)**	**(3.0 mg/Kg)**	**(15.0 mg/Kg)**	**dose-response + / -**
Number of dams examined		30	30	30	30	
Clinical findings during application of test substance						
Salivation post dose				↑	↑	+
Dilated pupils					↑	+
Mortality of dams *state %*	0/716	0 (0%)	0 (0%)	(0) 0%	1 (3.3%)	+
Necropsy findings in dams dead before end of test					No gross lesions	
Abortions		0	0	0	0	-
Body weight gain, unadjusted (g)						
day 0-6		35	36	37	36	-
day 6-9		10	9	7	-3**	+
day 9-12		16	13	15	0**	+
day 12-16		25	24	18*	18	+
day 16-20		63	66	62	51*	+
day 6-16		51	46	40**	17**	+
day 0-20		148	148	138	103**	+
Food consumption (g/animal/d)						
day 0-6		23.3	22.3	22.3	21.8	-
day 6-9		22.3	22.1	21.8	19.5**	+
day 9-12		24.9	23.3	23.7	12.9**	+
day 12-16		24.7	24.3	23.8	21.0**	+
day 16-20		27.2	26.7	26.6	24.8	-
day 6-16		24.0	23.4	23.2	18.2**	+

day 0-20		24.4	23.7	23.6	20.6**	+
Pregnancies (number)		27	30	25	28	-

* statistically significant different from control, p<0.05
** statistically significant different from control, p<0.01

Table 56c: Litter response (Caesarean section data) in the study ZnPT CAR Doc IIIA A6.8.1/02

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	**study**				
Mean number of corpora lutea per dam	16.8	17.2	17.5	17.1	17.7	-
Mean number of implantations per dam	15.0	15.3	16.0	15.2	16.0	-
Resorptions *total/number of dams*	1.2	0.81	0.80	1.44	3.67**	+
total number of fetuses *total/number of dams with viable fetuses*	8382/603	392/27	457/30	345/25	350/28*	+
pre-implantation loss (%)		10.8	8.6	11.0	9.4	-
post-implantation loss	1.1/dam	22/27	24/30	36/25	99/27**	+
total number of litters		27	30	25	24	-
fetuses / litter		14.5	15.2	13.8	12.5*	+
live fetuses / litter *state ratio*	13.9	14.5	15.2	13.8	12.5*	+
dead fetuses / litter *ratio*		0	0	0	0	-
fetus weight (mean) *[g]*	3.4	3.6	3.4	3.4	3.0**	+
Mean gravid uterus weight [g]	76.3	80.9	81.9	73.2	67.8	-
Fetal sex ratio *[ratio m/f]*	0.98	1.25	1.16	1.16	1.11	-

* statistically significant different from control, p<0.05
** statistically significant different from control, p<0.01

Table 56d: Examination of the fetuses in the study ZnPT CAR Doc IIIA A6.8.1/02

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	**study**				
Total fetuses (litters) with malformations	36 (33)	1 (1)	3 (2)	7 (6)*	168 (24)**	+

Total fetuses (litters) with developmental variations	900 (281)	79 (25)	78 (27)	56 (22)	171 (24)*	+

* statistically significant different from control, p<0.05
** statistically significant different from control, p<0.01

A GLP developmental toxicity study (ZnPT CAR Doc IIIA A6.8.1/01) according to US EPA 83-3, which complies with OECD 414, investigated the oral administration of zinc pyrithione by gavage to New Zealand white rabbits. The dose levels used were 0, 0.5, 1.5 and 3.0 mg/kg bw. Maternal toxicity was manifested as reduced food consumption (-13-31%, p<0.01 in high-dose and -14-23%, p<0.01, in mid-dose) and reduced body weight gain on days 6-19 (weight gain was 2% and 59% of control values in the high and mid-dose females, respectively, p<0.01). Weight gain increased during days 20-29 with average weight gain on days 0-29 being 85% and 83% of controls for the same groups (no statistically significant reduction), and no differences in average adjusted body weights (calculated as body weight minus gravid uterine weight) on days 0-29 were recorded between any of the dose groups.

Developmental toxicity in the high-dose group included a decrease in the number of pregnant animals (13 as compared to 17-18 in the other groups), increased post-implantation loss (65% compared to 11% in controls, dose-response but not statistically significant) and increased incidence of resorptions (mean 3.3 per pregnant dam compared to 0.8 in controls, dose-response but not statistically significant). Total litter resorption was observed in 5 high-dose and 1 mid-dose female. Moreover, one high-dose animal aborted at the end of pregnancy. Mean litter size was also reduced (2.0 compared to 6.2 in controls, p<0.05) as was the number of animals with viable foetuses (7/13 compared to 17/17 in controls, dose-response but not statistically significant). The litter resorptions were not the cause of the reductions seen in maternal body weight gain as weight gain was 1.4 g in the highest dose group on days 6-19 if calculated for the females without total litter resorption only.

Due to the high incidence in litter loss only 26 foetuses remained in the high-dose group compared to 105 in the control group making evaluation of teratogenic effects difficult. But 7/26 high-dose foetuses were malformed as compared to 7/105 in the control group. Several of the malformations are considered rare and severe. Multiple cephalic and limb malformations occurred in three foetuses from two of the seven litters examined in this treatment group.

In the mid-dose group, there was an increase in resorptions (a mean of 1.6 per pregnant dam compared to a mean of 0.8 in controls) and an increase in post-implantation loss (29% compared to 11% in controls).

Table 57: Maternal toxicity and signs of developmental toxicity observed in the study ZnPT CAR Doc IIIA A6.8.1/01

Reference	Maternal toxicity	Foetal viability	Malformations
ZnPT CAR Doc IIIA A6.8.1/01 Oral rabbit	<u>3.0 mg/kg bw/day:</u> ↓ food consumption (- 13-31%, p<0.01) ↓ body weight gain d 6-19 (-98%, p<0.01) ↓ mean gravid uterus weights (-32% but not statistically significant)	<u>3.0 mg/kg bw/day:</u> ↑ post-implantation loss (65% compared to 11% in controls, dose-response but not statistically significant) ↑ resorptions (mean 3.3 per pregnant dam compared to mean 0.8 in control group, dose-response but not statistically significant) ↑ total litter resorption (5) ↑ abortion at the end of	<u>3.0 mg/kg bw/day:</u> ↑ number (7/26, 27%) of examined foetuses with malformations compared to controls (7/105, 0.07%, p<0.05), mainly seen in 3 foetuses in 2/7 litters: • anencephaly (1) • hydrocephaly (1) • rigid flexure of shoulders and elbows (1)

		pregnancy (1) ↓ mean litter size (2.0 compared to 6.2 in controls, $p<0.05$) ↓ mean number of viable foetuses (2 compared to 6.2 in control, $p<0.05$) ↓ number of animals with viable foetuses (7/13 compared to 17/17 in controls, dose-response but not statistically significant)	• cleft palate (1) • microglossia (1) • malformed testis (1) • malformed skull bones (1) • craniorachischisis (1) • vertebral malformation with or without associated rib malformation (2) • ectrodactyly (1) • bent limb bone: tibiofibula (1) • malformed scapulae (1) • humerus and ulna absent (1)
	<u>1.5 mg/kg bw/day:</u> ↓ food consumption (-14-23%, $p<0.01$) ↓ mean gravid uterus weights (-32% but not statistically significant)	<u>1.5 mg/kg bw:</u> ↑ resorption (mean 1.6 per pregnant dam compared to mean 0.8 in control group, dose-response but not statistically significant) ↑ post-implantation loss (29% compared to 11% in control)	

Table 58a: Malformations observed per foetus and litter in the study ZnPT CAR Doc IIIA A6.8.1/01

Animal number	Foetus number	Malformation
45752	2	Interrupted ossification
45755	1	Malformed testis Craniorachischisis Ectrodactyly Malformed scapulae Humerus and ulna absent
	2	Anencephaly Bent limb bone: tibiofibula
	6	Vertebral malformation with associated rib malformation
45758	1	Hydrocephaly Rigid flexure of shoulders and elbows Cleft palate Microglossia
45759	1	Vertebral malformation without associated rib malformation
	2	Fused sternebrae

Table 58b: Maternal effects in the study ZnPT CAR Doc IIIA A6.8.1/01

Parameter	**control data**				

	historical	study	**low dose (0.5 mg/Kg)**	**medium dose (1.5 mg/Kg)**	**high dose (3.0 mg/Kg)**	**dose-response + / -**
Number of dams examined		20	20	20	20	
Clinical findings during application of test substance						
Incidence of red fluid in the refuse pan					↑	+
Defecation			↓	↓	↓	+
Mortality of dams *state %*	30/979 (3.1%)	0 (0%)	0 (0%)	(1) 5%	0 (0%)	-
Necropsy findings in dams dead before end of test						
Red discolorization in the lung				1		-
Tan discolorization in the liver				1		-
Abortions	36/979 (3.7%)	0	0	0	1	+
Body weight gain, unadjusted (g)						
day 0-6		299	314	256	304	-
day 6-12		139	140	50**	36**	+
day 12-19		138	147	99	-34**	+
day 19-24		123	103	156	208*	+
day 24-29		126	62	104	142	-
day 6-19		277	206	164**	2**	+
day 0-29	270	825	765	682	703	-
Food consumption (g/animal/d)						
day 0-6		231.7	230.9	213.0	221.9	-
day 6-12		203.2	209.2	175.1**	176.8*	+
day 12-19		196.2	191.4	151.6*	135.2**	+
day 19-24		191.7	198.3	192.5	196.0	-
day 24-29		155.1	155.5	181.3	190.2	-
day 6-19		199.5	199.6	168.2**	154.4**	+
day 0-29		197.1	198.2	183.1	182.7	-
Pregnancies (number)		17	18	17	13	-

* statistically significant different from control, p<0.05

** statistically significant different from control, p<0.01

Table 58c: Litter response (Caesarean section data) in the study ZnPT CAR Doc IIIA A6.8.1/01

Parameter	control data		low dose	medium dose	high dose	dose-response + / -
	historical	study				
Mean number of corpora lutea per dam	13.1	11.2	10.8	11.2	10.2	-
Mean number of implantations per dam	8.0	6.9	6.3	5.4	5.5	+
Resorptions *total/number of dams*		13/17	13/18	25/16	40/12	+
total number of fetuses *total/number of dams with viable fetuses*	5509/787	105/17	100/18	61/15	26/7	+
pre-implantation loss		38.2%	41.8%	50.0%	38.0%	-
post-implantation loss	0.9/doe	11.05	12.45	29.15*	64.95*	+
total number of litters		17	18	16	13	
fetuses / litter						
live fetuses / litter *state ratio*	7.0	6.2	5.5	3.8	2.0*	+
dead fetuses / litter *ratio*		0	0.055	0	0	-
fetus weight (mean) *[g]*	40.8	49.8	49.0	49.6	51.7	-
Mean gravid uterus weight [g]		406.3	374.7	278.0	278.0	
Fetal sex ratio *[ratio m/f]*	2913/2807	60/45	54/46	23/38	12/14	-

* statistically significant different from control, p<0.05
** statistically significant different from control, p<0.01

Table 58d: Examination of the fetuses in the study ZnPT CAR Doc IIIA A6.8.1/01

Parameter	control data		low dose	medium dose	high dose	dose-response + / -
	historical	study				
Total fetuses (litters) with malformations	215 (171)	7 (6)	12 (7)	5 (4)	7 (4)*	+
Total fetuses (litters) with developmental variations	4153 (770)	83 (17)	88 (18)	50 (14)*	21 (7)	+

* statistically significant different from control, p<0.05
** statistically significant different from control, p<0.01

A published study from 1979 (Nolen and Dierckman, 1979) where rats and rabbits were exposed to zinc pyrithione is included as supporting information. The study was not performed according GLP or any guideline but is roughly comparable to OECD 414. Notable omissions were clinical observations, food consumption, organ weights and individual animal data. In the rabbit experiment, 20 rabbits per group were dosed with 1.0, 2.5, and 5.0 mg/kg bw/day by gavage. Food consumption was reduced in the highest dose group compared to controls (-17%, p<0.05) and the maternal body weight gain during the dosing period decreased in a dose-related manner, with a statistically significant weight loss in the highest dosed group, -136 g compared to +175 g in controls. No information on total body weights was given but assuming a body weight of 4 kilos the weight change would represent approximately -3% while body weight gain was +4% for controls.

There was a dose-related increase in post-implantation loss (83% in high-dose, p<0.05, and 47% in mid-dose, not statistically significant, compared to 12% in controls). No treatment-related skeletal or visceral abnormalities and no effect on foetal body weights were observed in any of the dose groups, however, it is noted that due to the high incidence of post-implantation loss the number of foetuses available for foetal examination was very low at the high and intermediate dose levels.

Table 59: Maternal toxicity and signs of developmental toxicity observed in rabbit part of Nolen and Dierckman, 1979

Reference	Maternal toxicity	Foetal viability	Malformations
Nolen and Dierckman, 1979 Oral rabbit	5.0 mg/kg bw/day: ↓ food consumption compared to controls (-17%, p<0.05) ↓ body weight days 6-18 (-136g compared to +175g in controls) 2.5 mg/kg bw/day: ↓ body weight gain (-71% compared to controls, not statistically significant)	5.0 mg/kg bw/day: ↑ post-implantation loss (83%, p<0.05) 2.5 mg/kg bw/day: ↑ post-implantation loss (47% compared to 12% in controls, not statistically significant)	None observed.

In the rat part of the study, 10 rats were dosed with 7.5 and 15.0 mg/kg bw/day by gavage during days 6-15 of gestation. This is not in line with OECD 414 which requires testing of 20 animals/dose at three dose levels. Both dose groups showed dose-related decreases in weight gain compared to controls (-83% and -71% respectively, p<0.05), and 5/10 dams in each group showed hindlimb paralysis. No information on terminal body weights was available. There was a significant increase in the numbers of skeletal abnormalities in the highest dose group (82% compared to 45% in vehicle controls, p<0.05), and the foetal weights in this group were significantly lower than in the vehicle group (-23%, p<0.05). Skeletal abnormalities included forked ribs (11% compared to 0% in controls), missing ribs (18% compared to 0% in controls) and floating ribs (29% compared to 0% in controls). These effects may have been caused by the maternal toxicity.

Table 60: Maternal toxicity and signs of developmental toxicity observed in rat part of Nolen and Dierckman, 1979

Reference	Maternal toxicity	Foetal viability	Malformations
Nolen and Dierckman, 1979 Oral rat	15.0 mg/kg bw/day: ↓ body weight gain days 0-15 compared to vehicle controls (-83%, p<0.05) ↑ hind limb paralysis (5 compared to 0 in controls) 7.5 mg/kg bw/day: ↓body weight gain days 0-15 (-71%, p<0.05) ↑ hind limb paralysis (5 compared to 0 in controls)	15.0 mg/kg bw/day: ↓ foetal body weights (-23%, p<0.05)	15.0 mg/kg bw/day: (high-dose/controls) ↑ skeletal abnormalities (82/45%, p<0.05): • forked ribs (11%/0%) • missing ribs (18%/0%) • floating ribs (29%/0%)

A developmental toxicity study (ZnPT CAR Doc IIIA A6.8.1/03) with dermal exposure of zinc pyrithione to rats used dose levels of 0, 10, 15, 30 and 60 mg/kg bw. Maternal toxicity was manifested as decreased food consumption in the high-dose group (-21%, p<0.01) and decreased adjusted body weights (calculated as body weight minus gravid uterine weight) in the mid- and high-doses (-12% and -31%, respectively, p<0.01). Furthermore, limited use of hindlimbs (24/25 animals), shuffling gait (22/25) and no use of hind limbs (2/25) was observed in the high-dose group together with a significantly decreased muscle tone (21/25, p<0.01) and loss in muscle mass (12/25, p<0.01). None of these effects were seen in the controls. The animals in the highest dose level also exhibited emaciation, dehydration, ungroomed coat, urine-stained abdominal fur, low carriage, hunched posture, chromodacryorrhea and chromorhinorrhea.

Developmental toxicity was observed in the high-dose group only and included reduced foetal body weights (-21% for males, p<0.01, and -18% for females, p<0.01) and an increased number of foetuses with malformations or variations (12 compared to 6 in controls, p<0.01). Skeletal variations included wavy ribs (3) and incomplete ossification of sternal centra (3). A decrease in foetal ossification sites including caudal vertebrae (p<0.01), forelimb phalanges and metacarpals (p<0.05) and hindlimb phalanges (p<0.05) was also observed. Gross examination revealed medial rotation of both hindlimbs (1 in high-dose and 1 in mid-dose) and absent tail (1). Depressed eye bulges and microphtalmia (1) were observed at soft tissue examination. No effect on foetal viability was observed. The decrease in foetal weights and the associated skeletal variations that were recorded can probably be attributed to the significant maternal toxicity (-31% adjusted body weight) that was observed at the same dose level.

Table 61a: Maternal toxicity and signs of developmental toxicity observed in the study ZnPT CAR Doc IIIA A6.8.1/3

Reference	Maternal toxicity	Foetal viability	Malformations
ZnPT CAR Doc IIIA A6.8.1/03 Dermal rat	<u>60 mg/kg:</u> ↓ adjusted body weight compared (-31% (p<0.01)) ↓ relative food consumption compared to controls (-11-21%, p<0.01) ↓ mean gravid uterine weights compared to controls (-24%, p<0.01) ↓ muscle tone days 12-20 (16-21 rats compared to 0 in controls, p<0.01) ↓ muscle mass (6-12 rats compared to 0 in controls, p<0.01) ↑ number of rats with clinical observations compared to controls (p<0.01 in all cases):	<u>60 mg/kg:</u> ↓ foetal body weights compared to controls (♂: -21%, p<0.01; ♀: -18%, p<0.01) No effects on foetal viability or litter size were observed.	<u>60 mg/kg:</u> ↑ number of foetuses with any alteration (malformations or variations) (12% compared to 6% in controls, p<0.01) ↑ number of foetuses with skeletal variations (p<0.01): • wavy ribs (3%) • incomplete ossification of sternal centra (5%) ↓ foetal ossification sites averages compared to controls: • caudal vertebrae (p<0.01) • forelimb phalanges and metacarpals (p<0.05) • hindlimb phalanges (p<0.05) and metatarsals (p<0.01) ↑ number of foetuses with foetal gross external alterations: • medial rotation of both hindlimbs (1 in high-dose and 1 in mid-dose) • absent tail (1) ↑ number of foetuses with foetal soft tissue variations:

	• flaking grade 1 (14) • limited use of hindlimbs (24) • shuffling gait (22) • dehydration (21) • ungroomed coat (19) • urine-stained abdominal fur (12) • low carriage (11) • chromodacryorrhea (9) • emaciation (7) • chromorhinorrhea (8) • hunched posture (4) • no use of hindlimbs (2) <u>30 mg/kg:</u> ↓ adjusted body weight (-12%, p<0.01) ↑ limited use of hindlimbs (2/24)		• depressed eye bulges and microphtalmia(1)

Table 61b: Maternal effects in the study ZnPT CAR Doc IIIA A6.8.1/3

Parameter	control data		Grp II	Grp III	Grp IV	Grp V
	historical	**Grp I**				
Number of dams examined	not reported	23	24	24	24	25
Clinical findings during application of test substance: difficulty in movement, impairment of hindlimbs, hunched posture, emaciation.	/	-	-	-	-	+
Mortality of dams (%) **(Both females dying on-study appeared normal, gained weight, and no adverse clinical signs were apparent – deaths not believed related to test article)**	/	0	1	0	0	1
Abortions (%)	/	0	0	0	0	0
Body weight gain Day 3 Day 6 Day 7 Day 9 Day 13 Day 16 Day 21	/	- - - - - -	- - - - - -	- - - - - -	- - - - ↓ ↓ ↓ ↓	- - ↓ ↓ ↓ ↓ ↓

Food consumption Day 0-3 Day 6-9 Day 9-12 Day 12-15 Day 15-18 Day 18-20 Day 20-21 Day 0-21	/	- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - ↓ ↓ ↓ ↓	- - ↓ ↓ ↓ ↓ ↓ ↓ ↓
Water consumption	/	n.a.	n.a.	n.a.	n.a.	n.a.
Pregnancies (No. / %)	/	23	23	24	24	24
Litters with live foetuses	/	23	23	24	24	24
Necropsy findings in dams dead before end of test	/	n.a.	No adverse findings	n.a.	n.a.	No adverse findings

