Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON PYRITHIONE ZINC; (T-4)-BIS[1-(HYDROXY-.KAPPA.O)PYRIDINE-2(1H)-THIONATO-.KAPPA.S]ZINC

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance 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
Dossier submitter: Sweden

Note to the reader: there were two further targeted Public Consultations on additional data submitted later on in the process and, therefore, some of the responses to the initial comments may have been superseded. This refers primarily to the environment sections of the RCOM and, more specifically, to comments 80-83. For more details, please see the other PC comments and responses to these comments in the Records of the targeted public consultations (Annex 3 and Annex 4).

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Germany		Individual	1
Comment received				
As a reproductive toxicologist I am concerned about some misinterpretations leading to the wrong conclusion to label ZPT as a 1 B developmental toxicant. Having read the relevant study reports as well as the comprehensive report "Supportive document to the ZnPT Industry CLH Consortium comments on Reproductive Toxicity", and taking into account all available information, I disagree with the suggested classification 1 B. ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT comments Jochen Buschmann.pdf				
Dossier Submitter's Response				
Noted. Your comments (including those in the attachment) are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2017	Germany		Individual	2
Comment received				
As a toxicologist with more than 35 years of professional experience including issues of classification and labelling, I have read the proposal and want to provide the following summary comments. The CLH-report proposes to classify Zinc Pyrithione (ZPT) (CAS 13463-31-7) as a developmental toxicant category 1B. However, a need for classification as a category 1B developmental toxicant is not supported by the toxicity data on ZPT. Developmental toxicity by ZPT occurs only in the presence of excessive maternal toxicity and ZPT does not have an intrinsic, specific property to produce adverse effects on fetal development. The CLH proposal does not follow the procedures outlined in the CLP directive: • Integration of all available data using weight-of-evidence as required in the CLP directive is not performed • maternal toxicity is not adequately assessed • specificity of the adverse effects to the embryo is not assessed • key information (expert review on consequences of massive reductions in maternal body weight for study evaluation, results of food restriction studies) is not correctly interpreted Applying an adequate integration of the available information using weight of evidence, classification of ZPT as a reproductive toxicant category 1B is not supported by the large database on the toxicity of ZPT and other salts of PT. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Comment ZPT-ECHA.pdf				
Dossier Submitter's Response				
Noted. Your comments (including those in the attachment) are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium	EPDLA - European Polymer Dispersion and Latex Association	Industry or trade association	3
Comment received				
The European Polymer Dispersion and Latex Association (EPDLA) included its comments on the proposed classification of zinc pyrithione in the attachment. Zinc pyrithione is under review for approval as an in-can preservative (product-type 6) under the BPR and may be used as a co-biocide for such purpose in polymer dispersions. The members of the European Polymer Dispersion and Latex Association (EPDLA) have detailed their comments on the proposed classification of zinc pyrithione in the attachment. ECHA note – An attachment was submitted with the comment above. Refer to public attachment EPDLA-Comments on CLH of zinc pyrithione.pdf				

Dossier Submitter's Response
Thank you for your comment. In the attachment you wanted to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process. We do note that in your attachment, you support comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Support the comments of the DS and note the reply for comment #56.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany	Thor GmbH	Company-Manufacturer	4
Comment received				
Thor GmbH fully supports the detailed comments made by the "ZnPT Industry CLH Consortium" in the submitted document "Supportive Document to the ZnPT Industry CLH Consortium comments on Reproductive Toxicity" to which we contributed comments on the studies owned by Thor GmbH. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2017 07 07 Thor GmbH comments to ZnPT CLH report.pdf				
Dossier Submitter's Response				
Noted. Please see our response to the comments made by the ZnPT Industry CLH Consortium under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany		MemberState	5
Comment received				
The CLH proposal for classification and labelling is supported. The DS provides a sufficiently detailed CLH report addressing all the dossier endpoints with clear information presented in tables and summaries. Also the description of deviations from test guidelines and the modifications on each study protocol were useful to evaluate the study reliability. However, the ATP synthesis and energy metabolism may be involved in the mode of action of this substance and detailed information on clinical chemistry analysis (e.g haematocrit and glucose tabulated values) from each animal study would have been appreciated.				
Dossier Submitter's Response				
Thank you for your support. The ZnPT Industry CLH Consortium has provided the information on clinical chemistry analysis in conjuction with the mode of action of the pyrithiones. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2017	Netherlands		Individual	6

Comment received
I find it strange and rather unnerving that a substance that has been used in anti-dandruff shampoo for decades now suddenly is considered repro toxic.Shampoos are massaged into the skin and potentially available for inhalation and oral uptake. I find it also strange that the eCA has considered it would take some of the aspects of the late arrival reports and would not take others into account. It looks like those that were in favour of a predetermined possition were taken on board at those that would not fit that view were not. Thirdly I find the grounds for refusing to take on board test results on other pyrithiones awkward. Since the substance zinc is not fully understood, we don't want to use the other pyrithiones even where it handles on aspects where zinc is not considered a guilty party. Fourth and last it looks like the Swedish CA has a grudge against active substances and the biocidal products in which they are used. This shows in their current policies and also in evaluations of other substances under BPR.
Dossier Submitter's Response
Firstly, new studies performed with ZnPT were available in 2015 which have affected the classification. Secondly, all the reports received by the DS were taken into account and referred to in the CLH report. Thirdly, see our response under the comment number 56 concerning the results on other pyrithiones. Your sentence on zinc is uninterpretable. Fourthly, the Swedish CA's all policies are towards Health and Environmental Safety. Our evaluations are based on science and within the remit of respective chemical legislation.
RAC's response
RAC supports the efforts of the DS and considers the CLH report to be a balanced and clear description of all pertinent studies used in assessing the reproductive toxicity of zinc pyrithione.

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2017	Belgium	CEPE	Industry or trade association	7
Comment received				
Our input to the public consultation aims at raising awareness on the importance to take into account all scientific arguments because this substance is important to our business ECHA note – An attachment was submitted with the comment above. Refer to public attachment CEPE ZnPT Public Consultation final 20170621.pdf				
Dossier Submitter's Response				
Thank you for your comment. In the attachment you wanted to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process. We do note that in your attachment, you support comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.06.2017	United Kingdom	British Coatings Federation	Industry or trade association	8
Comment received				
We have detailed our concerns over the proposed classification in the attached position statement The statement refers to the specific arguments against the proposed classification, mentioning the assessments made by other global regulatory authorities and that ZnPT does not have an intrinsic property to produce adverse reprotoxic effects. It also details the use of ZnPT in the paints, coatings and inks industry, and reiterates the major concerns that our industry has regarding the progressive loss of active substances from the paint formulators toolbox, citing the **NINE** industry papers that have already been submitted on this subject over the past 3 years. ECHA note – An attachment was submitted with the comment above. Refer to public attachment BCF ZnPT - Public Consultation response June 2017 Final.pdf				
Dossier Submitter's Response				
Thank you for your comment. In the attachment you wanted to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process. We do note that in your attachment, you support comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Finland	Tikkurila.Oyj	Company-Downstream user	9
Comment received				
Zinc pyrithione (ZnPT) is used as film fungicide (PT7) and in-can biocide (PT6) in paints and coatings. We would like to take this opportunity to raise the attention that pyrithione family of biocides are essential for users of paints and coatings as these substances offer sufficient long-term preservation of our products. The proposed classification as Reprotoxic Category 1B means that ZnPT falls under the exclusion criteria in BPR (Article 5), and thus it will no longer be available for the paint and coating industry. Tikkurila Oyj, as a downstream user of ZnPT, supports the consolidated comments submitted by the ZnPT Industry CLH Consortium during this consultation. The socio-economic impact of losing suitable film-preservatives and the issue of key in-can biocide substances disappearing one after the other has been highlighted by CEPE. Tikkurila Oyj support their concerns.				
Dossier Submitter's Response				
Thank you for your comment. Implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation are not relevant to the current CLH process. Since you support comments made by the ZnPT Industry CLH Consortium, please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Japan	Mitsubishi Chemical Corporation	Company-Manufacturer	10

Comment received

Our concerns over the proposed classification as Repro. 1B are included in the attached position statement, alongside information on the importance of this substance to our business and long-standing use of the substance without concerns being raised.

The studies included in the CLH report that support classification as a Category 1B reprotoxicant all appear to have a test substance purity value of <99.9 %. With this substance it is often observed that heavy metals can potentially be present including Lead and Cadmium (amongst others) and accordingly the effects in the detailed studies may be as a result of the presence of such heavy metals which have well known and observed reproductive effects. The presence or absence of such impurities is lacking in the experimental details and if present, may have contributed to the noted effects.
For older data, there is also the possibility that the trace amounts of impurities are unknown, and that some commercial suspensions contained preservatives and dispersants which were considered to be CBI, with unknown reportoxicity details available.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment MCC ZnPT Public Consultaion July 2017.pdf

Dossier Submitter's Response

Please note that for the studies where purity of the test material was mentioned as >95% in the CLH report, the Companies owning those studies have claimed the exact purity value as confidential. Nevertheless, it is true as you state that the test substance purity in the studies is <99.9%. Your concern seem to be that heavy metals such as lead and cadmium may be present as impurities in the substance (i.e. ZnPT) that may come from the starting materials used in the production of ZnPT, and that these impurities may have contributed to the observed effects. Please note that the definition of a 'substance' in the CLP Regulation covers any impurities deriving from the manufacturing process.

