Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

CLH-O-0000001412-86-239/F

Adopted

14 September 2018

14 September 2018

CLH-O-0000001412-86-239/F

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

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

Chemical name: **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**

The proposal was submitted by **Sweden** and received by RAC on **22 May 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 May 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 July 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur, appointed by RAC: **Miguel A. Sogorb**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus.**

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

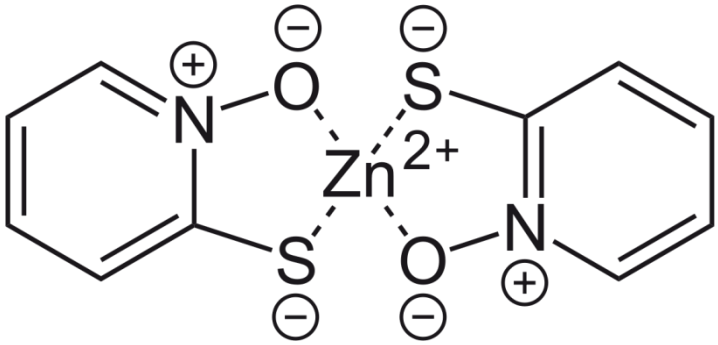
	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc	236-671-3	13463-41-7	Repr. 1B Acute Tox. 2 Acute Tox. 3 Eye Dam. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H330 H301 H318 H372 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H360D H330 H301 H318 H372 H410		M=1000 M=10	
RAC opinion	TBD	pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc	236-671-3	13463-41-7	Repr. 1B Acute Tox. 2 Acute Tox. 3 Eye Dam. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H330 H301 H318 H372 H400 H410	GHS08 GHS06 GHS05 GHS09 Dgr	H360D H330 H301 H318 H372 H410		oral: ATE = 221 mg/kg bw inhalation: ATE = 0.14 mg/L M=1000 M=10	
Resulting Annex VI entry if agreed by COM	TBD	pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc	236-671-3	13463-41-7	Repr. 1B Acute Tox. 2 Acute Tox. 3 Eye Dam. 1 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H330 H301 H318 H372 H400 H410	GHS08 GHS06 GHS05 GHS09 Dgr	H360D H330 H301 H318 H372 H410		oral: ATE = 221 mg/kg bw inhalation: ATE = 0.14 mg/L M=1000 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Zinc pyrithione (ZnPT) is an active substance in the meaning of Regulation EU No 528/2012 (private area and public health area disinfectants and other biocidal products, in-can, masonry, fibre, leather, rubber, polymerised materials and film preservatives and antifouling products). It has no current entry in Annex VI of the CLP Regulation and all hazard classes are open for assessment. This assessment is based purely on the zinc coordination complex. While read across may be useful in some circumstances each pyrithione species has distinct physicochemical and toxicological properties and the assessment of zinc pyrithione is based on the toxicological database specific to this particular substance.

ZnPT is useful as an antimicrobial agent active against gram-positive and -negative bacteria, fungi, and yeasts and is known for its uses in antifouling paints and as a topical treatment for some mild forms of dermatitis (e.g. seborrhoea, dandruff). ZnPT is thus used in rinse-off products (excluding oral hygiene products) and in leave-in hair products which are regulated under the Cosmetics Regulation 1223/2009.



The pyrithione ligands are chelated to Zn^{2+} via oxygen and sulfur centers in a metal coordination complex. In solution, a 'monomer' of zinc pyrithione may be considered to be comprised of one zinc atom chelated by two pyrithione units by way of 2 × S and 2 × O atoms. ZnPT only penetrates into cells when the coordination complex is intact. A natural equilibrium exists, however, in which some of the molecules are separated into component zinc and pyrithione portions, neither of which are as effective as the intact coordination complex.

ZnPT acts on microbial membranes to eliminate certain ion gradients that are used by bacteria to store energy and by fungi as the source of energy for nutrient transport. The unionized dimeric molecule is lipid soluble and will readily cross cell membranes (pyrithione is a weak acid), furthermore, because it also acts as a divalent cation ionophore it can transport Zn^{2+} and Cu^{2+} into cellular compartments. ZnPT is believed to act by catalysing the electro-neutral exchange of H^+ and other ions with K^+ across cell membranes, resulting in collapse of H^+ gradients (e.g. proton motive force), K^+ gradients and other cell ion gradients important to cell function, with consequences depending upon the environment and the organism.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification of ZnPT for physical hazards on the basis of the following results:

- Negative results in two different EEC A.14 studies for testing the capability of ZnPT to be explosive;
- Negative results in two different EEC A.10 studies for testing the flammability of ZnPT;
- Data (including an ECC A.16 test) indicating that ZnPT dust ignites at around 200°C;
- One EEC A.17 study indicating that ZnPT is not oxidising;
- Absence of data for the following hazards: self-reactive substances, phyrophoric solids, flammable gases and corrosive metals.

The DS also considered the following physical hazards not applicable to ZnPT: flammable gases, oxidizing gases, gases under pressure, flammable liquids, phyrophoric liquids, oxidizing liquids and organic peroxides

Assessment and comparison with the classification criteria

RAC supports the DS's proposal for no classification of ZnPT for physical hazards on the basis of negative results found in A.14, A.10, A.16 and A.17 tests, absence of data for the other 4 hazards and non-applicability for the remaining 7 hazards.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of ZnPT for Acute Tox. 3; H301 (Toxic if swallowed) on the basis of one study performed with rats, according to GLP and OECD TG 401, that yielded an LD_{50} of 221 mg/kg bw. It was supported by an acute neurotoxicity study performed in rats, according to GLP and OECD TG 424, showing a LD_{50} of 150 mg/kg and by the Scientific Committee on Consumer Safety (SCCS) opinion stating that LD_{50} values in the *"rat ranged from 92 to 266 mg/kg and in the mouse from 160 to 1000 mg/kg".*

The DS proposed no classification of ZnPT for acute dermal toxicity on the basis of a limit dose study, according to GLP and EPA guideline 81-2, where 2000 mg/kg bw caused no mortalities.

The DS proposes classification of ZnPT for Acute Tox. 2; H330 (Fatal if inhaled) on the basis of three OECD TG 403, GLP-compliant studies; the first showing an LD_{50} of 0.84 mg/L for male rats, the second one an LD_{50} higher than 0.61 and the third one an LD_{50} in a range between 0.05 and 0.5 mg/L. A fourth, less reliable study showed an LD_{50} of 0.14 mg/ml.

Comments received during public consultation

One company downstream user provided a general comment about natural presence of pyrithione in food. RAC noted that the mere presence of a substance in food does not provide information

about its intrinsic capability to induce a specific hazard, and therefore it is not relevant for classification purposes.

Three different Member State Comptent Authorities (MSCAs) supported the DS's proposal of classification for acute toxicity and a fourth one requested acute toxicity estimate (ATE) values to ensure consistent classification of mixtures containing ZnPT. The DS replied that their ATE proposals for acute oral toxicity was 221 mg/kg, while the ATE for acute inhalation toxicity was less clear because it is in the range between 0.05 and 0.5 mg/L.

Assessment and comparison with the classification criteria

The three tables below summarise the available acute toxicity studies for oral, dermal and inhalation routes, respectively.

Table: *Summary of the animal studies on acute oral toxicity studies with ZnPT.*

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>OECD TG 401

GLP

Reliability: 2 (purity of the test substance was not specified)

Wistar Albino rat

5 sex/dose

14 days post exposure period</td>
<td>125, 158, 200, 254 and 321 mg/kg bw</td>
<td>
<table>
<tr><th colspan="3">Mortalities</th></tr>
<tr><th>Dose (mg/kg bw)</th><th>Number dead/total</th><th>Day of death</th></tr>
<tr><td>125</td><td>1/10</td><td>1</td></tr>
<tr><td>158</td><td>2/10</td><td>1-3</td></tr>
<tr><td>200</td><td>3/10</td><td>1</td></tr>
<tr><td>254</td><td>5/10</td><td>1-5</td></tr>
<tr><td>321</td><td>6/10</td><td>1-4</td></tr>
</table>
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} = 221 mg/kg bw
</td>
<td>ZnPT CAR Doc IIIA A6.1.1/01*

Year: 1986</td>
</tr>
<tr>
<td>OECD TG 401

GLP

Reliability: 2 (purity of the test substance was not specified)

Sprague-Dawley CD rat</td>
<td>500, 707 and 1000 mg/kg bw</td>
<td>
<table>
<tr><th colspan="3">Mortalities</th></tr>
<tr><th>Dose (mg/kg bw)</th><th>Number dead/total</th><th>Day of death</th></tr>
<tr><td>500</td><td>0/10</td><td>-</td></tr>
<tr><td>707</td><td>3/5</td><td>-</td></tr>
<tr><td>1000</td><td>4/5</td><td>1-7</td></tr>
</table>
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
</td>
<td>Doc IIIA A6.1.1/02

Year: 1997</td>
</tr>
</table>

<table>
<tr>
<td>5 females /dose

5 males at 500 mg/kg bw

14 days post exposure period</td>
<td></td>
<td>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} = 774 mg/kg bw</td>
<td></td>
</tr>
<tr>
<td>Not specified: Guideline, GLP-compliance or not, animals/group, sex and observation period

Rat (strain not specified)</td>
<td>Dose level and purity not specified</td>
<td>LD_{50} = 92-266 mg/kg bw

(These studies were not available for evaluation by the DS, but it is noted that the studies in rats gave similar results as the evaluated studies.)</td>
<td>SCCS opinion on ZnPT

Year: 2014

(studies not assessed by the DS)</td>
</tr>
<tr>
<td>OECD TG 424

Acute neurotoxicity

Reliability: 1

Crl:CD®(SD)IGS BR VAF/Plus® rat</td>
<td>25, 75, 150 mg/kg bw

Purity: >95%</td>
<td>
<table>
<tr><th colspan="3">Mortalities</th></tr>
<tr><th>Dose (mg/kg bw)</th><th>Number dead/total</th><th>Day of death</th></tr>
<tr><td>25</td><td>0/20</td><td>-</td></tr>
<tr><td>75</td><td>2/20</td><td>3-4</td></tr>
<tr><td>150</td><td>4/20</td><td>2-3</td></tr>
</table>

<u>Clinical signs at 75 and 150 mg/kg bw:</u>
dehydration, urine-stained abdominal fur, soft or liquid faeces, localized alopecia on the underside and chromorhinorrhea, hunched posture, red substance on the fur of both forelimbs, excess salivation and red perioral substance in some moribund animals, reduced body temperature by 0.6-0.8 ºC, significantly decreased average hind limb grip test value at day 7 for males, reduced motor activity at 1 hour post-dosage.

Reduced body weight (7-18%) and food consumption.

No adverse necropsy findings.

4/10 females died at 150 mg/kg bw

LD_{40} for females= 150 mg/kg bw</td>
<td>ZnPT CAR Doc IIIA A6.9/01

Year: 2005</td>
</tr>
</table>

The CLH report cites an additional study in the Thor GmbH Art. 95 dossier (2014) for assessment of acute oral toxicity with ZnPT in the rat, performed according to OECD TG 423, and GLP-compliant, where the LD_{50} was found to be 300 mg/kg bw. However, this dossier was not accessible to RAC.

Table: *Summary of the animal studies on acute dermal toxicity studies with ZnPT.*

Study	Dose level	Results	Reference
US EPA 81- 2 GLP Reliability: 2 (purity of the test substance was not specified) Sprague-Dawley CD rat 5/sex 14 days post exposure period	2000 mg/kg bw Limit test	No signs of toxic effects were seen LD_{50} > 2000 mg/kg bw	ZnPT CAR Doc IIIA A6.1.2/01 Year: 1997

The CLH report cites an additional study in the Thor GmbH Art. 95 dossier (2014) for assessment of acute dermal toxicity with ZnPT in the rat, performed according to OECD TG 402 and GLP-compliant, where the LD_{50} was found to be higher than 2000 mg/kg bw. However, this dossier was not accessible to RAC.

Table: *Summary of the animal studies on acute inhalation toxicity studies with ZnPT.*

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>OECD TG 403

GLP

Nose-only

Reliability: 2

Sprague-Dawley CD albino rat

5/sex/dose

4 hours exposure

14 days post exposure period</td><td>1.82, 0.95 and 0.53 mg/L

MMADs 3.8, 3.5 and 3.3 µm

Purity of the test substance was not reported</td><td>
<table>
<tr><th colspan="3">Mortalities</th></tr>
<tr><th>Dose (mg/L)</th><th>Number dead/total</th><th>Day of death</th></tr>
<tr><td>0.53</td><td>1/10</td><td>1</td></tr>
<tr><td>0.95</td><td>5/10</td><td>1</td></tr>
<tr><td>1.82</td><td>8/10</td><td>1</td></tr>
</table>
Clinical signs: 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.</td><td>ZnPT CAR

Doc IIIA A6.1.3/01

Year: 1996</td></tr>
</table>

		Histopathological examination: 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. **LC_{50} males = 0.84 mg/L** LC_{50} females = 1.34 mg/L LC_{50} males + females = 1.03 mg/L	
Nose only US EPA 81-3, which complied with OECD TG 403 GLP Reliability: 1 Sprague-Dawley CD rat 5/sex/dose 4 hours exposure 14 days post exposure period	0.24 and 0.61 mg/L Purity: ≥95 % MMADs 1.9 and 2.3 µm	1/5 males died at 0.24 mg/L 1/5 males and 2/5 females died at 0.61 mg/L All deaths occurred at day 1 post-exposure Clinical signs: increased salivation, laboured breathing, decreased activity and tremors 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. **LC_{50} > 0.61mg/L**	ZnPT CAR Doc IIIA A6.1.3/03 Year: 1991
OECD TG 403 Nose-only Reliability: 1 Wistar Han rat 5/sex/dose 4 hours exposure 14 days post exposure period	0.05 and 0.5 mg/L Purity ≥95 % MMADs 2.7-4.4 µm	One male and 1 female dosed with 0.5 mg/L were sacrificed on day 1 due to ethical reasons. Two males and 2 females were found dead on day 2 and the remaining animals were sacrificed due to ethical reasons. No mortalities occurred at the other dose level At 0.5 mg/L, 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	Thor GmbH Art. 95 dossier Year: 2014

<table>
<tr><td></td><td></td><td>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.

LC_{50} within the range of 0.05-0.5 mg/L</td><td></td></tr>
<tr><td>Whole-body

US EPA 81-3, which complied with OECD TG 403

GLP

Reliability: 3 (according to DS it is likely that the test substance was orally ingested by preening)

Sprague-Dawley CD albino rat

5/sex/dose

4 hours exposure

14 days post exposure period</td><td>0.054, 0.14, 0.16, 0.82, 1.4 and 1.5 mg/L

Purity of the test substance was not reported

48% dispersion

MMADs 2.8-5.3 µm</td><td><table><tr><th colspan="3">Mortalities</th></tr><tr><th>Dose (mg/L)</th><th>Number dead/total</th><th>Day of death</th></tr><tr><td>0.054</td><td>1/10</td><td>During exposure</td></tr><tr><td>0.14</td><td>3/10</td><td>1</td></tr><tr><td>0.16</td><td>7/10</td><td>1-2</td></tr><tr><td>0.82</td><td>10/10</td><td>0-2</td></tr><tr><td>1.4</td><td>10/10</td><td>During exposure + days 1-3</td></tr><tr><td>1.5</td><td>10/10</td><td>1-3</td></tr></table>
Clinical signs of toxicity: 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.

LC_{50} = 0.14 mg/L</td><td>ZnPT CAR

Doc IIIA A6.1.3/02

Year: 1991</td></tr>
<tr><td>OPPTS 870.1300

Sprague-Dawley rat

5/sex/dose

4 hours of exposure</td><td>0.68, 1.19 and 2.25 mg/L

48% dispersion</td><td>LC_{50} = 5.08 mg/L</td><td>SCCS opinion on ZnPT

Year: 2014

(study not assessed by the DS)</td></tr>
</table>

*CAR refers to the Competent Authority Report under the biocide process

Comparison with criteria

According to the Guidance on the Application of the CLP Criteria (CLP guidance), classification for acute toxicity should be based on the lowest reliable LD/LC_{50}. For oral toxicity studies, the lowest LD_{50} in the study with the highest reliability was 221 mg/kg bw. According to the CLP Regulation, a substance should be classified as Acute Tox. 3 when the LD_{50} was higher than 50 mg/kg bw and lower than 300 mg/kg bw and therefore due to the LD_{50} of 221 mg/kg bw ZnPT should be classified in Category 3. The classification of the substance within this group is also supported by the SCCS opinion on ZnPT that reports lower LD_{50} for rats than for mice, ranging between 92 and 266 mg/kg bw, and by an acute neurotoxicity study reporting an LD_{50} of 150 mg/kg bw/d.

According to the CLP guidance classification for acute dermal toxicity is not warranted when the LD_{50} is higher than 2000 mg/kg bw. A limit dose of 2000 mg/kg of ZnPT caused no toxicity and therefore classification for acute dermal toxicity is not warranted.

According to the CLP guidance, classification for acute inhalation toxicity Category 2 is warranted for dusts and mists when the LC_{50} is between 0.05 and 0.5 mg/L; while an LC_{50} between 0.5 and 1.0 mg/L warrants classification in Category 3. The data base provides one study with an LC_{50} of 0.84 mg/L pointing towards Category 3; however, the purity of the ZnPT batch used in this study was not provided, which reduces the impact of this study. A second study yielded an LC_{50} higher than 0.61 mg/L, which would also indicate classification as Category 3. The third study showed, using only two doses, an LC_{50} between 0.5 and 0.05 mg/L; while in the fourth study, the purity of the substance was not provided and estimated, using 4 different doses, an LC_{50} of 0.14 mg/L. The last two studies provided reliable LC_{50} data and were considered for RAC for setting classification.

In conclusion, **RAC supports the DS's proposal for classification of ZnPT as Acute Tox. 2; H330 (Fatal if inhaled) and Acute Tox. 3; H301 (Toxic if swallowed).**

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

Summary of the Dossier Submitter's proposal

The DS proposed not to classify ZnPT for STOT SE since the available studies did not indicate a specific target organ toxicity at dose levels not causing general toxicity and mortality, and classification in Category 1 or 2 is therefore not considered warranted. In addition, according to the DS, the acute inhalation toxicity studies reports symptoms including laboured breathing, gasping, increased salivation, rales and noisy respiration. However, the DS considered these symptoms as indicative of respiratory tract irritation and noted that they were observed at lethal doses. Moreover, in repeated dose inhalation studies, local irritation was also observed but it was neither transient nor reversible and therefore the classification criteria for respiratory tract irritation (Category 3) were not considered to be fulfilled. Finally, 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 3 is not considered warranted.

Comments received during public consultation

No comments for this hazard were received during the public consultation.

Assessment and comparison with the classification criteria

RAC notes that:

- The clinical signs of toxicity observed in the acute oral toxicity studies were noted at or slightly below doses where mortality occurred and appear to be non-specific toxic effects. Ataxia and lethargy could be signs of a narcotic effect but could also have been caused by the general distress of the animals.
- In the acute neurotoxicity study, the deaths were not considered test substance-related. Clinical signs of toxicity did not indicate any clear neurotoxic effect (see above) and were observed within the dose range also causing general toxicity and mortalities. The effects are considered to be covered by the classification for acute toxicity.
- 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 and 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 STOT RE section).

In conclusion, to avoid classifying for effects already covered by acute toxicity, mortality cannot be considered as a critical effect for classification as STOT SE, and moreover no target organ could be identified after single administration of ZnPT. Therefore, the criteria for classification as STOT SE 1 or 2 are not met. Classification as STOT SE 3 are not warranted either since the narcotic and respiratory tract irritant effects can not be considered reversible and/or independent of general toxicity and lethality. Therefore, **RAC supports the DS's proposal not to classify ZnPT for STOT SE.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS proposed not to classify ZnPT for skin corrosion/irritation on the basis of an OECD TG 404 study conducted under GLP, which showed that 0.5 g of the substance did not induce erythema and oedema in the skin of rabbits.

Comments received during public consultation

One MSCA supported the proposal of no classification but requested consideration of the human data included in the SCCS/1512/13 report. The DS provided this information in the RCOM and it is displayed in the section 'Additional key elements' in the Background document (BD).

A downstream user highlighted that according to SCCS, ZnPT can be used for hair/ skin. RAC notes that the use of the substance is not relevant for classification purposes.

Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation study.

Table: *Summary of the animal study on skin corrosion/irritation with ZnPT.*

Study	Dose level	Results	Reference
OECD TG 404 GLP Reliability: 1 New Zealand Albino rabbit 3 females 4 h exposure	0.5 g (dry weight) Purity >95 %	Observations made at 1, 24, 48 and 72 hours. Erythema: 0 Oedema: 0	ZnPT CAR Doc IIIA A6.1.4/01 Year: 2001

No erythema or oedema were observed in a well-conducted GLP-compliant study according to OECD TG 404. The human data (only two positive case report together with a negative study with 8 individuals) were not robust enough to support classification. Therefore, the criteria for classification were not met and **RAC supports the DS proposal not to classify ZnPT as a skin irritant.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed classification of ZnPT for Eye Damage Category 1 (H318: Causes serious eye damage) on the basis of a GLP study performed following OECD TG 405 where there were severe corneal lesions and redness.

Comments received during public consultation

Three different MSCA supported the DS's proposal.

Assessment and comparison with the classification criteria

The table below summarises the available animal eye corrosion/irritation study.

Table: *Summary of the animal study on eye corrosion/irritation with ZnPT.*

Study	Dose level	Results	Reference
OECD TG 05 GLP Reliability: 1 New Zealand White rabbits 1 female 24 hours exposure Examinations: 1 and 2 h.	84 mg Purity > 95%	Corneal opacity at 24 h: 4 Iritis at 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

The CLH report cites an additional study in the Thor GmbH Art. 95 dossier (2014) for assessment of acute eye irritation/corrosion in the rabbit, performed according to OECD TG 405 and with GLP compliance, where ZnPT caused irreversible eye damage. However, this study was not accessible to RAC.

According to the CLP Regulation, classification for serious eye damage in Category 1 is warranted when the corneal opacity score was higher than 3. ZnPT induced corneal opacity with score of 4, and made the examination of iris impossible due to the severity of chemosis and corneal impairments. This study was also supported by the studies provided in the SCCS report (2014; see 'Additional key elements' section in the Background document). Therefore, **RAC agrees with the DS that classification of ZnPT for Eye Damage 1; H318, is warranted.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed not to classify ZnPT for skin sensitisation on the basis of a Guinea pig maximisation test in which only 10% of animals responded to the challenge during the first 24 hours. This response was reduced to 0 after 48 hours.

Comments received during public consultation

One MSCA supported the proposal not to classify.

One individual highlighted that the maintenance of ZnPT for its use as biocidal PT6 and PT7 is crucial for avoiding biologically contaminated paints on the market. RAC notes that this issue is not relevant for classification purposes.

One MSCA commented that the SCCS report (2014) contains human data on cosmetic formulations for at least the pyrithione moiety, confirming low potential of the substance to induce skin sensitisation in humans. The DS replied and agreed that the human data on pages 17 to 22 in the SCCS report can be included. RAC also notes this information as relevant and provides it in the section 'Additional key elements' in the Background document.

Assessment and comparison with the classification criteria

The table below summarises the available skin sensitisation study with animals.

Table: *Summary of the animal study on skin sensitisation with ZnPT.*

Study	Dose level	Results	Reference
OECD TG 406 Maximization test modified according to Maurer and Hess GLP Reliability: 1 Dunkin Hartley Guinea pig 20 females in test group 10 females in control group 24 h exposure	Epicutaneous induction of 25% (w/w) in white petrolatum Challenge: 10% (w/w) in white petrolatum	24 h: 2/20 48 h: 0/20 **Negative**	ZnPT CAR Doc IIIA A6.1.5/01 Year: 2002

The sensitisation potential of ZnPT was investigated in a maximisation study in accordance with GLP and OECD TG 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.

RAC notes that the human data provided in the SCCS report (SCCS/1512/13) shows that the potential for skin sensitisation of ZnPT (if any) is very weak. Moreover, according to the CLP Regulation, an incidence higher than 30% in a Guinea pig maximisation test is considered a positive response triggering classification. Since the incidence observed in the Guinea pig study was 10%, ZnPT did not fulfil the classification criteria under the conditions of the study. Therefore, **RAC supports the DS's proposal not to classify ZnPT for skin sensitisation.**

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

Summary of the Dossier Submitter's proposal

The DS proposed classification of ZnPT for STOT RE 1; H372 (Causes damage to organs through prolonged or repeated exposure) on the basis of the following results:

- Haemolytic anaemia in monkeys at 22 mg/kg bw/d after oral exposure for 28 days;
- Neurotoxicity in rats at 2.5 to 10 mg/kg bw/d and mortalities at 5 and 10 mg/kg bw/d after oral exposure for 90 days;
- Neurotoxicity in rats at 60 mg/kg bw/d 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.

The DS also 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.