Table 61c: Litter response (Caesarean section data) in the study ZnPT CAR Doc IIIA A6.8.1/3

Parameter	control data					
	historical	**Grp I**	**Grp II**	**Grp III**	**Grp IV**	**Grp V**
Corpora lutea	not reported	15.5	16.00	15.1	14.8	15.7
Implantations. mean per litter	not reported	14.5	14.7	14.0	14.0	14.1
Early Resorptions. mean per litter	not reported	0.80	0.50	0.50	0.60	0.70
Late Resorptions. mean per litter	not reported	0.00	0.00	0.00	0.10	0.00
Viable young. mean per litter	not reported	13.7	14.3	13.5	13.4	13.4
Percent males	not reported	51.9	52.2	52.1	47.6	47.5
Male fetuses weight /Litter weight (g)	not reported	5.52	5.41	5.44	5.44	4.39
Female fetuses weight/Litter weight (g)	Not reported	5.15	5.07	5.10	5.17	4.23
Mean foetal weight (g)	not reported	5.35	5.25	5.28	5.31	4.31
placenta weight (mean) [g]	not reported	not reported	not reported	not reported	not reported	not reported

Table 61d: Examination of the foetuses in the study ZnPT CAR Doc IIIA A6.8.1/3

Parameter	**control data**					
	historical	**Grp I**	**Grp II**	**Grp III**	**Grp IV**	**Grp V**
External anomalies / alterations	not reported					
Number of foetuses examined		315	328	310	323	308
Number of litters examined		23	23	23	24	23
No total abnormalities detected		0	0	0	0	1
Eye Bulge Depressed	not reported					
Litter incidence		0	0	0	0	1
Fetal incidence		0	0	0	0	1
Hindlimb (s): Rotated Medially	not reported					
Litter incidence		0	0	0	1	1
Fetal incidence		0	0	0	1	1
Tail: Absent	not reported					
Litter incidence		0	0	0	0	1
Fetal incidence		0	0	0	0	1
Soft Tissue anomalies / alterations	not reported					
Number of foetuses examined		151	157	149	155	148
Number of litters examined		23	23	23	24	23
Eyes: Retina Folded	not reported					
Litter incidence		1	1	1	0	0
Fetal incidence		1	1	1	0	0
Eyes: Microphthalmia	not reported					
Litter incidence		0	0	0	0	1
Fetal incidence		0	0	0	0	1

Skeletal anomalies / alterations Number of foetuses examined Number of litters examined	not reported	164 23	171 23	161 23	168 24	160 23
Skull: Sygomatic – incomplete ossified						
Litter incidence	not reported	0	0	0	0	1
Fetal incidence		0	0	0	0	1
Skull: Squamosal, incomplete ossified	not reported					
Litter incidence		0	0	0	0	1
Fetal incidence		0	0	0	0	1
Cervical vertebrae: Cerv rib @ 7th verteb						
Litter incidence	not reported	2	0	1	1	2
Fetal incidence		2	0	1	1	2
Thoracic Vertebrae: Centrum, Bifid						
Litter incidence	not reported	1	0	1	0	1
Fetal incidence		2	0	1	0	1
Caudal Vertebrae: 3 Present						
Litter incidence	not reported	0	0	0	0	1
Fetal incidence		0	0	0	0	1
Ribs: Wavy						
Litter incidence	not reported	0	0	0	0	2
Fetal incidence		0	0	0	0	3
Ribs: Short						
Litter incidence	not reported	0	1	0	0	0
Fetal incidence		0	2	0	0	0
Ribs: Incompletely Ossified						
Litter incidence	not reported	1	0	0	0	0
Fetal incidence		1	0	0	0	0
Sternal Centra: Incompletely Ossified						
Litter incidence	not reported	0	0	0	0	3
Fetal incidence		0	0	0	0	5
Pelvis: Ischium, incompletely ossified						
Litter incidence	not reported	0	0	0	0	1
Fetal incidence		0	0	0	0	2

In a prenatal developmental toxicity study performed according to the guidelines (OECD 414/EPA OPPTS 870.3700/EU B.31) and with GLP, zinc pyrithione (purity: >95%) was given to mated female Wistar Han rats by diet at 0, 5, 15, and 25 ppm, corresponding to mean intakes of 0, 0.4, 1.18, and 1.68 mg/kg bw, from GD 6-20 (Thor GmbH Art. 95 dossier, 2015). Maternal toxicity was observed in the high-dose group as decreased body weight gains (ranging -36 to -69%) from GD 15-20 and decreased relative food consumption (ranging -21 to -45%) from GD 14-20. Most of the high-dose animals showed clinical signs of abnormal gait, piloerection and pale faeces. One female on a single occasion had hunched posture. Mean foetal body weights in the high-dose group were statistically significantly lower (-9% for females and -8% for males). The author considered this as secondary to the observed maternal toxicity. No other treatment related maternal or developmental findings were found in other dose groups. The results are summarised in the table below.

Table 62a: Maternal toxicity and signs of developmental toxicity in the oral rat study from Thor GmbH Art. 95 dossier

Reference	**Maternal toxicity**	**Foetal viability**	**Malformations**
Thor GmbH Art. 95 dossier, 2015 Oral rat	1.68 mg/kg bw: • Abnormal gait, piloerection and pale faeces was noted in most animals. One female on a single occasion had hunched posture. • ↓ absolute bw, bw gains (ranging -36 to -69%), and adjusted bw gains from GD 15-20 • ↓ absolute and relative food consumption (ranging -21 to -45%) from GD 14-20	1.68 mg/kg bw: Statistically significant ↓ mean foetal body weights (♀: -9%; ♂: -8%)	None

Table 62b: Further presentation of maternal and developmental effects in the oral rat study from Thor GmbH Art. 95 dossier

Dosage (ppm in diet)	0	Low-dose (5 ppm)	Mid-dose (15 ppm)	High-dose (25 ppm)
Maternal effects				
Body weight gain (g/animal), gestation day 6-20	84	89	86	39*
Abnormal gait	--	--	--	3/22*
Developmental effects				
Fetal weight (g)	3.5	3.6	3.5	3.3*
Resorptions (%/litter)	8.1	3.4	5.4	6.1
Malformed fetuses	1	2	3	2

*significantly different from control (P<0.05)

Table 62c: Summary of maternal survival and pregnancy status in the oral rat study from Thor GmbH Art. 95 dossier

DOSE GROUP :	1		2		3		4	
	NO.	%	NO.	%	NO.	%	NO.	%
FEMALES ON STUDY	22		22		22		22	
FEMALES THAT ABORTED OR DELIVERED	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT DIED	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT ABORTED	0	0.0	0	0.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT WERE EUTHANIZED	0	0.0	0	0.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES EXAMINED AT SCHEDULED NECROPSY	22	100.0	22	100.0	22	100.0	22	100.0
NONGRAVID	1	4.5	1	4.5	0	0.0	1	4.5
GRAVID	21	95.5	21	95.5	22	100.0	21	95.5
WITH RESORPTIONS ONLY	1	4.8	0	0.0	0	0.0	0	0.0
WITH VIABLE FETUSES	20	95.2	21	100.0	22	100.0	21	100.0
TOTAL FEMALES GRAVID	21	95.5	21	95.5	22	100.0	21	95.5

1- 0 PPM 2- 5 PPM 3- 15 PPM 4- 25 PPM

Table 62d: Summary of foetal data at scheduled necropsy in the oral rat study from Thor GmbH Art. 95 dossier

GROUP		SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1	TOTAL	118	125	243	0	11	1	12	255	274	19	NA	21
	MEAN	5.6	6.0	11.6	0.0	0.5	0.0	0.6	12.1	13.0	0.9	3.5	
	S.D.	2.42	2.29	3.09	0.00	0.68	0.22	0.75	3.04	2.75	1.09	0.26	
2	TOTAL	126	130	256	0	9	0	9	265	285	20	NA	21
	MEAN	6.0	6.2	12.2	0.0	0.4	0.0	0.4	12.6	13.6	1.0	3.6	
	S.D.	2.39	2.04	2.46	0.00	0.75	0.00	0.75	2.44	2.34	1.66	0.18	
3	TOTAL	121	147	268	0	13	1	14	282	295	13	NA	22
	MEAN	5.5	6.7	12.2	0.0	0.6	0.0	0.6	12.8	13.4	0.6	3.5	
	S.D.	1.79	2.08	2.26	0.00	0.73	0.21	0.73	2.04	1.87	0.73	0.33	
4	TOTAL	113	134	247	0	18	0	18	265	277	12	NA	21
	MEAN	5.4	6.4	11.8	0.0	0.9	0.0	0.9	12.6	13.2	0.6	3.3	
	S.D.	1.77	1.86	2.79	0.00	1.01	0.00	1.01	3.12	2.84	0.75	0.27	

NA = NOT APPLICABLE

1- 0 PPM 2- 5 PPM 3- 15 PPM 4- 25 PPM

In another prenatal developmental toxicity study performed according to the guidelines (OECD 414/EPA OPPTS 870.3700/EU B.31) and with GLP, zinc pyrithione (purity: >95%) was given to mated female New Zealand White rabbits by oral gavage from GD 7-28 at doses of 0, 0.5, 1.5, and 4 mg/kg (Thor GmbH Art. 95 dossier, 2015). Maternal toxicity was observed in the high-dose group in the form of red/orange discolouration of the urine (in 10 animals), statistically significantly reduced absolute body weight (ranging -8 to -9% during GD 20-29) & body weight gains (ranging -55 to -100% during GD 13-29) and reduced absolute (ranging -15 to -32% during GD 10-23) & relative (ranging -16 to -28% during GD 10-20) food consumption. The study author considered the maternal toxicity to be an indirect effect due to a high incidence of resorptions in this group. There was a statistically significant increase in post-implantation loss (67% compared to 8% in controls) and decrease in mean of viable foetuses (33% compared to 92% in controls) in the high-dose group. Such developmental toxicity was also observed in the mid-dose group in the absence of maternal toxicity, i.e. statistically significant increase in post-implantation loss (23% compared to 8% in controls) and decrease in mean of viable foetuses (77% compared to 92% in controls). However, for 6 of the 21 does in the mid-dose group the body weight gain was statistically significantly lower during GD 7-29 (58% of the controls) and most of the post-implantation losses in this group were seen in those six does (see Table 63c). The high-dose group had only 9 litters with viable foetuses compared to 19 litters with viable foetuses in the mid-dose group and in controls.

Adverse effects on foetal morphology were observed in both mid- and high-dose groups. External malformations of omphalocele were observed in two foetuses from two litters in the high-dose group and also in two foetuses from two litters in the mid-dose group. Two foetuses (one each from mid and high-dose group) among the four affected foetuses also had an absent tail. These external malformations were not found in controls and in only one historical control foetus. The author considered these to be treatment related. The foetus in the high-dose group with omphalocele and absent tail also had several urinary tract malformations/variations (absent right kidney and ureter, dilated left ureter and absent urine bladder). These were not observed in controls and only one historical control foetus had absent urine bladder. The malformations of omphalocele in the mid-dose group were observed only in the litters of six does that had statistically significant decrease in body weight gain (see Table 63c).

In the high-dose group, statistically significant increase in skeletal malformations (11 foetuses from 5 litters) were observed compared to controls (2 foetuses from 2 litters) including fused sternebrae (15.9%/litter), rib anomaly (6.5%/litter), vertebral anomaly with/without rib anomaly (6%/litter) and single findings of fused skull bone, costal cartilage anomaly and bent limb bones. Treatment related skeletal variations were also observed in six foetuses from five litters of the high-dose group. Those include branched sternebrae (3 foetuses from 2 litters) and vertebral supernumerary sites (3 foetuses from 3 litters). There was a statistically significant increase in litter incidences of 13th full rib and pelvic girdle caudal shift in mid- and high-dose groups. The author considered these to be not toxicologically relevant. One foetus in the mid-dose group showed visceral malformation consisting of teratology of fallot (i.e. pulmonary stenosis, ventricular septum defect, dextaposed aorta overriding the ventricular septum and thickened right ventricular wall). This incidental finding was not considered to be treatment related by the author.
There was no maternal or developmental toxicity in the low-dose group.
The results are summarised in the table below.

Table 63a: Maternal toxicity and signs of developmental toxicity in the oral rabbit study from Thor GmbH Art. 95 dossier

Reference	Maternal toxicity	Foetal viability	Malformations
Thor GmbH Art. 95 dossier Oral rabbit	4 mg/kg bw: • red or orange discoloration of urine in 10 animals (which all had early resorptions) • Urinalysis on a single day of four animals showed high levels of blood in the urine. One of these animals also had high levels of glucose in the urine. • five animals had ↓ faeces for 2-6 days • ↓ absolute bw (ranging -8 to -9%) during GD 20-29 and ↓ bw gains (ranging -55 to -100%) during GD 13-29. The author considered that this was caused by early resorptions in the animals which resulted in also ↓ mean uterus weight and ↑ corrected absolute bw gain which were not statistically significant. • ↓ absolute (ranging -15 to -32% during GD 10-23) and relative food consumption (ranging -16 to -28% during GD 10-20)	4 mg/kg: • statistically significant ↓ mean of viable foetuses (33% compared to 92% in controls) • statistically significant ↑ post-implantation loss (67% compared to 8% in controls; a mean of 4.5 per litter compared to a mean of 0.5 in both controls and historical controls)	4 mg/kg: • two foetuses (from two litters) had external malformations of omphalocele; tail was absent in one of these foetuses (these findings were not found in controls, were found only in one historical control foetus and also in the 1.5 mg/kg group; thus, the author considered these as treatment related) • Three foetuses (from two litters) had following visceral malformations (none in controls) - Urinary tract malformations/variations in one foetus such as absent right kidney and ureter, dilated left ureter and absent urine bladder (None in controls and only absent urine bladder finding in only one historical control foetus. These urinary tract malformations/variations were seen in the same foetus that showed omphalocele & absent tail. The author considered these as treatment related.) - one foetus showed absent lung lobe and one foetus had right-sided aortic arch (the author considered these as incidental findings) • eleven foetuses (from 5 litters) had following skeletal malformations (statistically significant) compared to 2 foetuses (from 2 litters) in controls - fused sternebrae (15.9%/litter; 7 foetuses from 5 litters)

			- rib anomaly (6.5%/litter; 2 foetuses from 2 litters) - vertebral anomaly with/without associated with rib anomaly (6%/litter; 2 foetuses from 3 litters) - single findings for the following: fused skull bones, costal cartilage anomaly and bent limb bones (the author considered these as treatment related as the litter incidences were well above historical control data) • Six foetuses (from 5 litters) had following treatment related (according to author) skeletal variations - branched sternebrae (3 foetuses from 2 litters) - vertebral supernumerary sites (3 foetuses from 3 litters) statistically significant ↑ in litter incidences of 13th full rib and pelvic girdle caudal shift (also found in 1.5 mg/kg group; the author considered these to be not toxicologically relevant)
	1.5 mg/kg bw: • red or orange discoloration of urine in 1 animal (which had early resorption) • there were no statistically significant changes compared to controls in body weights (gain) or (relative) food consumption • four animals had ↓ faeces for 3-6 days	1.5 mg/kg: • statistically significant ↓ mean of viable foetuses (77% compared to 92% in controls) • statistically significant ↑ post-implantation loss (23% compared to 8% in controls; a mean of 1.7 per litter compared to a mean of 0.5 in both controls and historical controls)	1.5 mg/kg: • two foetuses (from two litters) had external malformations of omphalocele; tail was absent in one of these foetuses (these findings were not found in controls, were found only in one historical control foetus and also in the 4 mg/kg group; thus, the author considered these as treatment related) • one foetus showed incidental visceral malformation (the author did not consider it as treatment related) consisting of teratology of fallot (i.e. pulmonary stenosis, ventricular septum defect, dextaposed aorta overriding the ventricular septum and thickened right ventricular wall) • statistically significant ↑ in litter incidences of 13th full rib and pelvic girdle caudal shift (also found in 4 mg/kg group; the author considered these to be not toxicologically relevant)

Table 63b: Further presentation of maternal and developmental effects in the oral rabbit study from Thor GmbH Art. 95 dossier

Dosage (mg/kg/day)	0	0.5	1.5	4

Maternal effects				
Body weight gain (g/animal), gestation day 7-29	359	391	322	163*
Body weight gain (g/animal) Gestation day 7-20	143	169	115	-12*
Food consumption (g/animal/day), gestation day 7-29	131	124	121	113*
Food consumption, (g/animal/day), gestation day 7-20	138	128	127	108*
Developmental effects				
Fetal weight (g)	47	47	47	44
Viable fetuses per litter	7.3	7.9	6.4	2.5*
Pos-implantation loss (%/litter)	7.9	5.5	23.2*	66.8*
Fetuses with malformations /total fetuses	2 /146	5 /157	5 /127	11 /47*

*statistically different from control (P<0.05)

Table 63c: Maternal and developmental toxicity data for control and 1.5 mg/kg/day group, dividing the latter into the six does with high maternal toxicity and the 15 without, in the oral rabbit study from Thor GmbH Art. 95 dossier

Dose (mg/kg/day)	0	1.5 (15 does without high toxicity)	1.5 (6 does with high toxicity)
Maternal weight gain, (g/animal) gestation day 7-20	143	156	26*
Maternal weight gain (g/animal) gestation day 7-29	359	365	208*
Post-implantation loss (%)	7.9	7.7	55.8*
Fetuses with malformations (total fetuses)	2 (146)	3 (106)	2 (21)*

* Statistically different from control (P<0.05)

Table 63d: Summary of maternal survival and pregnancy status in the oral rabbit study from Thor GmbH Art. 95 dossier

DOSE GROUP :	1 NO.	1 %	2 NO.	2 %	3 NO.	3 %	4 NO.	4 %
FEMALES ON STUDY	22		22		22		22	
FEMALES THAT ABORTED OR DELIVERED	0	0.0	0	0.0	1	4.5	1	4.5
FEMALES THAT DIED	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT ABORTED	0	0.0	0	0.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES THAT WERE EUTHANIZED	0	0.0	0	0.0	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	0	0.0
FEMALES EXAMINED AT SCHEDULED NECROPSY	22	100.0	22	100.0	21	95.5	21	95.5
NONGRAVID	2	9.1	2	9.1	1	4.8	2	9.5
GRAVID	20	90.9	20	90.9	20	95.2	19	90.5
WITH RESORPTIONS ONLY	1	5.0	0	0.0	1	5.0	10	52.6
WITH VIABLE FETUSES	19	95.0	20	100.0	19	95.0	9	47.4
TOTAL FEMALES GRAVID	20	90.9	20	90.9	21	95.5	20	90.9

1- 0 MG/KG 2- 0.5 MG/KG 3- 1.5 MG/KG 4- 4.0 MG/KG

Table 63e: Summary of foetal data at scheduled necropsy in the oral rabbit study from Thor GmbH Art. 95 dossier

GROUP		SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1	TOTAL	74	72	146	0	7	3	10	156	180	24	NA	20
	MEAN	3.7	3.6	7.3	0.0	0.4	0.2	0.5	7.8	9.0	1.2	46.5	
	S.D.	2.18	1.96	2.43	0.00	0.99	0.49	1.05	2.24	1.92	1.70	3.73	
2	TOTAL	68	89	157	1	9	0	10	167	178	11	NA	20
	MEAN	3.4	4.5	7.9	0.1	0.5	0.0	0.5	8.4	8.9	0.6	45.5	
	S.D.	1.19	2.16	1.98	0.22	1.05	0.00	1.15	2.06	1.68	1.00	4.98	
3	TOTAL	54	73	127	0	33	0	33	160	176	16	NA	20
	MEAN	2.7	3.7	6.4	0.0	1.7	0.0	1.7	8.0	8.8	0.8	46.8	
	S.D.	2.05	2.01	3.10	0.00	2.43	0.00	2.43	2.36	1.74	1.24	6.38	
4	TOTAL	22	25	47	1	85	0	86	133	153	20	NA	19
	MEAN	1.2	1.3	2.5**	0.1	4.5	0.0	4.5	7.0	8.1	1.1	44.3	
	S.D.	1.50	1.92	3.03	0.23	3.37	0.00	3.31	2.33	2.17	1.87	4.41	

** = Significantly different from the control group at 0.01
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG 2- 0.5 MG/KG 3- 1.5 MG/KG 4- 4.0 MG/KG

In two recent rat whole embryo culture (rWEC) assays, sodium pyrithione and 2-MSP, the principle metabolite of pyrithione, were tested at concentrations of 0.15, 0.46, 0.92 or 2.3 µM and 3, 6, 12 or 30 µM, respectively, to determine whether the toxic moiety pyrithione has an intrinsic developmental hazard (article in manuscript). The reports of these assays were provided by the zinc pyrithione task force to the DS in August 2016. The pyrithione moiety (i.e. in analogy by testing sodium pyrithione and 2-MSP) was concluded as not embryotoxic in these assays by the zinc pyrithione task force. In the sodium pyrithione assay, sporadic effects were observed in some experimental groups but without a dose-response relationship and the highest concentration did not show effects. However, the results of these rWEC assays are not completely relevant to conclude that zinc pyrithione is not directly embryotoxic as the toxicological significance of Zn^{2+} in synergy with the pyrithione is not addressed in these assays. Moreover, it should be noted that even though the WEC assay is validated by ECVAM, the predictability and applicability domains are not yet sufficiently defined for regulatory implementation (Adler et al., 2011).