"'substance' means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition;"

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Japan	<confidential>	Company-Downstream user	11

Comment received

Mitsubishi Chemical Corporation, our reliable suppler, has been producing this substance over forty years and selling globally.
This substance's application is for personal care product(e.g. hair shampoo) and for industrial(e.g. construction and coating). We have been selling this substances(50%

water solution) for customers long years, but we have not received any trouble report from them about toxicity to reproduction.
Dossier Submitter's Response
Thank you for your comment. Absence of 'any trouble report' does not mean there is no intrinsic hazard.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium	Cosmetics Europe	Industry or trade association	12
Comment received				
Cosmetics Europe welcomes the opportunity to contribute to the Public Consultation on the CLH Proposal for Zinc Pyrithione (ZnPT, CAS # 13463-41-7) as Reproductive toxicant 1B, H360D. We are aware that the purpose of this public consultation is to receive toxicological and scientific arguments relating to ZnPT with a view to the proposed classification. However, since there is no formal socio-economic analysis required under Article 15 of Regulation 1223/2009 on cosmetic products, we also would like to take this opportunity to highlight to authorities the significant impact that such a classification could ultimately have on the cosmetics industry through Article 15 of the Cosmetics Regulation. Therefore, in this submission we will address (1) Information about Cosmetics Europe, the Cosmetics Industry as well as the importance of Zinc Pyrithione in Cosmetic Products, (2) Global Regulatory Status of Zinc Pyrithione in Personal Care Products, (3) Impact of a Repr. 1B classification to the cosmetics industry, (4) Safety of Zinc Pyrithione in Cosmetics Products and (5) General Comments on the CLH Report. In addition, we will provide specific comments on the Endpoint of Reproductive Toxicity relating to the CLH proposal and dossier. In summary, Cosmetics Europe, representing its members, does not agree with the proposed classification and labelling of ZnPT as Reproductive Toxicant 1B and we are very concerned that this will likely lead to the loss of a key active for Anti-Dandruff and other cosmetic products as well as of a cosmetic preservative. Given the format of this public consultation and the web interface requirements, our submission is accompanied by 2 Attachments which contain more details as well as references and therefore need to be viewed together with the comments submitted here (1 – General Comments, 2 – Comments on the Reprotoxicity Endpoint). In addition, we want to stress that we support the detailed arguments presented during this consultation by the manufacturers of ZnPT and key stakeholders. We would like to draw attention particularly to the arguments presented by the ZnPT Industry CLH Consortium. 1. About Cosmetics Europe, the Cosmetics Industry and the importance of Zinc Pyrithione in Cosmetic Products Cosmetics Europe is the European trade association representing the interests of the cosmetics industry. Its membership consists of 27 national associations of the EU Member States and beyond, 17 major international companies, four supporting association members, four supporting corporate members and three correspondent associated members. Cosmetics Europe represents more than 4,000 companies throughout the EU via the active representation of its member national associations. For more information about "Cosmetics Europe", please consult our website: www.cosmeticseurope.eu The industry makes a significant contribution to the European economy across its value chain. It is estimated that the cosmetics industry brings at least €29 billion in added value to the European economy every year, of which approximately €8 billion is contributed				

directly by the manufacture of cosmetic products (the remaining €21 billion is generated indirectly through the supply chain). Small/Medium sized Enterprises (SMEs) are key drivers of innovation and economic growth. While there are more than 5,000 enterprises manufacturing cosmetics in Europe, the vast majority of these companies are SMEs. In 2015, there were 4,605 SMEs in Europe. Along the value chain, a wide variety of different types of enterprises are involved indirectly in the cosmetics industry. For example, there are over 100 companies manufacturing cosmetic ingredients in Europe, 20,100 enterprises involved in the wholesale of cosmetics and 45,700 specialist stores retailing cosmetics. About half a million hairdressing and beauty salons (the majority of which are also SMEs or micro-enterprises) also rely on the use of cosmetics and the number of European spas is also growing and may be a source of inward investment to Europe in the form of "wellness tourism". The industry supports millions of jobs. Including direct, indirect and induced economic activity, the industry supports at least 2 million jobs. Of these, 152,000 workers are employed directly in the manufacture of cosmetic products, and around 1.6 million workers are employed indirectly in the cosmetics value chain.

Zinc Pyrithione (ZnPT) is an important ingredient for the cosmetic industry and its use is extensive across major brands and store brands. Zinc Pyrithione has been used in cosmetics for more than 50 years and has been repeatedly assessed and confirmed to be safe by independent global safety authorities, including the US FDA and the EU Commission's Scientific Committee on Consumer Safety (SCCS). ZnPT has published uses in many personal care product forms globally - shampoos, conditioners, hair tonics, bar and liquid soaps, body washes; among these uses, use in Anti-dandruff Shampoos is most frequent (~85% of all ZnPT-based cosmetic products). A search in Mintel´s Global New Product Database (gnpd.com) indicated the launch of >550 new cosmetic products containing ZnPT to the European market in the past 5 years. Zinc Pyrithione is broadly used as antidandruff agent, preservative, conditioning agent and antiseborrhoeic agent (CosIng). ZnPT is regulated under the European Cosmetic Products Regulation (EU CPR 1223/2009) as a cosmetic preservative under Annex V/8; it is allowed for use in rinse-off hair products up to 1% and in other rinse-off products (except for oral products) up to 0.5%. Use in leave-on hair products is allowed under Annex III/101 up to a maximum level of 0.1%.
In their opinion SCCS/1512/13 (June 2014) the SCCS has concluded that "zinc pyrithione, when used in a concentration up to 2.0% as an anti-dandruff agent in rinse-off hair care products, is safe for the consumer".

2. Global Regulatory Status of Zinc Pyrithione in Personal Care Products

Zinc Pyrithione has been allowed for use in consumer, personal care products by many regulatory authorities around the globe based on its positive human and environmental safety profile. ZnPT-formulated personal care products are controlled globally as "over-the-counter" (OTC) drug, quasi-drug, cosmetics, or household product, through regulatory processes, such as product notifications, product registrations, or ingredient registrations.
ZnPT has a long history of use globally and is approved as a preservative, cosmetic biocide, and anti-dandruff ingredient. ZnPT has published uses in many personal care product forms including shampoos, conditioners, body washes, bar and liquid soaps, moisturizers, and make-up preparations.
ZnPT is regulated under the European Cosmetic Products Regulation (EU CPR 1223/2009) as a cosmetic preservative under Annex V/8; it is allowed for use in rinse-off hair products up to 1% and in other rinse-off products (except for oral products) up to 0.5%. Use in leave-on hair products is allowed under Annex III/101 up to a maximum level of

0.1%.

Globally, ZnPT is authorized for use in US, Canada, Japan, Korea, Switzerland, Russia, Saudi Arabia (and the Gulf States), China, ASEAN, India, Brazil, Mexico, Chile and other countries, whereby uses are similar to the ones authorized in the European Union or higher use levels (up to 2% in rinse-off hair products) are allowed.
For example, ZnPT is authorized in the US in the following personal care product forms:
• 0.3 – 2.0% as the active ingredient of Anti-Dandruff OTC products - rinse off products
• 0.1 -0.25% as the active ingredient of Anti-Dandruff OTC products - leave on products
• 0.95-2.0% as the active ingredient of OTC products for Seborrheic dermatitis – rinse off products.
• 0.1-0.25% as the active ingredient of OTC products for Seborrheic dermatitis – leave on products.