Comments received during public consultation

Comments from MSCAs

Three MSCAs supported the DS's proposal for classification for STOT RE Category 1. A fourth MSCA also supported the proposal but proposed a modification of the hazard statement, stating explicitly the damage on the nervous system instead of the more general proposal of the DS *"Causes damage to organs through prolonged or repeated exposure"*. The DS answered that the

rationale behind their proposal not to state neurotoxicity in the hazard statement is that mortalities were also observed in rats via oral and inhalation exposure and that haemolytic anaemia was observed in monkeys via oral exposure.

Comment from ZnPT Industry CLH Consortium *(comment number 75 in RCOM)*

The ZnPT Industry CLH Consortium submitted a long and detailed comment disagreeing with the proposed classification, and instead proposed classification as STOT RE 1 with the following hazard statement: *"Causes damage through prolonged or repeated exposure via the inhalation route"*. The rationale behind their proposal is summarised in the following paragraphs:

The finding of hind limb weakness has only been observed in rats (fully and completely reversible) and occasionally in rabbits, but has never been observed in mice. Moreover, no observation of skeletal muscle atrophy (hind limb weakness) has been observed in non-human primates; which shows NOAEL over 20 times greater than the NOAEL for rodents. The Consortium commented that this brings *into question the likelihood that pyrithione exposure produces neurotoxicity in humans*". They also considered that, for CLH purposes, primates are the more appropriate surrogate for humans and welcomed the opportunity to discuss this issue with RAC. Finally, they stated that in the available primate studies neurotoxic effects were not observed at the highest dose tested (22 mg/kg bw/d), and therefore they proposed that classification for STOT RE specifying the nervous system as the target organ is not warranted.

Regarding the haemolytic anaemia, the Consortium stated that the concurrent control values were higher than normal published data for the monkey species *Macaca fascicularis*, while the values seen in the 22 mg/kg bw/d group were within the published historical control data (HCD) range except for haemoglobin (Hb), which were lower. In the 28-d study in monkey, when the mean data, after 4 weeks' exposure, for both female and male groups separately, and female/male high-dose groups combined are compared to the equivalent control group data the following reduction in Hb levels were seen: Female (-22.1%), Male (-21.8%), Female/Male combined (-21.9%). However, when the comparison of the high-dose group data (week 4 of exposure) is made with the same animals at the start of the study (pre-dose), the reduction in Hb levels are calculated as: Female (-20.0%), Male (-15.4%), Female/Male combined (-17.7%). The Hb data for all animals in control and 22 mg/kg bw/d groups is provided in the 'Additional key elements' section in the Background document. Attending to these considerations the Consortium considers that classification as STOT RE on the basis of haemolytic anaemia should be reconsidered.

To further support their view to reconsider the proposed STOT RE classification, the Consortium had also evaluated two further studies on monkeys where haematological criteria were measured (one 90-d study with ZnPT and one 1-year study with NaPT). They concluded that the 90-d study should not be considered for classification purposes due to low reliability, as e.g. the purity of the test substance was not given, only a few animals were used, blood parameters were affected in control animals, animals from the lowest dose group were not necropsied, there was no significant difference between treated and control animals, and values in all dose groups were higher than published HCD values at the onset of the study. The Consortium considered that the study with NaPT meets the quality criteria and together with above stated considerations about the 28-days study in monkeys, would qualify as a key studies to support no classification for STOT RE on the basis of haemolytic anaemia.

The Consortium also proposed the use of an allometric factor to account for the differences in toxicity between rat and primate since the thresholds for classification for STOT RE are based on data from rat, according to the Consortium.

The Consortium considered that taken together, the studies discussed above do not support classification of ZnPT for STOT RE for haemolytic anaemia.

As was stated above, the Consortium proposed the use of primate as surrogate for human. No mortalities have been observed in primates treated with pyrithiones via the oral route at much higher doses than in rats, and therefore mortality is not considered to be an appropriate endpoint for the determination of a STOT RE by this route of administration.

No deaths have been observed in the repeated dermal studies with ZnPT in the rat and thus there is no direct evidence to indicate that classification via the dermal route for lethality would be warranted.

The Consortium agreed with the DS that mortalities in rats via the inhalation route warrant classification since this route has not been investigated in primates.

DS reply to the comments by the Consortium

For classification, generally the studies that would lead to the most severe classification are used in a weight of evidence evaluation. With ZnPT, neurotoxicity and mortalities observed in rats would lead to the most severe classification.

According to the CLP guidance (3.9.2.5.2; version 5.0) reduction in Hb levels greater than or equal to 20% is given as an example of haematological effects warranting classification as STOT RE. In the 28-d oral study in monkeys, even after comparing the Hb levels in the same animals at the start of the study (pre-dose) and end of treatment, the Hb reduction in females was 20%.

To the DS's knowledge, RAC doesn't apply allometric scaling for classification and labelling purposes.

Comment from a company manufacturer *(comment number 76 in RCOM)*

This company disagreed with the DS's proposal for classification of ZnPT for STOT RE 1 because no consistent specific target organ toxicity was observed in different species after oral application (rat, monkey) and because the primary effect is organ-independent and according to the CLP regulation: *"One shall carefully evaluate the data and, where possible, not include secondary effects"*.

According to this company, the proposed mode of action of pyrithione compounds is Krebs cycle arrest via aconitase inhibition. A consequence of this inhibition is an impaired energy production on cellular level, which has numerous downstream effects at physiological processes of an organism. Food conversion, which correlates food consumption to the body weight gain during a defined time period, is one of these downstream effects. Figures provided in the 'Additional key elements' section in the Background document show the effects of high-dose ZnPT and NaPT treatment, respectively, on food consumption (FC) and body weight gain (BWG) over the 90 days of the sub-chronic toxicity study.

When compared to the corresponding controls, the FC of both, male and female rats, did not change during the treatment with ZnPT (2.5 mg/kg bw/d). However, at the same time there was a decrease in BWG in males (-16%) and females (-36%) during the last two weeks of ZnPT treatment. For NaPT (5 mg/kg bw/d), the FC in male and female rats was again constant over the complete treatment duration. Notably, while the BWG of male rats did not differ over time, female rats showed a BWG decrement of 74% compared to the corresponding control group during the last two weeks of NaPT treatment.

The commenting company also stated that the inhibition of energy production is further supported by clinical observations of hind limb weakness and by microscopic findings of muscle atrophy and axonal degeneration in hind limb skeletal muscle, two organs with strongly energy depending physiological functions.

According to this company, taken together all the above observations support the mode of action of pyrithiones and consequently, effects on energy depending physiological functions and subsequent organ atrophy have to be considered secondary to the inhibition of the mitochondrial target enzyme aconitase (oxidative phosphorylation) and non-specific in nature and therefore STOT RE is not warranted.

DS reply to the above comment

According to the DS the inhibition of oxidative phosphorylation in the strongly energy depending organs provides information on the mode of action of adverse effects seen in these organs, confirming specific target organ toxicity.

Assessment and comparison with the classification criteria

The table below summarises the available oral repeated dose toxicity studies in animals.

Table: *Summary table for repeated dose toxicity studies in animals with ZnPT.*

Method	Results	Reference
40 CFR 798.2650 GLP Reliability: 2 (purity was not reported) ZnPT (not specified batch, not specified purity) Sprague-Dawley Crl:CD® BR rats 10/sex/dose Oral 0.2; 1; and 5 (2.5) mg/kg bw/d The highest dose level was reduced to 2.5 mg/kg bw/d from days 17 -18 onwards 90 days	<u>0.2 mg/kg bw/d:</u> No adverse effects <u>1 mg/kg bw/d:</u> ↑ clinical signs: increased salivation, isolated incidents of red/brown staining around the mouth ↓ plasma urea (females) ↑ inflammatory cell infiltrates in the forestomach (1 male) <u>2.5 mg/kg bw/d:</u> ↑ clinical signs: hunched posture, noisy respiration, pallor of extremities ↓ plasma urea (females) ↓ creatinine (females) <u>5 mg/kg bw/d:</u> 3 females killed on days 16-19 ↓ movement in hind limbs (6 females) ↑ clinical signs: increased salivation, noisy respiration, hunched posture, piloerection, dehydration, emaciation, tiptoe/high stepping gait, loss of righting reflex, lethargy, vocalisation ↓ body weight (females -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 (purity was not reported, there were deviations from OECD TG 408; e.g. histopathology of peripheral nerves were not performed, and and limitations in reporting)	<u>5 ppm:</u> No adverse effects <u>25 ppm:</u> ↓ bw (-10% in females) <u>125 ppm:</u> 33/39 dead animals ↓ movement of the hindlimbs progressing to complete paralysis ↓ bw (females: -69%, males: -85%) and food consumption ↑ tissue changes associated with marked growth suppression and cachexia	ZnPT CAR Doc IIIA A6.4.1/01 Year: 1973

ZnPT (batch specified, not specified purity) Charles River CD Albino rats 20/sex/dose Oral, in diet 5, 25 and 125 ppm (equivalent to 0.35, 1.75 and 10.04 mg/kg bw/d in males and to 0.39, 2.13 and 10.26 mg/kg bw/d in females) 94 days		
No guideline No GLP Reliability: 3 (deviations from OECD TG 452: 10 animals/sex instead of 20; no urinalysis; no clinical chemistry; no formal observations for clinical signs; body weight measured at 7 time points only; food consumption not measured; haematology was done at 11 and 24 months instead of every six months) ZnPT (not specified batch, not specified purity) 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 corresponded to approximately 4 mg/kg bw/d for females 2 years	<u>2, 5 and 10 ppm:</u> No adverse effects <u>25 ppm (~2 mg/kg bw/d):</u> ↑ mortality (females) ↑ hind limb paralysis (females) ↓ body weight gain (females) <u>50 ppm:</u> 10 females + 6 males dead between week 20 and 80 ↑ hind limb paralysis (males, females) ↓ body weight gain	ZnPT CAR Doc IIIA 6.5/03 Year: 1958
EC Guideline B.7 GLP	<u>5.5 and 11 mg/kg bw/d:</u> No effects <u>22 mg/kg bw/d:</u>	ZnPT CAR Doc IIIA A6.3.1/01

Reliability: 2 (as neurotoxicity not investigated) Purity: >95% Cynomolgus monkeys: 4 (low and mid dose) 6 (control and high-dose) 4 sacrificed after 28 days 2 observed during a 14-d recovery period Oral (gelatine capsule) 0, 5.5, 11 and 22 mg/kg bw/d 28 days Daily	↑ mortality in one animal (this animal vomited before dosing and may have been unhealthy at the onset of the study) ↑ clinical signs: vomiting, diarrhoea, decreased activity ↑ effects on haematology (e.g. ↓Hb 22%) ↓ food intake ↑ adrenal weight (47 %) (females)	Year: 1992
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) ZnPT (batch: specified, purity: not specified) Rhesus macaca mulatta monkeys 3/sex/dose (except low-dose: 4 males + 2 females) Oral (gavage) 0, 0.5, 2.0 and 8.0 mg/kg bw/d 93 - 94 days	<u>2.0 mg/kg bw/d + 8 mg/kg bw/d:</u> No effects on blood parameters were seen in this study as compared to controls; however reduced values for red blood cells (RBC), Hb and haematocrit (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 HCDs while the values at the end of the study were within the HCD values. <u>2.0 mg/kg bw/d:</u> ↑ vomiting day 1 ↓ relative uterus weight (-23%) <u>8.0 mg/kg bw/d:</u> ↑ 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 ZnPT (specified batch but not purity) 52.2% aqueous suspension	<u>20 and 100 mg/kg bw/d:</u> No adverse effects <u>1000 mg/kg bw/d:</u> ↓ body weight gain (females: -17%) ↓ food consumption (females: -23%)	ZnPT CAR Doc IIIA A6.4.2/01 Year: 1973

Sprague-Dawley rats 15/sex/dose Dermal 0, 20, 100 and 1000 mg/kg bw/d 90 days		
OPPTS 870.3700 GLP Reliability: 1 ZnPT (specified batch, purity >95%) Crl:CD (SD)IGS BR VAF/Plus rats 25 females/group 0, 10, 15, 30, 60 mg/kg bw/d 6 h/d on gestation days (GD) 0-21 Dermal	<u>30 mg/kg bw/d:</u> ↓ adjusted body weight (-12%, p<0.01) Limited use of hind limbs (2/24 animals) Shuffling gait (1/24 animals) <u>60 mg/kg bw/d:</u> ↓ 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) ↑ number of rats with clinical observations (p<0.01 in all cases) Limited use of hind limbs (24/25 animals) Shuffling gait (22/25 animals) No use of hind limbs (1/25 animals) Decreased muscle tone (21/25 animals, p<0.01) Loss in muscle mass (12/25, p<0.01). Clinical signs: emaciation, dehydration, ungroomed coat, urine-stained abdominal fur, low carriage, hunched posture, chromodacryorrhea and chromorhinorrhea.	ZnPT CAR Doc IIIA A6.8.1/03 Year: 2005
US EPA guideline OPPTS No. 870.3465 GLP Reliability: 2 ZnPT (specified batch, purity: >95%) Sprague-Dawley rats 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/d, 5 d/week	<u>0.002 mg/L:</u> No adverse effects <u>0.006 mg/L:</u> ↑ clinical signs: slight swelling around eyes, respiratory gurgles, gasping ↑ histopathological effects in lungs and larynx <u>0.0135 mg/L:</u> 2 dead animals (days 3 and 20) ↑ clinical signs: slight swelling around eyes, respiratory gurgles, gasping, decreased activity, hypothermia, tip-toe gait ↓ bw (males 10%)	ZnPT CAR Doc IIIA A6.3.3/01 Year: 2005
Comparable to US EPA guideline OPPTS No. 870.3465 GLP Reliability: 2 (as haematology, urinalysis, clinical chemistry and	<u>0.0005 mg/L:</u> ↑ Bronchoalveolar lavage fluid (BALF) parameters (↑ eosinophils, neutrophils, lymphocytes, lactate dehydrogenase (LDH), total protein, cell lysis) ↑ lung weight, microscopic findings in the lung (bronchointerstitial pneumonitis, smooth muscle hypertrophy) <u>0.0015 mg/L:</u>	ZnPT CAR Doc IIIA A6.3.3/02 Year: 2009

ophthalmology were not investigated) ZnPT (specified batch, purity >95%) Sprague-Dawley rats 15/sex/dose Inhalation (nose only) 0, 0.0005, 0.0015, and 0.005 mg/L 6 h/d, 5 d/week 28 days (interim sacrifice at 5, 10, 28 days)	↑ BALF parameters (↑ eosinophils, neutrophils, lymphocytes, LDH, total protein, cell lysis) ↑ lung weight, microscopic findings in the lung (bronchointerstitial pneumonitis, smooth muscle hypertrophy) ↑ lymphoid hyperplasia <u>0.005 mg/L:</u> 1 female mortality (day 15) ↓ bw (-15%) and food consumption ↑ hindlimb impairment (1 female) ↑ skeletal muscle degeneration (3 female) ↓ thymus weight (-40%) ↑ BALF parameters (↑ eosinophils, neutrophils, lymphocytes, LDH, total protein, cell lysis) ↑ lung weight, microscopic findings in the lung (bronchointerstitial pneumonitis, smooth muscle hypertrophy) ↑ lymphoid hyperplasia	
US EPA guideline 82-4 (subdivision F) GLP Reliability: 2 (the purity of the test substance was not stated) 52.2% ZnPT suspension (purity of active ingredient prior to suspension in water not specified) Dawley albino (Charles River CD) rats 0, 0.0005, 0.0025, 0.010 mg/L 15/sex/dose Inhalation (whole body) 90 days	<u>0.0005 mg/L:</u> No adverse effects <u>0.0025 mg/L:</u> 1 male + 1 female mortalities (weeks 3 and 13) ↑ clinical signs: laboured breathing, rales, increased salivation, decreased activity, dry red-brown material around the nose, hair loss ↑ inflammation of the lungs ↑ lung weight <u>0.010 mg/L:</u> 3 male + 4 female mortalities (weeks 4-12) ↑ clinical signs: laboured breathing, rales, increased salivation, decreased activity, and dry red-brown material around the nose ↓ bw and food consumption (females) ↑ lung weight	ZnPT CAR Doc IIIA A6.4.3/01 Year: 1993

The table below summarises all adverse effects reported at dose levels below the guidance values in the CLP Regulation for classification for STOT RE.

Table: *Summary of adverse effects reported within the guidance values in the repeated dose toxicity studies with ZnPT potentially relevant for classification as STOT RE*

Study	Effect	Dose	STOT RE classification
90-d in rat (oral)	Clinical signs 30% mortality in females + clinical signs + ↓ hind limb movements + reduced body weight	1, 2.5 mg/kg bw/d 5 mg/kg bw/d	Cat 1: ⩽ 10 mg/kg bw/d Cat 2: ⩽ 100 mg/kg bw/d
90-d in rat (oral)	85% mortality (combined males + females) + ↓ 69-85% body weight + reduced body weight	10 mg/kg bw/d	Cat 1: ≤ 10 mg/kg bw/d

			Cat 2: ≤ 100 mg/kg bw/d
2-year in rat (oral)	Mortality (unspecified incidence) + hind limb paralysis (females) 100% mortality in females and 60% males + hind limb paralysis (males + females)	≈ 2 mg/kg bw/d ≈ 4 mg/kg bw/d	Cat 1: ≤ 1.25 mg/kg bw/d Cat 2: ≤ 12.5 mg/kg bw/d
90-d in rat (dermal)	Reduced body weight	100 mg/kg bw/d	Cat 1: ≤ 20 mg/kg bw/d Cat 2: ≤ 200 mg/kg bw/d
21-d in rat (dermal) (developmental)	Limited or no use of hind limbs + clinical signs + reduced body weight	30-60 mg/kg bw/d	Cat 1: ≤ 80 mg/kd/d Cat 2: ≤ 800 mg/kd/d
28-d in monkeys (oral)	Haematological effects + clinical signs	22 mg/kg bw/d	Cat 1: ≤ 30 mg/kg bw/d Cat 2: ≤ 300 mg/kg bw/d
21-d in rats (inhalation)	10% mortality + clinical signs	0.0135 mg/l	Cat 1 ≤ 0.08 mg/L Cat 2 ≤ 0.8 mg/l
28-d in rats (inhalation)	Alterations in composition of broncho alveolar fluid 7% mortality in females + hind limb paralysis	0.0005, 0.0015, 0.05 mg/L 0.005 mg/L	Cat 1: ≤ 0.06 mg/L Cat 2: ≤ 0.6 mg/L
90-d in rats (inhalation)	13% mortality (combined males + females) + clinical signs+ lung alterations 23% mortality (combined males + females) + clinical signs + lung alterations	0.0025 mg/l 0.010 mg/l	Cat 1: ≤ 0.02 mg/L Cat 2: ≤ 0.2 mg/L

Decrease in body weight

A decrease in body weight has been consistently reported in oral (90-d) and dermal (90- and 21-d) studies in rat. These bodyweight reductions were reported concurrently with clinical signs. The Industry proposed a mechanism of action for ZnPT based on Krebs cycle arrest, making it impossible to transform food into energy, causing a reduction in body weight (see the figures above in the section Additional key elements). RAC noted that the most severe reduction was reported in a study with low reliability (reliability score=3); while in other cases the reduction was either not clearly stated in the CLH report or was moderate (around 20% reduction). Based on these data RAC does not consider the decreases in body weight as relevant for classification purposes.

Clinical signs

A wide array of clinical signs was reported in oral, dermal and inhalation studies. The main clinical effects after oral exposure were increased salivation, noisy respiration, hunched posture, piloerection, dehydration, emaciation, tiptoe/high stepping gait, loss of righting reflex, lethargy, and vocalisation. The main clinical effects reported after inhalation exposure were laboured breathing, rales, increased salivation, decreased activity, and dry red-brown material around the nose slight swelling around eyes, respiratory gurgles, gasping, decreased activity, hypothermia, and tip-toe gait. The main clinical signs after dermal exposure were emaciation, dehydration, ungroomed coat, urine-stained abdominal fur, low carriage, hunched posture, chromodacryorrhea and chromorhinorrhea. All these signs appeared at concentrations below the guidance values in the CLP Regulation. However, RAC notes that all these effects are unspecific and does not allow identification of a target organ, and moreover are potentially consistent with

the mechanism of action proposed by the Industry based in a reduction of ATP concentration in cells that might affect all tissues. Therefore, RAC does not consider the clinical signs as relevant for classification purposes.

Alteration in broncho alveolar fluid and other effects on lungs

The inhalation studies reported alterations in the composition of broncho alveolar fluid and other effects on lungs, such as lung weight, microscopic findings (bronchointerstitial pneumonitis, smooth muscle hypertrophy), increases of weight and inflammation. These effects might be consequences of irritation at the point of contact of the substance (as occurs with the gastrointestinal tract irritation reported in some oral studies) and the influence on pulmonary function is unclear with the available information. Therefore, RAC does not consider these effects as relevant for classification purposes.

Haematological effects

Haematological changes were reported in the 28-d oral toxicity study in monkeys. The effects on blood parameters consisted of reductions in Hb (-22%), RBC (-29%) and Hct (-16%) accompanied by an increase in mean corpuscular volume (MCV) (18%) at 22.0 mg/kg bw/d compared to control values. The control values were higher than HCD data for *Macaca fascicularis*, while the values reported in the 22.0 mg/kg bw/d dose group were within the HCD range except for Hb which were lower. The changes were statistically significant in the highest dose group (see table below).

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

Week	Control		5.5 mg/kg bw/d		11 mg/kg bw/d		22 mg/kg bw/d	
	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	6.5
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	
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 2 weeks recovery.; [2]HCD values from Fortman *et al.*, 2002
* Statistically significant (p<0.05); ** Statistically significant (p<0.01)

The ZnPT Industry CLH Consortium provided comments against considering these haematological effects as relevant for classification purposes. The Industry specifically considered that the reduction in Hb should be estimated using the values recorded for the same group of animals before starting the dosing as controls. RAC noted that in this case, indeed, the reduction in Hb content was lower (tables in section Additional key elements) than estimated in the table above. Another argument put forward by the Industry was the fact that a 90-day study in monkeys did not provide support for classification on the basis of haematological alterations. Finally, the Industry also proposed the use of an allometric factor for considering the inter-species differences between rat and monkeys, since the guidance values for classification are set for effects in rats.

RAC noted regarding these comments by the ZnPT Industry CLH Consortium that:

- The classification is usually based on the most severe effects. In the case of Hb concentration, this is for females monkeys exposed to 22 mg ZnPT/kg bw/d where a reduction of either 22.1 or 20% was reported. However, the CLP guidance specifically states a reduction of Hb concentration of 20%, as relevant for classification purpose, which is the most severe effect reported.
- At this dose of 22 mg/kg bw/d, also a severe reduction (29%) in RBC was reported in females. This reduction is also relevant for classification purposes.
- Even when considering the values proposed by the Industry arguing that classification is not warranted, RAC notes that it might be a borderline case. 22 mg/kg bw/d is lower than the guidance valus of 30 mg/kg bw/d and a reduction in Hb was seen at 22 mg/kg bw/d.
- RAC agrees that the 90-d study in monkeys does not provide strong support for classification on the basis of haematological impairments. However, RAC notes that in this study the haematological assessment was not reliable since the values reported in controls were below the HCD. Moreover, in this study the dose was lower than the dose inducing haematological alterations in the 28-d study.
- The use of allometric factors is not considered for classification purposes since it is focused on hazard identification and not risk assessment.

In conclusion, RAC considers the haematological effects as relevant for classification purposes.

Neurotoxicity

Neurotoxicity was consistently reported in rat studies using oral, dermal and inhalation routes. The Industry provided comments arguing that nervous system is not a specific target of repeated toxicity for ZnPT. Their opinion was based on the following facts:

- The reversibility of the effects in rat;
- Monkeys were considered a more appropriate model for assessing the effects of ZnPT in humans;
- Neurotoxicity was reported only in rats and not in monkeys;
- The neurotoxicity is a secondary effect of the mechanism of action consisting in a ATP reduction as consequence of the Krebs cycle arrest induced by ZnPT.

However, RAC notes that no support was provided for the statement that monkeys are better models than rodents for assessment of ZnPT, especially taking into consideration that neurotxicity was not assessed in some of the studies in monkeys. RAC also notes that Annex I: 3.9.1.1 of the CLP guidance specifically states that for STOT RE classification *"All significant health effects that can impair function, both reversible and irreversible"* should be considered. In conclusion, RAC considers the neurotoxicity effect as relevant for classification purposes.

Mortalities

Mortalities were observed in oral and inhalation studies in rat. The mortalities appeared in the oral studies at doses between 4 and 10 mg/kg bw/d and between day 16 (in the 90-da study) and week 20 (in the 2-year study). RAC notes that the oral LD_{50} in rodents was 221 mg/kg bw; which is a dose much higher than the lethal doses after repeated exposure. Therefore, taking into consideration this oral LD_{50} and that lethality always appeared after a high number of repeated doses, RAC does not consider that the lethality after oral exposure can be attributed to acute toxicity and considers it relevant for classification purposes.