10.10.6 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Annex I (3.7.2.1.1) a substance should be classified in Category 1B for reproductive toxicity when the following applies:

> *"The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*

Effects on foetal viability were observed in four of the seven studies available. In the oral rat study of high reliability (ZnPT CAR Doc IIIA 6.8.1/02), mean post-implantation loss was 3.7 compared to 0.8 in controls ($p<0.01$) with a reduction in the mean number of viable foetuses per litter (12.5 compared to 14.5, $p<0.05$) and whole litter resorptions observed in 3 dams. Maternal toxicity in this study was seen as reduced food consumption (up to -48%), reduced adjusted body weight at gestation day 20 (-8% compared to controls) and reduced adjusted body weight gain (-38% compared to controls) but was not likely the cause of the effect on foetal viability. According to OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (number 43[12]), a feed restriction study clearly showed that severe weight loss or decrease in body weight gain per see induced minor changes in skeleton development but no effects on viability or malformations in the rat (Fleeman, 2005). Although not statistically significant, post-implantation loss was also seen in the intermediate dose group (1.4 compared to mean 0.8 in controls) in the absence of maternal toxicity.

Effects on foetal viability were also seen in the three studies in rabbits. In the first study (ZnPT CAR Doc IIIA A6.8.1/01) one high-dose doe aborted at day 17 and whole litter resorptions occurred in one mid-dose and 5 high-dose does. In the high-dose group, there was an increase in the mean post-implantation loss (mean 3.3 (65%) compared to 0.8 (11%) in controls, dose-response but not statistically significant) together with a reduction in the mean number of viable foetuses per litter (2.0 compared to 6.2 in controls, $p<0.05$). In the mid-dose group, there was an increase in resorptions (a mean of 1.6 per pregnant dam compared to a mean of 0.8 in controls) and an increase in post-implantation loss (29% compared to 11% in controls). Maternal toxicity in the high-dose group was seen as reduced food consumption (-13-31%) and severely reduced body weight gain (-98% compared to controls) during GDs 6-19. Maternal toxicity in the mid-dose group was also seen as reduced food consumption (-14-23%) and reduced body weight gain (-41%) during GDs 6-19.

In the second rabbit study (Nolen and Dierckman, 1979), dose-related post-implantation loss was also seen (83% in high-dose and 47% in mid-dose compared to 12% in controls, $p<0.005$ in high-dose but not statistically significant in mid-dose). Maternal toxicity was less marked in this study and manifested as reduced food consumption (-17%, $p<0.05$) and maternal body weight gain (-136 g compared to +175 g in controls, dose-response and statistically significant in the highest dosed

[12] OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment, Number 43. 24 July 2008.

group). No information on total body weights was given but assuming a body weight of 3.5 kilos the weight changes would represent about ±5%.

In the third rabbit study (Thor GmbH Art. 95 dossier, 2015), there was a statistically significant decreased mean of viable foetuses (33% compared to 92% in controls) and statistically significant increase in post-implantation loss (67% compared to 8% in controls; a mean of 4.5 per litter compared to a mean of 0.5 in both controls and historical controls). Maternal toxicity in the high-dose group was observed mainly as decreased absolute body weight (ranging -8 to -9%) during GDs 20-29, body weight gains (ranging -55 to -100%) during GDs 13-29, absolute food consumption (ranging -15 to -32% during GDs 10-23) and relative food consumption (ranging -16 to -28% during GDs 10-20). Adverse effects on the foetuses were also observed in the mid-dose group. There was a statistically significant decreased mean of viable foetuses (77% compared to 92% in controls) and statistically significant increase in post-implantation loss (23% compared to 8% in controls; a mean of 1.7 per litter compared to a mean of 0.5 in both controls and historical controls). There was no significant maternal toxicity at this dose at the whole group level. However, 6 of the 21 does in this group had decreased body weight gain that was statistically significantly lower during GDs 7-29 (58% of the controls) and most of the post-implantation losses in this group were seen in those 6 does.

A feed restriction study by Cappon (2005[13]) investigated the effects of maternal weight loss on embryo-foetal development in rabbits. The weight loss in the most severe restricted dose group was more than 200 g, *i.e.* more severe than that seen in the studies with zinc pyrithione, and in spite of the maternal weight loss no statistically significant increases in pre- or post-implantation loss or in the number of viable foetuses were observed in the Cappon study, indicating that the post-implantation loss seen in the zinc pyrithione studies were not a secondary effect to maternal toxicity.

Reductions in foetal body weights were seen in all four studies in rats but can probably be explained by the maternal toxicity also seen in the studies.

Malformations were seen mainly in three oral studies of high reliability, one in rats and two in rabbits. In the rat study (ZnPT CAR Doc IIIA 6.8.1/02), malformations were seen in all 24 litters examined (168 foetuses compared to 1 in controls). The most common malformation observed was a vertebral malformation with or without an associated rib malformation observed in 153 (89%) of the high-dose foetuses examined. A high incidence of fused sternebrae (30 foetuses in 14 litters) and other sternebral malformations (35 foetuses in 13 litters) was observed in the high-dose group. Additional malformations in high-dose foetuses included ulna or radus missing, adactyly, ectrodactyly, syndactyly and polydactyly. The incidence of these malformations was 1 or 2 except for ectrodactyly that were recorded in 9 foetuses from 5 litters. In addition, four cases of soft tissue malformations were observed: dimorphism (2), malformed brain (1) and renal hypoplasia (1). None of these malformations were found in the controls.

Rare and severe malformations were observed in the rabbit study with zinc pyrithione (ZnPT CAR Doc IIIA A6.8.1/01), although incidences were low. It should be noted however that due to the high foetal toxicity, only 26 foetuses remained for examination and the incidence noted may be an underestimation. Single cases were recorded in the highest dose of anencephaly, hydrocephaly, craniorachischisis, rigid flexure of shoulders and elbows, cleft palate, microglossia, interventricular septal defect, malformed testis, malformed skull bones, ectrodactyly, malformed scapulae, bent limb bone (tibiofibula) and humerus and ulna absent. These malformations were seen mainly in three foetuses from two different mothers. In total 7/26 foetuses were affected compared to 7/105 in controls. The delayed ossifications recorded in the high-dose group are considered to have been

[13] Cappon, G.D., Fleeman, T.L., Chanin, R.E., Hurtt, M. E.: Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. Birth Defects Res B Dev Reprod Toxicol. 2005 Oct;74(5):424-30.

caused by the maternal toxicity, but the malformations observed are considered to have been caused by a specific teratogenic effect of zinc pyrithione.

In another rabbit study with zinc pyrithione (Thor GmbH Art. 95 dossier, 2015), external malformations of omphalocele were observed in two foetuses from two litters each of mid- and high-dose groups. It should be noted that high-dose group had only 9 litters with viable foetuses compared to 19 litters with viable foetuses in the mid-dose group and in controls. Two foetuses (one each from mid- and high-dose group) among the four affected foetuses also had an absent tail. These external malformations were not found in controls and in only one historical control foetus. The foetus in the high-dose group with omphalocele and absent tail also had several urinary tract malformations/variations (absent right kidney and ureter, dilated left ureter and absent urine bladder). These were not observed in controls and only one historical control foetus had absent urine bladder. In the high-dose group, statistically significant increase in skeletal malformations (11 foetuses from 5 litters) were observed compared to controls (2 foetuses from 2 litters) including fused sternebrae (15.9%/litter), rib anomaly (6.5%/litter), vertebral anomaly with/without rib anomaly (6%/litter) and single findings of fused skull bone, costal cartilage anomaly and bent limb bones. One foetus in the mid-dose group showed visceral malformation consisting of teratology of fallot (i.e. pulmonary stenosis, ventricular septum defect, dextaposed aorta overriding the ventricular septum and thickened right ventricular wall).

According to OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (number 43), a feed restriction study clearly showed that severe weight loss or decrease in body weight gain per see induced minor changes in skeleton development and in rabbits abortions occurred in the most severe restricted dose group but no malformations (Cappon, 2005).

Malformations were not seen in the other studies (one dermal study in rats and two oral studies of low reliability in rat and rabbits, respectively). Skeletal abnormalities and incomplete ossification were observed but can probably be attributed to maternal toxicity. A slight increase in alterations/ variations was seen in the dermal study with rats where medial rotation of both hindlimbs was seen in both mid and high-dose and single cases of absent tail, depressed eye bulges and microphthalmia was seen in high-dose.

In the zinc pyrithione developmental toxicity review paper (June 2016) submitted to the DS by the zinc pyrithione task force, reference is made to the maternal toxicity workshops in 2009 (Beyer et al., 2011) wherein a decrease in body weight gain of 20% was considered excessive. The participants of the workshops recommended that all relevant information should be considered for good dose selection for developmental and reproductive toxicology studies with an intent to avoid marked maternal toxicity leading to mortality or decreased body weight gains of greater than 20% for prolonged periods. However, “several participants considered maternal toxicity to be an indicator to stop dose escalation, but generally do not consider maternal toxicity as a reliable explanation for developmental toxicity”.

Annex I to CLP, section 3.7.2.4.2 states “*...Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies*”. With the available information, it cannot be unequivocally demonstrated that the developmental effects of zinc pyrithione are secondary to maternal toxicity.

Classification for zinc pyrithione in Category 1A is not applicable since there is no evidence from humans. The classification for zinc pyrithione in Category 2 is not applicable either as there is no mechanistic information that raises doubt about the relevance of the effects for humans and evidence from experimental animals is sufficiently convincing to place it in Category 1.

Therefore, classification in Category 1B is proposed for zinc pyrithione based on the malformations and post-implantation losses seen in three independent guideline studies in two different species.

10.10.7 Adverse effects on or via lactation

No data is available.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Classification in Repr. 1B (hazard statement H360D – May damage the unborn child) is proposed for zinc pyrithione.

The route cannot be specified as there is no data available with inhalation exposure.

10.11 Specific target organ toxicity-single exposure

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

Oral route of exposure

Acute toxicity data are available from two oral LD_{50} studies (please refer to section 10.1) and one acute neurotoxicity study in rats. Results of the acute oral toxicity studies are presented in tables therein.

Table 64: Mortalities and clinical signs of toxicity observed in acute oral toxicity study ZnPT CAR Doc IIIA A6.1.1/01

Dose [mg/kg]	Number of dead / number of investigated	Time of death	Observations
125	1/10	Day 1	Ptosis, piloerection, brown stain on anogenital area
158	2/10	Day 1-3	Emaciation, lethargy, diarrhea, anogenital area soiled, anogenital area wet, body surfaces soiled, chromodacryorrhea, right eye partially closed, chromorhinorrhea, ptosis
200	3/10	Day 1	Emaciation, lethargy, body surfaces soiled, piloerection, anogenital area soiled, anogenital area wet, anogenital area stained brown, chromorhinorrhea, ptosis, alopecia, chromodacryorrhea
254	5/10	Day 1-5	Emaciation, lethargy, anogenital area wet, piloerection, diarrhea, anogenital area soiled, anogenital area stained brown, body surfaces soiled, chromodacryorrhea, right eye partially closed, chromorhinorrhea, ptosis, alopecia, chromodacryorrhea
321	6/10	Day 1-4	Ataxia, emaciation, lethargy, diarrhea, piloerection, chromodacryorrhea, ptosis, chromorhinorrhea, bloated abdomen, alopecia, anogenital area wet, anogenital area stained brown

Table 65: Mortalities and clinical signs of toxicity observed in acute oral toxicity study ZnPT CAR Doc IIIA A6.1.1/02

Dose [mg/kg]	Number of dead / number of investigated	Time of death	Observations
500 (male)	0/5	n.a.	Ataxia, decreased respiratory rate, diarrhoea, diuresis, hunched posture, lethargy, laboured respiration
500 (female)	0/5	n.a.	Lethargy, decreased respiratory rate, diarrhoea, diuresis, hunched posture
707	3/5	Day 1-7	Ataxia, lethargy, decreased respiratory rate, diuresis, hunched posture, laboured respiration, ptosis, piloerection, red/brown stains around the eyes and snout, splayed and tiptoe gait
1000	4/5	Day 3-4	Ataxia, lethargy, decreased respiratory rate, diarrhoea, diuresis, hunched posture, laboured respiration, red/brown stains around snout, fur stained by test substance

The clinical signs of toxicity observed in the acute oral toxicity studies were noted at or slightly below-dose levels where mortality occurred and appear to be non-specific toxic effects. Ataxia and lethargy could be signs of a narcotic effect but could also be have been caused by the general distress of the animals.

Results of the acute neurotoxicity study are summarised in table 66.

Table 66: Mortalities and clinical signs of toxicity observed in acute neurotoxicity study ZnPT CAR Doc IIIA A6.9/01

Dose [mg/kg]	Number of dead / number of investigated	Time of death	Observations
25	0/20	n.a.	Transient reduction in number of movements at 1 hour post-dosage in females. Not statistically significant for the 1.5 hour session.
75	2/20	Day 3, 4	Dehydration, urine-stained abdominal fur and soft or liquid faeces. Coldness to the touch (2f) and ptosis (1f) in moribund animals. Reduced body weight (5-6%) and food consumption. Reduced body temperature by 0.6ºC. Significantly decreased average hind limb grip test value at days 7 and 14 for females. Reduced motor activity at 1 hour post-dosage. No adverse necropsy findings.
150	7/20 (3 deaths were due to injury during dosing)	Day 2-3	Dehydration, urine-stained abdominal fur, soft or liquid faeces, localized alopecia on the underside and chromo-rhinorrhea. Hunched posture, red substance on the fur of both forelimbs, excess salivation and red perioral substance in some moribund animals. One animal that died showed no adverse clinical signs. Reduced body weight (7-18%) and food consumption. Reduced body temperature by 0.6-0.8ºC. Significantly decreased average hind limb grip test value at day 7 for males. Reduced motor activity at 1 hour post-dosage. No adverse necropsy findings.

In the acute neurotoxicity study (ZnPT CAR Doc IIIA A6.9/01) rats were given a single dose of zinc pyrithione followed by a 14-day observation period. The study was performed in accordance with GLP and OECD 424. Two females at 75 mg/kg and one male and six females at 150 mg/kg did not survive to scheduled sacrifice; however the male rat and two of the six female rats in the 150 mg/kg dosage group were injured during dosage administration and the deaths were not considered test substance-related. Clinical signs of toxicity were noted in males at 150 mg/kg and females at 150 mg/kg and 75 mg/kg (see table 66) but did not indicate any clear neurotoxic effect.

Effects on body weight were observed in males of all dose groups and the severity and duration of the effect increased with increased dose. Absolute body weights in males were reduced with 5-6 % in the intermediate dose group during days 3-4 and 7-18% in the high-dose group during days 2-10. The effect on body weight was less severe in females with no statistically significant changes in absolute body weight at any dose level compared to control.

At one hour post-dosage, body temperatures were reduced by 0.6ºC in high-dose males and by 0.6ºC and 0.8ºC in the intermediate and high-dose group females, respectively. On day 7 post dosage males at 150 mg/kg had a significantly decreased average hind limb grip test value, however there were no effects observed in any dose group in the hindlimb evaluation. No test substance-related microscopic lesions were revealed by the neurohistological examination.

Motor activity measurements revealed significant decreases in the total time spent in movement for the 1.5 hour session in male and female rats in the 75 and 150 mg/kg dosage groups when tested one hour post dosage. Within this session the number of movements and the time spent in movement were reduced or significantly reduced at the 5 minute through 30 minute intervals at both dose levels. Female rats in the lowest dose group also exhibited a decrease in both time spent in movement and number of movements at several time points during the one hour post dosage session. These effects were not seen at 7 days. There was an effect on movement seen in females at 25 mg/kg one hour post-dosage that was statistically significant at some of the measuring points but did not greatly affect the overall result of the session (number of movements at 0/25/75/150 mg/kg: 672/600/378/371).

Zinc pyrithione is irritating to the gastro-intestinal tract and it is considered possible that gavage administration of such a substance would cause a reduction in spontaneous movement in the animals, making these findings of questionable relevance for classification.

In summary, indications of a possible neurotoxic effect were seen but these were of low magnitude and transient or did not occur at the highest dose. Furthermore, as they were observed within the dose range also causing general toxicity and mortalities they are considered to be covered by the classification for acute toxicity.

Dermal route of exposure

No adverse effects were noted in the acute dermal toxicity study (see section 10.2).

Inhalation route of exposure

Three studies are available on the acute inhalation toxicity of zinc pyrithione in rats (please refer to section 10.3).

Results of the studies are presented in tables 67, 68, and 69.

Table 67: Mortalities and clinical signs of toxicity observed in acute inhalation toxicity study ZnPT CAR Doc IIIA A6.1.3/01

Dose [mg/L]	Number of dead / number of investigated	Time of death	Observations
0.53	1/10	Day 1	Ataxia, decreased and increased respiratory rate, hunched posture, laboured, gasping and noisy respiration, lethargy, pallor of extremities, piloerection, ptosis, red/brown staining around the eyes and snout, wet fur. Macroscopic observations: Lungs: swollen, pale, dark patches and foci. Pale liver, congestions in the small intestine.
0.95	5/10	Day 1	Ataxia, decreased respiratory rate, hunched posture, laboured, gasping and noisy respiration, lethargy, pallor of extremities, piloerection, ptosis, red/brown staining around the eyes, mouth and snout, wet fur. Macroscopic observations: Lungs: swollen, pale, abnormally dark, dark patches. Excessive fluid in the thoracic cavity. Liver: dark and pale, patchy pallor and accentuated lobular pattern. Gaseous distension, congestions and reddening in the gastro-intestinal tract.
1.82	8/10	Day 1	Ataxia, decreased respiratory rate, hunched posture, laboured, gasping and noisy respiration, increased salivation, lethargy, pallor of extremities, piloerection, ptosis, red/brown staining around the eyes and snout, wet fur. Macroscopic observations: Lungs: swollen, pale, abnormally red and dark, dark patches and foci. Liver: dark and pale, patchy pallor and accentuated lobular pattern. Pale kidney and congestions and reddened small intestine.

Table 68: Mortalities and clinical signs of toxicity observed in acute inhalation toxicity study ZnPT CAR Doc IIIA A6.1.3/03

Dose [mg/L]	Number of dead / number of investigated	Time of death	Observations
0.24	1/10	Day 1	Increased salivation, laboured breathing, decreased activity and tremors on day of exposure. Macroscopic observations: Congested or discoloured (red) lungs were noted at necropsy for all animals dying on study. Necropsy observations for all animals surviving to study end appeared normal.
0.61	3/10	Day 1	Increased salivation, laboured breathing, gasping, decreased activity and tremors. Macroscopic observations: Congested or discoloured (red) lungs were noted at necropsy for all animals dying on study. Necropsy observations for all animals surviving to study end appeared normal.