3. Impact of a Reproductive Toxicant 1B classification on cosmetic industry
A classification of Zinc Pyrithione as Reproductive Toxicant 1B would mean that ZnPT is banned for use in cosmetic products. This follows from the European Cosmetic Products Regulation (EU CPR) which states in Article 15.2 that "the use in cosmetic products of substances classified as CMR category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008 (CLP) shall be prohibited". Exemptions to the CMR 1A and 1B ban are possible, however they can only be granted in very exceptional cases, when a series of stringent conditions are fulfilled (safety dossier favorably reviewed by the SCCS, compliance with food safety requirements, absence of suitable alternatives, specific uses…). In case an exemption would not be granted for use of ZnPT in cosmetic products, a significant number of cosmetic products would be impacted (see section 1 above).
In addition, Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD) currently does not cover reprotoxicants; in other words, a Repro 1B classified ZnPT would not be regulated under this Directive in its current state. However, an amendment to this Directive to include reprotoxicants under its scope is under discussion and may be in effect at the time of a potential classification of ZnPT. Should the CMD be such amended, specific measures would need to be adopted for manufacturing of ZnPT containing products (once classified), despite no benefit to worker safety.
4. Safety of Zinc Pyrithione in Cosmetic Products
As a general comment, we state that there are no human safety concerns from use of cosmetic products such as shampoo or leave-in antidandruff treatments, or body cleansers, containing the broad-spectrum antimicrobial/fungicide ZnPT.
For the calculation of the overall Margin of Safety the SCCS, in their 2014 opinion and for doubling the concentration, used 0.5 mg/kg bw/day as a LOAEL, with an adjustment factor of 3 to make the NOAEL 0.167 mg/kg bw/day (see SCCS/1512/13, page 80). The CLH Dossier on ZnPT references three developmental toxicity studies as well as a 90-day study, which were conducted after the 2014 SCCS opinion (by Thor GmbH). The Cosmetics Europe Working Group on Zinc Pyrithione has reviewed the study summaries of the Thor data. We conclude that these data do not change the safety assessment of ZnPT in cosmetics – as confirmed by SCCS in 2014, and confirm the NOAEL for ZnPT to be 0.5mg/kg bw. Because the NOAEL remains the same, the risk assessment and favorable opinion not impacted by the "new" data. In other words, the overall conclusion of the SCCS remains valid: "Based on the scientific data provided the SCCS considers that zinc pyrithione, when used in a concentration up to 2.0% as an anti-dandruff agent in rinse-off hair care products, is safe for the consumer".
The SCCS has provided a summary assessment of maximum exposure in this opinion. It concludes that and internal dose of 5.25 ug/kg/day represents a worst-case estimate of human exposure from consumer products. The opinion states that this value is based on

three highly conservative assumptions:
1. that use includes both rinse-off and leave-on products;
2. it represents the upper boundary of absorption, based on human clinical studies, and
3. it adds "one standard deviation" to the measured internal dose.

Even with this conservatism, this worst-case value for human exposure, 5.25 ug/kg/day, is 100 times lower than the oral NOAEL for maternal toxicity (0.5 mg/kg bw/day) based on any developmental reproductive toxicity studies (even the "new" data). Therefore, there is no concern for maternal toxicity occurring in humans with cosmetic product use. Accordingly, the lack of intrinsic embryotoxicity of pyrithione, and the negligible exposure from product use by consumers, leads us to conclude that ZnPT does not have the potential to affect human development, directly or indirectly.

5. General Comments on the CLH Report

Page 6: Cosmetic Europe notes, that, while the CLH Dossier Submitter referred to the SCCS opinion in relation to the uses of ZnPT in cosmetic products, the CLH dossier does not discuss and consider the conclusions drawn by the SCCS including but not limited too developmental effects (Chapter 3.4.8, pp 50ff). We want to stress that the conclusions drawn by the SCCS (2014), MAK (2012) and HSE (2003) are appropriate, relevant and thus need to be considered.

Page 7: A toxicological review of ZnPT needs to consider all relevant data incl data on related salts such as NaPT which has e.g. been recognized by the SCCS (2014, p 34): "A read across from sodium pyrithione to ZPT is considered appropriate based on the following reasoning: data on metabolism of ZPT (see section "toxicokinetics" and see also SCCNFP/0671/03) demonstrate that Zn is cleaved from the molecule after uptake and that ADME of the metal ion and the pyrithione moiety is different. Studies performed in pigs using NaPT and ZPT pointed to a common metabolic pathway (references B.68, B69, B.70). Further, both Zn2+ and Na+ ions are not considered to be neurotoxic. Thus, it can be assumed that neurotoxic effects observed after ZPT exposure are due to the pyrithione moiety. It can thus be concluded that results from other pyrithione-liberating salts might support the findings obtained with ZPT." Cosmetics Europe notes that, while the CLH Dossier Submitter "acknowledges that zinc pyrithione shows some structural similarity to sodium pyrithione (EC 223-296-5) and copper pyrithione (238-984-0), in that they share the common organic moiety i.e. Pyrithione", the available data on these salts are not considered in the classification assessment.

In addition, data from whole embryo culture assays were cited (page 81/82) but not considered in a weight-of-evidence assessment. Here, we want to state that the CLP requires a Weight-of-Evidence approach using all available data: "Appropriate classification will always depend on an integrated assessment of all available data and their interrelationship using a weight of evidence approach." (Guidance on the Application of the CLP Criteria, Version 4.1, Chapter 3.7.2.3., page 398). In this context, the conclusions of the SCCS are valid and relevant and data on NaPT and CuPT as well as the data from whole embryo culture need to be considered.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CE ZnPT Public Consultation.zip

Dossier Submitter's Response

Thank you for your comment including the attachment. You want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the Cosmetics Regulation while you do realise that these are not relevant to the current CLH process. We do note your support for comments made by the ZnPT Industry CLH

Consortium which also covers your general comments on the CLH report and specific comments on the toxicity to reproduction endpoint. Please see our response under the comment number 56.
RAC's response
Extensive and relevant comments but we must focus on the hazard assessment of zinc pyrithione for classification purposes.

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Germany	<confidential>	Company-Downstream user	13
Comment received				
We are a major manufacturer of paints, coatings and related products for uses in the construction sector. We support and refer to the comments of our European and German industry associations (CEPE and VdL, respectively). Furthermore we would like to address the individual sight of our company regarding the proposed classification of Zinc Pyrithione (ZnPt). ZnPt is used as dry film and in-can preservative for water based coatings. The proposed classification as repr. 1B would have the legal consequence that ZnPt would no longer be available as biocidal active substance due to the exclusion criteria as laid down in the Biocide Product Regulation (Regulation (EU) No. 528/2012). We want to highlight this "automated" consequence of the proposed new classification as this would lead to consequences which are not proportionate and excessive. As a manufacturer of paints and coatings we are not in a position to comment on the proposed classification of ZnPt as repr. 1B. However, we do have sufficient knowledge about the uses of this substance as dry film and in-can preservative in our products. These uses do not pose any risk to health or environment as there is no relevant exposure to ZnPt. Thus, applying the criteria as laid down in REACH regulation, these uses have to be considered as "safe". We know of course that the harmonized classification of substances under CLP is solely hazard-based. However, it is important to bear in mind that this approach may lead to consequences which are not appropriate, especially in case of "automated" consequences under other legislation (Biocide Product Regulation, see above). We are already facing a very challenging situation with the in-can preservative Methylisothiazolinone (MIT). The proposed harmonized classification and labelling for MIT (RAC opinion) is including a SCL of 15 ppm for skin-sensitizing properties, which is expected to be transferred to the biocidal active approval as concentration limit for use in products for the general public. As 15 ppm are well below the concentration needed for MIT to be effective as in-can preservative, we assume that MIT will not be available in future for such use. The loss of the broad effective in-can-preservative MIT has to be compensated. One of the few remaining in-can-actives will be ZnPt. If ZnPt would also be lost for this use the already small portfolio of in-can-actives will be so reduced that in-can-preservation of water-based construction products will be almost impossible. Thus for the evaluation of the C&L proposal for ZnPt such "automatic" consequences should also be taken into account. Consequences which might lead to a ban of ZnPt as a dry film and in-can preservative – although these uses are "safe" due to the lack of any relevant exposure.				
Dossier Submitter's Response				
Thank you for your comment. You want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process. Since you also support				

and refer to the comments of the European and German industry associations, please see our response under the respective comments.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Sweden		Individual	14
Comment received				
I do not agree with the proposed classification and labelling of Zinc Pyrithione (ZnPT) as reproductive toxicant Cat 1B; scientifically important results in studies have not been taken into account and the classification proposal doesn't follow CLP regulations.				
Dossier Submitter's Response				
Noted. However, this comment does not specify which results have not been taken into account and which aspect of the CLP Regulation is not followed.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Norway	Jotun A/S	Company-Downstream user	15
Comment received				

Jotun A/S comment on the Public Consultation on the proposed classification of zinc pyrithione

The Jotun Group is a leading supplier of decorative, protective and marine paints, and powder coatings, worldwide. Jotun operate in the decorative, protective, marine and powder segment.

With regard to the proposed reprotox classification and the assignment of M-factors for zinc pyrithione Jotun is aware of and support the comments given by the ZnPT Industry Consortium. If the proposal is adopted as is this will have big consequences for our industry. We therefore ask that these comments are taken seriously into account during the evaluation.

Zink pyrithione is an important dry-film preservative (PT7) that prevents growth of algae and fungi on coated surfaces and an important in-can preservative (PT6) preventing bacterial spoilage of wet paint. For antifouling (PT21) it is one of the few remaining co-biocides for high quality products reducing problems with fouling on ships (invasive species, maneuverability, fuel consumption and air pollution).

The proposed classification as Reprotoxic Category 1B means that zinc pyrithione falls under the exclusion criteria in BPR (Article 5), meaning that zinc pyrithione will no longer be available for the paint and coating industry.

Furthermore, we are concerned that the classification of zinc pyrithione as Reprotoxic Category 1B will affect the future use of copper pyrithione (PT21) and sodium pyrithione (PT6), leaving the industry with even fewer options to ensure the quality and functions of its products.