The mortalities appeared in the inhalation studies at doses between 0.0025 and 0.0135 mg/L and between day 3 (in the 21-d study) and week 3 (in the 90-d study). RAC notes that the inhalation LD_{50} in rodents was 0.05 mg/L; and that in the 90-d study some mortalities were seen

at doses 20 times lower than the LD_{50} and after 3 weeks of repeated exposures. Therefore, taking into consideration this inhalation LD_{50} and that the mortality always appeared after a high number of repeated doses, RAC does not consider that the mortality after inhalation can be attributed to acute toxicity and considers it relevant for classification purposes.

Comparison with criteria

In summary, the effects warranting classification are:

- Haematological alterations, borderline between Category 1 and 2 after oral exposure in monkeys;
- Neurotoxicity warranting Category 1 in the oral 90-d and in the inhalation 28-d studies in rat;
- Neurotoxicity warranting Category 2 in the oral 2-year study in rat and in the dermal developmental study (also in rats);
- Mortalities warranting Category 1 in the oral 90-d study in rats and in the 21-d, 28-d and 90-d inhalation studies in rats;
- Mortalities borderline between Category 1 and 2 (but with very high incidence of mortality) in a second oral 90-d study in rats;
- Mortalities warranting Category 2 in the 2-year oral toxicity study in rats.

Therefore, using a weight of evidence approach, **RAC supports the DS's proposal for classification of ZnPT as STOT RE 1; H372.**

Regarding the route of exposure **RAC also supports the DS's proposal for no specification of the route of exposure** since mortalities were seen by two different routes (oral and inhalation) and neurotoxicity were seen in two routes (oral and dermal).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS included the following genotoxicity tests in the CLH report:

- One negative *in vitro* gene mutation in *S. typhimurium* (reliability 1);
- One positive *in vitro* chromosomal aberration assay in mammalian cells (V79 Chinese hamster lung fibroblasts) (reliability 1);
- One *in vitro* gene mutation in mammalian cells (V79 Chinese hamster lung fibroblasts) with equivocal results (reliability 1);
- One positive *in vitro* gene mutation in mammalian cells (L5178Y mouse lymphoma) (reliability 1);
- One positive *in vitro* Comet assay with human epithelial keratinocytes (no assigned reliability because it is a study published in open scientific literature);
- One negative *in vivo* Crl:NMRI BR mice erythrocyte micronucleus test (reliability 2);
- One negative *in vivo* Sprague-Dawley mice erythrocyte micronucleus test (no assigned reliability);
- One negative *in vivo* chromosome aberration test with Cynomolgus monkeys (reliability 3);

- One negative *in vivo* Comet assay with Wistar Han rats (reliability 1).

With this database, and taking into consideration the absence of positive results *in vivo* to confirm the negative results found *in vitro*, the DS proposed not to classify ZnPT for germ cell mutagenicity.

Comments received during public consultation

One MSCA supported the DS's proposal not to classify ZnPT for germ cell mutagenicity.

Assessment and comparison with the classification criteria

The table below summarises the results of the available mutagenicity and genotoxicity tests.

Table: *Summary table of relevant in vitro mutagenicity studies with ZnPT.*

Method	Test system	Tested concentrations	Results	Remarks	Reference
In vitro gene mutation in bacteria OECD TG 471 GLP Reliability: 1	*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100, TA 102	Assay 1: strains TA 1535, TA 1537, TA 98, TA 100: 0; 6.25; 12.5; 25.0; 50.0; 100 µg/plate Assay 1: strain TA 102: 0; 3.13; 6.25; 12.5; 25.0; 50 µg/plate Assay 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 Assay 2: strain TA 102: 0; 1.56; 3.13; 6.25; 12.5; 25.0; 35.0 µg/plate Purity: >95 %	**+ 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
In vitro chromosomal aberration assay in mammalian cells OECD TG 473 GLP	Chinese hamster lung fibroblasts (V79 cell line)	Assay 1 (+/- S9): 0, 0.0488, 0.0977, 0.195, 0.395, 0.781, 1.56, 3.13 and 6.25 µg/mL Assay 2 (-S9): 0, 0.12, 0.023, 0.047, 0.094, 0.188, 0.375,	**+ S9: Positive** **- S9: Positive**	The incidences in aberrations exceeded the historical values for background controls of the laboratory. The positive results were	ZnPT CAR Doc IIIA A6.6.2/01 Year: 2002

Reliability: 1		0.75, 1.5 and 3.0 µg/mL Assay 2 (+S9): 0, 0.047, 0.094, 0.188, 0.375, 0.75, 1.5, 3.0, 6.0 and 12.0 µg/mL Purity: >95 %		found at doses causing reductions in cell viability of 43, 56 and 62%.	
In vitro gene mutation in mammalian cells OECD TG 476 GLP Reliability: 1	Chinese hamster V79 cells	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 Assay 2 +S9: 1.23, 1.85, 2.78, 4.17 and 6.25 µg/mL Purity: >95 %	**Equivocal**	Cytotoxicity + S9: 6.25 µg/mL: 22% relative survival Cytotoxicity - 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 TG 476 GLP Reliability: 1	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 Concentrations tested +S9: 0, 0.4, 0.6, , 1, 1.5, 2, 2.5, 3 and 3.5 µg/mL Purity >95%	**+ S9: Positive** **- S9: Positive**	Cytotoxicity -S9: 0.5 µg/mL: 12% relative survival Cytotoxicity+S9: 3.5 µg/mL: 11% relative survival	Thor GmbH Art 95 dossier Year: 2014
In vitro Comet assay	Human epithelial keratinocytes	Doses levels/sampling times:	**Positive**	Cytotoxicity: 100 nM: 91% (24h)	Lamore SD, Cabello CM, Wondrak GT

Published study No guideline		100 nM: 1, 3, 12 h 500 nM: 1, 3, 12 h		500 nM: 92% (1h); 90% (6h); 75% (12 h)	(2010). The topical antimicrobial ZnPT 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.

The CLH report cites an additional mutagenicity *in vitro* test from the Thor GmbH Art. 95 dossier (2014) performed according to OECD TG 471 and with GLP compliance in *Salmonella typhimurium* and *Escherichia coli*. The result of this study was negative; however, this dossier was not available to RAC. Anyway, the negative result of this test was concordant with the *in vitro* gene mutation test in the *Salmonella typhimurium* also performed according to GLP and OECD TG 471 (ZnPT CAR Doc IIIA A6.6.1/01; table above).

ZnPT 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 TG 473 and with GLP compliance (Thor GmbH Art. 95 dossier, 2014). Nevertheless, this study was just cited in the CLH report but could not be assessed by RAC.

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-h sampling time and in the absence of S9 metabolism at the dose level selected for scoring at the 31-h sampling time. The incidences in aberrations exceeded the HCD values of the laboratory. ZnPT was thus found to be clastogenic under the conditions of the study. Nevertheless, RAC notes that positive results were found only in presence of significant cytotoxicity.

ZnPT 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 TG 476. In the presence of S9 metabolic activation, a statistically significant effect was observed in the ANOVA analysis performed by the laboratory ($p<0.001$ in Assay 1 and $p<0.01$ in Assay 2) (see tables below); 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 HCD mean value (8.80) but still within the HCD range. In Assay 2 the value was higher than the recorded HCD range. The result of the study should therefore be considered positive. However,

RAC notes that the fact that the mutation frequency in treated samples was within the HCD makes the result of this study equivocal.

***Table**: 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				**Without metabolic activation**			
Dose level (µg/mL)	**% RS**	**MF day 6**	**MF day 9**	**Dose level (µg/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
+ control	74	1137	1155	**+ control**	81	695	702
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:** 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				**Without metabolic activation**			
Dose level (µg/mL)	**% RS**	**MF day 6**	**MF day 9**	**Dose level (µg/mL)**	**% RS**	**MF day 6**	**MF day 9**
0.0	100	25.68	24.68	**0.0**	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
+ control	104	1467	1246	**+ control**	102	1170	1193
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							

In a second *in vitro* mutagenicity study performed in accordance with GLP and OECD TG 476 the mutagenicity at the TK locus in L5178Y mouse lymphoma cells was studied (Thor GmbH Art 95 dossier). In absence of metabolic activation, ZnPT induced an up to 6.7-fold dose related increase in the mutation frequency, which was well above the HCD range. Both small and large colonies were increased. The relative total growth at the highest dose was reduced by 88%, which is acceptable by the test guideline. In the presence of metabolic activation, ZnPT induced an up to

8.3-fold dose related increase in the mutation frequency, which was also outside the HCD range. Both small and large colonies were increased. The relative total growth at the highest dose was reduced by 89%, which is acceptable by the test guideline. Both small and large colonies were increased. ZnPT was therefore considered to be mutagenic in both the absence and presence of metabolic activation under the conditions of the test. The table below summarises the results of this study.

Table: *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
+ control	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
+ control	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 ZnPT for 1, 3 or 12 hours. Cells treated with hydrogen peroxide served as positive controls and untreated cells as negative controls. 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. In a second part of the study, primary human epidermal melanocytes were exposed to 500 nM ZnPT 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 ZnPT was considered positive for induction of comet assays under the conditions of the study.

The table below summarises the results of the available mutagenicity and genotoxicity tests.

Table: *Summary table of relevant in vivo mutagenicity studies with ZnPT.*

Method	Test system	Tested concentrations	Results	Remarks	Reference
Mammalian erythrocyte micronucleus test OECD TG 474 EPA 84-2 GLP Reliability: 2 (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)	Crl:NMRI BR mouse 5/sex/dose	800, 1000 and 1300 mg/kg Single dose Gavage Purity >95 % 24 and 48 hours	**Negative**	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 score is given because the study has not been evaluated by the DS. Only a short summary is available with no information regarding the purity.	Sprague-Dawley mouse 5/sex/dose	0, 11, 22, 44 mg/kg Single i.p. injection 24, 48 and 72 hours	**Negative**		Arch registration dossier Year: 1990
In vivo chromosome aberration test	Cynomolgus monkey	0, 5.5, 11 and 22 mg/kg bw/d	**Negative**	One female in the 22.0	ZnPT CAR Doc IIIA A6.6.5/01

Japanese MITI guideline GLP The method is similar to EC method B.10 and OECD TG 473 Reliability: 3 (there was no information on the average cell cycle length of the lymphocytes; the cells were cultivated longer than appropriate and no positive control was used)	4/sex/group	Purity >95% Oral (capsule), once daily for 28 days		mg/kg bw/d 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	Year: 1992
In vivo Comet assay ICH S2 (R1), 2012 GLP Reliability: 1	Wistar Han rats 5/sex/group Tissues investigated: liver, blood, and duodenum	0, 25, 50 and 100 mg/kg bw/d Oral (gavage), once daily for 3 days Purity: >95%	**Negative**	Viability of cells of all dose levels was 94-100%. Doses were chosen based on a range-finding test where 3/6 animals died at 200 mg/kg bw/d The DNA damage in blood (20.5%) and duodenum cells (42.02%) from vehicle treated animals was higher than the	Thor GmbH Art 95 dossier Year: 2014

				acceptance criteria (<15%). However, the positive control clearly induced DNA damage according to the acceptance criteria.	

ZnPT was investigated in a micronucleus study (ZnPT CAR Doc IIIA A6.6.4/01) performed according to OECD TG 474 and GLP. There was no statistically significant increase in the numbers of micronucleated polychromatic erythrocytes at any dose level tested at any time point for males (see table below). The results were within the range of historical negative controls. In females (see second table below) of the high-dose group sacrificed at 48 h post-administration, 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.

T*able:* *Micronucleus study in mice (ZnPT CAR Doc IIIA A6.6.4/01).* Summary 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 times (h)		24	48	24	24	24	48	24
% 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: *Micronucleus study in mice (ZnPT CAR Doc IIIA A6.6.4/01).* Summary 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 times (h)		24	48	24	24	24	48	24
% 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*	13.0*
	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 ZnPT is available from the registration dossier and has not been evaluated by the DS. The reporting is very limited and shows deficiencies. Therefore, it is only included as supporting information. The result of the study is reported to be negative.

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

ZnPT was also investigated in a chromosomal aberration study (ZnPT CAR Doc IIIA A6.6.5; Table 18) performed according to GLP but not following any OECD test guideline. There were no significant differences in the average frequencies of structural aberrations or polyploidy between the ZnPT treated monkeys and the negative control group and therefore ZnPT was considered negative for clastogenicity. The study was found to be of limited quality.

An *in vivo* comet assay is available from a dossier on ZnPT. The mean tail intensity in liver, blood, and duodenum cells of the negative controls was 9.72%, 20.50% and 42.02%, respectively. 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 ZnPT does not cause biologically relevant DNA damage.

Comparison with criteria

According to the CLP guidance, classification of a substance as mutagenic for germ cells is warranted when there is evidence obtained from somatic cell mutagenicity tests *in vivo* in mammals or other *in vivo* somatic cell genotoxicity tests, supported by positive results from *in vitro* mutagenicity assays.

The available results show that ZnPT was able to induce chromosomal aberrations and gene mutations in V79 Chinese hamster lung fibroblasts under *in vitro* conditions. ZnPT was also able to break DNA strands *in vitro* in human epithelial keratinocytes. However, none of these positive results could be confirmed *in vivo* in two different mammalian erythrocyte micronucleus tests (one in mice and one in rats), in one chromosome aberration test in monkey and in one comet assay in rats with liver, blood and duodenum.

In conclusion, the criteria for classification for germ cell mutagenicity have not been met and consequently **RAC supports the DS's proposal not to classify ZnPT for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS presented an old study (1958) with serious limitations that revealed no increases in tumour formation in the CLH dossier. The DS also summarised carcinogenicity studies performed under OECD TG 453 with NaPT, showing no NaPT-induced tumour increases. Taking this into account, the DS proposed not to classify ZnPT for carcinogenicity.

Comments received during public consultation

One CA supported the DS's proposal not to classify ZnPT for carcinogenicity.

Assessment and comparison with the classification criteria

There is no robust substance-specific data available to assess the carcinogenic potential of ZnPT. Information of some relevance for this endpoint is available in a chronic toxicity study in which histopathological examinations were included. However, this study was performed in 1958, before GLP or any test guidelines were established, and the study is poorly reported, lacking information on purity, unclear dose levels and has several deviations from OECD TG 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). The results of this study is summarised in the table below.

Table: *Summary table of animal carcinogenicity studies.*

Method, guideline, deviations	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
No specific guideline No GLP Reliability: 3	Rat Strain not stated 10/sex/dose	ZnPT Batch: Not specified Purity: Not specified	0, 2, 5, 10, 25, 50 ppm Oral, in diet Food consumption per day not specified Daily treatment 10 ppm Approx. ≈ 1.3 mg/kg bw/d for males and females 2 years	**No increase in tumour formation** <u>2, 5 and 10 ppm:</u> No adverse effects <u>25 ppm:</u> ↑ mortality (females) ↑ hind limb paralysis (females) ↓ BWG (females) <u>50 ppm:</u> ↑ mortality (10 females, 6 males) ↑ hind limb paralysis (males, females) ↓ BWG	ZnPT CAR Doc IIIA 6.5/03 Year: 1958

The histopathological examinations included 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.

RAC notes that although the study provides some information on the carcinogenic potential of ZnPT, the results of the study are not considered sufficiently reliable and robust to serve as key data and therefore **RAC supports the DS's proposal not to classify ZnPT for carcinogenicity based on lack of data.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The effects of Zinc Pyrithione were well described by the DS in the CLH report. Fertility/sexual function was investigated in a two-generation reproductive toxicity study performed according to the guidelines (OECD TG 416/EPA OPPTS 870.3800/EU B.35) and with GLP. ZnPT (purity >95%) was given to Han Wistar rats by daily oral gavage at dose levels of 0, 0.2, 0.5, and 2.5 mg/kg bw/d (Thor GmbH Art.95 dossier), see Background document, table 52. This study was considered to be acceptable by the DS with a reliability factor of 1.

Dosing schedule

For the parental generation the minimum number of doses was 70 (pre-mating) plus 15 (mating). In the case of females, the dosing continued until lactation day 21-23. For F1 animals the dose started after weaning with a similar pattern to that described for the parental generation.

Observed Effects

F0 parental effects/toxicity

General toxicity was reported only at the top dose, and the reported effects included:

- Hunched posture, piloerection and lean appearance in 6/24 females;
- ↓ body weight gain (during pre-mating treatment, days 22-64) relative to controls in females from day 22 (10%) to day 64 (20%);
- ↓ size of skeletal muscle in 3/24 females;
- ↑ 8% relative liver and ↑ 11% spleen weight (no histopathological findings);
- 10 females with muscle histopathological findings (atrophy in 7/24; fat replacement in 6/24 and axonal degeneration in 4/24);
- ↓ epididymal sperm concentration (statistically significantly but within normal ranges for age and strain).

According to the study author, there were no adverse effects on any of the reproduction parameters or on pup development that were attributable to the treatment. Nevertheless, the following effects were detected: total litter loss in 3/24 females at 2.5 mg/kg bw/d; 2 non-pregnant females at 0.5 mg/kg bw/d and 1 female with implantation sites only and 1 non-mating female at the low dose of 0.2 mg/kg bw/d.

F1 pup effects/toxicity

The effects found in F1 pups included:

- vaginal patency in the females of all treated groups delayed by an average of two days compared to control group;
- the mean litter size (9.7 g) of the high-dose pups was lower than the control (10.9 g) and the HCD (11.5) means;
- nine pups were dead at the high-dose at the first check (6 of those pups were from the female with total litter loss and 3 from a second female);
- four pups from a top-dose litter were lost from PND 7-14;
- one pup from the top-dose group was euthanized on PND 22 after signs of piloerection, lethargy, swelling of the head, pale appearance, a wound, and skin abnormalities.

F1 parents effects/toxicity

The findings reported for the F1 parents were:

- one top-dose female was euthanized after total litter loss (author did not consider this finding treatment-related);
- statistically significant lower absolute body weights (but not body weight gains) for the top-dose females on GD 4-20;
- higher incidence of fluid in the uterus in mid- and top-dose females (author did not consider this finding treatment-related or toxicologically relevant);
- skeletal muscle histopathological findings in 10/24 top-dose females (including 1 case of atrophy);
- the mean litter size (10.2) of the top-dose pups was lower than the control (11.3 g) and the HCD (11.5) means.

F2 pups effects/toxicity

There was no treatment-related or toxicologically relevant effects on the developmental parameters of the F2 generation.

Conclusions

In summary, the DS considered there were no treatment related adverse effects on sexual function and fertility.

Development

The effects of ZnPT on development were extensively described by the DS in the CLH report (overview of studies in table 53 of the Background document). In total six reports specifically on ZnPT were available to the DS, including a topical application study and one published peer review journal article that investigated the effects in both rats and rabbits by the oral (diet, gavage) and dermal routes (Nolen & Dierckman, 1979). It should be noted that the DS only considered the oral routes from the Nolen & Dierkman (1979) publication. These studies were considered sufficient to form the basis of a classification proposal for ZnPT. The summaries presented below outline the most relevant effects for consideration of classification for reproductive toxicity. Additional tables in the in the Background document give greater detail if required, see tables 56, 57, 58, 59, 60, 61, 62, and 63.

Study Summaries

The results from each study were described in detail by the DS and the most relevant toxicological effects can be briefly summarised as follows:

Study 1: Rabbit developmental toxicity study (1993) - CAR A6.8.1/01

Doses: [0; 0.5; 1.5; 3.0] mg/kg bw/d
A GLP-compliant developmental toxicity study according to US EPA 83-3, which complies with OECD TG 414, investigated the oral (by gavage) administration of ZnPT to NZW rabbits. Application of dose was on GD 6-18 post artificial insemination. There were no treatment-related deaths. There were no reported effects on mobility or skeletal muscle.

Maternal toxicity:

1. ↓ food consumption GD 6-19 [controls; +0.1%; -16%**; -23%**]
2. ↓ food consumption GD 0-29 [controls; +0.6%; -7%; -7%]
3. ↓ bw gain GD 6-19 [controls; +3%; -41%**; -99% (highly variable)**]
4. ↓ bw gain GD 0-29 (corrected): [controls; -6%; -2%; -1%]
5. Corrected terminal bw [controls; +1%; +0.7%; +0.5%]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Limited evidence of maternal toxicity at the doses tested. The greatest changes in food consumption (g/animal/d) and body weight gain were from GD 12-19. Weight gain increased when the period of dosing was finalised during days 20-29, with the overall mean weight gain on days 0-29 being 85% and 83% of controls for the 1.5 mg/kg bw/d and 3.0 mg/kg bw/d dose groups respectively. There were no differences in average adjusted body weights (calculated as body weight minus gravid uterine weight) on days 0-29 between any of the dose groups.

Developmental toxicity:

1. ↓ number of pregnant animals [17; 18; 16; 13]
2. ↑ post-implantation loss [11%; 12%; 29%*; 65%*]
3. ↑ early resorptions – mean per doe/litter [0.8; 0.7; 1.6; 3.3]
4. ↑ whole litter resorptions [0; 0; 1; 5]
5. ↓ mean litter size – live foetuses per litter [6.2; 5.5; 3.8; 2.0*]
6. ↓ animals with viable foetuses [17/17; 18/18; 15/16; 7/13]
7. ↓ number of viable foetuses [105; 100; 61; 26]
8. ↑ incidence of malformations [7/105; 12/100; 5/61; 7/26]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Foetal viability was adversely effected. ZnPT was clearly embryotoxic. The assessment of teratogenic effects is difficult because of the low surviving numbers of foetuses in the top dose tested. On a proportional basis, malformations were increased in the top-dose group (foetuses effected: 7/26 vs. 7/105 in the control group).

Study 2: Rat developmental toxicity study (1993) - CAR A6.8.1/02

Doses: [0; 0.75; 3.0; 15.0] mg/kg bw/d
A developmental toxicity study was performed with SD rats exposed to ZnPT by the oral route (oral gavage), in accordance with GLP and OECD TG 414. Application of dose was on GD 6-15. Sacrifice was on GD 20. The only clinical signs observed were increased salivation and dilated pupils; no effects on hind limbs were recorded. One high-dose dam was found dead on GD 16.

Maternal toxicity:

1. ↓ food consumption GD 6-16 [controls; -2.5%; -3.3%; -24%**]
2. ↓ food consumption GD 0-20 [controls; -3%; -3%; -25.6%**]
3. ↓ bw gain GD 6-16 [controls; -9.8%; -22%**; -67%**]
4. ↓ bw gain GD 0-20 (corrected) [controls; -1.5%; -3%; -37%**]
5. Corrected terminal bw [controls; -1%; +0.3%; -8.4%**].

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Limited evidence of maternal toxicity. The greatest changes in food consumption (g/animal/d) were from GD 9-12 and GD 6-9 and GD 9-12 for body weight gain. Weight gain increased when the period of dosing was finalised during days 16-20 with the overall mean corrected weight gain on days 0-20 being 63% and 97% of controls for the 15 mg/kg bw/d and 3.0 mg/kg bw/d dose groups respectively. There was little difference in the average adjusted body weight (calculated as body weight minus gravid uterine weight) on days 20 between the top dose group (92%) and the concurrent controls.

Developmental toxicity:

1. ↑ post-implantation loss [5.3%; 5.0%; 9.4%; 22.9%**]
2. ↑ early resorptions – mean per dam/litter [0.8; 0.8; 1.44; 3.7**]
3. ↑ whole litter resorptions [0; 0; 0; 3]
4. ↓ number of viable foetuses/litter [14.5; 15.2; 13.8; 12.5*]
5. ↓ mean foetal weights [3.6g; 3.4g; 3.4g; 3.0g**]
6. ↑ incidence of malformations, foetuses (litters) [1 (1); 3 (2); 7 (6); 168 (24)**]
7. ↑ incidence of variations, foetuses (litters) [79(25); 78(27); 56(22); 171(24)*]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Developmental toxicity was manifested by increased early resorptions and reduced foetal body weights. The reduction in mean foetal body weights was significant (-15% in females, $p<0.01$, and -17% in males, $p<0.01$). Foetuses of the top-dose group exhibited a high incidence of malformations (168 of examined foetuses compared to 1 in controls, $p<0.01$). These included 153 (24 litters) cases of "vertebral malformation with or without an associated rib malformation"; 30 (14 litters) cases of "fused sternebrae #1, #2, #3, #4 and/or #5"; and 35 (13 litters) cases of "sternal malformation #1, #2, #3, #4 or #5 absent". These are in contrast to zero incidences of these malformations in all other dose groups and concurrent controls. There were also significant numbers of developmental variations in the top-dose group (171 of examined foetuses compared to 79 in controls, $p<0.05$). The majority of these variations involved the sternebrae and numbers of pairs of full ribs.

Study 3: Rat developmental toxicity study (2005) - CAR A6.8.1/03

Doses: [0; 10; 15; 30; 60] mg/kg bw/d
A developmental toxicity study was performed with SD rats exposed to ZnPT by the dermal route (topical application, 6 hours daily), in accordance with GLP and OECD TG 414. Application of dose was on GD 0-20. Sacrifice was on GD 21. Significant clinical signs were observed affecting mobility and skeletal muscle with hind limb involvement that increased with the duration of exposure. There were no test substance related deaths.