Table 69: Mortalities and clinical signs of toxicity observed in acute inhalation toxicity study ZnPT CAR Doc IIIA A6.1.3/02

Dose [mg/L]	Number of dead / number of investigated	Time of death	Observations
0.054	1/10	During exposure	Laboured breathing, gasping, material around eye, material around mouth, material around nose. Macroscopic observations: Trachea: solid white material in lumen, trace.
0.14	3/10	Day 1	Laboured breathing, material around eye, material around mouth, material around nose, urine stained abdomen, rales. Macroscopic observations: Lungs: moderate multilobar congestion; red multilobar foci (0.2 - 0.3), mild. Trachea: solid white material in lumen, mild; clear fluid in lumen, mild. Mandibular lymph node: enlarged 4x, bilateral, moderate. Uterus: generalized dilation, mild.
0.16	7/10	Day 1, 2	Laboured breathing, gasping, material around eye, material around mouth, material around nose, urine stained abdomen, rales, nasal discharge, staining around mouth, staining around nose, lachrymation. Macroscopic observations: Lungs: mild to severe multilobar congestion; red multilobar foci (0. - 0.4 cm). Trachea: solid white material in lumen, mild; fibrin clot in lumen, severe. Thoracic cavity: 2 mL of clear fluid, mild.
0.82	10/10	Day 0 -2	Laboured breathing, gasping, material around eye, urine stained abdomen, nasal discharge, staining around mouth, staining around nose, lachrymation. Macroscopic observations: Lungs: mild to moderate multilobar congestion; mild to moderate generalized and multilobar red discoloration; mild red multilobar foci (0.1 - 0.2 cm). Trachea: solid white material in lumen, mild to moderate. Larynx: white material in lumen, mild. Kidney: right pelvis, dilated, mild.
1.4	10/10	During exposure, day 1, 3	Hunched posture, trembling, laboured breathing, material around eye, body surface stained. Macroscopic observations: Lungs: moderate to severe multilobar congestion; severe multilobar and multifocal white discoloration. Trachea: solid white material in lumen, mild to severe. Esophagus: solid white material in lumen, mild to severe.
1.5	10/10	Day 1, 3	Hunched posture, lethargy, trembling, laboured breathing, material around nose, material around eye, prostration, body surface stained. Macroscopic observations: Lungs: moderate multilobar congestion; generalized severe red discolouration. Trachea: solid white material in lumen, mild. Esophagus: solid white material in lumen, severe. Larynx: moderate occlusion by white viscous material. Stomach glandular: solid white material in lumen, moderate.

In the acute inhalation toxicity studies clinical signs including laboured breathing, gasping, increased salivation, rales and noisy respiration were noted. These symptoms indicate an irritation of the respiratory tract but were observed at lethal doses. As no non-lethal doses were investigated no information is available on possible respiratory tract irritation at lower doses.

No adverse effects were noted after the first dose administration in the subchronic studies in rats (see section 10.12).

10.11.2 Comparison with the CLP criteria

Regulation EC No 1272/2008 (CLP), section 3.8.1 states that:

> *"Acute toxicity refers to lethality and STOT-SE to non-lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such case the most appropriate class should be assigned."*

The available studies do not indicate a specific organ toxicity occurring at dose levels not causing general toxicity and mortality and classification in Category 1 or 2 is therefore not considered warranted.

Table 3.8.1 describes Category 3:

> *"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."*

The specific criteria for respiratory tract irritation are (section 3.8.2.2.1):

> *"There are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation.*
>
> *This special classification would occur only when more severe organ effects including in the respiratory system are not observed."*

The data from the acute inhalation toxicity studies show symptoms including laboured breathing, gasping, increased salivation, rales and noisy respiration. These symptoms are indicative of respiratory tract irritation but were observed at lethal doses. In repeated dose inhalation studies, local irritation was also observed but was not transient nor reversible. The classification criteria for RTI are thus not considered to be fulfilled.

The specific criteria for narcotic effects are (section 3.8.2.2.1):

> *"Narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure."*

The clinical signs of toxicity observed in both oral and inhalation studies often included lethargy and ataxia but they occurred at doses which also caused systemic toxicity and mortality and could therefore also have been caused by the general distress of the animals. The classification for acute toxicity is considered to cover these effects and classification with STOT SE is not considered warranted due to these effects.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification is proposed for zinc pyrithione.

10.12 Specific target organ toxicity-repeated exposure

Table 70: Summary table of animal data on STOT RE

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
40 CFR 798.2650 GLP Reliability: 2, as purity was not reported	Zinc pyrithione Batch: not specified Purity: not specified	Rat Sprague-Dawley Crl:CD® BR 10/sex/dose	Oral	0.2; 1; and 5 (2.5) mg/kg bw/day The highest dose level was reduced to 2.5 mg/kg from days 17 -18 onwards 90 days	<u>0.2 mg/kg bw:</u> No adverse effects <u>1 mg/kg bw:</u> ↑ clinical signs: increased salivation, isolated incidents of red/brown staining around the mouth ↓ plasma urea (females) ↑ inflammatory cell infiltrates in the forestomach (1m) <u>2.5 mg/kg bw/day:</u> ↑ clinical signs (hunched posture, noisy respiration, pallor of extremities) ↓ plasma urea (females) ↓ creatinine (females) <u>5 mg/kg bw/day:</u> ↑ mortality (3 f killed *in extremis*) ↓ movement in hind limbs (6 f) ↑ clinical signs: increased salivation, noisy respiration, hunched posture, piloerection, dehydration, emaciation, tiptoe/high stepping gait, loss of righting reflex, lethargy, vocalisation ↓ body weight (f: -21 %) ↓ food consumption (females) ↑ gastric and GI tract irritation	ZnPT CAR Doc IIIA A6.4.1/03 Year: 1997
No guideline No GLP Reliability: 3, as purity was not reported, there were deviations from	Zinc pyrithione Batch: specified Purity: not specified	Rat Charles River CD Albino 20/sex/dose	Oral, in diet	5 ppm (m: 0.35 mg/kg bw; f: 0.39 mg/kg bw) 25 ppm (m: 1.75 mg/kg bw, f: 2.13 mg/kg bw) 125 ppm (m: 10.04	<u>5 ppm:</u> No adverse effects <u>25 ppm:</u> ↓ bw (-10% in females) <u>125 ppm:</u> ↑ mortality (33/40 rats) ↓ movement of the hindlimbs	ZnPT CAR Doc IIIA A6.4.1/01 Year: 1973

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
OECD 408 (e.g. histo-pathology was not made of peripheral nerves) and limitations in reporting.				mg/kg bw, f: 10.26 mg/kg bw) 94 days	progressing to complete paralysis ↓ bw (f: -69%, m: -85%) and food consumption ↑ tissue changes associated with marked growth suppression and cachexia	
No guideline No GLP Reliability: 3 Deviations from OECD 452 and EC B.30: 10 animals/sex instead of 20; no urinalysis; no clinical chemistry; no formal observations for clinical signs; body weight 7 time points only; food consumption not measured; haematology was done at 11 and 24 months instead of every six months.	Zinc pyrithione Batch: Not specified Purity: Not specified	Rat (Strain not stated) 10/sex/dose	Oral, in diet	0, 2, 5, 10, 25, 50 ppm (food consumption per day not specified) Daily treatment 25 ppm corresponds to approx. 4 mg/kg bw/day for females 2 year	<u>2, 5 and 10 ppm:</u> No adverse effects <u>25 ppm ~2 mg/kg bw/day:</u> ↑ mortality (f) ↑ hind limb paralysis (f) ↓ body weight gain (f) <u>50 ppm:</u> ↑ mortality (10f, 6 m) ↑ hind limb paralysis (m, f) ↓ body weight gain	ZnPT CAR Doc IIIA 6.5/03 Year: 1958
EC Guideline B.7 GLP Reliability: 2, as neuro-	Zinc pyrithione Batch: specified	Monkey Cyno-molgus 4 (low and mid-dose) 6 (control	Oral (gelatine capsule)	0, 5.5, 11 and 22 mg/kg bw/day 28 days Daily	<u>5.5 and 11 mg/kg bw/day:</u> No effects <u>22 mg/kg bw/day:</u> ↑ mortality in one animal* ↑ clinical signs: vomiting, diarrhoea, decreased activity	ZnPT CAR Doc IIIA A6.3.1/01 Year: 1992

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
toxicity not investigated	Purity: >95%	and high-dose), 4 sacrificed after 28 days, 2 observed during a 14-day recovery period			↑ effects on haematology (e.g. Hb -22%) ↓ food intake ↑ adrenal weight (47 %) (females) *this animal vomited before dosing and may have been unhealthy at the onset of the study	
No guideline No GLP Reliability: 3, as purity was not reported, few animals were used and animals from the lowest dose group were not necropsied	Zinc pyrithione Batch: specified Purity: not specified	Monkey Rhesus Macaca mulatta 3/sex/dose, except low-dose: 4 m + 2 f	Oral (gavage)	0, 0.5, 2.0 and 8.0 mg/kg bw/day 93 - 94 days	2.0 mg/kg bw/day: ↑ vomiting day 1 ↓ relative uterus weight (-23%) 8.0 mg/kg bw/day: ↑ vomiting day 2 ↓ relative uterus weight (-55%) ↑ testis weight (20%)	ZnPT CAR Doc IIIA A6.4.1/02 Year: 1973
US EPA FIFRA Guideline 82-3, Equivalent to EC method B.28 Pre-GLP Reliability: 1	Zinc pyrithione Batch: specified 52.2% aq suspension Purity of active ingredient prior to suspension in water not specified.	Rat Sprague-Dawley 15/sex/dose	Dermal	0, 20, 100 and 1000 mg/kg bw/day 90 days	20 and 100 mg/kg bw/day: No adverse effects 1000 mg/kg: ↓ body weight gain (f: -17%) ↓ food consumption (f: -23%)	ZnPT CAR Doc IIIA A6.4.2/01 Year: 1973
US EPA guideline OPPTS NO. 870.3465 GLP Reliability: 2	Zinc pyrithione Batch: specified Purity: >95%	Rat Sprague-Dawley 20/sex/dose	Inhalation (nose only)	0, 0.002, 0.006, and 0.0135 mg/L 21 days, interim sacrifice at 5 days 6 h/day, 5 days/week	0.002 mg/L: No adverse effects 0.006 mg/L: ↑ clinical signs: slight swelling around eyes, respiratory gurgles, gasping ↑ histopathological effects in lungs and larynx	ZnPT CAR Doc IIIA A6.3.3/01 Year: 2005

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
					0.0135 mg/L: ↑ mortality (5 animals, 3 may not have been treatment-related) ↑ clinical signs: slight swelling around eyes, respiratory gurgles, gasping, decreased activity, hypothermia, tip-toe gait ↓ bw (m: -10%)	
Comparable to US EPA guideline OPPTS NO. 870.3465 GLP Reliability: 2, as haematology, urinalysis, clinical chemistry and ophthalmology were not investigated.	Zinc pyrithione Batch: specified Purity: >95%	Rat Sprague-Dawley 15/sex/dose	Inhalation (nose only)	0, 0.0005, 0.0015, and 0.005 6 h/day, 5 days/week 28 days, interim sacrifice at 5, 10, 28 days	0.0005 mg/L: ↑ BALF parameters (↑ eosinophils, neutrophils, lymphocytes, LDH, total protein, cell lysis) ↑ lung weight, microscopic findings in the lung (broncho-interstitial pneumonitis, smooth muscle hypertrophy) 0.0015 mg/L: ↑ BALF parameters (↑ eosinophils, neutrophils, lymphocytes, LDH, total protein, cell lysis) ↑ lung weight, microscopic findings in the lung (broncho-interstitial pneumonitis, smooth muscle hypertrophy) ↑ lymphoid hyperplasia 0.005 mg/L: ↑ mortality (1f) ↓ bw (-15%) and food consumption ↑ hindlimb impairment (1f) ↑ skeletal muscle degeneration (3f) ↓ thymus weight (-40%) ↑ BALF parameters (↑ eosinophils, neutrophils, lymphocytes, LDH, total protein, cell lysis) ↑ lung weight, microscopic findings in the lung (broncho-interstitial pneumonitis, smooth muscle hypertrophy) ↑ lymphoid hyperplasia	ZnPT CAR Doc IIIA A6.3.3/02 Year: 2009
US EPA guideline 82-4, subdivision F GLP Reliability:	Zinc pyrithione Batch: specified	Rat Sprague-Dawley albino (Charles River CD) 15/sex/do	Inhalation (whole body)	0, 0.0005, 0.0025, 0.010 mg/L 90 days	0.0005 mg/L: No adverse effects 0.0025 mg/L: ↑ mortality (1m, 1f) ↑ clinical signs: laboured	ZnPT CAR Doc IIIA A6.4.3/01 Year: 1993

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
2, as the purity of the test substance was not stated. This study cannot be used for estimation of systemic toxicity through inhalation exposure due to likelihood of oral ingestion through preening.	52.2% aq suspension Purity of active ingredient prior to suspension in water not specified.	se			breathing, rales, increased salivation, decreased activity, dry red-brown material around the nose, hair loss ↑ inflammation of the lungs ↑ lung weight 0.010 mg/L: ↑ mortality (3m, 4f) ↑ clinical signs: laboured breathing, rales, increased salivation, decreased activity, and dry red-brown material around the nose ↓ bw and food consumption (f) ↑ lung weight	

Table 71: Summary table of human data on STOT RE

No data.

Table 72: Summary table of other studies relevant for STOT RE

No data.

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

Oral route of administration

In a 90-day oral toxicity study performed according to GLP and guideline 40 CFR 798.2650, 10 rats/sex/dose were administered doses of 0.2, 1.0 and 5.0 mg zinc pyrithione/kg bw/day by gavage. Three females of the highest dose were sacrificed for humane reasons on days 16 and 19. Clinical signs were observed as limited movement or loss of movement of the hind limbs, increased salivation, noisy respiration, hunched posture, piloerection, dehydration, emaciation, tiptoe/high stepping gait, loss of righting reflex, lethargy and vocalisation. Due to the severity of these effects, the highest dose level was reduced from 5.0 to 2.5 mg/kg bw/day from days 17-18 onwards. After this reduction and a cessation of dosing on days 21 and 22 a steady regression in clinical signs was detected so that by day 36 surviving females showed incidents of hunched posture, noisy respiration and pallor of the extremities. The increased salivation, noisy respiration, hunched posture, fur wetting and staining seen at 5 mg/kg bw/day were considered to be a result of the oral administration of an irritant test material. Macroscopic findings in the gastric epithelium and GI tract supported this conclusion. Despite the limited/loss of movement of the hind limbs observed in a majority of the females at 5.0 mg/kg bw/day indicating a neurotoxic effect no effects were noted in the FOB evaluations at any of the dose levels but it should be noted that these were performed after the reduction of the highest dose level (days 27, 51 and 86). Zinc pyrithione seems to have a steep dose response curve and it is likely that the LAEL for neurotoxic effects would be somewhere between 2.5 and 5.0 mg/kg bw/day.

In a 94-day oral toxicity study not performed according to GLP or any specific guideline but mainly following OECD 408, 20 rats/sex/dose were administered zinc pyrithione in diet at concentrations of 5, 25 and 125 ppm corresponding to approximately 0.35/0.39, 1.75/2.13 and 10.04/10.26 mg/kg bw/day in males/females. Females at 25 ppm exhibited a 10% reduction in body weight. All 20 females and 19 of 20 males in the 125-ppm dose group died during the dosing period, with 33 of the 39 deaths attributed to the dose. Common symptoms in most rats prior to death and in the one survivor before autopsy were depression of respiratory rate, weakness, emaciation, and paralysis of the hind legs. Body weights were severely depressed. All females in the high-dose group had died at week 13 but at week 9 body weight was reduced by 69% compared to controls; in the one surviving male body weight was reduced by 85% at week 13 compared to controls at the same time point. Food consumption was similarly severely reduced and the histopathologic evaluation revealed numerous changes attributable to prolonged severe inanition and emaciation.

Zinc pyrithione was further investigated in a chronic toxicity/carcinogenicity study (ZnPT CAR Doc IIIA 6.5/01). The study was conducted in 1958 and is considered to be of low reliability as it is limited in scope and reporting, not performed in accordance with any guideline nor GLP and the purity of the test substance was not specified. It can therefore only be used to support findings observed in other studies. Rats were exposed to 2, 5, 10, 25 and 50 ppm zinc pyrithione in the diet. Food consumption was not measured but using default values[14], 25 ppm would equal approximately 2 mg/kg bw/day for females (see study summary). In male rats, no effects were noted on survival, whereas in female rats at 25 and 50 ppm, numbers of survived animals markedly decreased with four females of the highest dose group having died at 20 weeks and no females surviving at 80 weeks. Hind limb paralysis and reduced body weight gain was noted in females at 25 and in males and females at 50 ppm.

In a study in monkeys (ZnPT CAR Doc IIIA A6.3.1/01) performed in accordance with GLP and EC Guideline B.7, animals were exposed to 0, 5.5, 11 and 22 mg/kg bw/day for 28 days followed by a 2-week recovery period. One high-dose animal died in the study, but this animal vomited prior to dosing on day 1 and it is possible that it was unhealthy at the onset of the study. Animals showed decreased activity and reduced food intake. The main effects observed were gastrointestinal effects (vomiting and diarrhoea) and effects on blood parameters. The effects on blood parameters consisted of reductions in Hb (-22%), RBC (-29%) and Hct (-16%) accompanied by an increase in MCV (18%) at 22.0 mg/kg bw/day compared to control values. The control values were higher than published normal data[15] for *Macaca fascicularis*, while the values detected in the 22.0 mg/kg bw/day dose group were within the published normal range except for Hb which were lower. The changes were statistically significant in the highest dose group. See table 73.

[14] Technical Guidance Document on Risk Assessment (TGD), 2003. Annex VI, Default reference values for biological parameters. Tables 2 and 3.

[15] Fortman, J. D., Hewett, T. A. and Bennett, B. T.: The laboratory nonhuman primate, in The Laboratory Animal Pocket Reference Series, CRC Press LLC, 2002.

Table 73: Results of haematology evaluations in 28-day oral toxicity study in monkeys (ZnPT CAR Doc IIIA A6.3.1/01)

Week on test	Control		5.5 mg/kg bw/day		11 mg/kg bw/day		22 mg/kg bw/day	
	M	F	M	F	M	F	M	F
Total RBC (x10[6]) *Normal range[2]: 5.3 – 6.3*								
0	6.8	6.6	7.0	6.6	6.9	7.0	7.0	6.6
4	7.2	6.9	6.5	6.2	6.5	6.6	5.6**	4.9**
6[1]	7.3	7.1	-	-	-	-	6.4	6.2
Hb (g/100 mL) *Normal range: 11.0 – 12.4*								
0	12.9	12.0	13.4	12.9	13.4	13.5	13.0	12.4
4	14.0	12.7	12.4	12.1	12.6	12.2	11.0**	9.9**
6[1]	14.0	13.0	-	-	-	-	12.4	13.0
Hct (%) *Normal range: 33.1 – 37.5*								
0	45	43	47	45	46	46	46	43
4	47	44	45	43	44	44	40**	37*
6[1]	47	45	-	-	-	-	46	44
MCV (fl) *Normal range: 59-66*								
0	65.7	65.3	67.0	67.5	66.8	66.0	65.0	65.5
4	65.5	64.5	68.3	69.8	68.0	66.3	71.3**	76.2**
6[1]	65.0	63.0	-	-	-	-	71.5	71.5

[1]After recovery 2 weeks.

[2]Normal range values from Fortman et al, 2002

* Statistically significant (p<0.05)

** Statistically significant (p<0.01)

Zinc pyrithione was also investigated in a 90-day oral toxicity study in monkeys (ZnPT CAR Doc IIIA A6.3.1/01) not performed according to GLP or any specific guideline. Three animals/sex/dose (4m + 2f in low-dose) were given 0.5, 2.0 and 8.0 mg/kg bw/day by gavage. No effects on blood parameters were seen in this study as compared to controls, however reduced values for RBC, Hb and Hct were noted for all dose groups including controls as compared to pre-dosing. The reductions in Hb were in the range of 20–25 % in both high-dose animals and controls, but the initial values were higher than the published normal values while the values at the end of the study were within the historical range for normal values. Vomiting was observed on days 1-2 as well as reduced uterus weights at both 8.0 (-55%) and 2.0 (-23%) mg/kg bw/day. There was no information on the stages of the oestrus cycle of the animals. A 20% increase in testis weight was also observed in males at the highest dose level. The reliability of the study was considered to be low since the purity of the test substance was not given, only few animals were used, blood parameters were affected in control animals and animals from the lowest dose group were not necropsied.