The socio-economic impact of losing suitable film-preservatives and the issue of key in-can biocide substances disappearing one after the other has already been highlighted by CEPE. We support their concerns. We remain available to provide further information.
Dossier Submitter's Response
Thank you for your comment. You want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation, however, these are not relevant to the current CLH process. Since you support also the comments made by the ZnPT Industry CLH Consortium, please see our response under the respective comments.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	United Kingdom		Individual	16
Comment received				
CHL Report(2016) regrettably: 1. neglects to use read across (although read across is clearly valid within the metal-pyrithione family; 2. discounts the in vitro studies 3.fails to consider the mode of action information 4. uses very limited and questionable survey of literature				
Dossier Submitter's Response				
For your first three points, please see our response under the comment number 56. Your fourth point does not specify the alleged limitation and the questions concerning the survey of literature.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	United Kingdom		Individual	17
Comment received				
These comments are regarding Reproductive Effects. 1. Consideration of all available data and consideration of all aspects of the CLP Regulation and guidance is necessary. For instance, consideration of secondary effects, mode of action and importantly, taking a weight of evidence approach. The Regulation and guidance are very clear on this. 2. The classification proposal for reproductive effects ignores the data that indicates that the reproductive effects observed are seen only at maternally toxic doses and moreover, that these effects are secondary to maternal toxicity and not a direct effect on the foetus. There is data on the mode of action and data from other pyrithione compounds that are part of the category/read across among the various pyrithiones (Cu, Na & Zn). Data on the other pyrithiones add to the weight of evidence that reproductive effects observed are seen only at maternally toxic doses and are secondary to maternal toxicity and are key in considering the mode of action.				

3. These aspects are extensively covered in the comments submitted by the ZnPT Industry CLH Consortium which gives this information in detail and should be considered fully
Dossier Submitter's Response
As you mentioned, your comments are covered by those made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	Netherlands	PPG	Company-Downstream user	18
Comment received				
Zinc Pyrithione (CAS No 13463-41-7) is of high importance for many of our products, because it is one of the most important active substances for in-can preservation, for dry-film preservation and for anti-fouling (Product Type 6, 7 and 21 respectively, under the Biocide Product Regulation 528/2012 (BPR)). Even though the public consultation should refer to toxicological arguments on inherent properties, we feel the need to consider other aspects of a potential classification as well. Zinc Pyrithione is increasingly used as in-can preservatives due to the substitution of other biocidal substances, many of which faced comparable classification activities. Dry-film preservation is very important for coatings as it prevents the growth of microorganisms on coated surfaces, such as the facades of buildings. Next to the decorative aspect, this also extends the durability of the coatings themselves as well as of the painted substrates. Also here the portfolio of available substances is getting smaller. For anti-fouling it is even one of the few remaining co-biocides. Less performing antifouling products increase the fouling of ship hulls, thereby increasing the fuel consumption and cleaning costs. The proposed classification as reprotoxic category 1b would mean that Zinc Pyrithione would fall under the exclusion criteria of article 5 of the BPR. The proposed classification will then probably result in the substance not being available for the paint industry anymore. Because there are not many actives available with the required functional properties, and because these substances have their own limitations, the possibilities to protect the paints and the substrates become too small. The same applies to the options for antifouling products on sea going vessels. Hence, we believe that the proposed classification of ZnPT would endanger the product- and the substrate preservation as well as anti-fouling properties.				
Dossier Submitter's Response				
Noted. You want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number

03.07.2017	Belgium	Procter & Gamble Manufacturing Belgium N.V.	Company-Downstream user	19

Comment received

Procter & Gamble, as a downstream user of ZnPT, fully supports the consolidated comments submitted by the "ZnPT Industry CLH Consortium" during this consultation. Whilst we understand the need for harmonised classification of active substances as per Article 36.2 of the CLP, we are concerned about the proposed classification, particularly for reproductive toxicity (Repr. 1B) which we believe is not warranted. Indeed, in our opinion, the CLH report evaluation did not consider all the evidence required to assess developmental toxicity as per the CLP Regulation. We provide below specific comments on the reproductive toxicity for which we would appreciate your consideration. These specific comments are related to the influence of maternal toxicity in the ZnPT prenatal studies which, we believe, has been incompletely addressed in the CLH Report . The review of prenatal developmental toxicity studies and repeat-dose toxicity studies on ZnPT, NaPT and/or CuPT in a weight-of-evidence approach indicates that developmental effects are only observed in the presence of excessive maternal toxicity. Conversely, developmental effects are not observed when excessive maternal toxicity is absent.

As a responsible company committed to the highest quality for our products, P&G has always placed safety to health and the environment as a key priority, applying recognised and agreed science-based approaches to evaluate the safety of our ingredients.

Dossier Submitter's Response

Noted. Please see our response under the comment number 56.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Germany	German Paint and Printing Ink Association (VdL)	Industry or trade association	20

Comment received

Zinc pyrithione (ZnPT – CAS No 13463-41-7) is a biocide active, which is of high importance for the paint and coatings industry in Germany. We are aware that the public consultation on the proposed classification should only consider toxicological arguments on inherent properties and we refer to the work done by the corresponding industry consortia. Nevertheless, we would like to highlight the severe impact, which the supposed classification would have on our industry and especially the deco paint sector.

ZnPT is one of the key actives for dry-film preservation (PT 7). Dry-film preservation is most important for organic resin-based coatings and prevents the growth of microorganisms like algae and fungi on coated surfaces, such as the facades of buildings. The proposed classification as reprotoxic category 1B has the legal consequence that ZnPT would fall under the exclusion criteria under the biocides legislation (regulation (EU) No. 528/2012). Thus, the proposed classification will most likely result in ZnPT being no longer available.

CLP classification is hazard-based and hence the actual risk is not considered. BPR exclusion criteria are also hazard-based. The use of ZnPT as biocide active substance (PT6 and PT7, see below) is considered as safe as there is no relevant exposure - neither

during the application of the paint nor during the service life of a painted surface. The proposed CLP classification would thus lead to the ban of a safe use, which is unreasonable and disproportionate.

ZnPT is mainly used due to its fungicidal properties. Currently there are only very few substances left, which can be used as film preservatives and which act as fungicides, but which are also under pressure due to the CLH and BPR processes. Thus, one has to fear that the proposed classification of ZnPT would endanger the film-preservation as a whole. The socioeconomic impact of losing suitable film-preservatives is highlighted in the contribution of CEPE.

Apart from its use in PT 7, ZnPT is also increasingly employed as an in-can preservative. Over 70% of the production of paints and printing inks in Germany is water-based. Most of these products need preservatives to prevent microbial growth. We estimate that alone in the German market for paints and printing inks a business volume of around 2.6 billion € is relying on in-can preservatives. With the isothiazolinones being subject to severe restrictions and the formaldehyde releasers being under pressure due the classification of formaldehyde, ZnPT is one of the very last remaining alternatives. Although it is not suitable for all applications, it is essential to have this option available. Furthermore ZnPT is non-volatile, which has advantages especially for indoor applications. With more and more substances being no longer available due to the review process, the future of water-based dispersion paints is in danger.

We remain available to provide further information.

The German paint and printing ink association (VdL) represents over 180 – mostly mid-sized – manufacturers of paints, coatings and printing inks. The VdL stands for nearly 90 percent of this industry in Germany. In 2016 the German manufacturers of paints, coatings and printing inks realized sales of ca. 8 billion euros and employed ca. 25,000 staff.

Dossier Submitter's Response

Thank you for your comment. We note that you want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Germany	Procter and Gamble Manufacturing GmbH	Company-Downstream user	21

Comment received

SUMMARY

Pyrithione and pyrithione-like compounds (di(2-pyridyl) disulfide N,N'-dioxide, also called pyrithione disulfide, and metal salts of pyrithione) have been identified in plants that are ingested by humans. The bulbs of the plant Allium Stipitatum or Persian shallot, are one of the most frequently consumed alliciaceous species in Turkey, Iran and other central Asia countries. The plant Polyalthia nemoralis has been used as a traditional Chinese medicine. Additionally, Pyrithione disulfide has been found in a member of the mushroom family.

BACKGROUND

The genus Allium comprises hundreds of species including garlic, onion, shallot, leeks and chives. The characteristic odor that comes from this genus is produced by compounds derived from cysteine. Allium Stipitatum (syn. Allium hirtifolium Boiss), also known as Persian shallot, is one of four plants in this group which Pyrithione and Pyrithione Disulfide have been identified (1-3). It is consumed in Iran and neighboring countries in various dishes including as a flavoring agent in yogurt. Additionally, Stipitatum extracts have been used for centuries to treat a variety of conditions such as tuberculosis, bronchitis, gout, diarrhea, stomach pain, psoriasis and as an analgesic (4-6).

Allium is not the only genus in which pyrithione has been identified. Polyalthia nemoralis, a member of the Annonacae family, is found in areas of southern China and has been used as a traditional Chinese medicine (TCM) for centuries and has been found to contain Pyrithione and Pyrithione copper and zinc salts (7-10). Its alleged use is as an antimalarial treatment and for the treatment of stomach aches (11-12).

Additionally, Pyrithione disulfide has been found in a member of the Basidiomycete (Mushroom) family, specifically Cortinarius Speciosissimus (13). Although not an edible mushroom, the finding does demonstrate an even wider presence of Pyrithione-like materials in plant species (13).