Maternal toxicity:

1. ↓ food consumption (g/d) GD 0-21 [controls; -2%; -4%; -7%; -23%**]

2. ↓ bw gain GD 0-21 (corrected) [controls; -11.3%; -13%; -53%**; -143%**]
3. ↓ corrected terminal bw [controls; -2.5%; -2.5%; -12%**; -30.7%**]
4. ↑ animals with low muscle tone [0/23; 0/24; 0/23; 2/24; 21/23**]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Evidence of maternal toxicity with significant reductions in food consumption and body weight parameters.

Developmental toxicity:

1. Post-implantation loss – not increased
2. Early resorptions – less than or similar to controls
3. Whole litter resorptions – not observed
4. Number of viable foetuses/litter – similar to controls
5. ↓ mean foetal weights [5.35g; 5.25g; 5.28g; 5.31g; 4.31g**]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Developmental toxicity was limited and confined to the top-dose level. It included reduced foetal body weights (-21% for males, p<0.01, and -18% for females, p<0.01; -19.5% combined, p <0.01). There was no effect on foetal viability. The DS described an increased number of foetuses with malformations or variations (12 compared to 6 in controls, p<0.01) but this was not clearly defined. According to the DS, the decrease in foetal weights and the associated skeletal variations could be attributed to maternal toxicity.

Study 4a: Rat oral study; Nolen and Dierckman, (1979)

Doses: [0; 7.5; 15.0] mg/kg bw/d
This study is presented as supporting information, it was not performed according to GLP or any guideline but may be roughly comparable to OECD TG 414. Notable omissions were clinical observations, food consumption, organ weights and individual animal data. The study was performed with a limited number of animals (10 per dose). SD rats were exposed to ZnPT by oral gavage on GD 6-15. Sacrifice was on GD 20. Effects on hind limbs were recorded.

Maternal toxicity:

1. ↓ bw gain GD 0-15 [controls; -71%; -83%*]
2. Corrected terminal bw – no data
3. Hindlimb paralysis [0; 5/10; 5/10]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Evidence of maternal toxicity. No information on terminal body weight.

Developmental toxicity:

1. Post-implantation loss – no data
2. Early resorptions – less than or similar to controls
3. Whole litter resorptions – not observed
4. Number of viable foetuses/litter – similar to controls
5. ↓ mean foetal weights [5.50g; 4.85g; 4.23g*]
6. ↑ incidence of skeletal variations.

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Developmental toxicity was limited to a reduction in mean foetal body weights which was significant (-23%, p≤0.05). There was a significant increase in the numbers of rib abnormalities in the highest dose group. The DS considered that the maternal toxicity in the form of maternal body weight decrements may be sufficient to explain the effects.

Study 4b: Rabbit oral study; Nolen and Dierckman, (1979)

Doses: [0; 1.0; 2.5; 5.0] mg/kg bw/d
This study is presented as supporting information, it was not performed according to GLP or any guideline but may be roughly comparable to OECD TG 414. The study was performed with 20 animals per dose. New Zealand White rabbits were exposed to ZnPT by oral gavage on GD 6-18. Sacrifice was on GD 29. Effects on hind limbs were recorded.

Maternal toxicity:

1. ↓ food consumption (g) GD 6-18 [controls; -15%; -11%; -17%*]
2. ↓ body weight gain GD 6-18 [controls; -28.6%; -71.4%; -178%*]
3. Corrected terminal bw – no data

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Evidence of maternal toxicity. No information on terminal body weight. There was a statistically significant weight loss in the highest dosed group, -136 g compared to +175 g in controls.

Developmental toxicity:

1. ↑ post-implantation loss [12%; 24%; 47%; 83%**]
2. ↑ early resorptions [0.8; 2.3; 4.0; 6.8*]
3. ↓ number of viable foetuses/litter [5.8; 7.4; 4.1; 1.4*]
4. Mean foetal weights – no effect.
5. Incidence of skeletal/visceral abnormalities – No treatment-related effects.

*p ≤ 0.05; **P ≤ 0.01

Conclusion: There was a dose related increase in post-implantation loss (83% in top-dose, p<0.05, and 47% in the mid-dose 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.

Study 5: Rat developmental toxicity study (2015) - Thor GmbH Art. 95 dossier

Doses: [0; 0.4; 1.2; 1.7] mg/kg bw/d
A developmental toxicity study was performed with Wistar Han rats exposed to ZnPT in the diet, in accordance with GLP and OECD TG 414. Application of dose was on GD 6-20. Sacrifice was on GD 20. There were clinical signs of abnormal gait, piloerection and pale faeces. All animals survived until scheduled necropsy.

Maternal toxicity:

1. ↓ food consumption GD 14-20, top dose relative to controls [-21 to -45%%**]
2. ↓ bw gain GD 15-20, top dose relative to controls [-36 to -69%**]
3. ↓ bw gain GD 6-20 [controls; +6%; +2%; -54%*]
4. ↓ bw gain GD 0-20 (corrected) [controls; +5.5%; -8.6%%; -109%*]
5. ↓ corrected terminal bw [controls; -1.3%; -3.4%; -20%**]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Some evidence of maternal toxicity. Changes in food consumption were significant on GD 14-20. There was a statistical difference in the average adjusted body weight (calculated as body weight minus gravid uterine weight) on GD 20 between the top dose group (-20%) and the concurrent controls.

Developmental toxicity:

1. Post-implantation loss – no effect.
2. Early resorptions – mean per dam/litter – no effect.
3. Whole litter resorptions – no evidense
4. Number of viable foetuses/litter – no effect
5. ↓ mean foetal weights [3.5g; 3.6g; 3.5g; 3.3g*]
6. No treatment related malformations.
7. No treatment related variations.

*p ≤ 0.05; **P ≤ 0.01

Conclusion: The only developmental effect reported was slightly lower foetal body weights. Mean foetal body weights in the high-dose group were statistically significantly lower (-9% for females and -8% for males). The DS considered this as secondary to the observed maternal toxicity. No other treatment related developmental findings were found in other dose groups.

Study 6: Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier

Doses: [0; 0.5; 1.5; 4.0] mg/kg bw/d
A GLP-compliant developmental toxicity study according to the guidelines (OECD TG 414/EPA OPPTS 870.3700/EU B.31), investigated the oral (by gavage) administration of ZnPT to NZW rabbits. Application of dose was from GD 7-28 post-coitum. There were no treatment related deaths. There were no reported effects on mobility or skeletal muscle. All animals surviving to day 29 post-coitum were subjected to necropsy.

Maternal Toxicity:

1. ↓ food consumption (g/animal/d) GD 7-20 [controls; -7%; -8%; -22%*]
2. ↓ bw gain GD 7-20 [controls; +18%; -20%; -108%*]
3. Corrected terminal bw – [controls; -2%; -2.4%; -0.6%]
4. ↑ red / orange discoloration of urine (animal incidence) [1/22; 0/22; 1/22; 10/22*]

*p ≤ 0.05; **P ≤ 0.01 Both absolute and relative food consumption was reduced significantly on days GD 7-20 only

Conclusion: Limited evidence of maternal toxicity at the doses tested. The greatest changes in food consumption and body weight gain were generally from GD 7-20 at the top dose. There was no change in corrected terminal body weight (according to RAC's evaluation of the data).

Note: In 6 of the 21 does in the mid-dose group (1.5 mg/kg bw/d) the body weight gain was statistically significantly lower during GD 7-29 (58% of the controls) but most of the post-implantation losses in the whole group were seen in those 6 does. However, the corrected maternal weight gain was in the same range as the controls in all these 6 does. Therefore, the maternal toxicity (decrease in weight gain) in these 6 does was due to the high incidence of resorptions in this group (similar to the high-dose group).

Developmental Toxicity:

1. Number of pregnant animals – not effected
2. ↑ post-implantation loss [8%; 6%; 23*%; 67%**]
3. ↑ early resorptions – mean per doe/litter [0.4; 0.5; 1.7**; 4.5**]
4. ↑ whole litter resorptions [1; 0; 1; 10]
5. ↓ mean litter size – live foetuses per litter [7.3; 7.9; 6.4; 2.5**]
6. ↓ animals with viable foetuses [19/20; 20/20; 19/21; 9/20]
7. ↓ number of viable foetuses [146; 157; 127; 47]
8. ↑ incidence of malformations [2/146; 5/157; 5/127; 11/47]
    a. External: omphalocele foetuses (litters) [0; 0; 2(2); 2(2)]
    b. Skeletal: combined, foetuses (litters) [2(2); 0; 2(2); 11(5)]

*p ≤ 0.05; **P ≤ 0.01

Conclusion: Developmental toxicity was observed following treatment at both 1.5 and 4 mg/kg bw/d. Foetal viability was adversely effected. Zinc Pyrithione was shown to be clearly embryotoxic. The assessment of teratogenic effects is complicated because of the low surviving numbers of foetuses in the top dose tested. Rare and severe malformations were observed that on a proportional basis were increased in the top dose group (foetuses effected: 11/47 (9 litters) vs. 2/146 (19 litters) in the control group). External malformations of omphalocele were observed in 2 foetuses from 2 litters each of mid- and top-dose groups. Two foetuses (1 each from mid- and top-dose group) among the 4 affected foetuses also had an absent tail.

HCDs: These external malformations were not found in concurrent controls and in only one HCD foetus. HCD data was supplied with the 2015 rabbit study report mentioned below, there were 15 studies (2008 – 2012), 2205 foetuses from 279 litters. Omphalocele (abdominal organs outside the body, intestinal loops herniating from around the umbilicus) and absent tail are rare malformations with just a single foetal occurrence for each noted from the supplied HCD. RAC notes that the HCD supplied with the earlier (CAR A6.8.1/01, 1993) rabbit study report also supports this incidence for omphalocele in rabbits. According to this HCD, included with the original study report, the omphalocele incidence was 3 foetuses in 3 litters out of a total of 56 studies (1985 – 1990) with 5872 foetuses from 806 litters

RAC further notes that the report amendment 1 to the final 2015 study report, as well as the additional information report that were subject to a targeted public consultation, contain additional HCD and that e.g. omphalocele was suggested to be a non-specific response to maternal stress. According to the report amendment 1 to the final study report, omphalocele was observed in 16 studies out of 40 (distributed equally among all treatment groups, not just controls, same testing laboratory) when the so called "complete HCD data set" (years 2006 to 2017) was re-examined for omphalocele. However, RAC is of the opinion that, in line with the CLP guidance (Version 5, July 2017), the HCD should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study). RAC further considers that the true HCD database should not include generic treatment groups due to the unforeseen effects from these treatments and the unquantifiable nature of the ensueing added maternal stress from substance dosing. A brief analysis of this report amendment from industry shows omphalocele was observed in 4 out of the 40 rabbit developmental toxicity studies when considering only the true controls.

Other Studies

Two recent rat whole embryo culture (rWEC) assays provided by the ZnPT task force, using either sodium pyrithione (NaPT) or 2-MSP (the principle metabolite of pyrithione), were also described by the DS (report LTS-16006, 2016; report LTS-16005, 2016). NaPT or 2-MSP 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 had an intrinsic developmental hazard. Sprague Dawley CD rat embryos were explanted on GD 9.5 and cultured for 44 hours. In the NaPT assay, sporadic effects were observed in some experimental groups but without a dose response relationship, and the highest concentration did not show effects. In summary, there were no effects on growth or morphological development for either NaPT or 2-MSP. The DS concluded that these rWEC assays were not completely relevant to conclude that ZnPT is not directly embryotoxic because the toxicological significance of Zn^{2+} in synergy with the pyrithione was not tested.

Conclusions

In summary, the DS considered the data for ZnPT sufficient to propose classification in Category 1B for development based on (1) the malformations and (2) post-implantation losses seen in three independent guideline studies in two different species.

Comments received during public consultation

Comments from MSCAs

Four MSCAs supported the proposal of the DS for classification with Repr. 1B.

Other Commentators (Individuals, Consultants, Industry or trade association, Company-Manufacturer, Company-Downstream users)

There was an extensive collection of comments and opinions supplied from a variety of commentators during the public consultation. Most of these comments were similar and involved only a few key points which are broadly summarised below. For more detail please consult the response to comments (RCOM) document.

- One comment routinely repeated was how the CLH proposal did not follow the procedures outlined in the CLP Regulation:
    - integration of all available data using weight-of-evidence as required in the CLP directive was not performed;
    - maternal toxicity was not adequately assessed;
    - specificity of the adverse effects to the embryo was not assessed;
    - key information (expert review on consequences of massive reductions in maternal body weight for study evaluation, results of food restriction studies) was not correctly interpreted.

- The CLH report neglected to use read across (although read across is clearly valid within the metal-pyrithione family). The use of all available data in a weight of evidence approach should therefore apply to all available studies with other salts of pyrithione and not just ZnPT. Another point made was that the ECHA read across framework justifies this approach.

- The CLH report fails to consider the mode of action information. The mode of action is defined in terms of inhibition of one of the key enzymes in the Krebs cycle and that the foetus being less reliant on oxidative metabolism cannot be a direct target for ZnPT.

- The CLH report discounts the rWEC *in vitro* studies. The results from the rat whole embryo culture (rWEC) study show clear evidence that pyrithione (and its metabolite, 2-(methylsulfonyl)pyridine), is not a direct acting developmental toxicant, *i.e.* it has no intrinsic, specific property to produce an adverse effect on the embryo.

- Developmental toxicity by ZnPT occurs only in the presence of excessive maternal toxicity and ZnPT does not have an intrinsic, specific property to produce adverse effects on fetal development. Furthermore, because there was excessive maternal toxicity, developmental effects observed in foetuses were secondary to those effects in the dams/does and should not be used as the basis for classification.

- Historically, ZnPT has been in use as personal care products for decades with no evidence of harmful effects.

Comments from the Targeted public consultation

Industry submitted a revised version of the study report for the rabbit study from 2015; Amendment No. 1 to the final study report on "Prenatal developmental toxicity study of ZnPT in rabbits by oral gavage" (Thor GmbH Art. 95 dossier, 2015). A targeted public consultation was held from 07/03/2018 to 21/03/2018. The targeted public consultation invited comments only on the Amendment to the final study report and on an additional information report (additional information report, 2018), both made available for consultation.

- Three MSCAs commented and continued to support the classification proposal from the DS: Repr. 1B (H360D)

- Nine comments from industry or industrial organisations and four comments from individuals all expressed disagreement with the proposed classification. The arguments were no different from those presented during the standard public consultation on the CLH proposal (opened from 23/05/2017 until 7/07/2017). There were some additional documents submitted, not related to the Amendment to the final study report and an additional information report (Thor, 2018) which was the basis for the targeted public consultation.

- RAC notes that the amendment to the final study report (also summarised in the additional information report, 2018) contains detailed evaluation of the maternal body weight individual data, particularly referring to the six animals in the mid-dose group and references to some HCD in particular on the malformation omphalocele (2006-2017).

Assessment and comparison with the classification criteria

Introduction

The CLH proposal and this opinion are in line with the requirements defined in the CLP Regulation regarding integration of all relevant information using a weight of evidence approach to conclude on classification. These requirements are:

1. A weight of evidence evaluation of the **relevant** toxicological database;
2. A detailed assessment of potential confounding of the outcome of developmental and reproductive toxicity studies by maternal toxicity;
3. Identification of an intrinsic property of the chemical to affect the embryo/foetus;
4. Integration of expert judgment in the final conclusion.

Scope of the developmental toxicity assessment

This opinion is based purely on the chemical species zinc coordination complex of pyrithione (CAS no. 13463-41-7). While read across may be useful in some circumstances (such as when specific

toxicological data is scarce), each pyrithione species has distinct physicochemical and toxicological properties and the assessment of the classification of ZnPTis based largely on the toxicological database specific to this particular speciation. The weight of evidence approach appears to have been misunderstood by several commentators; in this assessment weight of evidence refers to the totality of information specific to investigations using the Zn^{2+} coordination complex and not to all studies performed on all possible forms of pyrithione. RAC supports the DS and considers the available toxicological data package for ZnPT on reproductive effects sufficient to form an opinion.

Dose selection and general toxicity

Zinc pyrithione is a challenging substance. It has an extremely steep dose response toxicity curve (see CLH report, 10.12.1) that is also particularly noteworthy in the pregnant rabbit. This led to the selection of dose levels in prenatal studies that were very low relative to the doses of substances normally encountered during this evaluative process. These dose levels produced general toxicity (noted by effects on body weight gain and food intake) in excess of what is prescribed in the CLP guidance as target toxicity level, but not to the extent that there were clear indications of toxicity and that developmental toxicity was produced solely as a secondary consequence of maternal toxicity.

There were no developmental effects below 3 mg/kg bw/d for rat and below 1.5 mg/kg bw/d for rabbit. Higher dose levels, as recorded by some studies in range-finding experiments, lead to excessive maternal toxicity, particularly for the rabbit. Repeated doses above 8 mg/kg bw/d in the rabbit resulted in excessive lethality for example (CAR A6.8.1/01 1993 study referencing IRDC study number 397-054, and supported by results reported by Nolen and Dierckman, 1979). Such results are also supportive of STOT RE 1 (lethality, most sensitive species), but there is no firm data on rabbits available to RAC at this time to present this argument in the STOT RE section of this opinion document.

Mechanism of Action and nature of the toxophore

The proposed mechanism of toxicity of ZnPT, *i.e.* the inhibition of oxidative metabolism, produces adverse effects on the adult animal. In contrast to the opinion of the ZnPT Industry CLH Consortium Comments (June 2017), RAC holds the view that oxidative metabolism is as important in the embryo and foetus as in the adult animal and any perturbation of Krebs cycle, mitochondrial integrity or the proton motive force can have dire consequences for the developing organism. There is no data in this case to indicate that a metabolic disturbance in the pregnant animal is adverse to the embryo by indirect means, i.e. developmental effects are non-specific secondary consequences of maternal toxicity. The experiments using intact rat embryos in culture, away from maternal influences, appear to demonstrate that neither pyrithione nor its principal metabolite, have the ability to directly affect embryonic development. However, it is noted by RAC that the toxicity, bioavailability and antifungal activity of ZnPT can be highly dependent on the availability of Zn^{2+} ions as well as other divalent cations.

It is therefore important to highlight the importance of the zinc component and note that the DS is correct in its assertion that the rWEC studies did not take into account the effect zinc in combination with the pyrithione moiety may exert on the growth or morphological development of these rat embryos. Pyrithione is a divalent cation ionophore and in the presence of Zn^{2+} will chelate and form membrane permeable lipophilic zinc coordination complexes (in an equilibrium reaction), that enable intracellular access for the metal. Zinc is recognised as an important intracellular messenger and integral component of many proteins with diverse activities. Perturbations in Zn^{2+} homeostasis can induce cell death.

RAC notes that the mechanism of action (effects at the molecular level) for ZnPT is not well characterised and may involve multiple molecular targets since both pyrithione and Zn^{2+} (and

other divalent cations), will contribute to the overall toxicity profile. A mode of action (histological or functional changes at the cellular level) has been postulated - inhibition of oxidative phosphorylation with decreased intracellular energy supply presumably involving aconitase inhibition (and the possible inhibition of other Fe-S protein complexes). However, no mechanistic studies with ZnPT are available to substantiate these potential modes or mechanisms of action. There is no demonstrated link between aconitase inhibition in the maternal animals and indirect embryotoxicity. Thus, there is no mechanistic information that raises doubt about the relevance of the effects for humans. The requirements to fulfil Annex I, section 3.7.2.4.2 of CLP are not met and it is not possible to disregard the observed developmental effects.

Summary of the ZnPT studies submitted for assessment of developmental toxicity:

The developmental studies evaluated by the DS and assessed by RAC are summarised briefly in the table below. The Background document (table 53) gives greater detail to individual effects but the table here gives an overview of the main characteristics of each study and indicates where the DS finds support for Repr. 1B for development.

***Table**: Summary description of developmental toxicity studies.*

Study	Comments	DS C&L	Reference
(1) Rabbit, oral; strain: NZW	Oral gavage: 0, 0.5, 1.5, 3.0 mg/kg bw/d 20 x female per dose Dosing days 6-18 post artificial insemination ***Acceptable.*** GLP - Yes Guidelines – Yes, EPA 83-3 (OECD 414) Aqueous suspension of technical grade ZnPT. (52.2% a.i.)	Yes, Repr. 1B	CAR A6.8.1/01. Schardein JL. Report No. 397-056 (1993).
(2) Rat, oral; strain: Sprague - Dawley Crl:CD VAF/plus	Oral gavage: 0, 0.75, 3.0, 15.0 mg/kg bw/d 30 x female per dose Dosing days 6-15 post mating ***Acceptable.*** GLP - Yes Guidelines – Yes, EPA 83-3 (OECD 414) Aqueous suspension of technical grade ZnPT. (52.2% a.i.)	Yes, Repr. 1B	CAR A6.8.1/02. Schardein JL. Report No. 397-055 (1993).
(3) Rat, dermal; strain: Crl:CD (SD)IGS BR VAF/Plus	Topical: 0, 10, 15, 30, 60 mg/kg bw bw/d 23-25 x female per dose 6 hours per day on GD 0 through GD 20 ***Acceptable.*** GLP - Yes Guidelines – Yes, U.S. EPA (1998) ZnPT powder (98.3% a.i.)	No.	CAR A6.8.1/03. Barnett BS (2005) Report No. AEN00006.

(4a) Rat (SD) repro / fertility / developmental toxicity study	Topical: 0, 2.5, 7.5, 15 mg ZPT/kg bw/d Oral gavage: 0, 7.5, 15 mg ZnPT/kg bw bw/d 10 x female per dose Dosing days 6-15 post mating ***Supplemental.*** GLP - No Guidelines – No 48% aqueous suspension of ZnPT.	Supporting information only. Significant maternal toxicity, may account for effects in rats.	Nolen and Dierckman, (1979) Published paper: *Reproduction and teratology studies of ZnPT administered orally or topically to rats and rabbits. Food and Cosmetics Toxicology 1979 Dec; 17(6): 639-49*
(4b) Rabbits repro / fertility / developmental toxicity study	Topical: 0, 25, 50, 100 mg ZnPT/kg bw/d Oral gavage: 2 oral studies: 5, 10, 20 mg ZPT/kg bw/d and 0, 1, 2.5, 5 mg/kg bw/d 20 x female per dose Dosing days 6-18 post mating ***Supplemental.*** GLP - No Guidelines – No 48% aqueous suspension of ZnPT.	Supporting information only. Results support those in the CAR A6.8.1/01 1993 study. Significant post-implantation loss.	3. Nolen and Dierckman, (1979)
(5) Rat, oral; strain: Crl:WI (Han)	Oral (diet): 0, 0.4, 1.18, 1.68 mg ZnPT/kg bw/d 22 x female per dose Dosing days 6-20 post mating ***Acceptable.*** GLP - Yes Guidelines – Yes, EPA OPPTS 870.3700 (OECD 414) ZnPT powder, purity 97.55%.	No.	4. Thor GmbH, Art. 95 dossier (2015)
(6) Rabbit, oral; strain: NZW	Oral gavage: 0, 0.5, 1.5, 4 mg ZnPT/kg bw/d 22 x female per dose Dosing days 7-28 post mating ***Acceptable.*** GLP - Yes Guidelines – Yes, EPA OPPTS 870.3700 (OECD 414) Zinc pyrithione powder, purity 97.55%.	Yes, Repr. 1B	Thor GmbH, Art. 95 dossier (2015)

Notes:
1. Original study report quotes a range-finding study by the sponsor, IRDC study number 397-054 was used to set the dosage levels. In the range finding study "excessive mortality was present at dosage levels of 8.0 and 12.0 mg/kg bw/day". No further details were available. These findings are supported by the Nolen and Dierckman (1979) study.

2. A low reliability, dermal absorption study (CAR A6.2/03, *in vivo*, rat) indicates low potential for dermal absorption (0.4-0.7%)

3. Two oral gavage studies in rabbits and a range-finding study. Excessive death in the first oral study, results not suitable for classification assessment. ZnPT administered orally to groups of 15 pregnant rabbits from day 6 to day 18 of pregnancy was lethal to 6 at the 5-mg/kg level, 9 at 10

mg/kg and 15 at 20 mg/kg in the first study. All of these does died after 3-5 daily treatments. No histological lesions observed in these animals. There were no viable foetuses at the 10 mg/kg bw dose. Repeated with lower doses of 1, 2.5 and 5 mg/kg bw/d and the results of the second oral study were reported in the CLH report.