A 90-day oral toxicity study combined with a neurotoxicity study with zinc pyrithione in the rat is available in the Thor GmbH Art. 95 dossier (2014). The study was performed according to OECD 408 & 424 and with GLP compliance. Zinc pyrithione was given at 0, 0.2, 0.5 and 2.5 mg/kg bw by daily gavage for 90 days followed by a 14-day recovery period. The NOAEL in this study was set at 0.5 mg/kg bw based on the following effects at the next dose level: clinical signs, lower body weight/weight gains and effects on the hindlimb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration.

Dermal route of administration

In a 13-week dermal toxicity study (ZnPT CAR Doc IIIA A6.4.2/01) performed according to GLP and EPA FIFRA 82-3, 15 rats/sex/dose were administered zinc pyrithione at doses of 20, 100 and 1000 mg/kg bw/day. The concentration of the test substance was 52.2% and the doses were prepared based on this fraction. The only adverse effects observed were reductions in body weight gain and food consumption in the highest dose group. NOAEL was found to be 100 mg/kg bw/day.

A developmental toxicity study (ZnPT CAR IIIA A6.8.1/03, also summarised in section 10.10) performed according to GLP and OPPTS 870.3700 with dermal exposure of zinc pyrithione to rats used dose levels of 0, 10, 15, 30 and 60 mg/kg bw. The dosage volume was 1 mL/kg, adjusted daily on the basis of the individual body weights recorded immediately before administration. The rats were exposed to the test substance for 6 hours each day. During the exposure period, the rat's back was wrapped and an Elizabethan collar was placed on the rat to prevent oral ingestion of the test substance. After the completion of the exposure period, the wrap and collar were removed, the back of each rat was washed and the cage cleaned.

The study is considered to be of high reliability and included detailed daily examinations of the animals with an additional assessment of muscle tone and mass on DGs 8, 12, 16 and 20. There were no test substance-related mortalities at any dose level. Animals dosed with 60 mg/kg bw exhibited reduced food consumption (-21%, $p<0.01$) adjusted body weight (-31%, $p<0.01$), limited use of hindlimbs (24/25 animals), shuffling gait (22/25 animals) and no use of hind limbs (1/25 animals) together with a significantly decreased muscle tone (21/25 animals, $p<0.01$) and loss in muscle mass (12/25, $p<0.01$). None of these effects were noted in the control animals. Clinical signs consisting of emaciation, dehydration, ungroomed coat, urine-stained abdominal fur, low carriage, hunched posture, chromodacryorrhea and chromorhinorrhea were also noted. At the 30 mg/kg bw dose level, reduced adjusted body weight (-12%, $p<0.01$) and food consumption (DGs 18-21, $p<0.01$) and low incidences of limited use of hindlimbs (2/24 animals) and shuffling gait (1/24 animals) were noted.

Inhalation route of administration

In a 21-day inhalation toxicity study (ZnPT CAR Doc IIIA A6.3.3/01) performed with nose-only exposure and according to GLP and OPPTS 870.3465, 20 rats/sex/dose were administered zinc pyrithione at doses of 0.002, 0.006 and 0.0135 mg/L for 6 hours daily. Substance related mortality was observed in two animals (days 3, 20) at 0.0135 mg/L; in addition three animals (2 at 0.0135 mg/L at and 1 at 0.006 mg/L) were thought to have died due to suffocation in the exposure tubes. One of the substance-related mortalities was considered to be due to laryngeal inflammation (although this was not firmly concluded since some of the surviving animals exhibited more severe laryngeal inflammation) while the cause of death for the second animal could not be established. At this dose level, clinical signs such as respiratory gurgles, gasping, decreased activity, hypothermia and tip-toe gait were observed. At the intermediate dose level, local effects in the respiratory tract were noted as an increase in numbers of alveolar macrophages, inflammation of the nasal mucosa and interstitium around the bronchioles and vessels of the lung, inflammation of the larynx which was ulcerative in some rats, mucous cell hypertrophy of the nasal and bronchial mucosa, squamous metaplasia of the nasal mucosa, larynx and trachea, and smooth muscle hypertrophy of the alveolar ducts. These effects were also seen at 0.0135 mg/L, increasing modestly with dose. No adverse effects were noted at 0.002 mg/L which was found to be the NOAEC of the study.

In a 28-day inhalation toxicity study (ZnPT CAR Doc IIIA A6.3.3) performed with nose-only exposure and according to GLP and OPPTS 870.3465, 15 rats/sex/dose were administered zinc pyrithione at doses of 0.0005, 0.0015 and 0.005 mg/L for 6 hours daily. One female exposed to 0.005 mg/L died on day 15 and again the cause of death was undetermined. Clinical observations consisting of thin body condition (3 females) and impaired use of the hind limb (1 female) were observed as well as reduced body weights (-15%).

At the intermediate and low-dose levels, local effects only were observed and included bronchoalveolar lavage fluid parameters (increased eosinophils, neutrophils, lymphocytes, LDH, total protein, cell lysis), increased lung weights and microscopic findings in the lungs. These included broncho-interstitial pneumonitis and smooth muscle hypertrophy of alveolar ducts, the latter observed as early as day 5 with increased incidence and severity with increasing dose and time on study.

In a 90-day inhalation toxicity study (ZnPT CAR Doc IIIA A6.4.3/01) performed with whole body exposure and according to GLP and US EPA 82-4, 15 rats/sex/dose were administered zinc pyrithione at doses of 0.0005, 0.0025 and 0.010 mg/L for 6 hours daily. Mortality was observed in 7 animals (weeks 4-12) at 0.010 mg/L and 2 animals (weeks 3, 13) at 0.0025 mg/L. These animals exhibited clinical signs of toxicity including laboured breathing, rales, increased salivation, decreased activity and dry red-brown material around the nose. Severe chronic active inflammation of the tracheal mucosa was observed in 2/4 high-dose rats that died on study. In both instances it was considered the mechanism of death as there was a considerable reduction in capacity of the remaining airway. One of the animals died of kidney inflammation but the cause of death of the remaining 6 animals was unknown. Lung weight increases were noted at 0.0025 mg/L and 0.010 mg/L which were related to pulmonary inflammation and medial hypertrophy of the pulmonary arteries that was noted microscopically. No adverse effects were noted at 0.0005 mg/L which was found to be the NOAEC of the study. It should be noted that the dose levels in this study were unreliable as the whole body exposure means that the test substance could have been orally ingested through preening.

10.12.2 Comparison with the CLP criteria

Regulation EC No 1272/2008 (CLP), Annex 1: 3.9.2.7.3, states for STOT RE:

> *"All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals: (a) morbidity or death resulting from repeated or long-term exposure. (b) significant functional changes in the central or peripheral nervous systems… (c) any consistent and significant adverse change in clinical biochemistry, haematology or urinalysis parameters."*

Oral route of administration

Repeated dose oral toxicity was investigated in rats and monkeys. In rats, the main effects noted after repeated oral exposure were local irritation, hindlimb impairment and/or mortalities. Hind limb impairment was observed in all three subchronic oral studies performed in rats and occurred at dose levels of 2.5 to 10 mg/kg bw/day. Mortalities were observed in two subchronic oral studies performed in rats at 5 and 10 mg/kg bw/day. In a supportive 2-year study with limited reporting, there was an effect on survival in the females within the 25 ppm (2 mg/kg bw/day) and 50 ppm (4 mg/kg bw/day) dose groups over the 2-year period. In the high-dose group 4 females had died at 20 weeks. Hind limb paralysis was reported to occur in females prior to death. No information is given in the study on the time these effects were observed but this study is considered to support the occurrence of both mortalities and neurotoxicity at low-doses as seen in two subchronic studies.

In contrast, the main effects noted in monkeys were local irritation and effects on haematology. In the 28-day study, Hb was reduced by 22% compared to controls, with reductions also in RBC (-29%)

and Hct (-16%) and an increase in MCV (18%). There were no other indications of haemolytic anaemia but according to Guidance on the Application of the CLP Criteria section 3.9.2.5.2, a reduction in Hb at ≥20% is sufficient for classification with STOT RE. According to the table 3.9.2, classification in Category 1 is warranted when significant toxic effects are observed at or below 10 mg/kg bw/day in a 90-day study. According to Annex 1, 3.9.2.9.5, for a 28-day study the guidance values are increased by a factor of 3, i.e. to 30 mg/kg bw/day. As the effect occurred at 22 mg/kg bw/day, classification in STOT RE 1 is thus warranted due to these observations.

In the 94-day monkey study there was a reduction in haematology parameters within each dose group compared to pre-dosing but no significant difference between treated and control animals. Values in all dose groups were also higher than published normal values at the onset of the study. This study is therefore not considered reliable for the purpose of evaluation of haematology and cannot be used for classification of this effect.

According to the Guidance on the Application of the CLP Criteria table 3.9.2, classification in Category 1 is warranted when significant toxic effects are observed at or below 10 mg/kg bw/day in a 90-day study. As neurotoxicity was observed at 2.5 to 10 mg/kg bw/day, classification in Category 1 is warranted for oral toxicity based on these effects.

Dermal route of administration

No specific target organ effects were noted in the subchronic dermal toxicity study at doses up to 1000 mg/kg bw/day but neurotoxic effects were seen at 60 mg/kg bw/day in a dermal developmental toxicity study. These manifested as limited use of hindlimbs (24/25 animals), shuffling gait (22/25 animals) and no use of hind limbs (1/25 animals) together with a significantly decreased muscle tone (16-21/25 animals, $p<0.01$) and loss in muscle mass (6-12/25, $p<0.01$). None of these effects were noted in the control animals. Clinical signs consisting of emaciation, dehydration, ungroomed coat, urine-stained abdominal fur, low carriage, hunched posture, chromodacryorrhea and chromorhinorrhea but there were no deaths at any dose level that were attributed to the test substance. At the 30 mg/kg bw dose level, low incidences of limited use of hindlimbs (2/24 animals) and shuffling gait (1/24 animals) were noted.

The diverging results seen in the two dermal studies indicate an increased sensitivity in pregnant animals. The developmental study was performed in 2005 and was considered to be of high reliability. Oral ingestion was prevented and can therefore not explain the higher level of toxicity seen. The subchronic study was performed in 1993 and the test substance was a 52.2% aq suspension, but except for the lack of information on purity of the active substance (prior to suspension in water), no major deficiencies were identified in the study. In any case, the effects seen in the developmental study were significant and the same as those observed in oral toxicity studies and are therefore considered relevant for classification of zinc pyrithione.

In the dermal developmental toxicity study, clear neurotoxic effects were noted at 60 mg/kg bw/day with low incidences seen at 30 mg/kg bw/day. The test substance was administered on GDs 0 through 20 in this study, *i.e.* for 21 days. According to the Guidance on the Application of the CLP Criteria table 3.9.2, classification in Category 1 is warranted when significant toxic effects are observed between ≤20 mg/kg bw/day in a 90-day study. According to Annex 1, 3.9.2.9.5, for a 28-day study the guidance values are increased by a factor of 3, i.e. to ≤60 mg/kg bw/day for Category 1. Classification in STOT RE 1 is thus warranted for dermal exposure.

Inhalation route of administration

The adverse effects noted in the repeated dose inhalation toxicity studies were primarily mortality and signs of local irritation. Hind limb impairment was noted only in one female in one of the studies.

Mortalities occurred at 0.0135, 0.005 and 0.0025 mg/L after 21, 28 and 90 days, respectively. However, it should be noted though that the dose levels in the 90-day study were unreliable as the whole body exposure means that the test substance could have been orally ingested through preening. The classification proposal is therefore based on the first two studies.

Mortalities was noted after considerably higher doses after acute exposure (1/10 animals at 0.53 and 0.24 mg/L, respectively). The effects seen in the repeated dose studies are therefore not considered to be acute effects and a separate classification for repeated dose effects is considered appropriate.

According to the Guidance on the Application of the CLP Criteria table 3.9.2, classification in Category 1 is warranted when significant toxic effects are observed at or below 0.02 mg/L in a 90-day study. As the mortalities were observed at concentrations far below this limit classification in Category 1 is warranted for inhalation toxicity.

10.12.3 Conclusion on classification and labelling for STOT RE

Zinc pyrithione caused the following effects in the studies:

Haemolytic anaemia in monkeys at 22 mg/kg bw/day after oral exposure for 28 days.

Neurotoxicity in rats at 2.5 to 10 mg/kg bw/day and mortalities at 5 and 10 mg/kg bw/day after oral exposure for 90 days.

Neurotoxicity in rats at 60 mg/kg bw/day after dermal exposure for 21 days.

Mortalities and a single case of neurotoxicity in rats at 0.0135 and 0.005 mg/L after 21 and 28 days, respectively, after inhalation exposure.

Each of these effects justify classification in STOT RE 1 (with adjusted guidance values for shorter exposure times). As the dose-response curve is steep, mortalities were seen in rats at comparable dose levels as the other effects.

It is proposed not to specify the route of exposure as mortalities were seen after both oral and inhalation exposure and it appears likely that it would occur also after dermal exposure at higher doses.

Classification in STOT RE 1 (hazard statement H372 – Causes damage to organs through prolonged or repeated exposure) is proposed for zinc pyrithione.

10.13 Aspiration hazard

Hazard class not assessed in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 ACUTE AQUATIC HAZARD

The summaries and evaluations of the acute aquatic studies with zinc pyrithione, are taken from the draft zinc pyrithione (ZnPT) CAR Doc IIIA.

Several new studies, relevant for evaluation of the environmental hazards are available from a dossier on zinc pyrithione submitted by Thor GmbH as part of their Article 95 notification of the substance just before the CLH report was finalised by the dossier submitter (DS). These new studies are evaluated by the DS and briefly summarised in the table below. Among these, the studies that affected the conclusions on classification and labelling that were based on studies from ZnPT CAR Doc IIIA, amongst other, are described under the respective endpoint sections of this report.

Table 74: Summary of environmental studies in Thor GmbH Art. 95 dossier

Study	Guideline	Species, strain, sex / test system Dose/conc. levels	Dose descriptor/results	Described under the respective endpoint sections in this report (Yes/No/Briefly)
Emissions of zinc pyrithione from Facades of two Buildings Located at the Thor GmbH Site Speyer	No guideline	The determination of the content of ZnPT in the leachates was performed by means of HPLC/UV (calibrated to Thors GmbH method).	The cumulative emission expressed as percentage of the initial content of ZnPT in the façade ranged from 0.59% to 1.20% over a 21 months period for the laboratory building “Labor”. For the canteen building the cumulative emission was found to be 0.09% over a 17 months period	No
Zn(^{14}C) Pyrithione- Route and rate of Degradation in Four Soils Incubated Under Aerobic conditions	OECD 307	Four different test soils	Zinc (^{14}C)-pyrithione degraded instantaneously in three of four soils and was not detected at time 0. In one soil zinc pyrithione represented 17.1% of the amount applied which decreased after 6 h of incubation to 7.5% and was not detected at later intervals. The half-life of zinc (^{14}C)-pyrithione was considered to be lower than 30 minutes, which represent the time to perform the first extraction step.	No
Zn(^{14}C) Pyrithione- Adsorption/Desorption in Five Soils	OECD 106	Test on five different soils	Using the McCall Classification scale to assess a chemicals potential mobility in	No

			soils (based on $K_{F,oc}$) Zn(^{14}C) pyrithione can be classified as having a low mobility in soils or being even immobile.	
Zn(^{14}C) Pyrithione: Aqueous Photolysis in Buffer and Natural Water	OECD 316	The photoloysis behaviour of Zn-Pyrithione was investigated in sterile buffer solutions at PH 7	Zn-pyrithione degraded instantly in irradiated and dark natural water system and irradiated buffer solution at pH 7. The substance was more stable in sterile buffer solution at pH 7 in the dark. Up to seven degradation products were detected the most predominant one was metabolite M4.	No
Zn(^{14}C) Pyrithione: Hydrolysis at three different pH values	OECD Guidelines for testing of Chemicals, 111, Hydrolysis as a Function of pH.	Hydrolysis was performed at pH 4, 7 and 9 in different buffer solutions.	The test item can be considered stable to hydrolysis at pH 4 and unstable at pH 7 and pH 9 conditions with DT50 values ranging from 9.7 to 79.4 days.	No
Zn (^{14}C) Pyrithione-degradation/Metabolism in two Aquatic Systems under Aerobic Conditions.	OECD Guideline for the testing of Chemicals, Guideline 308: Aerobic/Anaerobic Transformation in Aquatic sediment Systems.	Sediment and water were collected from a river and a pond in Switzerland	The rate of degradation of Zn (^{14}C) pyrithione was investigated in two different aquatic systems (a river and a pond) under aerobic conditions. Zn (^{14}C) pyrithione degraded in total aquatic system with DissT50 of 1.0 and 0.4 days for the river and pond systems, respectively. Corresponding DissT90 values were 3.4 and 1.3 days for the river and pond system, respectively. 11 metabolites were detected in the aqueous and sediment extract phase.	Yes (see Table 79)
Emissions of non-encapsulated Zinc Pyrithione (ZnPT) applied in Paint on Mineral Surfaces (Field Leaching Study over 20 months	No guideline	The emissions of non-encapsulated zinc pyrithione from paint on a mineral surface were determined under natural environmental conditions over a 20 months period.	The cumulative emission was 3.365 mg/m2 after 30 days application, and 3.386 mg/m2 after 12 months of application	No
Zn Pyritione: porous pot test method for assessing the	OECD 303	The biological test system was a consortium of microorganism	At the end of stabilization period DOC removal in all reactors	No

biodegradability of the test substance during waste water treatment simulation.		common to activated sludge from a primarily domestic wastewater treatment facility and radiolabelled test substance was applied. DOC and COD were determined	was stabilized at the level of 38,7% and 41.0% and COD removal was 95% to 97%.	
Zinc pyrithione: Effect on terrestrial Non-Target plants-Seedling Emergence test	OECD 208	Seedling growth test on maize, tomato, turnip, pea and sunflower with the 5 different zinc pyrithione concentrations.	Zinc pyrithione did not have an effect on survival of maize, oat and tomato any concentration tested except for turnip. The most sensitive endpoint measured in the study was weight, where pea and tomato were the most sensitive species (EC50 = 15.7 mg zinc pyrithione/kg dry soil, respectively)	No
Acute toxicity (14 d) of zinc pyrithione on the Earthworm *Eisenia fetida* in Artificial Soil	OECD 207	Determination on the acute toxicity of zinc pyrithione on the earthworm *Eisenia fetida* after 14 days exposure to five concentrations, and to estimate the LC50.	The LC50 was 230.8 mg zinc pyrithione/kg dry soil, with 95%-confidence limits of 216.9 and 245.5 mg zinc pyrithione/kg dry soil.	No
Algae, Growth Inhibition Test 96 h with *Pseudokirchneriella subspicatus*	OECD 201	The study was conducted under static conditions with initial density of 10007 cells/ml. Based on preliminary test 5 concentrations level were tested	72 h E_rC_{50} = 35.8 µg/L 96 E_rC_{50} = 32.9 µg/L 72 h NOEC =14.9 µg/L 96 h NOEC =14.9 µg/L	Briefly (see also Table 75)
Acute Immobilisation test, 48 h to *Daphnia magna*	OECD 202	The study was conducted in the dark under semi-static conditions over a period of 48 h with 5 concentrations of ZnPT	48 h EC50 = 51.1 µg/L 48 h NOEC = 7.68 µg/L	Briefly (see Table 75)
Reproduction test ,21 days, with *Daphnia magna*	OECD 211	The study was carried out under semi-static conditions. Nominal concentrations of the test item ZnPT were selected based on the results of an acute immobilisation performed at the test facilities.	NOEC 21 d = 2.21 µg/L EC50 = 7.15 µg/L	Briefly (see Table 77)
Early-Life Stage Toxicity Test with Zebrafish (*Danio rerio*) under Flow-Through	OECD 210	A test was conducted under flow-through conditions with the nominal ZnPT	NOEC = 1.25 µg/L LOEC = 3.12 µg/L	Briefly (see Table 77)

Conditions.		concentrations of 0.200-0.500-1.25-3.12-7.81 µg/L. The test lasted for 35 days.		
Respiration Inhibition test with Activated Sludge	OECD 209	The Respiration Inhibition Test with activated sludge was carried out under static conditions with ZnPT concentrations 0.3125-0.625-1.25-2.5-5-10 mg/L	EC50 = 2.82 mg/L	Briefly (see text under section 12.1.6 Aquatic toxicity for other aquatic organisms)
Soil Micro-Organisms: Nitrogen Transformation Test	OECD 216	The metabolic activity of nitrogen-N-formation rate (nitrate)of soil-microorganism were determined with the test concentration of ZnPT 250-100-40-16-6.4-2.56 mg/kg soil dry weight and was measured after 0,7,14, 28 days.	EC50 =222 mg/kg soil dry weight. The NOEC after 28 days in the soil = 16 mg/kg soil.	No
Soil Micro-Organism: Carbon Transformation test	OECD 217	The metabolic activity of soil microorganisms were determined a with test concentrations of ZnPT 250-100-40-16-6.40-2.56 mg/kg soil dry weight and was measured after 0,7,14,28 days	No EC50 could be determined NOEC was 6.4 mg/kg soil dry weight on day 7, 40 mg/kg dry weight on day 14 and 100 mg/kg dry weight on day 28.	No

For all of the three "species" (fish, invertebrates and algae), valid acute toxicity tests with zinc pyrithione are available (see Table 75).