ANALYTICAL EVALUATION

Persian Shallot (Allium Stipitatum) - Pyrithione and Pyrithione disulfide were identified in extracts of the Persian Shallot by three separate groups and has been rigorously verified by synthesis of authentic materials, several forms of mass spectrometry, chromatography and nuclear magnetic resonance spectroscopy (1-4). Pyrithione is generated by the action of the allinase enzyme on the precursor compound (R)-S-(2-pyridyl)cysteine N-oxide (2-PyCNO) (Figure 1; see attached document). The resulting Pyrithione can then undergo further oxidation to give Pyrithione disulfide and other compounds resulting from further reactions (Figure 2; see attached document). It is possible that any plant containing 2-PyCNO and allinase enzyme has the potential to form Pyrithione and related materials.

Polyalthia Nemoralis – Chinese researchers identified Pyrithione and it zinc and copper salts in extracts of Polyalthia nemoralis in the early 1980s (7) using spectroscopic, mass spectrometry and nuclear magnetic resonance spectroscopy. Further characterization and identification work was conducted more recently in 1994 (9) and 2010 (10) on extracts confirming these early findings.

Cortinarius Speciosissimus – Pyrithione disulfide was detected in extracts of this mushroom and confirmed by several mass spectrometry approaches as well as nuclear magnetic resonance spectroscopy.

CONCLUSION

Pyrithione, including zinc pyrithione, has been reported to occur in plants where human consumption is documented.

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Novel Disulfide Metabolite from the New Zealand Basidiomycete (Mushroom) Cortinarius Species", J. Nat. Prod. 64, 341-344 (2001). ECHA note – An attachment was submitted with the comment above. Refer to public attachment Natural presence of pyrithione in food-Attachment-July 1,2017.pdf
Dossier Submitter's Response
Your comment (with the attachment) and the conclusion therein "Pyrithione, including zinc pyrithione, has been reported to occur in plants where human consumption is documented" is noted.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.07.2017	Belgium	The ZnPT Industry CLH Consortium	Industry or trade association	22
Comment received				
These comments are being sent on behalf of the following members of the ZnPT Industry CLH Consortium, an industrial consortium of the manufacturers of Zinc Pyrithione (ZnPT) and of downstream products made with ZnPT, namely P&G, Lonza and Janssen. The ZnPT Industry CLH consortium is concerned about the classification proposal on ZnPT. ZnPT is indeed an important component across a range of disparate industries for use as a preservative in diverse products such as cosmetics, paints and coatings and members of the ZnPT Industry CLH Consortium have always been committed to work towards high safety and environmental standards. ZnPT has a long history of safe use, having been widely used, globally, for over 50 years. Further, the SCCS which concluded on the evaluation of ZnPT in 2014 concluded that the substance is safe for its intended use. Specific comments on the classification proposal for reproductive toxicity, STOT RE and hazardous to the aquatic environment are provided in the relevant boxes of this ECHA webform. Due to the complexity of the reproductive toxicity classification scheme which requires an assessment of several parameters in a weight-of-evidence determination, using expert judgment, it is important for the ZnPT Industry CLH Consortium to present our rationale, which leads us to conclude that ZnPT is not a reproductive toxicant, to the greatest possible extent during this consultation. A summary of the comments on reproductive toxicity is provided in the "Specific comments" box while our full detailed review is submitted as "a supportive document" in "Public attachment". Similarly, for the STOT RE and hazardous to the aquatic environment endpoints, detailed comments are provided in "Public attachment" while a summary text is provided in the "Specific comments" boxes due to technical limitations in the webform. (All Public Attachments are being provided together in a zip file containing 4 documents in pdf form.) It is therefore important that the attachments are also reviewed by the Dossier Submitter and RAC members, as they contain all the details including key information in graphs, tables and diagrams that could not be published through the webform. ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT CLH Consortium Comments 4 attachments -June 30, 2017.zip				
Dossier Submitter's Response				

Noted. All attachments are reviewed by the DS. Please see our response under the comment numbers 56, 75 and 79.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Belgium	Procter & Gamble Co.	Company-Downstream user	23
Comment received				
The CLP guidance for CMR classification stipulates that chemicals should not be classified for reproductive toxicity if it can be demonstrated that the adverse effects are secondary to maternal toxicity (CLP Regulation (Annex 1, Sections 3.7.2.1.; 3.7.2.2.1.; 3.7.2.3.5.; 3.7.2.4.1.; 3.7.2.4.2.; 3.7.2.4.3.). We have demonstrated that pyrithione does not have any intrinsic ability to produce toxicity to rodent embryos (using rat whole embryo culture) even at concentrations that are highly toxic to the adult. Furthermore, we have established that the mode of action of pyrithione salts is the inhibition of aconitase, a Krebs cycle enzyme. Inhibition of aconitase stops oxidative metabolism, an effect that is toxic to the adult animal but not the embryo, which is not reliant on oxidative metabolism until later in development. In the detailed comments below, and in the pdf attachments, I provide details on the mdoe of action and lack of direct embryotoxicity of pyrithione. This includes supporting evidence from subchronic studies that show the extent of the metabolic disturbances in the adult organism that indirectly lead to adverse developmental effects. This approach is rigorous and fulfills the criteria for a substance that should not be classified. Much of this information is new and is not addressed in the CLH report. ECHA note – An attachment was submitted with the comment above. Refer to public attachment zptmoaforpdf.pdf				
Dossier Submitter's Response				
Thank you for your comment. It is true that the mode of action of pyrithione salts is not discussed in the CLH report for ZnPT. All your comments including that in the attachment are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response, including that on mode of action, under the comment number 56.				
RAC's response				
Noted. There are a lack of mechanistic studies for zinc pyrithione and not all of the molecular targets for this substance have been identified.				

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	United Kingdom	Lonza Cologne GmbH	Company-Manufacturer	24
Comment received				
These comments are being submitted on behalf of Lonza and Procter&Gamble, manufacturers of Zinc Pyrithione (ZnPT) and of downstream products made with ZnPT, respectively. A summary of the comments on use of all data related to pyrithione salts is provided in the "Reproductive Toxicology Specific Comments" box whilst our full detailed review is submitted as a supportive document in the "Public Attachment". It is, therefore, very important that the attachments are reviewed by the Dossier Submitter and RAC members, as they contain all the details including key information in graphs and tables				

that could not be published through the webform. ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT CLH response_Read across documents_June 2017.zip
Dossier Submitter's Response
All your comments including that in the attachment are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
26.06.2017	United Kingdom	Farrow & Ball	Company-Manufacturer	25

Comment received
Zinc Pyrithione, ZnPT (CAS No. 13463-41-7) As a UK paint manufacturer, we fully support the views and opinions submitted by The British Coatings Federation (BCF) on 16th June 2017 and CEPE on 21st June 2017. We understand that the purpose of the public consultation is to examine in detail all scientific and toxicological evidence relating to Zinc Pyrithione (ZnPT) and to assess the justification for the proposed classification of Repr. 1B. H360D. If this substance were to have the above proposed classification approved by the authorities, then this would fall within the scope of Art. 5 of the BPR (meet exclusion criteria), which would lead to severe usage restrictions and potential removal from our formulations. As the Biocidal Products Regulation does not necessitate any formal socio-economic analyses we feel compelled to bring to the attention of the authorities the importance of ZnPT as both a dry film (PT 7) and in-can (PT 6) preservative in our paint formulations. ZnPT is a key ingredient in providing dry-film preservation of exterior surfaces and certain interior environments (e.g. kitchens and bathrooms) against fungal and algal attack which if left untreated not only looks unsightly but can present potential microbial risks to human health. ZnPT is also used as a co-biocide (particularly with isothiazolinones) for the in-can preservation (PT 6) of paints to prevent spoilage during transportation and storage. We rely on a carefully selected combination of biocidal actives to provide adequate sterilisation and long-term preservation of our products, but with challenges in legislation leading to an ever-decreasing portfolio of biocidal substances available in our formulating toolbox, our options are now becoming severely limited. Any novel technologies which could be employed to replace substances such as ZnPT will need sufficient time to clearly demonstrate their effectiveness and compatibility. Long-term effectiveness cannot always be assured within the classification timescales proposed by the authorities which could lead to sub-standard product within the marketplace.
Dossier Submitter's Response
Thank you for your comment. We note that you want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Denmark		Individual	26
Comment received				
Pyrithione zinc is presently used as a PT6 and PT7 biocide for the preservation of paints and are one of the few biocides which can used for the coatings due to its good performance and stability in paint formulation. Many other actives approved are not usable for the different paints either due to compatibility, limited activity for the microorganisms in general towards the relevant organisms within the coating industry but also because of negative influence of properties. During the last years we have been faced with an increased number of restrictions for the use of biocides particular for in-can preservation, PT6. This is true for various types of isothiazolinones. Due to these restrictions only 4 to 5 biocides may be used in the future and further limitations of the number of biocides able to give in-can preservation to paints will cause difficulties. Pyrithione zinc has been and will be introduced as a proven alternative to particular isothiazolinones as it will serve as an effective PT6 biocide in combination with other actives but also lower the risk for allergy reactions. Furthermore building resistance towards the bacteria is of risk as only few biocides are approved. Bacteria will always be present in water borne paints due to the water but also fillers of various kinds as talc and calcium carbonate. Attempts to have more sterile raw materials have indicated very high costs involved and is therefore not an option. Also sterilisation or other methods for the reduction of the bacteria content in liquids used in other industries will not be relevant to the coating industry. It is therefore crucial that pyrithione zinc is maintained as an option for the use as a PT6 and PT7. It is very certain that without using pyrithione zinc more contaminated paints will be present on the markets which will results in higher costs but also an increased amount of paint waste.				
Dossier Submitter's Response				
Thank you for your comment. We note that you want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation, however, these are not relevant to the current CLH process.				
RAC's response				
Noted. The response of the DS is supported.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	27
Comment received				
We agree that no classification could be proposed for carcinogenicity due to lack of data				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC also supports no classification for CARC.				