4. Evidence of generalised maternal toxicity. No developmental toxicity.

Maternal Toxicity

The relationship between maternal toxicity and developmental toxicity is complex. It is recognised that some perturbations of maternal physiology can result in malformations (Beyer *et al.*, 2011). Some of these effects include changes in acid-base balance, chemically-induced maternal nutritional deficiencies and maternal diabetes (Carney, 1997, Taubeneck, 1994). Other effects on the maternal unit can have profound consequenses that result in embryonic hypoxia and include maternal anaemia, diminished cardiac function, or uterine blood vessel constriction (Danielsson, 2013). The main surrogate markers of maternal toxicity evident in the developmental toxicity studies involve significant reductions in food consumption and body weight gain. No further detail is available to determine what other effects occur that may impact on the developing foetus. There is no data to illustrate that secondary mechanisms may be responsible for the effects observed in both rat and rabbit foetuses.

Annex I to the CLP Regulation does not provide defined criteria for excessive maternal toxicity (except for mortality) but states that consideration of maternal body weight indices should always be taken into account. However, developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, particularly when irreversible effects such as malformations and embryo/foetal lethality are observed. To discount the foetal findings it must be demonstrated that the developmental effects are secondary to maternal toxicity. This has not been shown for ZnPT.

Several commentators during the public consultation suggested that the observed effects in rat dams and rabbit does constitute excessive maternal toxicity, and on that basis the developmental effects may be discounted because they are (presumably) causally related. RAC recognises significant effects in the maternal animals, but supports the DS view that the developmental effects are more significant and cannot be solely explained as a secondary consequence of maternal toxicity with the available data.

The maternal toxicity with respect to body weight parameters in each developmental study is summarised as follows:

Study 1: Rabbit, oral gavage (1993), key study for Repr. 1B: YES.

- ↓ bw gain GD 0-29 (corrected): [controls; -6%; -2%; -1%]
- Corrected terminal bw [controls; +1%; +0.7%; +0.5%]

Study 2: Rat, oral gavage (1993), key study for Repr. 1B: YES.

- ↓ bw gain GD 0-20 (corrected) [controls; -1.5%; -3%; -37%**]
- Corrected terminal bw [controls; -1%; +0.3%; -8.4%**]

Study 3: Rat, dermal (1979), key study for Repr. 1B: No.

- ↓ bw gain GD 0-21 (corrected) [controls; -11.3%; -13%; -53%**; -143%**]
- ↓ corrected terminal bw [controls; -2.5%; -2.5%; -12%**; -30.7%**]

Study 4a: Rat, oral gavage (1979), key study for Repr. 1B: No.

- ↓ body weight gain GD 0-15 [controls; -71%; -83%*]
- Corrected terminal bw – no data

Study 4b: Rabbit, oral gavage (1979), key study for Repr. 1B: Supportive.

- ↓ body weight gain GD 6-18 [controls; -28.6%; -71.4%; -178%*]
- Corrected terminal bw – no data

Study 5: Rat, oral diet (2015), key study for Repr. 1B: No classification.

- ↓ bw gain GD 0-20 (corrected) [controls; +5.5%; -8.6%%; -109%*]
- ↓ corrected terminal bw [controls; -1.3%; -3.4%; -20%**]

Study 6: Rabbit, oral gavage (2015), key study for Repr. 1B: YES.

- ↓ body weight gain GD 7-20 [controls; +18%; -20%; -108%*]
- Corrected terminal bw – [controls; -2%; -2.4%; -0.6%]

Excessive maternal toxicity remains not clearly defined; it remains unclear except for lethality (where guidance values have been accepted). In general, maternal toxicity is more of an expert scientific judgement, taken on a case-by-case basis. Effects which may be classified as excessive include:

- Lethality (at greater than 10%)
- Dramatic reductions in absolute body weight
- Coma
- Severe inanition
- Ataxia
- Organ failure
- Abortions in rabbits

Body weight changes *per se* are not sufficient in this case, unless accompanied by severe inanition or other toxicological effects that raises doubt about the nature of the developmental effects observed.

It is the opinion of RAC in this case that such excessive maternal toxicity has not been observed in the key studies that support classification for developmental effects.

It is clear that the dose selection in the relevant developmental toxicity studies had to be made very carefully. We can generalise the relationship between maternal toxicity and the selected doses to a limited extent for both rats and rabbits. Rabbits are clearly the more sensitive species. There is a very steep dose response with respect to severe parental toxicity (death) and developmental toxicity (increased post-implantation loss due to early resorptions, no dead foetuses to any extent indicating that the early embryo is the most susceptible target). The study authors were aware of this trend and final study doses were determined from range-finding studies. The majority of studies were scored a 1 or 2 in terms of reliability, the only exception being the published peer review research paper by Nolen and Dierckman (1979), which is considered supplemental and whose data does not contradict the results from the GLP-compliant and guideline studies presented by the DS in the CLH report. There is no evidence to suggest the guideline studies were conducted inappropriately, but the dosing was limited by the toxicity profile of ZnPT. RAC has tried to summarise these specific points in the table below.

Table: *Dose selection, study reliability and maternal toxicity at the top dose (in bold); data from all Reproductive toxicity studies in the CLH report.*

Study	Comments	GLP/Guideline	Maternal toxicity (top dose)
(1) Rabbit Dev; CAR A6.8.1/01 (1993).	Oral gavage: 0, 0.5, 1.5, **3.0** mg/kg bw/d *Range-finding study:* IRDC study 397-054, oral gavage: 0, 0.5, 2.0, 4.0, 8.0, 12.0 mg/kg bw/d Excessive mortality at 8, 12 mg/kg bw/d Significant bw loss at 4.0 and 8.0 mg/kg/d (no further data available)	GLP / EPA 83-3 (OECD TG 414) *Reliability:* eCA = 2 applicant = 1	Limited evidence: - ↓ food consumption (-23%**) - ↓ bw gain (-99%**) - no mortality
(2) Rat Dev; CAR A6.8.1/02 (1993).	Oral gavage: 0, 0.75, 3.0, **15.0** mg/kg bw/d *Range-finding study:* IRDC study 397-053, oral gavage: 0, 0.75, 2.0, 5.0, 10.0, 15.0 mg/kg bw/d No mortality. Reduced bw gain at 5.0 and 10.0 and 15.0 mg/kg bw/d (no further data available)	GLP / EPA 83-3 (OECD TG 414) Reliability: eCA = 2 applicant = 1	Limited evidence: - ↓ food consumption (-26%**) - ↓ adjusted bw gain (-38%**) - single mortality top dose (1/30).
(3) Rat Dev; CAR A6.8.1/03 (2005).	Topical dose: 0, 10, 15, 30, **60** mg/kg bw/d *Range-finding study:* Argus study AEN00005, dermal: 0, 15, 30, 100, 200, 400 mg/kg bw/d Rats in both high-dose groups were sacrificed after 11-14 daily doses. Dev tox observed in the top dose: - ↑ resorptions/litter 16% vs 0.6% controls - ↓ muscle tone in dams (10/10) - ↓ bw gain - ↓ corrected bw (-27% vs controls) - ↓ gravid uterine wt. (-30% vs controls)	GLP / EPA 83-3 (OECD TG 414) Reliability: eCA = 1 applicant = 1	Moderate evidence: - ↓ food consumption (-21%**) - ↓ adjusted bw (-31%**) - no mortality - limited use of hindlimbs (24/25) - shuffling gait (22/25) - decreased muscle tone (21/25) - loss in muscle mass (12/25) - no use of hind limbs (2/25)
(4a) Rat repro / fertility / developmental toxicity study (1979)	Oral gavage: 0, 7·5, **15** mg/kg bw/d	Non-GLP / Non-guideline study. Journal article	- ↓ bw gain (-83%*) - hindlimb paralysis (5/10)
(4b) Rabbits repro / fertility / developmental toxicity study (1979)	1. Oral gavage: 5, 10, 20 mg/kg bw/d [lethality too high: 6/15, 10/15. 15/15, not suitable for hazard assessment] 2. Oral gavage: 0, 1, 2.5, **5** mg/kg bw/d [no lethality]	Non-GLP / Non-guideline study. Journal article	- ↓ bw gain (-178%*) - ↓ food consumption (-17%*)
(5) Rat Dev; Thor (2015)	Oral (diet): 0, 0.4, 1.18, **1.68** mg/kg bw/d [0, 5, 15, 25] ppm	GLP / EPA OPPTS 870.3700 (1998) (OECD	- ↓ bw gain (-69%*) - ↓ rel. food consumption (-45%)

	<u>*Range-finding study:*</u> 1. Project 501664, oral (diet): [0, 5, 50, 200] ppm, 14 days 50/200 – "severe toxicity" (no data) 2. Project 501679, oral (diet): [0, 5, 15, 25, 35] ppm, 14 days Excessive mortality at 35 ppm (3/6),hind limb weakness, abnormal gait, lower bw, lower bw gain (no further data available). No developmental toxicity.	TG 414, 2001) <u>Reliability:</u> DS = 1	- abnormal gait - piloerection - pale faeces
(6) Rabbit Dev; Thor (2015)	Oral gavage dose: 0, 0.5, 1.5, **4** mg/kg bw/d <u>*Range-finding study:*</u> 1. Tolerability study 501674, oral (gavage): 1, 2, 4, 6 mg/kg bw/d At 6 mg/kg bw/d – 2/3 dead by day2 2. Dose range finding study 501675, oral (gavage): 0, 0.5, 1.5, 4 mg/kg bw/d. At 4 mg/kg bw/d – 1/6 dead	GLP / EPA OPPTS 870.3700 (1998) (OECD TG 414, 2001) <u>Reliability:</u> DS = 1	- red discoloration of urine (10/22) - ↓ bw gain (-100%*) - ↓ rel food consumption (-28%) - ↓ abs food consumption (-32%)
(7) Rat 2-gen study, Thor (2015)	Oral gavage: F0/F1; 0, 0.2, 0.5, **2.5** mg/kg bw/d *Dose levels based on:* 1. A 14-d *dose range-finding* study (project 503881), 0, 0.2, 0.5, 2.5 mg/kg bw/d 2. A 90-d oral study (project 501665), 0, 0.2, 0.5, 2.5 mg/kg bw/d	GLP / EPA OPPTS 870.3800 (1998) (OECD TG 416, 2001) <u>Reliability:</u> DS = 1	F0: - adverse effects on skeletal muscle (F: 10/24) - ↓ bw gain pre-mating days 22-64 (-10 to -20%) - minor clinical signs F1: - adverse effects on skeletal muscle (F: 1/24)

Developmental Effects with little to no maternal toxicity

Foetal and embryonic effects were observed without clear maternal toxicity at mid doses in two oral gavage studies;

- <u>Rabbit study (2015) (1.5 mg/kg bw/d):</u>
  - no statistically significant changes on bw (gain) or relative food consumption in the dams;
  - statistically significant ↓ mean of viable foetuses;
  - statistically significant ↑ post-implantation losses compared to both controls and HCD;
  - ↑ malformations (2 foetuses from 2 litters had external malformations of omphalocele);
  - statistically significant ↑ litter incidences of 13th full rib and pelvic girdle caudal shift.

- Rat study (1993) - DocIIIA A6.8.1/02 (3 mg/kg bw/d):
    - clinical signs in dams limited to salivation and no bw or bw gain differences were observed;
    - ↑ number of examined foetuses with malformations;
    - ↑ number of examined foetuses with skeletal malformation (fused ribs, pelvic malformation, tail malformation);
    - ↑ number of examined foetuses with soft tissue malformations (diaphragms hernia, anal atresia).

Developmental Toxicity – Foetal Viability

Effects on embryo/foetal viability were observed in four of the six studies available:

1. Rat developmental toxicity study (1993) - CAR A6.8.1/02
2. Rabbit developmental toxicity study (1993) - CAR A6.8.1/01
3. Rabbit oral studies - Nolen and Dierckman (1979)
4. Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier

Table: *Summary of studies with clear indications of embryotoxicity.*

Study	Comments
1. Rat Dev Tox (1993) - CAR A6.8.1/02	• ↑ mean post-implantation loss (23% compared to 5.3% in controls)** • ↓ mean number of viable foetuses per litter (12.5 compared to 14.5)* • ↑ whole litter resorptions (3 dams compared to 0 in controls) • ↓ mean foetal weights [3.6g; 3.4g; 3.4g; 3.0g**]
2. Rabbit Dev Tox (1993) - CAR A6.8.1/01	• ↑ mean post-implantation loss (65% compared to 11% in controls)* • ↓ mean number of viable foetuses per litter (2.0 compared to 6.2)* • ↑ whole litter resorptions (5 does compared to 0 in controls)
3. Nolen and Dierckman, (1979) – Rabbit oral studies	• ↑ mean post-implantation loss (83% compared to 12% in controls)** • ↓ mean number of viable foetuses per litter (1.4 compared to 5.8)*
4. Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier	• ↑ mean post-implantation loss (67% compared to 8% in controls)** • ↓ mean number of viable foetuses per litter (2.5 compared to 7.3)** • ↑ whole litter resorptions (10 does compared to 1 in controls)

Two feed restriction studies provided some data indicating that large decrements in maternal body weight *per se* are not associated with reduced foetal viability. RAC recognises the limitations of feed restriction studies to evaluate maternal toxicity if considering only the effect on body weight parameters alone. RAC notes that these studies provide only a crude tool in the assessment of body weight changes, and that all effects and available data are taken into account in evaluating maternal toxicity and whether there are direct effects on the developing organism.

A feed restriction study in rats clearly showed that severe weight loss or decrease in body weight gain induced minor changes in skeleton development but with no effects on viability or malformations in the rat (Fleeman, 2005). Up to a 15% maternal gestational body weight loss in this study had no effect on embryo viability in rats.

A feed restriction study by Cappon (2005) investigated the effects of maternal weight loss on embryo-foetal development in rabbits. 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.

Reductions in foetal body weights were seen in all four studies in rats. The DS suggested these can probably be explained by the maternal toxicity seen in the studies. RAC agrees with this assessment. The study by Fleeman, (2005) shows foetal growth is retarded in response to maternal gestational body weight change with reductions of foetal body weight of up to 24% when maternal body weight is reduced by 37% relative to controls (and where the dams show body weight loss of about 15% with respect to their pre diet-restricted body weight).

Developmental Toxicity - Malformations

Malformations were seen in three oral studies of high reliability, one in rats and two in rabbits:

1. Rat developmental toxicity study (1993) - CAR A6.8.1/02
2. Rabbit developmental toxicity study (1993) - CAR A6.8.1/01
3. Rabbit developmental toxicity study (2015) - Thor GmbH Art. 95 dossier

Table: *Summary of studies with foetal malformations.*

Study	Comments

1. Rat Dev Tox (1993) - CAR A6.8.1/02	• ↑ malformations were seen in all 24 litters of the top dose of 15 mg/kg bw/d (168 foetuses compared to 1 in controls)
	  ○ vertebral malformation with or without an associated rib malformation in 153 (89%) foetuses
	  ○ fused sternebrae in 30 foetuses (14 litters)
	  ○ other sternebral malformations in 35 foetuses (13 litters)
	  ○ ectrodactyly in 9 foetuses (5 litters)
	  ○ four cases of soft tissue malformations
	• None of these malformations were found in the controls
	• No strong dose response: but there were malformations in 7 foetuses (6 litters) at 3 mg/kg bw/d

2. Rabbit Dev Tox (1993) - CAR A6.8.1/01	• ↑ proportional incidence of malformations in the 3 mg/kg bw/d group (foetuses effected: 7/26 vs 7/105 in the control group) ○ Single instances of rare and severe malformations • No clear dose response

3. Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier	• ↑ incidence of malformations, foetal incidence [2/146; 5/157; 5/127; 11/47]
	  ○ External: omphalocele foetuses (litters) [0; 0; 2(2); 2(2)]
	  ○ Skeletal: combined, foetuses (litters) [2(2); 0; 2(2); 11(5)]
	• The external malformations were not found in controls and in only one HCD foetus (1/2205 foetuses in 279 litters).

The Cappon (2005) feed restriction study clearly showed that severe weight loss or decrease in body weight gain induced minor changes in skeleton development, and in rabbits abortions occurred in the most severe restricted dose group without malformations.

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.

Comparison with the classification criteria

According to the CLP criteria, classification in Category 1A must be based on evidence from human data, which were not present in the CLH report. Therefore, classification as Repr. 1A is not warranted.

Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants, respectively, and must be based on the presence of clear (Category 1B) or some (Category 2) evidence of alterations in sexual function, fertility, or development. In addition, such evidence must be present in the absence of other toxic effects (or if occurring together with other toxic effects the adverse effects on reproduction must be considered not to be a secondary non-specific consequence of the other concurrent toxic effects).

Adverse effects on Reproductive function and fertility

In the rat 2-generation study there were no treatment-related adverse effects on fertility or reproductive performance up to the top dose of 2.5 mg/kg bw/d.

Based on the available data and its interpretation, RAC agrees with the DS assessment that **no classification for adverse effects on reproductive function and fertility is warranted**.

Development

Evidence for developmental effects associated with ZnPT were observed in both the rat and rabbit.

Key points: Consistency of effect:

↑ post-implantation loss,
↓ embryo/foetal viability, and/or whole litter resorption
↑ skeletal/total/soft tissue malformations,

Table: *Summary of key foetal effects*

Effect	Comments
1. There was an increase in rat early resorptions and post-implantation loss	**[Rat Dev Tox (1993) - CAR A6.8.1/02]** • Resorptions: 0.8 vs 3.7** (controls vs top dose) [HCD: 0.6 – 2.1, mean 1.1] • Post-implantation loss: 5.3% vs 22.9%** (controls vs high-dose)
2. There was a reduction in rat mean live litter size at the top dose	**[Rat Dev Tox (1993) - CAR A6.8.1/02]** • Foetuses per litter: 14.5 – 15.2 – 13.8 – 12.5* [HCD: 12.1 – 15.9, mean 13.9]
3. There was an increase in rabbit early resorptions and post- implantation loss	**[Rabbit Dev Tox (1993) - CAR A6.8.1/01]** • Resorptions: 0.8 vs 3.3 (controls vs top dose) [HCD: 0.1 – 2.3, mean 0.6] • Post-implantation loss: 11% vs 65%* (controls vs top dose) [HCD: 2.4 – 23%] **[Nolen and Dierckman (1979) – Rabbit oral studies]** • Resorptions: 0.8 vs 6.8* (controls vs top dose), dose response: [0.8; 2.3; 4.0; 6.8*] • Post-implantation loss: 12% vs 83%** (controls vs top dose), dose response: [12%; 24%; 47%; 83%**]

	Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier • Resorptions: 0.4 vs 4.5** (controls vs top dose), dose response: [0.4; 0.5; 1.7**; 4.5**] • Post-implantation loss: 8% vs 67%** (controls vs top dose), dose response: [8%; 6%; 23*%; 67%**]
4. There was a reduction in rabbit mean live litter size at the top dose	**[Rabbit Dev Tox (1993) - CAR A6.8.1/01]** • Foetuses per litter: [6.2; 5.5; 3.8; 2.0*] [HCD: 5.5 – 9.1, mean 7.0] **[Nolen and Dierckman, (1979) – Rabbit oral studies]** • Foetuses per litter: [5.8; 7.4; 4.1; 1.4*] **Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier** • Foetuses per litter:: [7.3; 7.9; 6.4; 2.5**]
5. There was evidence of increased rat skeletal malformations	**[Rat Dev Tox (1993) - CAR A6.8.1/02]** • Total: foetuses (litters) [1 (1); 3 (2); 7 (6); 168 (24)**]
6. There was evidence of increased rabbit skeletal malformations	**[Rabbit Dev Tox (1993) - CAR A6.8.1/01]** • Total: foetuses (litter): [7/105; 12/100; 5/61; 7/26] **Rabbit Dev Tox (2015) - Thor GmbH Art. 95 dossier** • Total: foetuses (litter): [2/146; 5/157; 5/127; 11/47]

After careful consideration of all the data, RAC concludes there is sufficient evidence of a substance-mediated effect. Development of rat and rabbit foetuses was impaired at top dose levels. There was a significant increase in early resorptions which impacted on the rat post-implantation loss and this effect was also noted in the rabbit developmental studies. There were malformations in both rats and rabbits. RAC did not find evidence that the effect was not a direct effect on the developing foetus, as the maternal toxicity is considered insufficient to explain the effects observed in the foetuses from top dose dams/does. Overall, RAC concludes that there is clear evidence for adverse effects on development, albeit in the presence of maternal toxicity, in both rats and rabbits.

Category 2 classification for ZnPT may not be applicable as there is no mechanistic information that raises doubt about the relevance of the effects for humans. The evidence from experimental animals is sufficiently convincing to place it in Category 1B. It seems reasonable to conclude from the data that the mitochondrion (and possibly other intracellular components such as proteasomal deubiquitinases or DUBs) is the target of ZnPT and that because of the ubiquitous nature of this organelle in most eurcaryotic cells, toxicity in both the maternal and developing animals is of little surprise. It is not possible in this case to determine the relationship between maternal toxicity and developmental outcome. Indeed, the number of potential molecular targets for zinc (or other metal divalent-cation complex) pyrithione is unknown. Considering just one target such as the mitochondrion, one would suspect a steep dose toxicological response and predict that tissues with a high metabolic and oxidative respiratory requirement would be more sensitive to the effects of this active substance.

The generic nature of the maternal toxicity (loss of appetite, reduction in body weight gain, reduction in body weight, and at higher levels frank toxicity such as neuromuscular involvement and death) makes it very difficult to suggest there is a causal relationship between reproductive and parental toxicity. The generic nature of the parental toxicity suggests a universal target or targets are involved. A universal target would imply that the developing embryo/foetus may be just as susceptible to toxicity as the parental animal.

The specific developmental findings (rat and rabbit early resorptions, reductions in mean litter sizes, malformations in both species) make it difficult to argue for a category 2 classification. There is no mechanistic data available to indicate specific maternally-mediated mechanisms that give rise to secondary developmental effects in the offspring. There is no specific connection between the maternal effects and the effects in the developing animal. Consideration of these features in the presence of significant irreversible effects such as structural malformations and embryo lethality in two species supports Category 1B rather than Category 2.

Classification in Category 1B for development is concluded for ZnPT based on the malformations and post-implantation losses seen in three independent guideline studies in two different species.

Effects on or via lactation

No data is available for evaluation.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

1.1 Overview:

This opinion lays out a detailed record of the development of the environmental classification proposed for ZnPT. It involved a number of additional stages to the normal process, normally followed by RAC and the RAC Secretariat.

1.1.1 In the original CLH report the DS proposed:

Aquatic Acute 1, M-factor 1000 ($0.0001 < LC_{50} < 0.001$ mg/L) based on acute toxicity of the algae *Skeletonema costatum* 48 h LC_{50} = 0.0006 mg/L (Ward & Boeri, 2004).

Aquatic Chronic 1 with an M-factor 10 (0.001 < NOEC <= 0.01 mg/L) based on fish being the most sensitive species (*Pimephales promelas* 32 d NOEC = 0.00122 mg/L), the substance being considered not rapidly biodegradable.

1.1.2 Following the first public consultation, the DS proposed:

Aquatic Acute 1, M-factor 1000 ($0.0001 < LC_{50} < 0.001$ mg/L) based on acute toxicity of the algae *Skeletonema costatum* 48 h LC_{50} = 0.0006 mg/L (Ward & Boeri, 2004).

Aquatic Chronic 1 with an M-factor 100 (0.00001 < NOEC <= 0.0001 mg/L) based on *Skeletonema costatum* as the most sensitive species and that the substance being rapidly degradable.

Following public comments, the chronic classification proposal was based on the 48 hr NOEC of the Ward & Boeri *(2004) Skeletonema costatum* (NOEC=0.00004-0.000080 mg/L) study instead of the chronic study performed with *Pimephales promelas*. The DS also agreed that the algal studies were suitable for the assessment of chronic effects and may therefore be used as such in proposing a chronic classification. The ZnPT Industry CLH Consortium sent in a new ready biodegradability test according to OECD TG 301B, and this allowed the DS to consider the substance as rapidly biodegradable. The ZnPT Industry CLH Consortium also commented extensively on a second *Skeletonema costatum* study (*Rebstock, 2010*) indicating its preference for more reliable effect concentrations.

1.1.3 Information made available prior to RAC-45 and first plenary discussions on ZnPT

Following the first public consultation and prior to plenary discussion at RAC-45, two detailed reports (*Schamphelaere, 2018; Arts, 2018),* were made available discussing the merits and deficiencies of the *Ward & Boeri* (2004) and *Rebstock* (2010) studies (see section 3.3.1). In addition, preliminary reports of two new *Skeletonema costatum* experimental studies (*Goudie, 2018; Hoover, 2018*) were also supplied to the RAC.

1.1.4 Following the second (ENV), targeted public consultation, the opinion document proposed:

Following RAC-45 (June 2018), two new *Skeletonema costatum* studies (*Goudie, 2018*; *Hoover, 2018*) were supplied by the ZnPT Industry CLH Consortium (July 2018). These finalised reports constituted the reason for having an extra targeted public consultation, held from 18 July to 01 August 2018, and reassessment of all available data for *S. costatum* (see section 3.3.2).

Aquatic Acute 1, M-factor 1000 ($0.0001 < ErC_{50} < 0.001$ mg/L) based on acute toxicity of the algae *Skeletonema costatum* 72 hour ErC_{50} = 0.00088 mg/L (Goudie, 2018).