Further to this, acute toxicity tests are given for the relevant major organic metabolite PSA (pyridine sulphonic acid), pyrithione sulphonic acid (OMSA) and 2,2-(pyridyl-N-oxide) disulphide (OMDS). The reliability of the studies are taken directly from the ZnPT CAR Doc IIIA. The reliability scores correspond to Klimisch scores (1 = reliable without restriction, 2 = reliable with restriction, 3 = not reliable, 4 = not assignable).

Table 75: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference and Reliability
US EPA 72-1	Freshwater Fathead Minnow *Pimephales promelas*	Flowthrough with zinc pyrithione during 96 h	NOEC = 0.0011 mg/L LC50 = 0.0026 mg/L LC100>0.0079 mg/L	Key study	NOEC assigned to concentration that allowed at least 90% survival and did not cause sub lethal effects	ZnPT CAR Doc IIIA A7.4.1.1/01 Reliability: 2 1994

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference and Reliability
US EPA 72-1	Freshwater Rainbowtrout *Oncorchyncus mykiss*	Flow through with zinc pyrithione 96 h	NOEC = 0.0016 mg/L LC50 = 0.0032 mg/L LC100 = 0.0087 mg/L	Supportive study		ZnPT CAR Doc IIIA A7.4.1.1/03 Reliability: 2 1994
OECD 203 (equivalent to EC Directive 92/69/EEC method C.1)	Zebra fish *Danio rerio*	Static 96 h	LC50 = 0.0104 mg/L	Supportive study	GLP dose-response	Thor GmbH Art. 95 dossier
US EPA 72-1	Freshwater Fathead Minnow *Pimephales promales*	Flow through with the main metabolite PSA 96 h	NOEC = 48.7 mg/L LC50>48.7 mg/L			ZnPT CAR Doc IIIA A7.4.1.1/17 1994
US EPA-72-3(b)	Marine mysid *Mysidopsis bahia*	Flow through natural seawater diluted with tap water (zinc pyrithione) 96 h	NOEC = 1.6 µg/L LC50 =0.0063 mg/L	Key study		ZnPT CAR Doc IIIA A7.4.1.2/03 Reliability: 1-2 1993
OECD 202	*Daphnia magna*	Semi-static with zinc pyrithione 48 h	EC50 = 0.051 mg/L			Thor GmbH Art. 95 dossier
OECD 202 92-69/EEC, C.2	*Daphnia magna*	Flow though with zinc pyrithione 48 h	NOEC = 0.0056 mg/L LC50 = 0.050 mg/L LC100<0.1800mg/L			ZnPT CAR Doc IIIA A7.4.1.2/02 Reliability: 3 1994
US EPA-72-2	*Daphnia magna*	Flow through with zinc pyrithione 48 h	NOEC = 0.0011 mg/L LC50 = 0.0082 mg/L LC100>0.011 mg/L			ZnPT CAR Doc IIIA A7.4.1.2/01 Reliability: 3 1993

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference and Reliability
US EPA 72-3(b)	*Daphnia magna*	Flow-through 96 h PSA	EC50 = 71.6 mg/L			ZnPT CAR Doc IIIA A7.4.1.2/15 Reliability: 2
US EPA	*Crassostrea virginica* eastern oyster	Flow through with PSA 48h	NOEC=51.1 mg/L LC50 = 85.6 mg/L		Test method: Shell growth	ZnPT CAR Doc IIIA A7.4.1.2/04 Reliability: 2 1994
US EPA-123-2	Marine diatom *Skeletonema costatum*	Static zinc pyrithione 48 h	NOEC = .00004 mg/L (initital) NOEC = 0.000080 mg/L(TWA) EC50 = 0.0006 mg/L (initial)	Key study	Growth inhibition	ZnPT CAR Doc IIIA A7.4.3.1/04 Reliability: 2 2004
US EPA 122-2	Fresh water algae *Selenastrum capricornutum*	Static zinc pyrithione 120h	EC50 = 0.028 mg/L (120h) EC50 = 0.030 (72 h) mg/L EC50 = .100 (48 h) mg/L NOEC = 0.0091 mg/L		Growth inhibition	ZnPT CAR Doc IIIA A7.4.3.1/01 Reliability: 2 1994
OECD 201	Fresh green algae *Pseudokirchineriella subspicata*	Static zinc pyrithione 72 h and 96 h	NOEC = 0.0149 mg/L E_rC50 = 0.051 mg/L		Growth inhibition	Thor GmbH Art. 95 dossier

11.1.1 Acute (short-term) toxicity fish

The test was conducted according to EPA-FIFRA guideline 72-1 using a flow-through test-system and the fathead minnow (*Pimephales promelas*) as the test organism that was exposed to different concentration of zinc pyrithione. The LC0, LC50 and LC100 of the fish was measured after 96 hours.

The test substance was soluble at the concentrations employed in the study. Due to the lower measured concentrations compared with the nominal concentrations. LD50 values were based on the mean measured concentrations. The mortality in the controls was below 10% and the lowest dose showed no effects. 100% mortality occurred at the highest dose after 24 hours and in the second highest dose after 96 hours. The dissolved oxygen was 60% of the air saturation at the temperature used. Therefore, the validity criteria can be considered as fulfilled.

The dissolved oxygen concentration during the test sometimes exceeded 100% saturation, and the random arrangement of the test vessels was identical to the arrangement of the previous study. These deviations had no effect on the outcome. The reliability of the study was 1.

The results based on the mean concentration was LC0 (96 hours) =1.1 µg/L, LC50 (96 hours) =2.6 µg/L and LC100 (96 hours) =7.9 µg/L

Metabolites

The major metabolite pyridine sulphonic acid (PSA) showed in an acute toxicity study with fresh water fish *Pimephales promales* (Fathead Minnow) an 96 h LC50>48.7 mg/L which indicates that PSA is the most toxic metabolite (see table 75) but cannot be used for classification purpose since the EC50 is “>”. This is also the case for the metabolites pyrithione sulphonic acid (OMSA) and 2,2-(pyridyl-N-oxide) disulphide (OMDS) that are carried out with fish and have 96 h EC50s>46.9 mg/l which cannot be used for classification purpose (ZnPT CAR Doc IIIA A7.4.1.1/17, ZnPT CAR Doc IIIA A7.4.1.1/14, ZnPT CAR Doc IIIA A7.4.1.1/11, ZnPT CAR Doc IIIA A7.4.1.1/18, ZnPT CAR Doc IIIA A7.4.1.1/12, ZnPT CAR Doc IIA A7.4.1.1/15, ZnPT CAR Doc IIIA A7.4.1.1/16, ZnPT CAR Doc IIIA A7.4.1.1/13). Reliability scores of these studies are 2-3.

It can be concluded with the tests on the metabolites that all three metabolites (pyridine sulphonic acid (PSA), pyrithione sulphonic acid (OMSA) and 2,2-(pyridyl-N-oxide) disulphide (OMDS) have adverse effect on the survival of fish, but are probably less toxic than the mother substance. However, a more accurate test with higher reliability is needed for classification purpose for all relevant metabolites.

Summary

Overall it can be summarized that zinc pyrithione is very toxic to fish with LC50<1 mg/l with the most sensitive species *Pimephales promales (*Fathead minnow) with 96h LC50=0.0026 mg/L. The metabolites PSA, OMSA and OMSia have an adverse effect on the survival of fish, however they are probably less toxic than the mother substance. More accurate tests with higher reliability are however needed for classification purpose for all relevant metabolites.

11.1.2 Acute (short-term) toxicity to aquatic invertebrates

The marine mysid, *Mysidopsis bahia*, was exposed to zinc pyrithione and the test was conducted in accordance to US EPA 72-3b (comparable to EPA OPPTS 850.1035). A number of minor deviations from the protocol were reported, for instance that a range finding test was not performed (historical data used), no 48 h LC50 was determined, and the random arrangement of the test chambers was identical to the arrangement of a previous study. These deviations are not considered to have affected the outcome of the study. Flow through (6.3 per 24 h) of natural seawater diluted with tap water was used to maintain an even exposure of the pyrithione. The test showed that (zinc) pyrithione is toxic to the mysid, with a 96 h LC50 of 0.0063 mg/L, and a NOEC of 0.0016 mg/L. RMS considers this as the key study.

The acute toxicity of zinc pyrithione to invertebrates was also tested in one fresh water species, *Daphnia magna*.. All these tests were guideline studies, performed in accordance with US EPA 72-2 and OECD 202 92/EEC, C2.

The recent study on *Daphnia magna*, (Thor GmbH Art. 95 dossier, 2015). This OECD 202 compliant study was a semistatic test. The EC_{50} was 0.051 mg/l after 48 h.

There were other acute toxicity studies carried out with *Daphnia magna* (see below). Even if the reliability of these studies were 3 (not reliable) they are of interest since they are carried out with the common invertebrate test organism *Daphnia magna* and gives some supportive information on the toxicity of invertebrates.

There was one acute toxicity study according to OECD 202 with *Daphnia magna*. This study showed a 48 h EC50 value of 0.050 mg/L based on nominal concentrations.

Another study is a flow through test (48 h) carried out with zinc pyrithione and *Daphnia magna* according to US EPA-72-2 and resulted in an LC50=0.0082 mg/L (draft ZnPT CAR Doc IIIA – A7.4.1.2/01/).

Metabolites

The studies performed indicate that zinc pyrithione is very acutely toxic to invertebrates (*Mysidopsis bahia* 96h LC50 = 0.0063 mg/L) while the main metabolite PSA is only moderately toxic to invertebrates (*Mysidopsis bahia)* 96 h EC50 =71.6 mg/L (ZnPT,CAR Doc IIIA A7.4.1.1/15). Another flow-through test was carried out for 48 h on the main metabolite PSA on the eastern oyster *Crassostrea virginica* which showed an LC50= 85.6 mg/L (ZnPT CAR Doc IIIA A7.4.1.1/18). There was also a test with *Daphnia magna* but it could not be used for classification purpose since EC50 was >122 mg/L (TWA) (ZnPT CAR Doc IIIA A7.4.2/12).

For the metabolite OMSA the 96 h EC50 for *Mysidopsis baha* was 71.3 mg/l ((ZnPT CAR Doc IIIA A7.4.1.1/14). The other invertebrate tests could not be used for classification purposes since the EC50 was >127 mg/L (ZnPT CAR Doc IIIA A7.4.1.1/11), and EC50 for *Crassostera virginica* was 99.2 mg/L (TWA) (ZnPT CAR Doc IIIA A7.4.1.1/17).

For the metabolite OMDS the test is not reliable and cannot be used for classification purpose (ZnPT CAR Doc IIIA A7.4.1.2/10, ZnPT CAR Doc IIIA A7.4.1.2/13, ZnPT CAR Doc IIIA A7.4.1.1/16).

Summary

It can be concluded that zinc pyrithione is overall very toxic to invertebrates (LC50 < 1 mg/L) with the most sensitive species, the marine schrimp *Mysidopsis bahia* 96h LC50 = 0.0063 mg/L. The main metabolite PSA and OMSA is only moderate toxic to invertebrates with LC50>1 mg/L but some data could not be used for classification purpose since EC50 values were ”>”. The metabolite OMDS has an unreliable study and cannot be used for classification purpose.

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

This study was performed on the marine diatom *Skeletonema costatum* using zinc pyrithione, under static conditions (see Table 75 and for more detailed study description ZnPT CAR Doc IIIA A7.4.3.1/04). The study was conducted in accordance with US EPA 123-2. This guideline is comparable to EPA OPPTS 850.5400, “Alga Toxicity, Tiers I and II”, with the exceptions that the study lasts 120 hours instead of 96 hours, the initial cell concentrations are 1×10^4 instead of 7.7×10^4, and test cultures are dosed with pre-cultures that are 7–10 days old instead 3–7 days old. The study was carried out with five concentrations of ZnPT, a dilution control at 20 C. The dilution water was sterile marine medium adjusted to PH of 8.0. Nominal concentrations were 0, 0.3, 0.6, 1, 2, 2.4 and 4.8 µg/L. The algae were distributed among three replicates of each treatment at a rate of approximately 10 000 cells/mL. Two stability samples with nominal concentration of 4.8 µg/L were prepared and incubated with the vessels. One of the samples were exposed to light where the others were shielded from light. The vessels were 250 ml glass flask that contained 100 ml of test solution. The vessels were randomly arranged on a rotary shaker adjusted to 100 rpm and located in an incubator during the test. Test vessels were repositioned daily. A 24 h light and 0 dark photoperiod was automatically maintained with cool-white fluorescent lights with light intensity of approximately

3749 to 3790 lux. The validity criteria has been met. Measured concentration at the end of the test were less than 80% of nominal. This often occur in static systems and does not invalidate the test. Growth inhibition correlated with the dose and was greater than 50% of the highest dose. The reliability is 1-2.

The NOEC determined in this study was 0.220 µg/l based on initial concentration, and 0.040–0.080 µg/l based on a TWA estimate (details in ZnPT Car Doc IIIA). At 0 h the pH was 7.9–8.0, and it changed to 7.4–9.1 after 120 h.

Growth inhibition tests on algae are performed under static conditions. The test concentrations could not be kept stable in such a static system. The growth curves from some tests indicate that the inhibition lasts until around 48 hours after initiation of the test. After this time point, the cultures start to recover, and the inhibition is no longer seen. Nonetheless, the inhibition was real during the first 48 hours. RMS has therefore decided only to consider the first 48 hours of the tests. In order to determine the concentrations during the test period, the concentrations at 48 hours after initiation were estimated, assuming that the pyrithiones are degraded by single first order degradation (justified by SFO kinetics for photolysis, hydrolysis and "die-away" rates). Geometric mean exposure concentrations for the test period were thereafter calculated, using the start concentration and the estimated concentration (at 48 h). For more detailed calculations see ZnPT CAR Doc IIIA A7.4.3.1/04. The TWA for 48 hours NOEC is thereby 0.000040–0.000080 µg/l and the EC50 value was 0.00060 mg/L.

In conclusion, zinc pyrithione was shown to have an adverse effect on the growth and growth rate of the marine diatom, *Skeletonema costatum*. Exposure of the diatom to zinc pyrithione for 48 h resulted in a NOEC of 0.000040–0.000080 mg/l. The 48-h EC50 value for growth inhibition after 48 h was 0.00060 mg/l.

Another tests was conducted on the fresh water species *Selenastrum capricornutum* in accordance to US EPA 122-2 Growth inhibition tests on algae are performed under static conditions. In the algae test according and EPA 122-2 the EC50 was at 120 h=0.028 mg/L, 72 h=0.03 mg/L and 48 h=0.1 mg/L. The NOEC was at 72 h=0.0091 mg/L.

An 96 h acute toxicity study with the green algae *Pseudokirchneriella subspicata* carried out according to OECD 201 showed an E_rC50 = 0.051 mg/L after 72 h and a NOEC = 0.0149 mg/L (Thor GmbH Art. 95 dossier, 2015).

Metabolites

The effect of metabolites PSA, OMSA and pyrithione disulphide (see figure 11.4-1 for the proposed metabolic pathway) tested, using the freshwater alga *Selenastrum capricornutum* as test organism. In the tests performed with OMSA and PSA, the pH was much lower than what is prescribed in the guidelines. The low pH may have affected the growth of the algae. As 50% inhibition was not obtained in the relevant tests, the EC_{50}-values are expressed as greater than the highest concentration with an acceptable pH (ZnPT CAR Doc IIIA A7.4.1.3/11 and A7.4.1.3/12). These endpoints cannot be used for classification purposes.

The static test with the metabolite pyrithione disulphide showed a high toxicity with an EC50 after 120 days =0.140 mg/L (see Table 76) but these are nominal values and the test had reliability 3 according to RMS and can also not be used for classification purpose.

Table 76: Growth inhibition effects of 2,2' (pyridyl-N-oxide) disulphide on algae

Method	Species	Endpoint / Type of test	Exposure		Results (mg/L)		Reference
			design	duration	NOEC	EC_{50}	

US EPA-122-2	Fresh water algae *Selenastrum capricornutum*	Growth inhibition	Static	120 hours	0.080 0.140	ZnPT CAR Doc IIIA A7.4.1.3/010 1994

Summary

Overall it can be concluded that zinc pyrithione and the metabolite pyrithione disuphide are very toxic to algae with a LC50 < 1 mg/L. The most sensitive species to zinc pyrithione is the marine diatom *Skeletonema costatum* 48 h EC50 = 0.0006 mg/L.

For the metabolites the tests performed with OMSA and PSA, the pH was much lower than what is prescribed in the guidelines. The low pH may have affected the growth of the algae. As 50% inhibition was not obtained in the relevant tests, the EC_{50}-values are expressed as greater than the highest concentration with an acceptable pH. These endpoints cannot be used for classification purposes.

The static test with algae *Selenastrum capricornutum* performed with the metabolite pyrithione disulphide indicates a very high toxicity, with an EC50 after 120 days was 0.140 mg/L but the test is based on nominal value and has low reliability and cannot be used for classification purpose.

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

The effect of zinc pyrithione on microbial activity in water was assessed by determining the level inhibition of respiration of micro-organisms present in activated sludge (ZnPT CAR Doc IIIA). The test was performed in accordance with OECD 209. The results obtained in this study are somewhat uncertain: the final concentrations in the test system were near or above the limit of solubility for zinc pyrithione in the three highest doses. No insoluble material was observed in the test system; however, this may have been due to the dark colour of the system. The EC50 and NOEC of zinc pyrithione was determined after 30 minutes and 3 hours and were 5.8 and 2.4 mg/L, respectively.

Another respiration inhibition test with activated sludge from a municipal treatment plant, and according to OECD 209 was carried out with zinc pyrithione (Thor GmbH Art.95 dossier). The test was carried out under static conditions with the test item concentrations 0.3125, 0.625, 1.25, 2.5, 5 and 10 mg/L. The respiration rates of the control, solvent control, reference and test item replicates were measured after contact time of three hours, and the inhibitory effects of the test and reference item were determined in comparison to the pooled control respiration rates. The mean inhibition of respiration for the test items replicates ranged from 3% to 88% and the EC50 value for the reference item was 90.2 mg/L.

The test results were a NOEC for zinc pyrithione of 0.3125 mg/L and EC50 of 2.82 mg/L and of the test item 6.25 mg/L.

Summary

Overall zinc pyrithione has a moderate to high effect towards microorganisms with a range of NOEC = 0.3125 mg/L to 5.8 mg/L.

11.2 LONG-TERM AQUATIC HAZARD

The summaries and evaluations of the long-term aquatic studies with zinc pyrithione are taken from the draft ZnPT CAR Doc IIIA and Thor GmbH Art. 95 dossier (2015).