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	Netherlands	PPG	Company-Downstream user	28

Comment received
No specific comments
Dossier Submitter's Response
Noted.
RAC's response
Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	29
Comment received				
We agree that zinc pyrithione does not fulfil the classification criteria for germ cell mutagenicity				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC also supports no classification for germ cell mutagenicity.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Germany		Individual	30
Comment received				
Critical Review of Reproductive Toxicity data of Zinc pyrithione (ZPT) 1. Summary of available studies 1.1 Developmental toxicity studies Several oral developmental toxicity studies have been performed in both rats and rabbits. In general, doses of 1.5 mg/kg and above caused maternal toxicity. In fetus-es anomalies were observed in the presence of maternal toxicity only, but not at dos-es not causing maternal toxicity. These anomalies were mainly observed in the (axi-al) skeleton and comprised some visceral ones. Postimplantation losses were also increased in doses that caused maternal toxicity. No maternal and/or fetal effects were observed below 1.5 mg/kg, and a clear NOAEL of 0.5 mg was derived for both endpoints. Maternal toxicity, besides the clinical observation of hind limb weakness, was ex-pressed as a decrease in body weight gain, both over the total treatment period, but also, and even more pronounced, at earlier stages of pregnancy which are consid-ered critical for the induction of anomalies in the embryo. Regarding fetal effects, it can be concluded that they do not form a specific pattern which would be characteristic for a direct effect on the embryo/fetus and the frequency of most of them did not follow a dose response relationship. An important feature of maternal effects is an extremely steep dose response relationship. 1.2 Multi-generation studies Two two generation reproduction toxicity studies performed with sodium pyrithione, from				

which read-across to ZPT is considered appropriate, are available. The results of these studies confirm data from developmental toxicity studies in that they showed high parental toxicity at 3.5 mg/kg together with some retardation in pup development. Important to note that applying doses up to 1.5 mg/kg, in pups there were no effects on gestation success or duration, number of pups born, live births, indices of viability and lactation, cumulative survival or sex ratio. No effects on pup weight were observed. No pup abnormalities were found following necropsy. No effects on development of ear opening, righting reflex or eye opening were observed.

1.3 Repeated dose toxicity studies

Since developmental toxicity studies would normally not provide sufficient information on maternal effects other than body weight and feed consumption, it is appropriate to consider results from repeated dose toxicity studies in order to specify possible underlying mechanisms.
In addition to the hind limb weakness seen in all studies at high dose levels, a dra-matic decrease in food conversion, expressed by an increase in the number of grams of food required to produce one gram of body mass (which could also be shown for the developmental toxicity studies) was observed. This finding strongly indicates an influence on energy supply.
Another consistent observation in the pyrithione repeated-dose toxicity studies is a tendency to a decrease in hemoglobin and/or hematocrit, which may influence the oxygen supply.

1.4. Mechanistic studies

1.4.1 Whole embryo culture studies

In order to determine whether pyrithione is a direct embryotoxicant, it was evaluated in rat whole embryo culture studies. This test has been evaluated by ECVAM and found to be a valid method for assessing the direct effects of chemicals on mammali-an embryonic development. Sodium pyrithione was used as the test agent because it is soluble in the culture medium (rat serum) and because zinc and sodium pyrithione both dissociate in the gastric lumen and are absorbed as pyrithione so that pyrithione is the only toxicologically relevant moiety of ZPT. Concentrations tested were equivalent to the area under the time-concentration curve (AUC) for a 24 hour period at dose levels of 0, 1, 3, 6, or 15 mg/kg pyrithione. The equivalent concentrations tested were 0, 0.15, 0.46, 0.92 or 2.3 µM, respectively. Sprague Dawley CD rat embryos were explanted on gestation day 9.5 and cultured for 44 hours. AUC was selected as the in vivo pharmacokinetic parameter to match in the culture system based on the presumed mode of action of pyrithione. In addition, the principal metabolite of pyrithione, 2-(methylsulfonyl)pyridine (2-MSP), was also tested in two replicates.
The results indicated no effects on growth or morphological development. There was a non-dose-responsive increase in dysmorphology at the 0.46 uM concentration, but this was not treatment related. These results clearly indicate that pyrithione does not have embryotoxic potential, even at concentrations that were equivalent to plasma concentrations at excessively maternally toxic dose levels (0.46, 0.92, and 2.3 uM).

1.4.2 Study on aconitase inhibition

It was shown in a number of studies that pyrithione also inhibits aconitase in mammalian cells. Cells derived from rats, rabbits and humans were exposed to sodium or zinc pyrithione. At concentrations that are comparable to in vivo concentrations that produced

toxicity, pyrithione effectively inhibited aconitase activity in all three cell types. This effect is much more critical for the adult organism rather than the developing embryo, since the latter is much less dependent on Krebs' cycle metabolism than the adult. So a direct effect of aconitase suppression occurring in the embryo is most unlikely. However, due to this effect occurring in the dams' metabolism, energy supply to the fetus from the maternal organism could be affected.

2. Role of maternal toxicity

In developmental toxicity studies, one would normally assess maternal toxicity based on food consumption and body weight gain. It is important to keep in mind that a change in these parameters would not be causative for any fetal effects, for they are both expressions of more complex underlying mechanisms leading to disturbances in the maternal homeostasis. More complex, mechanistic studies are often required to specify the underlying mechanism which can link maternal and fetal findings together, rather than just trying to explain these were causative (all studies trying to show an influence of food restriction on embryo-fetal effects, therefore, must fail).
In order to try and link embryo-fetal findings to maternal ones, it is also useful to investigate, based on individual data, whether in groups displaying a high variety in body weight changes it could be shown that most affected fetuses originated from those dams that gained less weight in the study.
Important to state that this was performed in one of the rabbit studies, and the named correlation could be established.

3. Proposed mechanism of action

A number of guideline and mechanistic studies are available allowing to conclude on the mechanism of action of ZPT. Taking the above described results together, it can clearly be postulated:
ZPT primarily inhibits aconitase in adult animals, thus causing decreased energy supply in the dams. This leads to the observed reduction in food conversion which in turn is causative for the observed decreases in body weight of the dams after treatment with doses of 1.5 mg/kg or higher. Thus, decreased body weight gain is an expression of aconitase inhibition in the dams. This is also confirmed by hind limb weakness, for muscles are highly dependent on energy supply, which is decreased via aconitase inhibition.
A direct effect on the embryo/fetus can be excluded since the embryo is much less dependent on Krebs' cycle metabolism than the adult. This can also be shown when comparing maternal weights with fetal weights, showing the latter being much less influenced. However, aconitase inhibition will lead to a deceased maternal energy supply for the developing organism. This effect can then be even enhanced by the observed decrease in hemoglobin and/or hematocrit, reducing the oxygen supply in the embryo/fetus thus creating mild hypoxia. Decreased maternal energy supply in connection with hypoxia can well lead to an unspecific pattern of anomalies as it was observed in the developmental studies both in rats and rabbits.
The fact that the vast majority of fetuses with anomalies were from litters of dams with the lowest body weight gain further substantiates that the anomalies observed in fetuses are secondary to maternal toxicity.

4. Summary and conclusions

Based on these data and applying evidence of weight in interpreting them, it was

concluded that any effects on fetal development observed in developmental tox-icity studies are not direct effects, but solely secondary to maternal toxicity, as shown by the following facts:
1. In a whole embryo culture study using relevant concentrations, pyrithione (the only toxicologically relevant moiety of ZPT) was unequivocally shown not to have any intrinsic embryotoxic potential, even at concentrations that were equivalent to plasma concentrations at excessively maternally toxic dose levels.
2. Based on mechanistic studies, a clear mechanism of action could be derived which plausibly explains that the observed fetal effects are solely due to indirect effects via the dam rather than a direct effect of the compound in the embryo/fetus.
3. In summary, the available data strongly support the conclusion that ZPT does not have "an intrinsic property to produce an adverse effect on reproduction".
4. Consequently, the proposal to classify ZPT in Category 1B for developmental tox-icity is not scientifically justified and a classification of ZPT is not warranted based on the available information.