Aquatic Chronic 1 with an M-factor 10 ($0.0001 < ErC_{10} \leq 0.001$ mg/L) based on *Skeletonema costatum* 72 h ErC_{10} = 0.00068 mg/L (Goudie, 2018) and considering the substance to be readily biodegradable and, therefore, rapidly degradable.

1.1.5 Regarding the release of Zn^{2+} ions into the environment

The implications of the release of zinc ions into the environment were not explicitly considered in the CLH report and consequently are not considered in the opinion except to note that the Zn^{2+} contributes to the overall efficacy of the substance. RAC agrees with the DS in concluding that the coordination complex ZnPT (and therefore the contribution by the Zn^{2+} ion component) was tested adequately in the toxicity studies made available for this assessment.

Zn^{2+} is considered a category 1 hazard for both acute and chronic aquatic effects. However, other divalent cations are also present in the environment and we cannot be certain of their contribution to the observed effects. Pyrithione will act as a general divalent metal cation ionophore in aqueous solution so that Cu, Fe, Ca, Mg could all be considered as likely as Zn to influence toxicity. Studying the growth curves of *Skeletonema costatum* indicates that the degradation of the organic component (i.e. pyrithione) correlates with a return to growth after 48 hours. This would suggest that the biological effect absolutely requires the presence of the organic component because metal divalent cations cannot be degraded; only transformed by normal environmental processes which can either increase or decrease the bioavailability of the non-organic component. The DS and RAC consider that the biological effects are mediated predominantly by the organic component but also influenced by the availability of divalent metal cations in the environment.

1.2 Degradation:
Only the organic component of ZnPT was considered in detail in the CLH report by the DS, presumably due to the chelating nature of the substance and the high activity shown by the organic component of the substance.

ZnPT is hydrolytically stable. In a GLP study conducted according to US EPA guideline 161-1 (similar to OECD TG 111), hydrolysis half-lives were determined in pH 5, 7, and pH 9 buffers, and pH 8.2 in seawater. At the study termination 76–83% of the (zinc) pyrithione remained. The DT50 for ZnPT was 96-123 days (pH 5-9). In a second guideline GLP study at an initial ZnPT concentration of 5 mg/L, the DT50 was 63 days at pH 3, >1 year at pH 7, and 41 days at pH 11.

A number of studies were outlined by the DS (table 78, CLH report), the key study was identified as that by Clark (2002), ZnPT: Assessment of Ready Biodegradability; CO_2 Evolution Test (reliability/ Klimisch score 1). This ready biodegradability test according to OECD TG 301B was carried out with ZnPT (dose 13.2 mg/L, which is greater than the solubility limit of 4.93 mg/L) and activated sludge, CO_2 evolution was measured for 28 days. The degradation of ZnPT was 39% after 28 days (or according to the ZnPT Industry CLH Consortium, 49% once dissolved phase CO_2 was accounted for, which is in accordance with the OECD TG 301B method), and approximately 18% after 10 days incubation at 21°C. On the basis of these results ZnPT was **initially** considered not rapidly degradable.

The DS acknowledged in the response to comments (RCOM) document, following public consultation, that the ZnPT Industry CLH Consortium had provided a new 28 days ready biodegradability test according to OECD TG 301B, in June 2017 (Menzies, 2017). The study was well performed and conducted using radiolabelled ZnPT at test concentrations of 100, 210 and 520 µg/L. The test concentrations were selected in order to negate issues with inocula inhibition (see section 3.1.1), and at the same time were below the solubility limit for ZnPT (4.93 mg/L; Wenighofer, 2002). Mineralisation was evaluated by trapping the $^{14}CO_2$ generated during the study in base traps and using liquid scintillation counting to quantify percent of theoretical CO_2 evolution. At all tested concentrations, ZnPT exceeded the pass levels (≥ 60% $ThCO_2$, 28 days, respirometric methods) for ready biodegradability:

- 100 µg/L → 64.9 ± 0.4% CO_2 production in 28 d and met the 10 d window
- 210 µg/L → 65.7 ± 0.6% CO_2 production in 28 d and met the 10 d window
- 520 µg/L → 72.4 ± 2.1% CO_2 production in 28 d and met the 10 d window

The DS agreed with the ZnPT Industry CLH Consortium that this new ready biodegradability test fulfilled the CLP criteria of rapid degradability. The DS concluded ZnPT was demonstrated to be readily biodegradable - the pass level of the test (60% theoretical oxygen demand) was achieved within 10 days from the onset of biodegradation (see also section 3.1).

Table: *Summary of relevant OECD TG 301 ready biodegradation studies*

Guideline	Results	Key or supportive study	Remarks and initial concentration of test compounds	Reference
OECD TG 301B	^{14}C-ZnPT % of theoretical CO2 Evolution: 100 µg/L: 60.5 ± 0.1% 10 d window, 64.9 ± 0.4% 28 d 210 µg/L: 60.4 ± 0.1% 10 d window, 65.7 ± 0.6% 28 d 520 µg/L: 69.1 ±	Key study	Ready biodegradabilty test with activated sludge, measuring CO2 evolution over 28 d test period ZnPT: 100, 210 and 520 µg/L in accordance with Annex II, OECD TG 301 test guideline (below activated sludge respiration inhibition EC_{50}	Menzies, 2017 Reliability/ Klimisch score: 1
OECD TG 301B	ZnPT % of theoretical CO_2 evolution: 17% 6 d 49% 28 d	Supportive (formerly a key study)	Ready biodegradability test with activated sludge, measuring CO2 evolution after 28 d: ZnPT: 13.2 mg/L (significantly above activated sludge respiration inhibition EC_{50} and limit of	ZnPT PT21 submission A7.1.1.2.1/01 Clarke N, 2002 Reliability/ Klimisch score: 1

Conclusion

On **final** assessment by the DS, ZnPT was <u>considered rapidly degradable</u> for classification purposes.

1.3 Bioaccumulation

The DS described results from two sources (table 78, CLH report), one from the key study by Ward & Boeri (2001), Bioconcentration test with zinc [pyridine-2,6-^{14}C]Omadine and the oyster, *Crassostrea virginica* (reliability/ Klimisch score 2). The second study was with fish (Thor GmbH Art. 95 dossier, 2015, no further details). Radiolabelled test substance was used in each study, and the results were based on the total radioactivity in the tissues.

The oyster study with ZnPT was done according to OECD TG 305E using an intermittent flow-through system. A low- and a high-dose experiment was included. The bioconcentration factors (BCF) calculated by the steady-state and kinetic methods were similar and ranged from 7.8 to 11.0. A BCF calculated with QSAR gave a BCF of 1.4.

The n-octanol/water partition coefficient was determined according to OECD TG 117 (HPLC method) in the bioaccumulation study (Thor GmbH Art. 95 dossier, 2015) with fish. A log Pow of 1.21 was reported at pH 6.0+/-0.05 and 23ºC indicating no potential for bioaccumulation of ZnPT. The BCF factor for fish was estimated from log Pow = 1.21 and was 0.33.

The DS considered ZnPT to have low bioaccumulation potential.

1.4 Metabolites

The DS confirmed in the response to public comments document there was a data gap because of unreliable studies, when it comes to the identified metabolites PSA, OMSA, OMSiA and OTS. However, since the DS concludes that the substance is rapidly degradable this point is no longer of concern regarding the classification of the substance.

1.5 Aquatic toxicity

The available data in the CLH report on aquatic toxicity consist of 10 acute (see also table 75, CLH report) and 7 chronic (table 77, CLH report) studies. All studies are considered valid by the DS. The lowest acute and chronic test results for all three trophic groups are presented below.

1.5.1 Acute aquatic hazard

Valid acute toxicity tests with ZnPT are available for all three trophic levels (fish, crustaceans and algae).

Table: *Summary of relevant acute aquatic toxicity studies with ZnPT. Key study is in bold.*

Species	Test guideline	Test type, duration, ref	Result
Pimephales promelas (Fathead Minnow)	US EPA 72-1, GLP (comparable to OECD TG 203)	96h, flow through CAR Doc IIIA A7.4.1.1/01 Klimisch score 2 (1994)	NOEC = 0.0011 mg/L LC_{50} = 0.0026 mg/L
Oncorhynchus mykiss (Rainbow trout)	US EPA 72-1, GLP (comparable to OECD TG 203)	96h, flow through CAR Doc IIIA A7.4.1.1/03 Klimisch score 2 (1994)	NOEC = 0.0016 mg/L LC_{50} = 0.0032 mg/L
Danio rerio (Zebra fish)	OECD TG 203	96h, static Thor GmbH Art. 95 dossier Supportive	LC_{50} = 0.0104 mg/L
Americamysis bahia (Marine mysid)	US EPA-72-3(b), GLP	96h, flow through CAR Doc IIIA A7.4.1.2/03 Klimisch score 1-2 Boeri *et al*., (1993)	NOEC = 0.0016 mg/L LC_{50} = 0.0063 mg/L
Daphnia magna	OECD TG 202	48h, semi-static Thor GmbH Art. 95 dossier	EC_{50} = 0.051 mg/L
Daphnia magna	OECD TG 202, GLP	48h, flow through CAR Doc IIIA A7.4.1.2/02 Klimisch score 3 (**NA**) Smyth *et al*., (1994)	NOEC = 0.0056 mg/L EC_{50} = 0.050 mg/L
Daphnia magna	US EPA-72-2, GLP	48h, flow through CAR Doc IIIA A7.4.1.2/01 Klimisch score 3 (1994)	NOEC = 0.0011 mg/L EC_{50} = 0.0082 mg/L
Skeletonema costatum **(Marine diatom)**	**US EPA-123-2**	**48h, static** **CAR Doc IIIA A7.4.1.3/04** **Klimisch score 1-2** **Ward and Boeri (2004)**	**NOEC = 0.000220 mg/L (initial)** **NOEC (48 h) = 0.00004-0.00008 mg/L (TWA)**

			EC_{50} = 0.0006 mg/L (initial)
Raphidocelis subcapitata (fresh water algae)	US EPA-122-2, GLP	120h, static CAR Doc IIIA A7.4.3.1/01 Klimisch score 2 Boeri *et al*., (1994)	EC_{50} = 0.028 mg/L (120h) EC_{50} = 0.030 mg/L (72h) EC_{50} = 0.100 mg/L (48 h) NOEC = 0.0091 mg/L
Raphidocelis subcapitata (fresh water algae)	OECD TG 201	72h, 96h, static Thor GmbH Art. 95 dossier	NOEC = 0.0149 mg/L ErC_{50} = 0.051 mg/L

NA = not acceptable

Acute toxicity - fish:

ZnPT is very toxic to fish with LC_{50} <1 mg/L with the most sensitive species *Pimephales promelas* (Fathead minnow) with 96h LC_{50}=0.0026 mg/L.

Acute toxicity - aquatic invertebrates:

ZnPT, overall is very toxic to invertebrates (LC_{50} < 1 mg/L) with the most sensitive species, the marine shrimp *Mysidopsis bahia* 96h LC_{50} = 0.0063 mg/L.

Acute toxicity - to algae or other aquatic plants:

ZnPT was shown to have an adverse effect on the growth and growth rate of the marine diatom, *Skeletonema costatum*, the most sensitive species tested. Exposure of the diatom to ZnPT for 48h resulted in a NOEC of 0.000040–0.000080 mg/L. The 48h EC_{50} value for growth inhibition after 48h was 0.00060 mg/L. The DS concluded that ZnPT was very toxic to algae with a LC_{50} < 1 mg/L.

Acute toxicity - to other aquatic organisms:

ZnPT has a moderate to high effect towards microorganisms with a range of NOEC = 0.1 mg/L to 0.3125 mg/L. The effect of ZnPT on microbial activity in water was assessed by determining the level of inhibition of respiration of microorganisms present in activated sludge (Mead, 2001). The test was performed in accordance with OECD TG 209 and reported a 3h EC_{50} of 2.4 mg/L and a NOEC of 0.10 mg/L.

Another respiration inhibition test with activated sludge from a municipal treatment plant (Thor GmbH Art.95 dossier), 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 test results indicated a NOEC for ZnPt of 0.3125 mg/L and an EC_{50} of 2.82 mg/L.

Conclusion

The acute aquatic classification proposed by the DS was based on the Ward and Boeri (2004) algal toxicity study on the marine diatom *Skeletonema costatum*. **The DS proposed classification as Aquatic Acute 1, with an M-factor = 1000 (0.0001 < LC_{50} < 0.001 mg/L) based on the acute toxicity of the algae *Skeletonema costatum* 48 h LC_{50} = 0.0006 mg/L.**

1.5.2 Chronic aquatic hazard

Valid chronic toxicity tests with ZnPT are also available for all three trophic groups (fish, invertebrates and algae).

Table: *Summary of relevant chronic aquatic toxicity studies with ZnPT. The initial CLH report key study is in bold.*

Species	Test guideline	Test type, duration, ref	Result
Pimephales promelas **(Fathead Minnow)**	**OECD TG 210, GLP**	**32 d, flow through** **CAR Doc IIIA A7.4.3.2/01** **Klimisch score 1-2** **(1999)**	**NOEC = 0.00122 mg/L** **LOEC = 0.00282 mg/L**
Danio rerio (Zebra fish)	OECD TG 210, GLP	30 d, flow through Thor GmbH Art. 95 dossier	NOEC = 0.00125 mg/L LOEC = 0.00312 mg/L
Daphnia magna (Fresh water Daphnid)	US EPA-72-4(b), GLP	21 d, flow through CAR Doc IIIA A7.4.3.4/01 Klimisch score 2 Boeri *et al*., (1999)	[1.] NOEC = 0.0027 mg/L LOEC = 0.0058 mg/L EC_{50} = NA
Daphnia magna (Fresh water Daphnid)	OECD TG 211, GLP	21 d, flow through Thor GmbH Art. 95 dossier	NOEC = 0.0021 mg/L LOEC = 0.0039 mg/L
Americamysis bahia (Marine mysid)	US EPA-72-4(c), GLP	28 d, flow through CAR Doc IIIA A7.4.3.4/02 Klimisch score 2 Boeri *et al*., (1999)	NOEC = 0.00228 mg/L LOEC = 0.0042 mg/L EC_{50} = 0.0052 mg/L
Arbacia punctulata (sea urchin)	Non guideline, GLP	Fertilisation & Embryo Phase: Static Adult Phase: Flow-through Fertilisation Phase (FP): 3h Embryo Phase (EP): 48h Adult Phase (AP): 30 days Klimisch score 2 Boeri *et al*., (1999)	NOEC: FP = 0.0010 mg/L EP = 0.0290 mg/L AP = 0.0450 mg/L LOEC: FP = 0.0017 mg/L EP = 0.0600 mg/L AP = 0.0990 mg/L
Lemna gibba G3 (Duckweed)	US EPA-123-2, GLP	14 d, flow through (**7 d exposure**) 7 d (recovery) CAR Doc IIIA A7.4.3.5.2/01 Klimisch score 2 Ward et al., (1998)	NOEC = 0.0040 mg/L EC_{50} = 0.0096 mg/L

[1.] NOEC = 0.0022 mg/L and LOEC = 0.0049 mg/L reported in the CLH report but these differ to those reported in the CAR for the same study and evaluated by the CA: NOEC of 2.7 µg/L a LOEC of 5.8 µg/L based on the total numbers of living offspring per parent at test termination. This was the most sensitive indicator. The EC_{50}-value was calculated

using survival data, since no sub-lethal effects were observed at the end of the test. However this was not the case sublethal effects were observed and the EC_{50} was not considered valid.

NA = not acceptable

Chronic toxicity - fish

ZnPT has a high chronic toxicity to fish with 32d NOEC = 0.00122 mg/L with the most sensitive species *Pimephales promelas* (Fathead minnow).

Chronic toxicity - aquatic invertebrates

Tests were conducted in three different species: the Daphnid (*Daphnia magna*), the Mysid (*Americamysis bahia*) and the Sea urchin (*Arbacia punctulata*). ZnPT, overall has a high chronic toxicity towards invertebrates. The most sensitive species was *Daphnia magna* with NOEC 21d = 0.0021 mg/L (supported by results for *Americamysis bahia*).

Chronic toxicity - to algae or other aquatic plants

Toxicity to aquatic plants was tested in the fresh water species *Lemna gibba*, using ZnPT. ZnPT was shown to have an adverse effect on the growth rate, with 7d NOEC = 0.0040 mg/L.

Conclusion:

The DS **initially** proposed Aquatic Chronic 1, with an M-factor = 10 (0.001 < NOEC ≤ 0.01 mg/L) based on fish (*Pimephales promelas* 32d NOEC = 0.00122 mg/L) along with the conclusion that the substance was not rapidly biodegradable.

However, due to new information on biodegradation provided during the public consultation (see section 2.1.2), the DS proposed a different M-factor.

The **final** chronic aquatic classification proposed by the DS was based on the acute *Skeletonema costatum* study (*Ward & Boeri, 2004*). **The DS proposed classification as Aquatic Chronic 1, with an M-factor = 100 (0.00001 < NOEC ≤ 0.0001 mg/L)**.

Comments received during public consultation

2.1 First Public Consultation

2.1.1 ZnPT Industry CLH Consortia_Environmental Classification_Supportive Attachment June 2017.

The ZnPT Industry CLH Consortium provided comments targeted against the key study identified by the DS for their acute aquatic classification proposal. They also mentioned another study on *S. costatum* with ZnPT (Rebstock, 2010), that was not considered in the CLH report. They proposed:

- Aquatic Acute 1; H 400, M factor 100
- Aquatic Chronic 1; H410, M factor 1

The DS, however, justified their choice of the study with the algae *Skeletonema costatum* by Ward & Boeri (2004) as a key study, by referring to the CLP guidance. In particular, under section 4.1.3.2.4.3 (Guidance on WoE for substances for which more than one valid piece of data is available for a given data element), it is explicitly stated that "where more than one acceptable test is available for the same taxonomic group, the most sensitive (the one with the lowest $L(E)C_{50}$ or $NOEC/EC_{10}$) should be used". Both the Ward and Boeri (2004) algae study and the

Rebstock (2010) algae study had equal quality data. In their assessment, the DS simply chose the study with the most sensitive EC_{50} and NOEC.

In addition, the ZnPT Industry CLH Consortium supplied a new study following the OECD TG 301B, conducted using radiolabelled ZnPT (Menzies, 2017). The study demonstrated that ZnPT was readily biodegradable. The DS accepted this study and considered ZnPT to be rapidly degradable.

2.1.2 MSCA Comments

In total four MSCAs commented on hazards to the environment.

- 4 MSCAs agreed with category 1 classification for acute and chronic aquatic hazards.
- 3 MSCAs suggested the use of the NOEC from the acute study (Ward &Boeri, 2004) on *Skeletonema costatum* for chronic aquatic classification purposes - setting of revised chronic M-factor.
- Relevance of metabolites questioned by 1 x MSCA.
- 1 MSCA noted that the release of Zn should have been addressed in regarding the degradability of ZnPT and considered the release of zinc as a degradation product is in itself a reason to consider ZnPT as not rapidly degradable.
- 2 MSCAs supported ZnPT as not rapidly degradable, but this is assumed to be the case without their knowledge of the new study which was supplied around the time the comments were submitted.

Comments from several MSCA's pointed out (1) that algae are the most acutely sensitive species and that the 48h NOEC from the acute study is suitable for use as a chronic endpoint, and (2) according to the CLP Guidance, short-term algae tests also provide chronic endpoints (*i.e.* NOEC and EC_{10} values). Algae also cover diatoms and therefore, endpoints on *Raphidocelis subcapitata* as well as endpoints on *Skeletonema costatum* may be considered for chronic classification. Following this line of reasoning, the DS agreed in the RCOM document that the key study for the chronic classification should be the (acute) study on the marine diatom, *Skeletonema costatum.* The NOEC from this study as indicated in the CLH report is in the range of 0.000040 – 0.000080 mg/L based on time-weighted-average (TWA) measured concentrations. This NOEC range, along with the fact that the substance is considered as rapidly degradable, led the DS to propose a chronic M-factor of 100 (0.00001 < NOEC < 0.0001 mg/L).

2.2 Second, Targeted Public Consultation

2.2.1 Industry and MS comments on two new S. costatum studies

New (preliminary) information became available just prior to RAC-45 (June 2018). Two new GLP, guideline studies on *Skeletonema costatum* were briefly presented and the committee agreed to a new, targeted public consultation, which was held from xx to yy 2018, upon receipt of the final, validated/audited study reports. Please refer to section 3.3 for presentation of key data and analysis.

Following the targeted public consultation, there were six comments, two by Industry and four by MS. The two Industry comments considered the Ward & Boeri (2004) study fundamentally flawed and unreliable. Three MS commented, two supported the use of the new studies in determining classification while one MS sought further clarifications. There was some misunderstanding in these comments with regard to the chronic M factor determination with MS assuming ZnPT to be not rapidly degradable.

3.1 Rapid Degradation: Menzies (2017) 28 days ready biodegradability test (OECD TG 301B) on ZnPT

The pyrithione moiety is considered rapidly degradable based on the Menzies (2017) study performed according to OECD TG 301B. This recently completed study was submitted by the ZnPT Industry CLH Consortium. The protocol was modified according to the Annex II of the OECD TG due to the inhibitory effect of the test substance on sewage sludge microorganisms: the test concentration was reduced in line with the guideline suggesting 1/10 of the activated sludge respiration inhibition EC_{50}.

3.1.1 Justification of the use of low test substance concentrations in the OECD TG 301B study by Menzies (2017)

ZnPT is a biocidal substance and known to have both fungistatic and bacteriostatic properties so toxicity to activated sludge microorganisms (the inoculum) is an important consideration when performing a ready biodegradation study. Another point to consider is the low water solubility of the substance (4.93 mg/L). The normal concentrations used for ready biodegradation studies typically range from 10 – 20 mg DOC/L and so are not applicable for investigating the biodegradability of ZnPT. The inhibitory effects of the substance were highlighted both in the CAR and CLH report. A GLP ZnPT OECD TG 209 activated sludge respiration inhibition study (Mead, 2001) was briefly described by the DS in the CLH report. This was previously evaluated by the Rapporteur Member State (RMS) and given a reliability/ Klimisch rating of 2 and determined acceptable in the 2016 CAR (DOC IIIA; section A7.4.1.4/01). Furthermore, a second study by Weniger (2002) reported in the CAR (DOC IIIA; section A7.4.1.4/02, reliability/ Klimisch score 2, acceptable) substantiates the results of the Mead (2001) study indicating inhibition of test organisms at low levels of test substance.

Table: *Summary of relevant activated sludge respiration inhibition tests (nominal concentrations)*

Guideline	Results (mg/L)	Reference
OECD TG 209, GLP Purity 97.2%	EC_{50} (3h): 2.4 NOEC (3h): 0.1	Mead, 2001 CAR A7.4.1.4/01
OECD TG 209, GLP Purity a.i. 97.9%	EC_{20} (3h): 0.44 EC_{50} (3h): 1.84	Weniger, 2002 CAR A7.4.1.4/02

There was no internal toxicity test reported in the Menzies (2017) study to check the inhibitory effect of the test substance. The results from the OECD TG 209 tests (e.g. ZnPT inhibited respiration by 27% at the lowest test concentration of 0.69 mg/L in the Weniger, 2002 study), indicate strong inhibition of respiration. Due to this activity of the substance and its low intrinsic solubility, it was considered valid to have modified the protocol according to Annex II of OECD TG 301 such that the test concentration may be reduced to 1/10 of the activated sludge respiration inhibition EC_{50} (or at least below the EC_{20}). Furthermore, because the EC_{50} values are considerably less than 20 mg/L, low test concentrations and radiolabelled test substance were entirely appropriate as employed in the Menzies (2017) study.

Point 1 from Annex II of the OECD TG 301 specifying conditions under which the test concentrations can be modified could not be verified, this however is not considered to invalidate the study or detract from the final results. The recommended inocula have been satisfied for both OECD TG 209 tests described above (Mead, 2001; Weniger, 2002; suspended solids

concentrations of 2 – 4 g/L) and the OECD TG 301B test (suspended solids concentrations ≤ 30 mg/L) adhered to by Menzies (2017).

3.1.2 Investigations into the ready biodegradability of ZnPT:

In the initial dossier submission, the Dossier Submitter (DS) identified the Clarke (2002) study as the key ready biodegradability study in the CLH report. The ZnPT Industry CLH Consortium, during the first public consultation, submitted a recently completed OECD TG 301B Ready Biodegradability study as an alternative for consideration (Menzies, 2017).

In the Clarke (2002) study, the poor water solubility of the test item was not adequately considered; there was only a single test concentration of 13.2 mg/L investigated. The study did not appear to take the suspected toxicity to the inoculum into account. There is a clear lag phase of approximately 3-6 days in the biodegradation curve of the test sample. However, the toxicity control shows little indication of inhibition of respiration. This result is unclear. It may simply suggest that ZnPT as the sole source of carbon is difficult to utilise relative to the reference control (sodium benzoate). ZnPT showed significant mineralisation reaching 49% CO_2 evolution after 28 days. ZnPT failed to meet the OECD TG 301B Ready Biodegradation test pass criteria (60% CO_2 generation in 28 d and meeting the 10 d window).