Table 77: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference and Reliability
OECD 210 Early life stage toxicity	*Pimephales promelas* (Fathead Minnow)	Flow through Zinc pyrithione 32 days	NOEC = 0.00122 mg/L LOEC = 0.00282 mg/L	Key study on reproduction and growth rate	Survival and sublethal effect at hatch, days 7, 14, 21, 28 and the total length of survival fish at the end of test	ZnPT CAR Doc IIIA A7.4.3.2/01 Reliability: 1-2 1999
OECD 210 Early life stage toxicity	*Danio rerio* (Zebrafish)	Flow through Zinc pyrithione 30 d	NOEC = 0.00125 mg/L LOEC = 0.00312 mg/L		Survival(mortality) hatch and growth expressed in length and dry weight	Thor GmbH Art. 95 dossier
US EPA-72-4(b) Chronic toxicity	Freshwater Daphnid *Daphnia magna*)	Flow-through 21 days	NOEC = 0.0022 mg/L EC50 = 0.029 mg/L LOEC = 0.0049 mg/L	Key study	Mean young per surviving daphnids and meanlength of surviving adults	ZnPT CAR Doc IIIA A7.4.3.4/01 Reliability: 2 1999
OECD Guidelines 211	Fresh water Daphnid *Daphnia magna*	Flow-through 21 days	NOEC = 0.0021 mg/L LOEC = 0.00391 mg/L		Reduction of reproduction and survival	Thor GmbH Art. 95 dossier
US-EPA-72-4	Marine Mysid *Americamys bahia = Mysidopsis bahia*	Flow- through 28 d	NOEC=0.00228 mg/L EC50=0.00521 mg/L LOEC=0.0042 mg/L		Sea water salinity 15-16‰, DMF solvent	ZnPT CAR Doc IIIA A7.4.3.4/02 Reliability: 2 1999
No standard guideline	Sea urchin	Fertilization & Embryo Phase: Static Adult Phase: Flow-through Fertilzation Phase: 3 hrs Embryo Phase: 48 hrs Adult Phase: 30 days	NOEC Fertilization Phase: 1.0 µg/L Embryo Phase: 29 µg/L Adult Phase: 45 µg/L LOEC		Fertilization Phase: Successful Fertilization Embryo Phase: Normal embryos Adult Phase: Survival and diameter	ZnPT CAR Doc IIIA A.7.4.3.4/03 Reliability: 3 2004

			Fertilization Phase: 1.7 µg/L Embryo Phase: 60 µg/L Adult Phase: 99 µg/L			
US EPA 850 440	Duckweed *Lemna gibba G3*	Flow through 14 days (7 d exposure and 7 d recovery)	NOEC = 0.0040 mg/L (7d) EC50 = 0.0096 mg/L (7d)	Key study	Growth rate	ZnPT CAR Doc IIIA A7.4.3.5.2/01. Reliability: 1-2 1998

11.2.1 Chronic toxicity to fish

Chronic toxicity was tested in the freshwater species Fathead minnow (*Pimephales promelas*), in accordance with OECD 210. The measured endpoints were survival and sub-lethal effects at hatch and at days 7, 14, 21 and 28, as well as the total length of surviving fish at the end of the test. All measured endpoints proved to be equally sensitive. The author reported some minor deviations from the protocol, e.g. the continuously recorded temperature slightly exceeded the target range of 25 ± 2 ºC (the temperature measured in each test vessel each day during the test was never outside the 25 ± 2 ºC range) and one fish from one of the replicate test vessels in one of the test groups was accidentally excluded from the weight determinations. These deviations are not believed to have affected the outcome of the study.

Another supportive chronic toxicity study according to OECD 210 with the zebrafish *Danio rerio* showed in a 30 days post hatch test a NOEC = 0.00125 mg/L and a LOEC = 0.00312 mg/L.

The results indicate that zinc pyrithione has an adverse effect on juvenile fish in the chronic toxicity test.

Summary

It can be concluded that zinc pyrithione has a high chronic toxicity to fish with the most sensitive species *Pimephales promales* 32 d NOEC = 0.00122 mg/L.

11.2.2 Chronic toxicity to aquatic invertebrates

Chronic toxicity to invertebrates was investigated using zinc pyrithione. Tests were conducted in three different species: the Daphnid (*Daphnia magna*), the Mysid (*Americamysis bahia* or *Mysidopsis bahia*) and the Sea urchin (*Arbacia puntulata*).

The Daphnia test was performed according to FIFRA 72-4(b). The guideline is generally comparable to OECD 211. Significant differences include: EC50 is calculated based on first-generation survival; the end point for number of living offspring per parent animal is expressed as the maximum acceptable toxicant concentration (MATC); the requirement for mortality of the controls is ≥30% rather than >20%; the requirement for number of offspring per surviving parent is >40 rather than ≥60; the range of test concentrations includes concentrations that have a significant effect on adult survivability. Furthermore, the EC50 value cannot be used since it is based on the mortality, assuming no sub-lethal effects were observed. There were, however, sub-lethal effects and the EC50 value should have been based on these. The 21 days NOEC was 0.0022 mg/L and is acceptable.

Another chronic toxicity test was performed with *Daphnia magna* according to OECD 210 where the NOEC reproduction was measured based on the geometric mean concentration ZnPT and the NOEC was 0.00221 mg/L and the LOEC was 0.00391 mg/L.

A chronic toxicity study was performed on *Mysidopsis bahia*, in accordance with guideline US EPA 72-4, which is equivalent to OPPTS 850.1350 “Mysid Chronic Toxicity Test” with the exception that a 16-hour light/8-hour dark photoperiod is used instead of 14-hour light/10-hour dark, no data are recorded for the G2 mysids, and the salinity is 15 ppt instead of 20 ppt. The most sensitive measures of toxicity determined by statistical analysis of survival, growth and reproduction data were the mean length and mean dry weight. The NOEC was 0.00228 mg/L after 28 days.

An additional test on the Sea urchin *Arbacia punctulata* studied the effect of zinc pyrithione on fertilization, embryo survival and adult survival. The test was not a guideline study, but its results confirm the results obtained in the guideline studies. The most sensitive endpoint was the fertilization phase. No mortalities or other adverse effects were observed in any of the treatment groups in the long-term adult’s test. However, adult sea urchins failed to spawn when stimulated after a 30-day toxicity test.

The results from the test show that zinc pyrithione affects all the test organisms adversely and can be considered to exhibit a chronic toxicity towards invertebrates.

Summary

Overall zinc pyrithione has a highly chronic toxicity towards invertebrates with the most sensitive species of *Daphnia magna* and marine mysid *Mysidopsis bahia* with NOEC 21 d = 0.0022 mg/L and NOEC 28 d = 0.00228 mg/L, respectively.

11.2.3 Chronic toxicity to algae or other aquatic plants

Toxicity to aquatic plants was tested in the fresh water species *Lemna gibba*, using zinc pyrithione. The study was conducted in accordance with guidelines EPA-FIFRA 123-2 and US-EPA 850.4400. The 72 d NOEC was measured to 0.0040 mg/L.

The results obtained in the study suggest that zinc pyrithione can have an adverse effect on the growth rate of duck weed.

Summary

It can be concluded that zinc pyrithione can have an effect on the growth rate of *Lemna gibba* with 7 d NOEC = 0.0040 mg/L.

11.3 BIOACCUMULATION

The summaries and evaluations of the bioaccumulation studies with zinc pyrithione are taken from the draft CAR for zinc pyrithione and Thor GmbH Art. 95 dossier (2015).

Table 78: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study	Remarks	Reference and Reliability

Zinc pyrithione 14C Low dose High dose	Oyster *Crassostera virginica*	Log Kow: 0.99 (zinc pyrithione dossier) BCF QSAR (predicted from Kow): 1.4 BCF observed: Kinetic estimate Low dose: 11+-3.6 High dose: 8.6+-3.9 Steady state estimate Low dose: 8.3 High dose: 7.8	Key study		ZnPT CAR Doc IIIA A7.4.2/02 Reliability: 2 2001
Zinc pyrithione OECD 117 (HPLC method, 23ºC)	Fish-No species were presented	logPow: 1.21 BCF fish: 0.33 BCF for fish eating birds/predators: 0.33			Thor GmbH Art. 95 dossier

11.3.1 Estimated bioaccumulation

The log Kow (ZnPT CAR Doc IIIA, 2001) is 0.99 and log Pow = 1.21 (Thor GmbH Art. 95 dossier, 2015) indicated that zinc pyrithione has a low bioaccumulation potential for aquatic organisms.

11.3.2 Measured partition coefficient and bioaccumulation test data

Accumulation of pyrithione in aquatic species is a potential concern with its use in antifouling applications. To determine if the compound has a potential to bioaccumulate one study with zinc pyrithione in the oyster is available (ZnPT CAR Doc IIIA A7.4.2/02). Radiolabelled test substance was used in each study, and the results were based on the total radioactivity in the tissues (ng ZnPT equivalents). As a result, the bioconcentration factors does not distinguish concentrations of pyrithione from any metabolites that may have formed.

The oyster study with zinc pyrithione was done according to OECD 305E using an intermittent flow-through system. A low- and a high-dose experiment was included. The average of 5 measurements in replicates 1 & 2 during the uptake phase were used for the steady-state estimate of BCF. This average for the low dose water concentration was 0.0565 µg zinc pyrithione eq/l, and for the high dose it was 0.474 µg zinc pyrithione eq/l. The corresponding tissue concentration in the low dose was 468 pg zinc pyrithione eq/g, and 3.73 ng zinc pyrithione eq/g in the high. The LOD for water was 0.0315 µg zinc pyrithione eq/l, and for oyster tissue 315 pg zinc pyrithione eq/g. All concentration data were thereby above the LODs. The bioconcentration factors (BCF) calculated by the steady-state and kinetic methods were similar and ranged from 7.8 to 11.0 l/kg ww. A BCF calculated with QSAR gave a BCF of 1.4 (see table 78).

The bioaccumulation study (Thor GmbH Art. 95 dossier, 2015) with fish was only briefly presented. The n-octanol/water partition coefficient was determined according to OECD 117 (HPLC method) (equivalent to EC Directive 92/69/EEC method A). A log Pow of 1.21 was reported at PH 6.0+/-0.05

and 23ºC indicating no potential for bioaccumulation of zinc pyrithione The BCF factor for fish was estimated from log Pow = 1.21 and was 0.33.

Summary

Overall it can be concluded that zinc pyrithione has low bioaccumulation potential for aquatic organisms with a log Kow = 0.99 and log Pow = 1.21 and BCF = 7.8-11.0 in oyster and BCF = 0.33 in fish. The calculated BCF with help of QSAR was 0.33–1.4.

11.4 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES

The summaries and evaluations of the rapid degradability with zinc pyrithione are taken from the ZnPT CAR Doc IIIA. Furthermore, information from a dossier on zinc pyrithione submitted by Thor GmbH in June 2015 as part of their BPR (Regulation (EU) 528/2012) Article 95 notification of the substance is also considered. Information on the degradation of copper pyrithione, which undergoes a similar pathway than zinc pyrithione, is included in the following Table as supportive evidence. The reasons for the inclusion are additional insights into the route of aerobic degradation, common degradation product (e.g. PSA) and fate. See also the proposed metabolic pathway of zinc pyrithione in Figure 11.4-1.

Table 79: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks and initial concentration of test compounds	Reference and Reliability
OECD 301B	ZnPT 17% degradation after 6 d 39% after 28 days	Key study	Ready biodegradable test with activated sludge, measuring CO_2 evolution after 28 d, Zinc pyrithione :13.2 mg/l	ZnPT CAR Doc IIIA A7.1.1.2.1/01 Reliability: 1 2002
OECD 301B & 92/69/EECC.4-C	ZnPT 17% after 8 d 54% after 43 days	Supportive study	Ready biodegradable test with activated sludge, measuring CO_2 evolution after 44 d, Zinc pyrithione: 26 mg/l	ZnPT CAR Doc IIIA A7.1.2.1/02 Reliability: 1 1998
OECD 301B	Major metabolite PSA 49% after 6 d 64% after 14 d 73%after 28d		Ready biodegradable test with activated sludge, measuring CO_2 evolution after 29 d, PSA 26 mg/l	ZnPT CAR Doc IIIA A7.1.1.2.1/03 Reliability: 1 2002

U.S. EPA §162-4	ZnPT Pyrithione dissipation – data from CuPT. Mineralisation to CO_2 was insignificant during the timeframe 30 days ZnPT + OTS (data from CuPT (see below) CuPT + OTS Degradation, whole system $^{fomc}DT_{50}$ = 2.7d (first-order multi compartment kinetics (FOMC). $^{fomc}DT_{90}$ = 70 d $^{sfo}DT_{50}$ = 21 d (a worst case single-first order half-life (SFO)		Aerobic marine water sediment system (dark) - Pyrithione dissipation in aqueous phase and sediment; - Metabolite formation; - CO_2 evolution; - Bound residues accumulation 5 g dw marine sediment in 10ml water ZnPT 52.2 ng/g(ng per gram water and sediment; intitial and nominal.)	ZnPT CAR Doc IIIA A7.1.2.2.2/01 Reliability: 1 1999
OECD 308 & OPPTS 835,4300	Dis50 (river water sediment system/all glass metabolism flasks and overlaying water) Dis50 Pond water sediment system/all-glass metabolism flask containing sediment and overlaying water	Water phase:1.01 days Sediment:n.a System:1.01 days Water phase:0.39 days Sediment:n.a System:0.39 days	Zinc pyrithione in aquatic systems with ZPT in conc 0.251 mg/L and 0.253 mg/L	Thor GmbH Art. 95 dossier Reliability: 2
OECD 307	DT50 in soils-(dissipation half-life) Aerobic dark conditions	DT50<30 min		Thor GmbH Art. 95 dossier

11.4.1 Ready biodegradability

A ready biodegradability test according to OECD 301B was carried out with zinc pyrithione (dose 13 mg/l) and activated sludge and CO_2 evolution was measured for 28 days (see table 79 and ZnPT CAR Doc IIIA A7.1.1.2.1/01). The study was performed according to GLP standards and has reliability 1. The test substance was not inhibitory to the microorganism in the activated sludge. Variation in the degradation rates for the test substance on different sampling days was considered to be due to normal biological variation in the respiration rates between the control and test material vessels. This biological variation was exaggerated by the relatively low carbon concentration employed in the test. The degradation of zinc pyrithione was 39% after 28 days and approximately 18% after 10 days incubation at 21 ^{0}C. On basis of these results zinc pyrithione was considered not rapidly degradable (see Table 79).

This standardized ready biodegradable study was chosen to be the key study, since it follows OECD 301B which is a highly recommended guideline to show rapidly degradation for classification purposes and followed also GLP recommendations.

Another ready biodegradability study supported the key study and was also carried out according to OECD 301B with zinc pyrithione and activated sludge, but the CO_2 evolution was measured after 40 days with a higher test concentration of ZnPT than in the key study above (ZnPT CAR Doc IIIA A7.1.1.2.1/02). The test was otherwise carried out the same way as for the key study and followed GLP recommendations. This ready biodegradable test also has a reliability 1. The degradation of zinc pyrithione was 17% after 8 days and 54% after 43 days (see Table 79).

Another degradation tests of zinc pyrithione (Thor GmbH, Article 95 dossier, 2015) was performed according to OECD 303 (see Table 79).

However, this tests is not recommended test according to the CLP guidance document (November, 2012). The main reason for not using test 303 is that the microbial biomass in a STP is significantly different from the biomass in the environment. Also there is a considerable different composition of substrates, and that the presence of rapidly mineralized organic matter in waste water may facilitate degradation of the test substance by co-metabolism

Another degradation study with zinc pyrithione was performed according to OECD 307 with zinc pyrithione (Thor GmbH Article 95 dossier, 2015).This study was a dissipation study which is hard to interpret. The OECD 307 is also not a recommended guideline for classification purpose when it comes to showing rapid degradation.

The major non transient metabolite, pyridine sulphonic acid (PSA) was barely ready biodegradable 73% degradation within 28 days (see Table 79).

11.4.2 BOD_5/COD

No study performed.

11.4.3 Other convincing scientific evidence

Aerobic degradation

The information on the aerobic degradation are taken from three different studies, two evaluated in the CuPT CAR (2014) and described in more detail in ZnPT CAR Doc IIIA and one new study from Thor GmbH Article 95 dossier (that is presented in more detail below).

The route of aerobic degradation and metabolites

These aerobic seawater/sediment studies were conducted in the dark with 10 ml seawater and 5 g dw sediment kept in 50 ml test tubes. The copper pyrithione study (Doc IIIA A7.1.2.2.2/03) was carried out for a period of 84 days and the zinc pyrithione study (Doc IIIA A7.1.2.2.2/01) for a period of 30 days, both with an initial nominal concentration of 46–52 ng/g dw sediment (0.05 ppm dw sediment). The degradation rate of pyrithione and the formation of degradants were compared and found to be the same for both compounds (see RMS comment to IIIA A7.1.2.2.2/01). This was expected given the transchelation observed to occur with pyrithione.

The proposed aerobic degradation pathway for zinc and copper pyrithiones is given in Figure 11.4-1. Zinc pyrithione and copper pyrithione degrade through oxidation of the thiol group resulting in pyrithione sulfinic acid (OMSiA), pyrithione sulfonic acid (OMSA, also named OMSoA), and 2-pyridine sulfonic acid (PSA, also named PSoA). Reduction of the N-oxide also takes place, but to a lesser extent. Formation of pyrithione thiosulfate (OTS) also occurred, but the origin of OTS was unclear. It may have been an artefact of the extraction procedure, or it could have formed via reaction of a pyrithione disulfide (OMDS) intermediate with SO_2^{-3} in the sediment or directly from pyrithione in presence of SO_2^{-3} and Cu^{+2}. The metabolites OMSA, PSA, OMSiA and OTS where the most abundant aerobic degradation products (>10%).

The concentration of OMSA co-varied with redox potential (E_h) in water and sediment. The E_h in water and E_h in sediment were measured separately, and they co-varied strongly during all time, and both parameters seem to be correlated to pH.

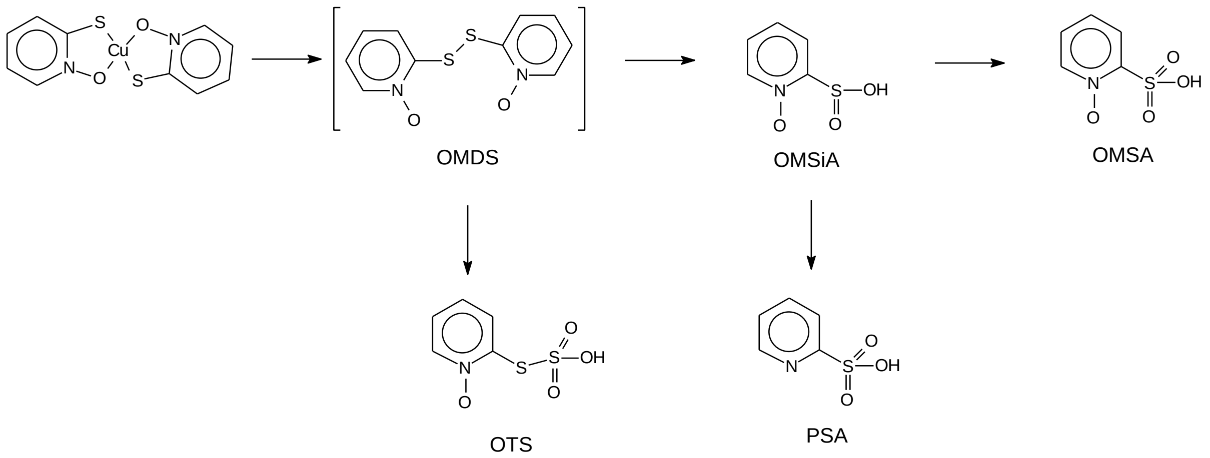


Figure 11.4-1. Proposed metabolic pathway for the aerobic aquatic metabolism of copper pyrithione (OMDS was not detected, but is shown in brackets as a possible intermediate). Zinc pyrithione has the same metabolic pathway as copper pyrithione. Zinc ions are also released.