Hannover, June 28th, 2017

Dr. Jochen Buschmann
Consultant in Reproductive Toxicology

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT comments Jochen Buschmann.pdf

Dossier Submitter's Response

Thank you for your comment and the attachment. All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.

RAC's response

Well formed arguments. But at the end of the day we have animal studies with an agent that results in clear foetotoxicity and malformations. The issue of maternal toxicity is a complex one but we must first take note of the effects on the foetus, and not automatically discount the effects as secondary to maternal toxicity without clear mechanistic evidence to do so.

Date	Country	Organisation	Type of Organisation	Comment number
28.06.2017	Germany		Individual	31

Comment received

Zinc pyrithione (ZPT) is a widely used antifungal agent that also has applications in dandruff control. The general toxicology database on ZPT and other salts of pyrithione is extensive. A proposal to classify ZPT as a developmental toxicant class IB (CLH-proposal) has been submitted to ECHA. The available information on the developmental toxicity of ZPT contains a number of studies performed according to regulatory guidelines in rats and rabbits. In addition, developmental toxicity studies on other pyrithione (PT) salts and detailed information on toxicokinetics and toxicity of ZPT after repeated dosing to animals are available for evaluation.
Application of ZPT to pregnant animals induces excessive maternal toxicity (reductions in weight gain well above 50 % during some periods of pregnancy) and non-dose related

increases in malformations in offspring at dose levels causing excessive maternal toxicity as indicated by the reduced weight gain. A detailed presentation of experimental evidence regarding the points raised in this expert review is available in the „SUPPORTIVE DOCUMENT TO THE ZNPT INDUSTRY CLH CONSORTIUM COMMENTS ON REPRODUCTIVE TOXICITY submitted in response to the CLH-proposal.

1. General comments on the CLH proposal
The CLH-Proposal on ZPT contains a detailed description of the available database on the developmental toxicity of ZPT and also covers most of the other relevant information. However, the proposal is not in line with the requirements defined in the CLP-directive regarding integration of all relevant information using a weight of evidence approach to conclude on a need for classification. These requirements are:
• A weight of evidence evaluation of the database integrating all relevant information,
• A detailed assessment of potential confounding of the outcome of developmental and reproductive toxicity studies by maternal toxicity,
• Identification of an intrinsic property of the chemical to affect the embryo/fetus
• Integration of expert judgment in the final conclusion
The CLH-Proposal does not adequately assess the role of maternal toxicity indicated by the massive reduction in maternal weight gain during relevant periods of embryonal development, does not adequately evaluate if the developmental effects are intrinsic effects to the embryo or secondary to maternal toxicity, and inappropriately dismisses several relevant dataset as being of little relevance for conclusions on the potential of ZPT to induce developmental toxicity. Therefore, the proposal to classify ZPT in Category 1B for developmental toxicity is not scientifically justified and the proposed classification is not warranted by the available information.

2. Specific comments on effects cited as supportive for classification
Inadequate integration of available information on the toxicity of ZPT in the CLH-proposal.
A number of repeated dose toxicity studies with ZPT have been conducted and the observations made in these studies provide relevant information on the toxicity of ZPT to evaluate maternal toxicity. The observations in the repeat dose studies with ZPT show that, as in the developmental toxicity studies, oral doses of ZPT in the range of 1 to 2 mg/kg bw/day are well tolerated. Above these dose levels, dose-response is very steep with excessive toxicity and mortality occurring at dose levels > 3 mg/kg bw/day. The results of these studies are described in detail in the CLH-proposal (table 70) and the steep dose-response after repeated administration is acknowledged (10.12.1) in the document. The integration of the results from the repeated dose studies in the weight of evidence approach is required since repeated dose studies assess systemic toxicity in much more detail as compared to the characterization of maternal toxicity in studies with a focus on developmental toxicity. Integration of the observations from the repeated-dose studies with ZPT and other PT salts therefore is required for a detailed assessment of maternal toxicity.
The CLH-proposal dismisses the use of toxicity information from other PT-salts without an adequate justification. In the CLH-report (under 10.9.1), the CLH-proposal briefly states that ZPT and salts of PT have different physicochemical properties that may influence toxicokinetics. This conclusion neglects the available information on the toxicokinetics of ZPT and other salts of PT, which clearly shows that only PT is bioavailable from ZPT after oral administration to rodents. Zinc present in ZPT is not absorbed from gastrointestinal tract after oral administration of ZPT and is thus excreted with feces. Other PT salts share identical toxicokinetic properties and metabolic pathways as ZPT and the active toxicant after administration of ZPT and other PT salts is identical (PT). Data on the toxicity of other PT salts will provide relevant information characterizing dose-response for effects on the embryo/fetus and the maternal organism and need to be integrated when

evaluating ZPT.
In contrast to statements in the CLH-report, zinc therefore has no relevance for systemic toxicity of ZPT since zinc is not bioavailable after oral administration of ZPT. As a consequence, the limitations of the whole embryo culture assays concluded in the CLH-report are not valid. In contrast, the information from these whole embryo culture assays is of high relevance for conclusions regarding classification of ZPT as developmental toxicant.

Maternal toxicity
Assessment of body weight gain during a toxicity study is a sensitive parameter to indicate toxicity. Reductions in weight gain during a toxicity study can be easily and precisely determined. For example, the US National Toxicology program defines the maximum tolerated dose (MTD) for a carcinogenicity study as a 10 % reduction in body weight gain in treated animals as compared to controls; in developmental toxicity studies, reductions in maternal weight gain of 10 to 20 % are considered to indicate excessive maternal toxicity (Beyer et al., 2011).
In developmental toxicity studies following regulatory guidelines, maternal body weight is determined in two to three day intervals. The CLH-proposal actually reports the detailed results on maternal body weight gain from the different developmental toxicity studies with ZPT under the heading "maternal effects". The results of the study described in table 56b of the CLH proposal and those from other studies (e.g. table 58b) show that maternal weight gain was reduced by > 50 % at high ZPT-doses. Animals in the high dose groups actually lost weight during time periods of gestation that are highly important for embryo/fetal development. Most of the developmental effects seen after high dose ZPT-administration are induced during the periods of gestation when maternal body weigh gain was most depressed.
The summary (10.10.6) in the CLH-proposal even concludes that ZPT induces "severely reduced body weight gain" but dismisses this indicator of pronounced maternal toxicity by citing results from a developmental toxicity study under food restriction. This study did not observe increased incidences of malformations, but also did not induce excessive maternal toxicity as does ZPT. Therefore, the only conclusion possible from the food restriction studies is that a reduction in maternal body weight does not induce developmental toxicity. Conclusions regarding effects of maternal toxicity on embryo/fetal development are not possible. As the severe reductions in body weight gain in the ZPT studies are indicative of excessive maternal toxicity that interferes with embryo/fetal development, depression of weight gain in the ZPT studies is secondary to toxicity and the comparison made is scientifically unsound.
Further support for a correlation between maternal weight gain decrements and developmental toxicity can be derived from an analysis of individual animal data from a rabbit developmental toxicity study administering ZPT at doses of 0, 0.5, 1.5 and 4 mg/kg bw/day from gestational day 7 – 28. Maternal weight gain was significantly reduced in the high dose animals, while, due to large variations in weight gain in the 1.5 mg/kg bw/day group, the effect on maternal weight gain was not significant on a group basis. Analysis of individual animal data shows that a weight gain of only 18 % relative to controls occurred in the most affected animals. Litters from these animals accounted for the vast major of developmental toxicity seen at this dose level.
In summary, developmental toxicity of ZPT is only observed at doses that induce severe maternal toxicity.
Inadequate evaluation if effects are an intrinsic property of the chemical to affect the embryo/fetus
The CLP-directive states "classification as a reproductive toxicant is intended to be used for substances that have an intrinsic property to produce an adverse effect on reproduction" and "substances shall not be classified if such an effect is solely produced