The toxicity control (containing the test material and sodium benzoate) outperformed the test sample and failed to demonstrate significant inhibition of the activated sludge microorganisms (73% degradation after 14 days and 71% degradation after 28 days). The biodegradation curve is remarkably similar to the sodium benzoate control with no lag phase. This would suggest some degree of toxicity to the inoculum, or possibly competition amongst different carbon sources for growth.

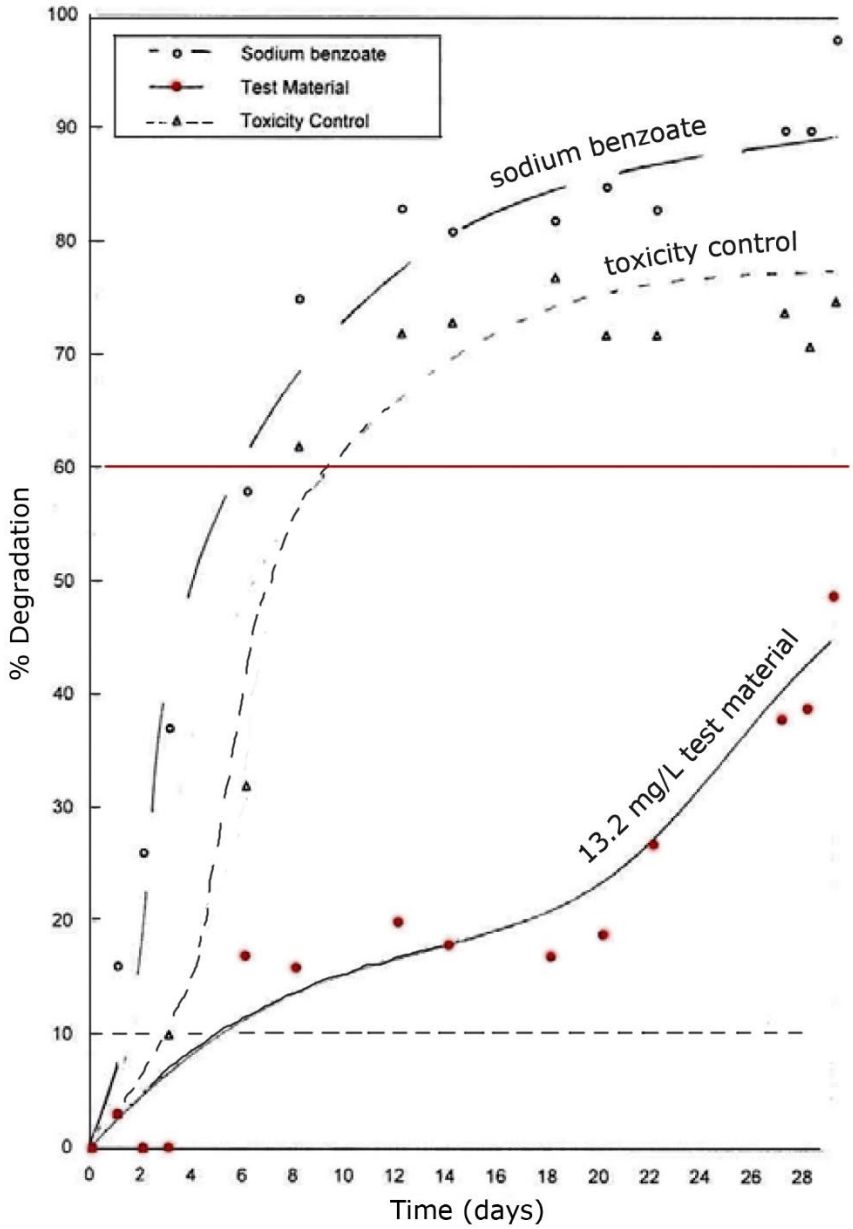


Figure 3: Biodegradation curves from *Clarke 2002*.

The Menzies (2017) study followed the OECD TG 301B and was conducted using radiolabelled ZnPT at test concentrations of 100, 210 and 520 µg/L.

At all test concentrations, ZnPT was readily biodegradable. 100 µg/L test systems reached 64.9 ± 0.4% CO_2 production in 28d and met the 10d window; 210 µg/L test systems reached 65.7 ± 0.6% CO_2 production in 28d and met the 10d window; 520 µg/L test systems reached 72.4 ± 2.1% CO_2 production in 28d and met the 10d window. All test samples showed a brief lag phase of approximately 4d demonstrating CO_2 production was delayed in comparison to the functional control, thus indicating that the test item caused inhibitory effects on the bacteria.

The validity criteria for ready biodegradability were met in this study:

- The differences in plateau values of the test replicates was less than 20%.

- The percentage degradation of the reference compound reached the pass levels well in advance of day 14 (on day 4, the reference material treatments reached 65.1 ± 1.0% $ThCO_2$ evolution).
- A concurrent toxicity test was not run, but results from other toxicity tests (OECD TG 209, GLP) indicated the test substance was inhibitory and as per Annex II of OECD TG 301, low concentrations of test substance were investigated.
- The inoculum blank was run in duplicate. One sample exceeded 40 mg/L total CO_2 evolution (rep 1: 47.8 mg/L) and was excluded from further consideration, while one did not (rep 2: 30.6 mg/L) after 28 days. The mean value, if calculated, would be still less than 40 mg/L and RAC considers this as an alternative argument to support validity instead of just dismissing one study as an "outlier" (validity criterion: < 40 mg/L after 28 days).
- The use of radiolabelled zinc ^{14}C-pyrithione allows for the specific measurement of evolved CO_2 from the degradation of test substance. The inorganic carbon (IC) content of the test substance suspension in the mineral medium at the beginning of the test was not measured or required.

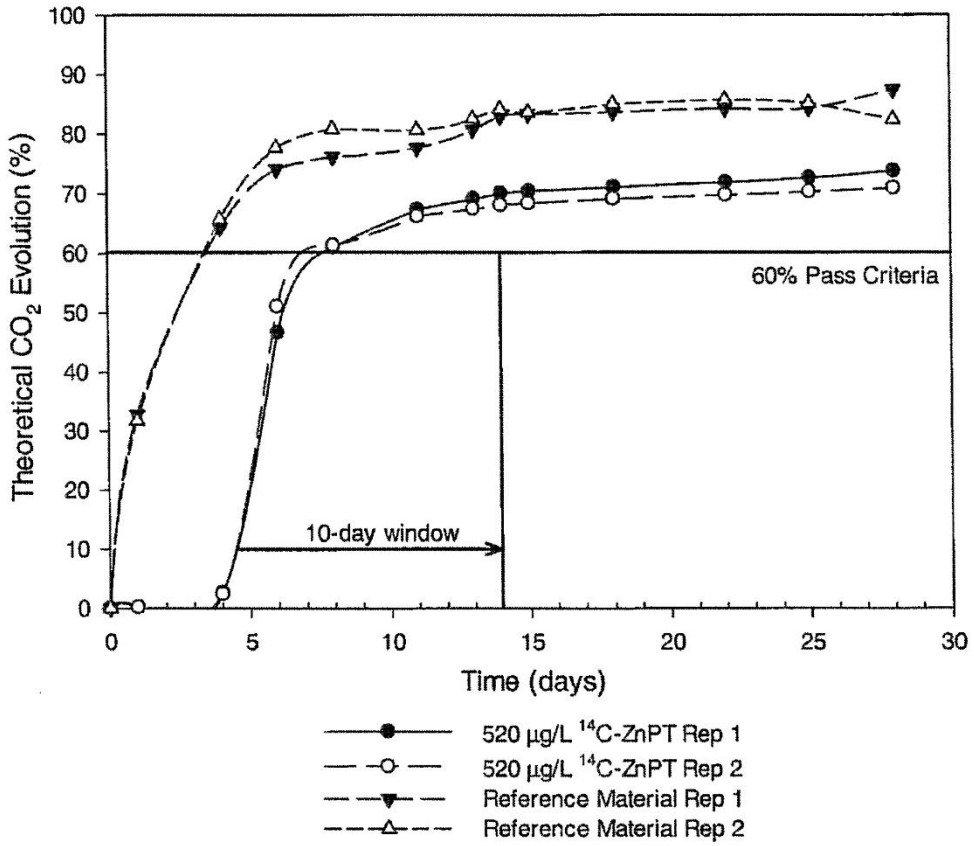


Figure 4: Biodegradation curves from Menzies 2017. All test samples had very similar plots. This plot was for the highest concentration of ZnPT tested (520 µg/L).

3.1.3 Conclusion

The evidence is sufficient to support rapid degradation of the organic part of the substance. In Section 4.1.3.2.4.5 of the CLP guidance document concerning evidence in degradation, it states that where conflicting datasets exist for a single chemical then the data of the highest quality and best documentation should be used in determining the rapid degradability of the test

substance, *cf.* "positive results in ready biodegradability tests can be considered valid, irrespective of negative results, when the scientific quality is good and the test conditions are well documented, i.e. guideline criteria are fulfilled, including the use of non-pre-exposed (non-adapted) inoculum." (Guidance on the Application of the CLP Criteria Version 5.0 – July 2017, Annex II.3.5). In this case, the Menzies (2017) study would appear to be the most appropriate. ZnPT is thus considered readily biodegradable, and therefore rapidly degradable for classification purposes.

3.2 CAR Section A7.4.1.3/04 Growth inhibition test on algae - Ward TJ, Boeri RL (2004)

This Section has now moved under 'Supplemental information - In depth analyses by RAC', with a conclusion that, in light of new data from studies only now available following the publication of the CLH report and the public consultation period, these estimates are superseded by endpoints based on actual measured data.

3.3 The most sensitive species for acute/chronic aquatic toxicity of ZnPT

The key question in the assessment of the aquatic toxicity of ZnPT asks "Is *Skeletonema costatum* the most sensitive species for assessment of acute and chronic aquatic toxicity studies?" It has been suggested that the Ward & Boeri (2004) study may be considered an outlier caused by poor technique and inadequate experimental design. A brief description of all the available (4) *Skeletonema costatum* studies can be seen in the following table.

Table: *Summary of Skeletonema costatum acute aquatic toxicity studies with ZnPT. Original Key study according to the DS in bold.*

Species	Test guideline	Test type, duration, ref	Result
***1. Skeletonema costatum* (Marine diatom)**	**US EPA-123-2** Control cultures only demonstrated exponential growth during the first 48 h. The DS suggested use of the 48 h EC_{50} for acute classification purposes (exposure period acceptable according to OECD TG 201 [typically 72h], but shorter than that recommended by US guidelines and ASTM E1218-04 for *S. costatum*, i.e. 96-120h). Many assumptions were made in extrapolating the TWA values from degradation rates, especially in reference to the Fenn, 2005 photolysis study. This introduces significant uncertainty in the final results.	**120h, static test (growth rate levelled off after 48h)** **CAR Doc IIIA A7.4.1.3/04** **Klimisch score 1-2** ***Ward and Boeri* (2004)**	**NOEC = 0.220 µg/L (initial)** **NOEC (48h) = 0.00004-0.00008 mg/L (TWA)** **ErC_{50} (48h) = 0.0006 mg/L**

	Of concern is the presence of copper contamination (Cu 40 µg/L) in the culture and test medium (5 times above the 772hr Q1 NOEC in the copper REACH dossier for this species) and the presence of particulate matter at 36 mg/L. In contrast to the guidelines, unfiltered natural seawater was used to formulate media. Measured concentrations were only available for the start and the end (120h) of the study. All measurements at the 120h time point were < LOQ (0.029 µg/L).		
2. Skeletonema costatum (Marine diatom)	US OPPTS 850.5400	120 h static test CAR Doc IIIA A7.4.1.3/55 Klimisch score 2 *Rebstock, (2010)* Endpoints were derived from mean measured concentrations.	NOErC (72h) = 0.31 µg/L ErC_{10} (72h) = 0.0019 mg/L (ZnPT Industry CLH Consortium, 2017) ErC_{50} (72h) > 0.0038 mg/L ErC_{10} (48h) = 0.0014 mg/L (*Schamphelaere, 2018*) ErC_{50} (48h) > 0.0060 mg/L (*Schamphelaere, 2018*)
3. Skeletonema costatum (Marine diatom)	U.S. EPA OCSPP 850.4500	120 h static test Goudie (2018) Report 86820 All endpoints derived from measured concentrations.	NOErC (72h) = 0.26 µg/L ErC_{50} (72h) = 0.00088 mg/L ErC_{10} (72h) = 0.00068 mg/L

4. Skeletonema costatum (Marine diatom)	U.S. EPA OCSPP 850.4500	120 h static test Hoover (2018) Report 86821 All endpoints derived from measured concentrations.	NOErC (72h) = 0.42 µg/L ErC_{50} (72h) = 0.00097 mg/L ErC_{10} (72h) = 0.00078 mg/L

3.3.1 Reliability of the Ward & Boeri (2004) and Rebstock (2010) algal toxicity studies.

The Dossier Submitter (DS) following the initial public consultation, based the acute aquatic classification proposal on the Ward & Boeri (2004) algal toxicity study using the marine diatom *Skeletonema costatum*. The DS proposed Aquatic Acute 1, with an M-factor = 1000 (0.0001 < LC_{50} < 0.001 mg/L) based on the acute toxicity of *Skeletonema costatum* 48 h EC_{50} = 0.0006 mg/L (initial concentration). Industry submitted an argument in support of the *Rebstock*, 2010 study instead of the Ward & Boeri (2004) study, outlining numerous problems with the latter study. The main Industry comments to reject the older study were based on poor technique, poor culture conditions and that the calculation of a time weighted average (TWA) concentration using results below the limit of quantitation (LOQ) was highly uncertain and the validity of a study in such cases requires further confirmation. Nevertheless, the Ward & Boeri (2004) study was evaluated in the BPR PT21 and rated as Klimisch 1-2 by the evaluating Competent Authority (eCA) at that time and was therefore considered acceptable for classification purposes by the DS in drafting the CLH report. The TWA was recalculated based on a better estimate for when LOQ is reached (using photolysis rate data derived from the Fenn, 2005 study) and was determined to be 0.000040 – 0.000080 mg/L.

An additional study on *S. costatum* with ZnPT (Rebstock, 2010) was submitted for evaluation and was considered reliable (RI=2) in the BPR PT21 evaluation of copper pyrithione, it was not included in the CLH report. The Industry argument that the Rebstock (2010) study with *Skeletonema costatum* is more reliable than the Ward & Boeri (2004) study on the same species appears to be a valid one. The growth rates in the control cultures of the Ward & Boeri (2004) study were variable and approached a plateau between 96 and 120 h (which appears to be the more appropriate timescale for endpoints for this species according to US guidelines). Indeed, growth was clearly not exponential (i.e. not log-phase) in both studies for the entire 120h. During the first 48h in the Ward & Boeri (2004) study there is a 33-fold increase (control) in the average sectional growth rate or 42-fold increase (solvent control), which would be sufficient to use the 48h data (instead of 72h according to OECD TG 201) for deriving the algae effect estimates. However, a new review report (*Schamphelaere, 2018*[1]), submitted by Industry after the public consultation period, cast further doubt on the validity of the study by *Ward & Boeri* (2004). According to the author, the biggest concerns for the Ward & Boeri (2004) study in comparison with the Rebstock (2010) study are methodological in nature:

i. 14h/10h light/dark in Rebstock (2010), as recommended in the US guidelines specifically for *S. costatum*,vs. 24h/0h in Ward & Boeri (2004), as recommended by default in OECD TG 201 for freshwater algae;

[1] Schamphelaere (2018) Comparative evaluation of two toxicity studies of Zinc-pyrithione to *Skeletonema costatum* for classification purposes.

ii. a different medium, i.e. synthetic seawater in Rebstock (2010) vs. natural unfiltered seawater with high particulate matter concentration (36 mg/L), 1.6 mg TOC/L and unintended Cu (40 ± 16 µg/L, 63µM) and Pb contamination (19.4 µg/L, 93.6 nM) in Ward & Boeri (2004);

iii. different initial algal cell densities, i.e. 77,000 cells/mL in Rebstock (2010) vs. 10,000 cells/mL in Ward & Boeri (2004);

iv. the complete loss in the Ward & Boeri (2004) study after 120 hours and the non-availability of intermediate time-points and final analytical determinations prohibits any reliable precise calculation of time-averaged concentrations. Hence, the NOECs and ErC50 reported in this study should be considered upper boundaries of the true values, i.e., they should be reported as "lower-than-values" ("<"-values) and not as "equal-to-values".

Overall the author concludes both studies have some design limitations, limitations in terms of exponential growth (control not always valid according to OECD criteria) and in terms of calculation methods of effect estimates. The points of most concern for the Ward & Boeri (2004) study is the presence of Cu in culture and test medium 5 times above the 72h NOEC in the REACH registration dossier for copper in *Skeletonema costatum* and the additional uncertainty imposed by modelling and extrapolating photolytic losses as applied in the biocide CAR, section A7.4.1.3/04. Factors such as light intensity and spectrum, attenuation by glassware and (growing) algae, influences of TOC and suspended matter, and test vessel dimensions were not reflected in such an estimate.

A second new review report was also submitted by Industry (Arts, 2018[1]). This report provided similar conclusions to that of Schamphelaere (2018). Both authors assessed both algal studies, evaluating them based on the Criteria for Reporting and Evaluating Ecotoxicity Data method (CRED) (Moermond *et al*., 2016[2]).

The DS commented on its preference for the Ward & Boeri (2004) study as the key study for *Skeletonema costatum* following the public consultation period. At that time there were only two available studies. The DS concluded that according to the CLP Guidance where there is more than one acceptable test for a particular taxonomic group, the most sensitive (the one with the lowest L(E)C50 or NOEC/EC10) should be used. RAC generally endorses this approach together with consideration of study reliability and quality of data. RAC recognises that there are some difficulties with both *Skeletonema costatum* studies, and neither are suitable as key studies in the context of newer data now available (see the following sections of the opinion).

3.3.2 Assessment of all Skeletonema costatum toxicity studies available (post-RAC-45), with final data from new studies

One RAC member commented that if Industry performed repeat studies with *Skeletonema costatum* then we might be in a better position to judge whether the Ward & Boeri (2004) study is indeed an outlier caused by poor technique. There is uncertainty in the results due to assumptions regarding the half-life of ZnPT and using photolytic degradation rates and the

[1] Arts (2018) Abstract of Summary and evaluation of Two algal inhibition studies investigating the toxicity of zinc pyrithione to the marine diatom *Skeletonema costatum*.

[2] Moermond *et al.*, (2016) CRED: Criteria for reporting and evaluating ecotoxicity data. Environmental Toxicology and Chemistry, 35 (5), pp. 1297-1309.

estimated time to an LOQ value to derive (TWA) NOECs. Industry submitted a new position paper (ZnPT Industry CLH Consortium, 2018[1]) making available additional (preliminary) information on two new GLP, guideline studies on *Skeletonema costatum.* These preliminary reports arrived with effect estimates derived from nominal concentrations rather than actual or measured concentrations. It was not possible at RAC-45 for an in-depth assessment of these studies. An initial assessment as presented by the ZnPT Industry CLH Consortium in their May 2018 position paper and a cursory overview of the preliminary study data by the RAC Rapps was however introduced at RAC 45. A new targeted public consultation was arranged on receipt of the finalised, audited versions of the two new *S. costatum* studies (Goudie, 2018; Hoover, 2018).

These two new GLP studies on *Skeletonema costatum* were conducted in 2018 to further clarify the sensitivity to ZnPT towards this species. The studies were conducted using recommended culturing conditions for *Skeletonema costatum* as described in the US guidelines (OSCPP 540.5400) and met reporting requirements and validity criteria according to OECD TG 201. The initial results (with reported nominal concentrations only), from both studies did not appear to support the effect concentrations determined from the Ward and Boeri (2004) study. However, on receipt of the final measured analytical results from both of the new studies a strong conclusion may be drawn which supports the original *Ward & Boeri* (2004) assumption in that *S. costatum* should be considered the most sensitive taxon for acute and chronic aquatic toxicity studies with ZnPT. The two 2018 studies allow for an accurate and reliable determination of the preferred ErC10 value rather than an uncertain NOErC value for assessment of chronic effect levels.

There are in total four studies available to characterise the toxicity of ZnPT to *S. costatum*:

1. *Ward and Boeri* (2004)
2. *Rebstock* (2010)
3. Report 86820, *Goudie* (2018)
4. Report 86821, *Hoover* (2018)

Ward & Boeri (2004) and Rebstock (2012) have been commented on in detail and both are found to have deficiencies e.g. in respect of their design, limitations in terms of exponential growth of the controls and OECD validity criteria, and contamination of growth media.

The two new studies (Goudie, 2018; Hoover, 2018) were designed primarily according to US guidelines (OSCPP 540.5400) but are also consistent with OECD TG 201. Both studies are identical in study design and chosen test concentrations and sponsored by the same Industry partner and performed by the same testing laboratory. They were conducted by different study directors and run at different times using different batches to guarantee independence of results. The studies were conducted using study conditions appropriate for *S. costatum*, included daily analytical determinations, and produced robust statistical results with low variability, suggesting optimal algal growth conditions.

Similar to the Rebstock (2012) study, the test media were prepared by the addition of appropriate reagent grade salts to filtered synthetic sea water. Seven nominal test concentrations (0.20, 0.40, 0.80, 1.6, 3.2, 6.4 and 12.8 µg ZnPT/L) were prepared using DMF as solvent and tested in triplicate. The test concentrations were analysed for ZnPT after 0, 24, 48, 72, 96, and 120h exposure by HPLC/MS/MS. The studies were conducted using 3 days old algal culture at test

[1] ZnPT INDUSTRY CLH CONSORTIUM COMMENTS ON ENVIRONMENTAL TOXICITY on the 1st draft RAC Opinion (ODD) of 17 April 2018 from the Rapporteur proposing harmonised classification and labelling at EU level on pyrithione zinc; (T-4)-bis[1-(hydroxy-.kappa.O)pyridine-2(1H)-thionato-.kappa.S]zinc.

initiation with an initial algal cell concentration of approximately 10,000 cells/mL. The tests were maintained at 20°C in a 14h light: 10h dark photoperiod as is standard for *S. costatum* and appropriate for light-sensitive substances. There were no statistical significant differences in the algal cell concentration results between the control and vehicle control samples from either study.

A comparison of the key design features of the four studies, including the culturing conditions, availability of analytical data and incorporation of exposure concentrations into the reported results, are shown below.

Table: *Summary of Test conditions and reporting in the four studies on S. costatum*

Species	**Initial cell density**	**Test medium**	**Contaminants**	**Light : dark cycle**	**Analytical Time points**	**Results**
Ward & Boeri (2004)	10,000 cells/mL (estimated)	Unfiltered sea water	Particulate: 36 mg/L Cu: 40 µg/L Pb: 19.4 µg/L	24:0	0 and 120h	Nominal
Rebstock (2010)	77,000 cells/mL (estimated)	Synthetic marine medium	Filtered Cu: <10 µg/L Pb: <2 µg/L	14:10	0, 24, 48, 72, 96 and 120h	Mean measured
Goudie (2018)	9,260 cells/mL (measured)	Synthetic marine medium	Filtered Cu: <5 µg/L Pb: <1 µg/L	14:10	0, 24, 48, 72, 96 and 120h	Mean measured
Hoover (2018)	8,890 cells/mL (measured)	Synthetic marine medium	Filtered Cu: <5 µg/L Pb: <1 µg/L	14:10	0, 24, 48, 72, 96 and 120h	Mean measured

A comparison of all four studies based on mean measured concentrations is not possible, since such measurements were not done in the *Ward & Boeri* study and there were differences in [light : dark] cycles, initial cell densities and exponential growth rates at different time periods.

The cell density in the controls was greater than 16 times initially inoculated at 72 hours and the guideline criteria were met with the coefficients of variation for cell density in both studies. The other validity criteria according to guidelines OECD TG 201 and U.S. EPA OCSPP 850.4500 were also met sufficiently.

3.3.2.1 Goudie (2018) Zinc Pyrithione: Static Growth Inhibition Test with the Marine Diatom, Skeletonema costatum (study no. 86820).