Zinc pyrithione degraded through dissociation of the pyrithione molecule and successive oxidation of the sulphur to form pyrithione sulfinic acid (OMSiA) and pyrithione sulphonic acid (OMSA). Reduction at the N oxide group resulted in the formation of pyridine sulphonic acid (PSA) as a terminal metabolite. Mineralization to CO_2 was insignificant during the timeframe of the study; however, the percentage of the dose remaining as bound residues in the sediment increased to ~29% of the dose by day 30. The fraction of the dose found in the water column increased from 17% day 0 up to 37% day 30, and further up to 40% day 83.

Pyrithione thiosulfate (OTS) was seen in the sediment extracts. This may have formed through reaction of pyrithione, or possibly an intermediate pyrithione disulfide (OMDS), with endogenous sulphite in the sediment. It could not be ruled out that at least some of the OTS seen in the extracts actually formed from pyrithione originally present in the sediment during extraction with KOH solution. Degradation of the pyrithione was therefore based on the combined amounts OTS and pyrithione in order to obtain the most conservative dissipation times.

Degradation of the pyrithione was therefore based on the combined amounts OTS and pyrithione in order to obtain the most conservative dissipation times. The applicant fitted the first time points (0–3 days) to single first-order kinetics (SFO), and the later time points (3–84 days) to first-order multi compartment kinetics (FOMC). Such a combination of kinetic fittings is not suitable for deriving input parameters to a fate model which execute calculations based on only the one type of kinetic fitting (EFSA PPR, 2005). This is the case with the MAMPEC and EUSES/TGD models, which need SFO-based degradation input parameters. Hence, the applicant's rate constants from the study report/summary could not be used in these PEC models. RMS therefore re-interpreted the degradation data. The aim was to derive SFO-based degradation parameters, which can be used as input for PEC modelling in MAMPEC, EUSES/TGD.

The bi-phasic degradation curve could be described by a first-order multi compartmental degradation half -life $^{FOMC}DT_{50}$ of 2.7 days, with a corresponding $^{FOMC}DT_{90}$ of 70.1 days (alpha 0.546, beta 1.05). According to FOCUS (2006, page 53 & 114), a worst case single-first order half -life $^{SFO}DT_{50}$ can be estimated from: $^{SFO}DT_{50}$ = $^{FOMC}DT_{90}$ / 3.32; which in this case gave 21 days. This is for the total system, and does not differentiate degradation rate in water from that in sediment. Such a distinction was not possible because in a sediment/water system the degradation rate in water and in sediment is always influenced by the transfer rates between these compartments. These transfer rates must be constrained in order to quantify the degradation rates. This can possibly be done by experiments with sterilized water and sediment, in which only the transfer rates are determined (FOCUS, 2006, Chapters 8–10 in the CuPT CAR (2014)), but not from the data presented in these studies alone. The system specific SFO degradation half-life of 21 days ($^{sfo}k_{deg,\ syst}$ = 0.033 d^{-1}) is only for modelling purposes, and does not describe the actual degradation curve from the experiment, which could only be done using the FOMC parameters.

Other relevant studies on aerobic degradation.

Aerobic study with two different aquatic systems

Material and Methods

Another aerobic degradation study with ^{14}C zinc pyrithione was carried in two different sediment systems: a river (Rhein, Switzerland) and a pond (Fröschweither Switzerland. ^{14}C Zinc pyrithione was applied at a rate between 175.6 µg and 177.0 µg test item per system. This resulted in an applied concentration of 250.9 µg/L to 252.9 µg/L.

The aquatic sediment systems were incubated under aerobic conditions in the laboratory in the dark at 20.8-+0.1 C for 28 days. Treated samples were continuously ventilated with moistened air and the outlet air was passed through a trapping system consisting of flasks with ethylen glycol and sodium hydroxide in series. Duplicate samples were taken foe analysis immediately after application (time 0), after 6 hour, and after 1, 3, 7, 15 and 28 days of incubation.

The aqueous phase was withdrawn from the flasks and the radioactivity measured by Liquid Scintillation Counting (LSC). Chromatographic profiling was performed by High performance Liquid Chromatograohy (HPLC). Sediments were extracted up to four tomes with 0.1M KOH, followed by Soxhelets extraction with acetonitrile and selected samples. Extracts were combined and analysed by LSC for recovery. Chromatographic profiling was performed by HPLC. Non-extractable radioactivity was determined by combustion of homogenised sediments. Volatile radioactivity trapped in ethylene glucol and sodium hydroxide was determined by LSC. Total mean recoveries were 94.4-+4.0 % and 98.1-+3.0% of applied radioactivity (AR) for the river and pond water/system, respectively.

Results and conclusion

Immediately after treatment (day 0) 98.4% and 97.7%AR was presented in the eques phase of river and pond systems. Following treatment, the amount of radioactivity in the aqueous phase of the river system steadily decreased to a minimum of 2.0% AR after 28 days of incubation. In the pond system, the amount of radioactivity in the aqueous phase rapidly decreased to 37.2% AR on day 7, followed by a slow decline to 33.7% AR until the end of the incubation (day 28).

The total amount of radioactivity extracted from the river sediment increased from 1.6% AR on day 0 to a maximum of 20.2% AR sampling day 3, followed by a decrease to 10.3% AR at the end of the 28-day incubation period. For the pond system, the amount of extractable radioactivity increased from 2.1% AR on day 0 to a maximum of 32.7% AR on sampling day 15, an accounted for 25.7% AR at the end of the incubation period. The amount of non-extractable radioactivity (bound residues) in the river and pond sediments reached a maximum of 23.2% AR and 34.9% AR respectively. For the pond system, the non-extractable radioactivity at the last sampling interval was submitted to fractionation of the sediment organic matter. The fulvic acids fraction accounted for 11.4% of applied radioactivity, while 12.3 AR was found in the humic acids fraction and 11.2% AR was associated with humin fraction.

The mineralisation of the test item in the two test systems differed strongly. In the system, a strong formation of $^{14}CO_2$ was observed from sampling day 3 onwards, increasing to a maximum of 59.3% AR at the end of the incubation period. In the pond system, only a minor amount of radioactivity was trapped in the sodium hydroxide solution, reaching a maximum of 1.9 % AR on sampling day 28. In both test systems, organic volatile products absorbed in the ethyl glycol traps did not exceed 0.1% AR at any sampling interval. Zn-Pyrithione was only detected in the aqueous phase of both systems. In the river system, the amount of parent test items decreased from 67.2% on day 0 to 7.7% AR on day 3, and was not detected at later intervals. In the pond system, the amount decreased from of parent test item decreased from 60.0% AR on day 0 to 11.0% AR on day 1, and was not detected at later intervals. The calculated DT_{50} and DT_{90} values for ^{14}C zinc pyrithione are based on single first- order kinetics and are presented in the table 78.

A total of 11 metabolites were detected in the aqueous phase and sediment extract phase, of which metabolite M2 was characterized as pyridine-2- sulfonic acid. In the river system, two major metabolites exceed 10% of the radioactivity applied, i.e. M1 and M4. The corresponding half-lives of M1 and M4 were 1.3 and 6.2 days, respectively. The degradation ultimately proceed by the formation non-extractable radioactivity applied, i.e. metabolites M2, M3, M4, M6 and M7. For three of them, the half-lives of 8.1 days (M4), 17,8 days (M6) and 34.8 days (M7) could be calculated. The degradation proceeded mainly via formation of metabolite M4, M7, M6 and M3, and ultimately by formation of metabolite M2 and non-extractable radioactivity.

In conclusion, this aerobic degradation study with two different aquatic systems follows the OECD 308 mainly, except information is missing if the substance concentration is realistic for the environment or not. No information is given about the concentration of the inoculum (cells/ml) and if it is relevant for the aquatic environment. There is no degradation pathway described in more detail and with all identified metabolites.

The primary degradation of zinc pyrithione seem to go fast in the water phase (half- life <16 d) and 11 different metabolites are formed, however only one of the metabolites are identified (pyridine-2-sulfonic acid (PSA)). No information of the half-life of the degradation of PSA was given.

There were at least 6 more metabolites that were not identified (M1, M3, M4, M5, M6, M7) and two of them were more persistent (M6 and M7).

In the CAR for copper pyrithione, see Chapter 4.1.7.1, "Metabolites observed in abiotic and biotic fate studies" informs you, that under environmentally relevant concentrations of total pyrithione (>µg/l) the metabolite PSA forms at the single highest percent. This is demonstrated in OECD 303A (Thor GmbH Art. 95 dossier, 2015) but also in other tests (non-guidance) with natural seawater, natural pond water and artificial water. The accumulation is seen both in systems with only water and in systems with water and sediment.

In water-sediment microcosms under such conditions, PSA accumulates and hence indicates persistence. This behaviour is further supported by field data from the Swedish Screening Programme on biocides (Swedish Environmental Protection Agency, 2014). The metabolite is however not a P-substance since it passes the ready degradation test (CuPT CAR, 2014).

The metabolite PSA was ready biodegradable, but it is at the time the most stable metabolite in the simulation tests. This is not a contradiction. The test result that a chemical is "ready biodegradable" does not mean that it is totally non-persistent. The degradation rate is still a finite value, perhaps corresponding to 15-150 d half-life in water as proposed in the TGD (Part II, Table 24). Others parts of the TGD (Part II, page 54-57, Table 8) also discusses how ready biodegradability compares with half-life in soil and sediment (as a function of sorptivity). Judging from the TGD (Part II, Table 8) we estimate that PSA would have a half-life of 30–300 days in soils and sediments. In the case that its half-life is this long in water and sediments, then the accumulation seen in the microcosms and water-sediment studies is not unexpected (CuPT CAR, 2014).

Summary

Overall the two readily biodegradable tests according to OECD 301B showed less than 70% of zinc pyrithione degraded in 28 days which shows that zinc pyrithione is not rapidly degradable and that the major transient metabolite, pyridine sulphonic acid (PSA) was ready biodegradable 73% after 28 d.

In two aerobic degradation studies with water/sediment systems, it seem that zinc pyrithione is primarly degraded (half-life <16 d) in to the main metabolite (PSA). In one of the aerobic degradation study also three other metabolites; OMSA, OMSiA and OTS were identified.

Also, both aerobic degradations studies with water and sediment systems, showed no ultimate degradation, with no CO_2 development in two out of three test systems. The worst case DT50 in one aerobic study was calculated for the whole system, a worst case single-first order half -life $^{SFO}DT_{50}$ =21 days.

However, it is still unclear if PSA and the other metabolites are not rapidly degradable.

The main metabolite PSA seem to be ready biodegradable (however, barely passing the 70% after 28 days level) but is still frequently found in the water coming in and going out from the sewage treatment plant in Sweden which would indicate persistency. This screening program of the biocides was performed in Sweden from 2000-2013 where ZnPT was included (Swedish Environmental Protection Agency, 2014, report 6634).

In the CAR of CuPT (2014), a half-life of 15-150 d in water is proposed of PSA and also that it is the most stable metabolite in simulation test. This also might indicate that PSA is not so rapidly degradable.

Also, there are still not enough reliable degradation test when it comes to classify the other metabolites. Thus, the results from the two ready biodegradation tests on ZnPT, together with the fact that it cannot be demonstrated that the degradation products from the primary degradation studies do not fulfill the criteria for classification as hazardous to the aquatic environment, lead to the conclusion that the substance is not rapidly degradable (Guidance on the Application of the CLP Criteria, Version 4.1, June 2015, Annex II, II.4). See also conclusion on Section 11.6.2.

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

Not relevant

11.4.3.2 Soil and sediment degradation data

Not relevant.

11.4.3.3 Hydrolysis

The hydrolysis of zinc pyrithione are presented in the draft CAR for zinc pyrithione. In the study zinc pyrithione was hydrolysed from an initial concentration of 2 mg/l. It was a GLP study conducted according to US EPA guideline 161-1 (similar to OECD 111). Hydrolysis half-lives were determined in pH 5, 7, and pH 9 buffers, and pH 8.2 in seawater, respectively. At the study termination 76–83%

of the (zinc) pyrithione remained, and hence the same uncertainty apply to this study regarding extrapolated half-lives. The metabolite OMDS reached 21% in pH 5 and 16% in 7 at day 30 of the experiment. In pH 9 and in ASW (pH 8.2) OMDS was below 10%, but OMSiA reached 12% in pH 9, and 17% in ASW. In this study the hydrolysis rate seemed to have no correlation with pH. The DT50 for zinc pyrithione was 96-123 days (pH 5-9).

In a second guideline GLP study at an initial zinc pyrithione concentration of 5 mg/l, the DT_{50} was 63 days at pH 3, >1 year at pH 7, and 41 days at pH 11. The hydrolysis reaction was first order at pH 3 and pH 11, whereas no significant hydrolysis was observed at pH 7.

In summary, zinc pyrithione is hydrolytically stable, but the rate is faster at lower concentration. The hydrolytical stability does not mean they do not change their complexation with metals in solution, in essence the chemical speciation pattern.

Summary

It can be conclude that hydrolysis DT50 of zinc pyrithione varies with concentration and seem also to vary with pH .The DT50 varies between 41 days and more than a year (pH 3-11) which shows that zinc pyrithione is hydrolytically stable.

11.4.3.4 Photochemical degradation

Not relevant.

11.5 ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION

A screening investigation of ZnPT and the main metabolite PSA was carried out 2000-2013 in the program of biocides dispersal in the environment and their health and environmental risks. This investigation was published in The Swedish Environmental Protection Agency report 6634 by Staffan E. Tjus (October 2014).

In the incoming water from the sewage plants PSA was detected in all samples (73-480 ng/L) and outgoing water from the sewage plant PSA was found in all samples except one to concentrations somewhat lower than for the incoming water 4-330 ng/L. In the sewage sediment from the sewage plant PSA was detected in all samples (25-280 µg/kg TS).

This investigation indicates that PSA might not be rapidly degradable since it is found frequently in the sewage plants in both the incoming sewage water and outgoing and also in the sediment of the sewage plant.

11.6 COMPARISON WITH THE CLP CRITERIA

11.6.1 Acute aquatic hazard

Zinc pyrithione fulfils the classification criteria for Aquatic Acute 1, since its toxicity to aquatic organisms from all three trophic levels (fish, crustacea and algae) is below 1 mg/l (EC50 < 1 mg/l).

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

Zinc pyrithione fulfills the criteria for classification as Aquatic Chronic 1 since its chronic toxicity to aquatic species from three trophic levels is below 1 mg/l (fish as the most sensitive species *Pimephales promales* 32 d NOEC = 0.00122 mg/L and invertebrate *Daphnia magna* 21 d NOEC = 0.002 mg/L and the marine mysid *Mysidopsis bahia* with a NOEC 21 d =0.00228, and aquatic plants

Lemna gibba 7 d NOEC = 0.0040 mg/L) combined with that the substance is not rapidly biodegradable.

Based on log Kow = 0.99 values and BCF = 7.9-11.0 in oyster and BCF = 0.33 in fish, zinc pyrithione is considered to have a low bioaccumulation potential in aquatic organisms.

For classification purpose it is applicable to classify zinc pyrithione as not rapidly degradable (<70% degradation within 28 days) according to the ready biodegradable tests OECD 301B, that showed 49 % degradation after 28 days. This study was supported by the same readily biodegradable test where a different concentration of zinc pyrithione was used. Zinc pyrithione degraded 54% after 43 days in this test.

Also the abiotic degradation showed that the hydrolysis of zinc pyrithione is stable with a DT50 that varies with a 41 days to more than a year depending on pH which supports to classify zinc pyrithione as not rapidly degradable.

According to the decision scheme (see Section II.4 in the guidance on the application of the CLP Criteria, Version 4.0, November 2013) the substance could still be regarded as rapidly degradable if it could be demonstrated that:

a) the substance undergoes a fast degradation (DT50<16 d) and

b) the degradation products do not fulfill the criteria for classification as hazardous to the aquatic environment.

The water/sediment studies available (see section 11.4 Rapid degradability of organic substances table 79 and section 11.4.3 other convincing scientific evidence) show that zinc pyrithione undergoes a fast primary degradation DT50<16 days and forms the metabolites PSA, OMSA, OMDS and OTS. There are reliable aquatic toxicity data for one of three tropic levels for the metabolite PSA. The aquatic toxicity data for the other metabolites are of too low reliability to be used for classification purpose (see section for the metabolites 11.1.1 fish studies, 11.1.2 invertebrate studies and 11.1.3 algae studies).

Therefore, due to lack of data, it cannot be demonstrated that the metabolites are classifiable and zinc pyrithione is thus regarded as not rapidly biodegradable.

11.7 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Zinc pyrithione can be classified as Aquatic Acute 1, with a M-factor 1000 (0.0001 < LC50 < 0.001 mg/L) based on acute toxicity of algae *Skeletonema costatum* 48 h LC50 = 0.0006 mg/L.

Zinc pyrithione can be classified as Aquatic Chronic 1 with an M-factor 10 (0.001 < NOEC <= 0.01 mg/L) based on fish with the most sensitive species *Pimephales promales* 32 d NOEC = 0.00122 mg/L and that the substance is not rapidly biodegradable.

Hazard statement codes: *Hazardous to the aquatic environment*

Aquatic Acute 1; H 400, M-factor 1000

Aquatic Chronic 1; H410, M-factor 10

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Hazard class not assessed in this dossier.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V, Andersen KE, Angers-Loustau A, Aptula A, Bal-Price A, Benfenati E, Bernauer U, Bessems J, Bois FY, Boobis A, Brandon E, Bremer S, Broschard T, Casati S, Coecke S, Corvi R, Cronin M, Daston G, Dekant W, Felter S, Grignard E, Gundert-Remy U, Heinonen T, Kimber I, Kleinjans J, Komulainen H, Kreiling R, Kreysa J, Leite SB, Loizou G, Maxwell G, Mazzatorta P, Munn S, Pfuhler S, Phrakonkham P, Piersma A, Poth A, Prieto P, Repetto G, Rogiers V, Schoeters G, Schwarz M, Serafimova R, Tähti H, Testai E, van Delft J, van Loveren H, Vinken M, Worth A, Zaldivar JM. Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Arch Toxicol. 2011 May;85(5):367-485.

Beyer, B.K., Chernoff, N., Danielsson, B.R., Davis-Bruno, K., Harrouk, W., Hood, R.D., Janer, G., Liminga, U.W., Kim, J.H., Rocca, M., Rogers, J. & Scialli, A. R. 2011. ILSI/HESI maternal toxicity workshop summary: Maternal toxicity and its impact on study design and data interpretation. Birth Defects Research Part B - Developmental and Reproductive Toxicology, 92**,** 36-51.

Bowen, D.E. (2011), Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the Comet assay and the flow-cytometric peripheral blood micronucleus test, Mutation Research, Vol. 722/1, pp. 7-19.

Cappon, G.D., Fleeman, T.L., Chanin, R.E., Hurtt, M. E.: Effects of feed restriction during organogenesis on embryo-fetal development in rabbit. Birth Defects Res B Dev Reprod Toxicol. 2005 Oct;74(5):424-30.

Copper Pyrithione (PT21). Document II A:Effect assessment for the active substance. Rapporteur Member State: Sweden. Final CAR 2014.

Exposure of biocides to the environment and their health and environmental risks (Screening year 2000-2013) Report 6634. Swedish Environmental Agency, October 2014.

ICH S2(R1): Guidnace on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (2012).

Nolen, G.A. and Dierckman, T.A. 1979 Reproduction and teratology studies of zinc pyrithione administered orally or topically to rats and rabbits. Food and Cosmetics Toxicology 1979 Dec;17(6):639-49.

OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment, Number 43. 24 July 2008.

SCCS (Scientific Committee on Consumer Safety), Opinion on Zinc pyrithione, 18 June 2013, SCCS/1512/13, revision of 18 June 2014.

Smith,C.C, et al. (2008), Recommendations for design of the rat Comet assay, Mutagenesis,Vol. 23/3, pp. 233-40.

Tice, R.R.et al.(2000), Single cell gel/Comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environmental and Molecular Mutagenesis, Vol. 35/3, pp. 206-21.

15 ANNEXES

The study summaries from draft ZnPT CAR Doc IIIA referred to in this report and the developmental toxicity review paper from the zinc pyrithione task force are provided as confidential appendices to the IUCLID file. Furthermore, the full study reports of the unpublished developmental toxicity studies on zinc pyrithione are also provided as confidential appendices to the IUCLID file.