Table: *Summary of the measured concentration of ZnPT in the growth medium*

	Zinc Pyrithione Concentration (µg/L)						
Sample	Nominal	Time-0	24 hours	48 hours	72 hours	96 hours	120 hours
Control	0.00	(0.01)	<LOD	(0.02)	<LOD	(0.02)	<LOD
Vehicle control	0.00	(0.01)	(0.01)	(0.02)	<LOD	<LOD	<LOD
Level 1	0.20	0.13	(0.05)	(0.03)	(0.01)	(0.02)	(0.01)
Level 2	0.40	0.26	0.08	(0.04)	(0.03)	(0.02)	(0.02)
Level 3	0.80	0.56	0.14	0.06	0.05	(0.04)	(0.04)
Level 4	1.60	1.13	0.29	0.12	0.12	0.11	0.08
Level 5	3.20	2.87	0.63	0.39	0.32	0.18	0.15
Level 6	6.40	4.64	1.75	0.85	0.71	0.50	0.36
Level 7	12.80	9.31	4.79	3.13	2.41	2.17	1.27
Low spike 1	0.16	0.13	0.14	0.13	0.12	0.13	0.13
Low spike 2	0.16	0.14	0.12	0.14	0.12	0.12	0.13
High spike 1	15.4	11.98	11.69	11.57	11.64	11.25	0.26
High spike 2	15.4	11.58	12.10	11.72	12.15	11.09	0.26

<LOD (Limit of Detection) = value below 0.0115 µg/L; Values in parentheses between LOD (0.0115 µg/L) and LOQ (Limit of Quantitation, 0.0517 µg/L)

Validity Criteria according to OECD TG 201:

(1) The number of cells in the controls showed a 3x, 10x, 25x, 53-55x and 109-117x increase by 24, 48, 72, 96 hours and 120 h, respectively. The growth rate in controls was considered sufficient to verify logarithmic phase from the 72hr time point and meets the validity criteria for biomass increase in OECD TG 201 (minimum 16-fold increase during exposure duration). See also figure 5 for growth curves over 72 hours at measured concentrations of ZnPT.

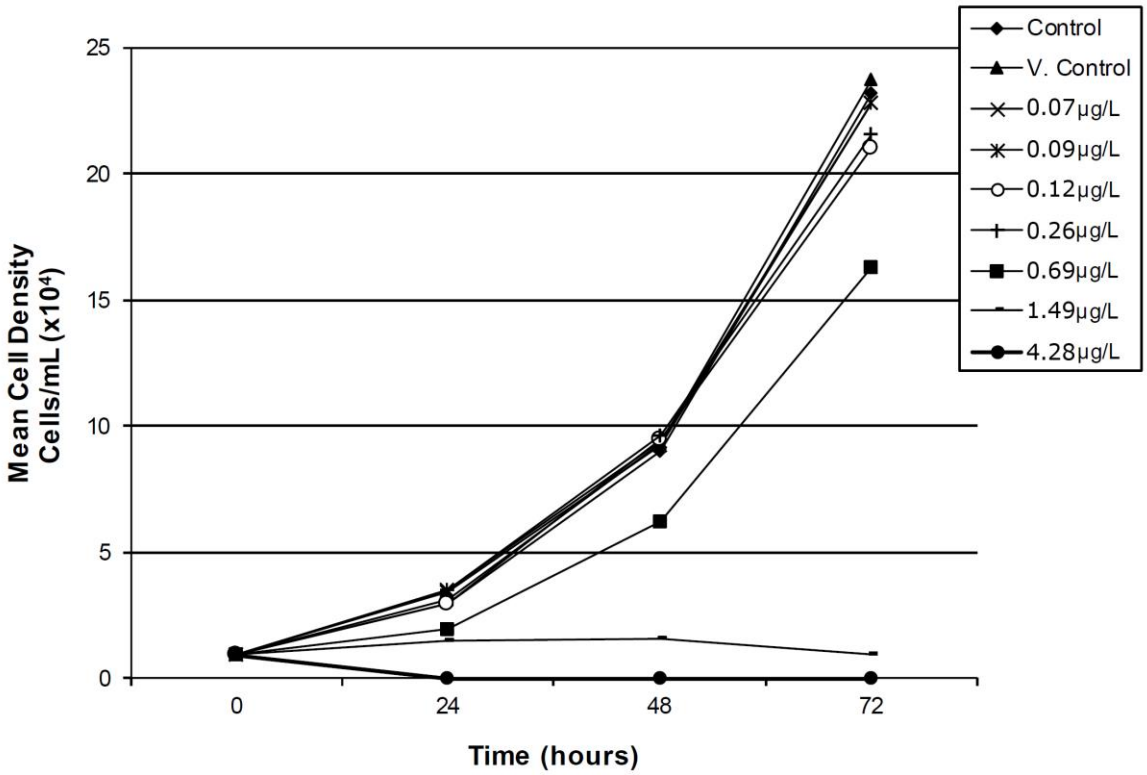


Figure 5: Growth Curves for *Skeletonema costatum* during a 72-hour exposure to ZnPT (geometric mean measured concentrations).

(2) The variability of the cell density and the growth rates in the control replicates were low. The reported CVs are 5 to 8% for cell density and 2% for average specific growth rate at 72 hours. This meets the validity criteria in OECD TG 201 (CV should not exceed 10% for less frequently tested species).

(3) The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for the 72 hours test) in both controls did not exceed 10%, being 4-5%, 6-9% and 4-7% for each respective interval. This meets the validity criteria in OECD TG 201 (CV should not exceed 35% for a 72 hours test).

Table: *Goudie (2018) Summary of the effect concentrations (i.e., ErC) based on growth rate. Results are based on geometric mean measured concentrations (µg a.i./L)*

EC type	48-hour	72-hour	96-hour
ErC10	1.35	0.68	0.518
ErC50	1.68	0.88	0.645
NOEC	0.34	0.26	0.22

This study satisfied the OECD TG 201 guideline validity requirements for a growth inhibition test with *Skeletonema costatum*. Choosing a time point other than the recommended 72-hours was not warranted. The effect concentrations associated with the 72-hour period are taken forward for the assessment of aquatic toxicity classification.

3.3.2.2 Hoover (2018) Zinc Pyrithione: Static Growth Inhibition Test with the Marine Diatom, Skeletonema costatum (draft report, study no. 86821).

Table: *Summary of the measured concentration of ZnPT in the growth medium*

	Zinc Pyrithione Concentration (µg/L)						
Sample	Nominal	Time-0	24 hours	48 hours	72 hours	96 hours	120 hours
Control	0.00	(0.02)	<LOD	<LOD	(0.01)	<LOD	<LOD
Vehicle control	0.00	(0.01)	(0.01)	<LOD	(0.01)	(0.01)	<LOD
Level 1	0.20	0.17	0.07	(0.04)	(0.03)	(0.03)	(0.02)
Level 2	0.40	0.41	0.09	(0.05)	(0.04)	(0.04)	(0.04)
Level 3	0.80	0.67	0.19	0.11	0.09	0.07	0.06
Level 4	1.60	1.37	0.42	0.28	0.19	0.13	0.12
Level 5	3.20	2.18	0.96	0.60	0.40	0.33	0.27
Level 6	6.40	5.39	2.67	1.98	1.40	1.21	0.90
Level 7	12.80	13.19	5.90	5.07	3.71	3.37	2.41
Low spike 1	0.16	0.16	0.16	0.16	0.16	0.18	0.17
Low spike 2	0.16	0.16	0.17	0.16	0.18	0.17	0.17
High spike 1	15.4	13.60	13.93	14.63	13.77	15.08	14.39
High spike 2	15.4	13.53	13.97	14.29	13.72	14.57	14.15

<LOD (Limit of Detection) = value below 0.0115 µg/L; Values in parentheses between LOD (0.0115 µg/L) and LOQ (Limit of Quantitation, 0.0517 µg/L)

Validity Criteria according to OECD TG 201:

(1) The number of cells in the controls showed a 4x, 12-13x, 47x, 97-99x and 163-175x increase by 24, 48, 72, 96h and 120h, respectively. The growth rate in controls was considered sufficient to verify logarithmic phase from the 72h time point and meets the validity criteria for biomass increase in OECD TG 201 (minimum 16-fold increase during exposure duration). See also figure 5 for growth curves over 72h at measured concentrations of ZnPT.

(2) The variability of the cell density and the growth rates in the control replicates were low. The reported CVs are 5 to 7% for cell density and 1-2% for average specific growth rate after 72h. This meets the validity criteria in OECD TG 201 (CV should not exceed 10% for less frequently tested species).

(3) The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for the 72h test) in both controls did not exceed 12%, being 7%, 8-12% and 2-9% for each respective interval. This meets the validity criteria in OECD TG 201 (CV should not exceed 35% for a 72h test).

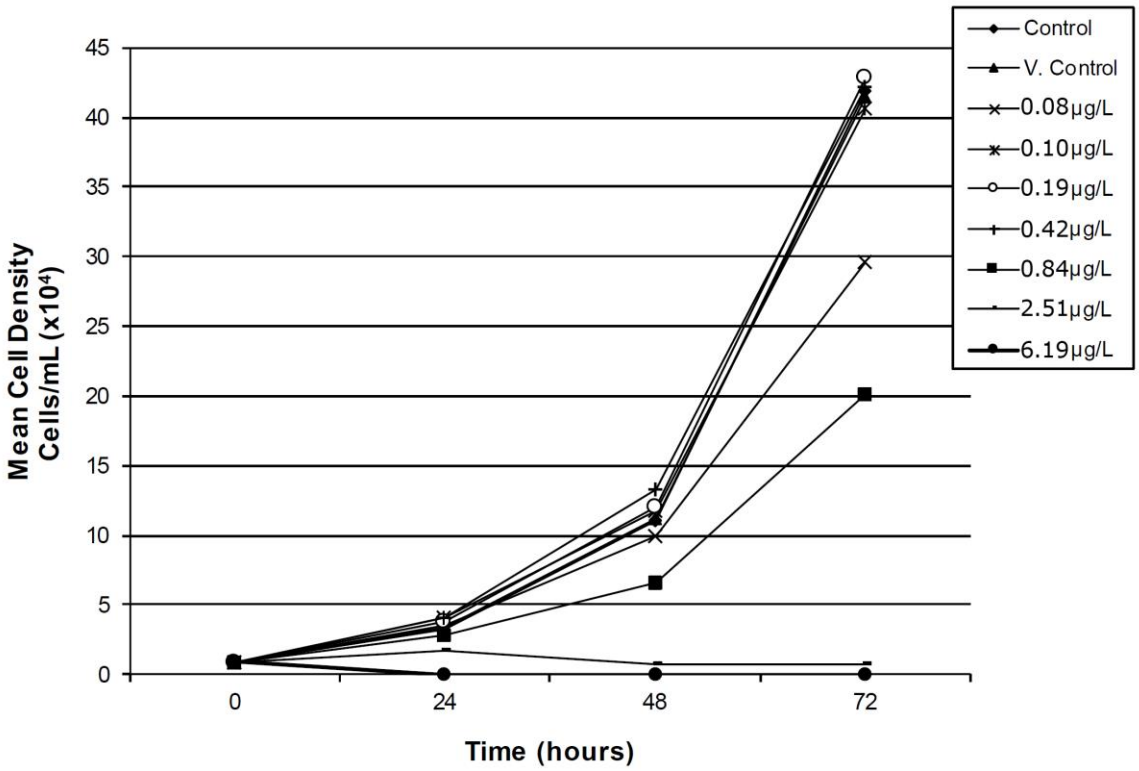


Figure 6: Growth Curves for *Skeletonema costatum* during a 72h exposure to ZnPT (geometric mean measured concentrations).

Table: Hoover (2018) Summary of the effect concentrations (i.e., ErC) based on growth rate. Results are based on geometric mean measured concentrations (µg a.i./L)

EC type	48-hour	72-hour	96-hour
ErC_{10}	0.991	0.778	0.686
ErC_{50}	1.23	0.969	0.854
NOEC	0.54	0.42	0.33

This study satisfied the OECD TG 201 guideline validity requirements for a growth inhibition test with *Skeletonema costatum*. Choosing a time point other than the recommended 72h was not warranted. The effect concentrations associated with the 72h period are taken forward for the assessment of aquatic toxicity classification.

3.3.3 Impact on acute (short-term) toxicity classification

The Ward and Boeri (2004) algal toxicity study was evaluated in the BPR PT21 and rated as Klimisch 1-2 by the eCA and would normally be considered acceptable for classification purposes. RAC notes that concerns were raised by Industry with regards to the methodological conduct of this study. Further concerns over variable growth rates in the controls and the choice of the 48 h time point for toxicity endpoints were not addressed by the DS. With only one other study to compare arising from input into the initial public consultation and no other data, RAC initially agreed with the DS to default to the most conservative values. However, results from two new studies (Hoover, 2018 and Goudie, 2018) are now available and indicate that *Skeletonema costatum* is the most sensitive taxon for the assessment of both acute and chronic aquatic toxicity. These two new studies are in agreement with each other, are well performed and, for the reasons outlined earlier in the opinion and the Background document to the opinion, address

all previous concerns with the two older studies in the *Skeletonema costatum* toxicity database. Alone, they are sufficient to propose classification with associated M-factors.

Conclusions:

(1) Based on the weight of evidence from comparing the four *Skeletonema costatum* studies and the studies conducted on all other aquatic taxonomic groups, *Skeletonema costatum* may be considered the most sensitive species for acute aquatic toxicity.

(2) In light of the more complete data package now available for *Skeletonema costatum*, RAC proposes suitable effect concentrations at 72h that will form the basis for the proposed classification:

ErC50: 0.00088 mg/L (0.88 µg/L) to 0.00097 mg/L (0.97 µg/L)

3.3.4 Impact on chronic aquatic toxicity classification

The final chronic aquatic classification proposed by the DS and agreed amongst MSCAs was also based on the *Skeletonema costatum* study by Ward & Boeri (2004). In its final assessment following public consultation, the DS proposed Aquatic Chronic 1, with an M-factor = 100 (0.00001 < NOEC ≤ 0.0001 mg/L) for a rapidly degrading substance.

The new studies presented to RAC confirm that *Skeletonema costatum* may be considered the most sensitive species to ZnPT. Reviewing the most conservative effect estimates suitable for a chronic assessment using this species presents the following data:

- ErC_{10}:
  - 0.0014 (48h) / 0.0019 mg/L (72h), (Schamphelaere, 2018; ZnPT Industry Consortium, 2017);
  - **0.00068/0.00078** mg/L (72h) (Goudie, 2018; Hoover, 2018)

- NOErC:
  - 0.0003 mg/L (72h) (Rebstock, 2010, CAR A7.4.1.3/55, RMS);
  - **0.00026/0.00042** mg/L (72h) (Goudie, 2018; Hoover, 2018)

Modern test guidelines for all aquatic taxa, including algae, recommend use of the EC_{10} as the effect parameter in preference to the NOEC. Regression-based effect estimates such as ErC_{10} and ErC_{20} are preferred because regression based estimates are less influenced by dose selection and make full use of the dose response curve. The most appropriate endpoint is the 72 h ErC_{10} = 0.00068/0.00078 mg/L derived from the *Goudie* (2018) and Hoover (2018) studies.

The latest report from Industry, dated 31 July 2018[1] suggested the 48h results from the *S. costatum* studies with ZnPT were the most appropriate for classification, claiming this time-point best balances the statistical considerations for growth rate with the decreasing certainty in the results as ZnPT degrades. RAC however does not see any fundamental difficulty with accepting the 72h results of the new studies in preference to other time points as recommended by OECD TG 201 while at the same time fulfilling all validation criteria at the 72h time point. Validation criteria (e.g. minimum multiplication factor of 16 reached in control cells) are not fulfilled at the 48h time point in either of the two new studies.

[1] SUPPORTIVE DOCUMENT TO THE ZnPT INDUSTRY CLH CONSORTIUM COMMENTS ON: The targeted consultation on harmonised classification and labelling of zinc pyrithione: assessment of the two new algal toxicity studies conducted in *Skeletonema costatum.*

Conclusions

(1) Based on the weight of evidence from comparing the four *Skeletonema costatum* studies and the studies conducted on the other aquatic taxonomic groups, *Skeletonema costatum* may be considered the most sensitive species for chronic aquatic toxicity.

(2) In light of the more complete data package now available for *Skeletonema costatum*, RAC proposes suitable effect concentrations at 72h that will form the basis for the proposed classification:

ErC_{10}: 0.00068 mg/L (0.68 µg/L) to 0.00078 mg/L (0.78 µg/L)

3.3.5 Overall conclusions

(1) The marine diatom *Skeletonema costatum* may be considered the most sensitive species for aquatic toxicity to ZnPT.

(2) The 72h ErC_{50} of 0.00088 mg/L for *Skeletonema costatum* (Goudie, 2018) supports classification as Aquatic Acute 1.

(3) The 72h ErC_{10} of 0.00068 mg/L for *Skeletonema costatum* (Goudie, 2018) supports classification as Aquatic Chronic 1.

Assessment and comparison with the classification criteria

4.1 Degradability

According to Section 4.1.3.2.4.5 of the CLP Guidance document, on weight of evidence for degradation, where even in cases of conflicting results in ready biodegradability tests, the study with data of the highest quality and the best documentation is considered sufficient to determine whether a substance is rapidly biodegradable or not. In this case positive results can outweigh negative results when the scientific quality is acceptable.

The Menzies (2017) study was a guideline compliant (OECD TG 301B), GLP Ready Biodegradability study with ZnPT. At the end of the 10-day window, 60.5 ± 0.1, 60.4 ± 0.1 and 69.1 ± 1.4% theoretical amount of evolved carbon dioxide ($ThCO_2$) was reached in the 100. 210, and 520 µg ^{14}C-ZnPT/L test treatments, respectively. At the end of the 28-days test, the mean biodegradation was 64.9 ± 0.4, 65.7 ± 0.6, and 72.4 ± 2.0% ThCO2 for the 100, 210, and 520 µg ^{14}C-ZnPT/L test treatments, respectively.

RAC considers ZnPT is readily biodegradable, and therefore **rapidly degradable** for classification purposes.

4.2 Bioaccumulation

There seems to be insufficient information in the CLH report to make any independent assessment of the reliability of the fish and oyster BCF studies, or the QSAR prediction. However, from the available information, ZnPT has a low bioaccumulation potential with a log Kow = 0.99 and log Pow = 1.21 (cut-off value for classification log Kow ≥ 4), and BCF = 7.8-11.0 in oyster and BCF = 0.33 in fish (cut-off value for classification ≥ 500 for the whole fish). The calculated BCF (QSAR) was 0.33–1.4. ZnPT is **not considered to be a bioaccumulative** substance for classification purposes.

4.3 Acute aquatic toxicity

RAC is of the opinion that ZnPT fulfils the classification criteria for Aquatic Acute 1, since its toxicity to aquatic organisms from all three trophic levels (fish, crustacea and algae) was below 1 mg/L (EC_{50} < 1 mg/L).

For algae and aquatic plants an ErC50 based on growth is preferred in the classification criteria. The lowest acute aquatic toxicity data was a 72 h ErC_{50} of 0.00088 mg/L for the marine diatom *Skeletonema costatum* based on the study by Goudie (2018). The lowest acute aquatic toxicity value 0.00088 mg/L is in the 0.0001 < $L(E)C_{50}$ ≤ 0.001 range giving an acute M-factor of 1000.

Based on an acute toxicity of ZnPT to *Skeletonema costatum* with a 72h ErC_{50} = 0.00088 mg/L (0.88 µg/L), ZnPT can be classified as Aquatic Acute 1, with an M factor of 1000 (0.0001 < ErC_{50} ≤ 0.001 mg/L).

4.4 Chronic aquatic toxicity

There are chronic toxicity data available for all three trophic levels. Most of the chronic studies supported Aquatic Chronic 1 since their NOEC < 0.01 mg/L. According to the CLP Guidance, NOEC and EC10 values from short-term algae tests are both accepted as chronic endpoints. Algae also cover diatoms and therefore, endpoints on *Skeletonema costatum* may be considered valid for chronic classification. On the basis that the two new *S. costatum* studies are reliable and with good data, RAC agrees with the DS on the chronic classification as Aquatic Chronic 1, but proposes a chronic M-factor of 10 (0.0001 < ErC_{10} ≤ 0.001 mg/L) based on *Skeletonema costatum* 72h ErC_{10} = 0.00068 mg/L (Goudie, 2018) and that the substance is rapidly degradable.

4.5 Conclusion

The marine diatom *Skeletonema costatum* may be considered the most sensitive species for acute aquatic toxicity assessment. Based on an acute toxicity of ZnPT with a 72 h ErC_{50} = 0.00088 mg/L (0.88 µg/L), ZnPT should be classified as Aquatic Acute 1, with an M-factor 1000 (0.0001 < ErC_{50} ≤ 0.001 mg/L).

The most sensitive trophic level for chronic toxicity assessment is also algae. The classification is again based on the marine diatom *Skeletonema costatum*. The lowest chronic 72 h ErC_{10} value is 0.00068 mg/L supporting classification as Aquatic Chronic 1. The effect concentration falls in the [0.0001 < ErC_{10} ≤ 0.001 mg/L] range giving a chronic M-factor of 10 for a rapidly degradable substance.

RAC therefore concludes that ZnPT should be classified as follows:

Aquatic Acute 1; H400 with an **acute M-factor** of **1000** and
Aquatic Chronic 1; H410 with a **chronic M-factor** of **10**

Supplemental information - In depth analyses by RAC

CAR Section A7.4.1.3/04 Growth inhibition test on algae - Ward TJ, Boeri RL (2004)

Background and introduction

The biocides reviewers' comments following the 2013 peer review are reproduced below to explain how the time-weighted average endpoints were derived from the Ward & Boeri (2004) study.

"Regarding the time-weighted average (TWA) for this study, we agree that it is not known when LOD was reached, it may very well (as one MS points out) have been reached earlier than at 120

h which is the so far used assumption. We have therefore made a new TWA, which is based on a better estimate for when LOD is reached.

In this we use a more realistic degradation rate based on expected light condition in the algae test. Photolysis under algae test conditions would be fully dominating, and we therefore neglect any biological and hydrolytic activity. The light intensity in the algae test was 3749 to 3790 lux (see RMS evaluation box of Doc III, Arch/EZPTF/ESPTF, A7.4.1.3/04/Ward & Boeri, 2004). Our expert on algae cultivation judge that this corresponds to ~80 µE/m^2/s (which is 1/20 to 1/25 of full daylight intensity (outdoors summer), ~2000 µE/m^2/s).

We further assume, that the photolysis tests (Arch/EZPTF A7.1.1.1.2/03/Fenn RJ, 2005) were done in full daylight. The longest half-life observed in these photolysis studies was 33 min (this was for winter outdoor light). On basis of 25 times lower light intensity, we assume 25 times slower degradation rate in this Skeletonema test, in essence a half-life of 750 min (= 12.5h). In order to incorporate conservatism, also a higher degradation rate is considered. We judge that 10 times slower rate can represent this, in essence a half-life of 330 min (= 6h). This would correspond to a case where the laboratory light intensity is related to winter outdoor light conditions in Fenn's photolysis studies, i.e. approximately 800 µE/m^2/s.

We can now estimate how long it takes to reach LOQ of 0.0088 µg/L in this *Skeletonema* study:

CLOQ = C0 × e^(–kphotolysis × t)

⇒ t = 56h (if DT50 is 12.5 h)

⇒ t = 27h (if DT50 is 6 h).

This enables calculation for TWA factors (at 48 h):

fTWA = (1 – e^(–k × t)) / (k × t):

⇒ fTWA = 0.35 (if DT_{50} is 12.5h)

⇒ fTWA = 0.18 (if DT_{50} is 6h).

And new TWAs of the NOEC:

=> TWA NOEC = 0.22 µg/L × 0.35

= 0.077 µg/L (DT_{50} is 12.5h)

⇒ TWA NOEC = 0.22 µg/L × 0.18

= 0.040 µg/L (DT_{50} is 6 h)."

Therefore, the calculated ^{twa}NOECs range from 0.040 – 0.077 µg/L (equivalent to ^{twa}NOEC= 0.000040-0.000080 mg/L)."

The chemical analyses performed in the original study of Ward & Boeri (2004) are not adequate for reliable calculations of precise effect concentrations (NOECs, ErC_{10}, ErC_{50} etc.). The data for algae samples at the 120hr time point were all below the limit of quantification and do not allow the calculation of degradation rates or reliable time-weighted average concentrations. Hence the approach taken above using data from the Fenn 2005 study. At the end of the Ward & Boeri (2004) experiment all ZnPT was lost in all treatments that included algae. In "stability samples"

kept in the dark ZnPT decreased from 5.1 to 1.1 µg/L in 5 days (and to below the LOQ of 0.029 µg/L in the samples with light). These results suggest that the initial loss (approximately 77%), can be attributed to non-photolytic degradation or (slow) adsorption to particulate matter or test walls, with the remainder due to photolysis.

Conclusion

This approach to deriving effect concentrations is acceptable if no other data is available. However, the nature of these estimates introduces uncertainty into the final endpoints. In light of new data from studies only now available following the publication of the CLH report and the public consultation period, these estimates are superseded by endpoints based on actual measured data.

Additional references

Amendment No. 1 to the final study report on "Prenatal developmental toxicity study of zinc pyrithione in rabbits by oral gavage" (Thor GmbH Art. 95 dossier, 2015)

Additional information report, 2018. Additional information report for zinc pyrithione. Available on ECHA website at https://echa.europa.eu/fr/harmonised-classification-and-labelling-previous-targeted-consultations.

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ANNEXES:

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

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC

Annex 3 Records of the targeted public consultation on human health

Annex 4 Records of the targeted public consultation on aquatic hazard