as a non-specific secondary consequence of other toxic effects". The CLH-proposal does not address this issue nor perform the required weight of evidence assessment.
As described above, maternal toxicity is inappropriately dismissed in the CLH-proposal despite clear evidence that developmental toxicity only occurs at doses of ZPT that induce excessive maternal toxicity.
PT, at concentrations relevant to those occurring in intact animals under the dosing conditions of the toxicity studies, PT did not induce toxicity in rat embryos in culture. The outcome of the embryo culture study is dismissed in the CLH-proposal based on the argument that the embryo culture study did not apply ZPT, but PT and that "the predictability and applicability domains (of the model) are not yet sufficiently defined for regulatory implementation". These are not valid points. Using PT is appropriate since only the PT moiety of ZPT is bioavailable and thus exposure of the embryo in vivo occurs only to PT or its metabolites. Moreover, concentrations of PT applied in the whole embryo studies were selected based on toxicokinetic consideration. The whole embryo culture has also been qualified "as ready to be considered for regulatory purposes" and is widely applied to characterize toxicity on the embryo and its mechanisms (Tonk et al., 2013; Dimopoulou et al., 2016; Dimopoulou et al., 2017). Therefore, the absence of toxicity in this assay shows that PT does not have a specific embryotoxic effects.
Further support for the conclusion that ZPT does not have an "intrinsic property to produce and adverse effect on reproduction" are the absence of dose response regarding developmental endpoints in individual studies and the absence of a specific pattern of developmental effects across studies. A dose response regarding incidence of developmental effects is expected if ZPT (or PT) is inducing direct effects on embryo/fetal development. However, there is no relationship between types of effects or severity of developmental toxicity in the studies with ZPT. In addition, there is no specific pattern of developmental effects across the studies. A specific pattern of malformations should be observed when a direct effect on the embryo is the basis for the developmental toxicity.
Comparison of maternal weight gain and fetal weights from the available developmental toxicity studies with ZPT alos show that maternal weight gain indicative of excessive maternal toxicity follows a steep dose response with weight gain reduction starting at dose levels in the range of 2 to 3 mg/kg bw/day reaching only about 40 % at a dose level of 8 mg/kg bw/day. In contrast, fetal weigh gain as sensitive parameter of fetal toxicity is little affected at these doses and starts to decline in a dose range well above that.
A mode of action (MoA) for toxicity of ZPT has been developed. This MoA involves inhibition of aconitase by PT as key step with subsequent inhibition of oxidative phosphorylation as shown in vitro with IC50s in the low □M range. The pattern of toxicity of ZPT and other PT salts with the steep dose response from doses without observable effects to doses inducing lethality is very similar to dose response curves observed in repeated dose studies with the prototypical aconitase inhibitor fluoroacetate (Eason et al., 1999; Eason and Turck, 2002); ZPT also induces a pronounced reduction in feed conversion efficiency in repeated dose studies (with NaPT) supporting this MoA. Food conversion efficiency is another metric of inhibited energy production. The embryo is much less dependent on Kreb's cycle metabolism until relatively late in gestation than the adult since its primary source of energy is glycolytic. Inhibitors of aconitase such as fluoroacetate have much less effect on embryonic mitochondria than adults (Neubert et al., 1971; Spielmann and Lucke, 1973; Spielmann et al., 1973).
In summary, the available data strongly support the conclusion that ZPT does not have "an intrinsic property to produce an adverse effect on reproduction". In contrast, effects are mediated by the excessive maternal toxicity due to a much higher sensitivity of the maternal organism to inhibition of oxidative phosphorylation.

3. Conclusions
The available data show that ZPT only induces developmental toxicity at doses that cause

excessive maternal toxicity expressed by decrements in maternal weight gain by well above 50 % as compared to controls. The available data also strongly support the conclusion that developmental toxicity of ZPT is not due to an in intrinsic property of ZPT to induce effects on the embryo, but secondary to non-specific effects on the maternal organism due to the high toxicity of ZPT. Therefore, classification of ZPT as a reproductive toxicant category 1B is not supported by a weight of evidence approach as required by the CLP-directive. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Comment ZPT-ECHA.pdf
Dossier Submitter's Response
Thank you for your comment and the attachment. All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany	Thor GmbH	Company-Manufacturer	32
Comment received				
Thor GmbH disagrees with the data submitter's (DS's) proposal for classification in Reproductive Toxicity 1B. The compound's intrinsic properties should be assessed in a weight of evidence approach covering all pyrithione compounds, namely sodium, copper and zinc pyrithione. The developmental effects were observed in presence on excessive maternal toxicity. No specific developmental effect, was identified, i.e. the occurred malformations do not correspond to any recognisable syndrome; hence, suggesting developmental findings are secondary to underlying mode of action leading to (maternal) toxicity. - Zinc pyrithione: Rat dietary developmental toxicity study (OECD 414) (Thor GmbH Art.95 dossier; 2015) The DS correctly presented the results of the teratogenicity study in Wistar Han rats. Maternal toxicity was observed only at the highest dose level (1.68 mg ZnPT/kg bw/d). Here, the maternal effects were observed in three dams showing clinical signs such as abnormal gait, a body weight gain decrement of > 50% over the dosing period as well as decreased food consumption. Effects on the foetuses were limited to a decrease in foetal weight in the high dose group, i.e. in the presence of maternal toxicity. In summary, there was no evidence of developmental toxicity in the absence of maternal toxicity. The mode of action of the pyrithione moiety (cf. "Supportive Document to the ZnPT Industry CLH Consortium comments on Reproductive Toxicity", July 2017) and the steep dose-response-curve for ZnPT explain the maternal toxicity and the foetal effects can be interpreted as secondary to maternal toxicity. In conclusion, the recent guideline conform teratogenicity study in rat (Reliability 1) provides evidence that ZnPT holds no intrinsic property to act as reproductive toxicant. - Zinc pyrithione: Rabbit oral (gavage) developmental toxicity study (OECD 414) (Thor GmbH Art.95 dossier; 2015) Maternal and developmental toxicity was observed in the high (4 mg ZnPT/kg bw/d) and mid dose (1.5 mg ZnPT/kg bw/d) groups. The biologically most relevant effect in the dams was decreased body weight gains compared to the control group, being statistically				

significant in the high dose group. There was no body weight gain in the high dose group during the first two weeks of dosing and body weight gain over the entire dosing period was decreased by more than 50% at the high dose compared to control. This decrement in body weight has to be attributed to an excessive maternal toxicity, which when occurring to such an extent justifies to disregard the developmental data in the relevant groups (ILSI/HESI workshop, Beyer et al, 2011). The toxicity of ZnPT shows a steep dose-response curve. It is noteworthy that in the dose range finding study in pregnant animals, one rabbit was found dead the morning after the first dose of 4 mg ZnPT/kg bw/d. This incident clearly supports the postulation of a steep dose-response and shows that the selected high dose for the main study was too high.
By contrast, there was a lack of statistical significance of the body weight gain reductions in the mid dose group (1.5 mg ZnPT/kg bw/d); this was attributed to the high variability of the dam's body weights in this group. Here, a more detailed evaluation of the individual results revealed six animals that were more adversely affected by the ZnPT treatment compared to the other dams in the same dose group (cf. "Supportive Document to the ZnPT Industry CLH Consortium comments on Reproductive Toxicity", July 2017). The biological relevance of the excessive maternal toxicity (mean: over 70% body weight gain decrement after the first two weeks of treatment) observed in these six animals cannot be dismissed. Importantly, the majority of the developmental effects in the mid dose group, including external malformations of omphalocele, can be attributed to the litters of these most affected dams in mid dose level group. Overall, the developmental effects in the high and mid dose group of the rabbit teratogenicity study were observed in presence of excessive maternal toxicity (>20% body weight gain reduction; see table 63c, page 80, CLH report).
Although the DS managed to describe the excessive maternal toxicity in the CLH report, it failed to appraise these effects relevant for the evaluation of the developmental effects. Furthermore, the DS failed to acknowledge that no specific developmental effect of ZnPT has been identified in any of the referred studies, i.e. that the occurred malformations do not correspond to any recognisable syndrome.

For more details please see the following documents:
- "Zinc Pyrithione (CAS: 13463-41-7): Assessment of Developmental Effects" (June 2016) provided by the DS in the confidential appendix of the CLH report and
- "Supportive Document to the ZnPT Industry CLH Consortium comments on Reproductive Toxicity" (July 2017) submitted by the "ZnPT Industry CLH Consortium" during the public consultation phase.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2017 07 07 Thor GmbH comments to ZnPT CLH report.pdf

Dossier Submitter's Response

Thank you for your comment and the attachment. All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany		MemberState	33
Comment received				
Pages 52-85. The classification proposed by the DS as Repr. 1B (hazard statement H360D – May damage the unborn child) is supported.				

• In two of the oral gavage studies on developmental toxicity with rabbits and rats, foetal and embryonic effects were observed without clear maternal toxicity already at mid doses tested (1.5 mg/kg bw – Thor GmbH dossier 2015; and 3.0 mg/kg bw –DocIIIA A6.8.1/02 respectively). At the 1.5 mg/kg bw dose from the rabbit study, there was no statistically significant changes on bw (gain) or relative food consumption in the dams; there was a statistically significant decrease in the mean of viable fetuses, statistically significant increase in post-implantation losses compared to both controls and historical control data, increased number of malformations (2 foetuses from 2 litters had external malformations of omphalocele) and statistically significant increase in litter incidences of 13th full rib and pelvic girdle caudal shift; these malformations were considered treatment related also by the study author. At the 3.0 mg/kg bw dose from the rat study, the dams' only clinical sign was salivation and no bw and bw gain differences were observed. There was an increase in the number of examined fetuses with malformations, increased number of examined fetuses with skeletal malformation (fused ribs, pelvic malformation, tail malformation) and an increased number of examined fetuses with soft tissue malformations (diaphragms hernia, anal atresia).
• In most of the 10 developmental studies available via oral (diet or gavage) and dermal route, these effects were either statistically significant, or dose-response dependent or above the historical control data.
• The pyrithione moiety can also not be considered the only impacting factor on the foetal toxicity from the developmental studies with zinc pyrithione. That is because literature is controversial on zinc as a supplemental heavy metal during pregnancy and it may interfere with maternal iron metabolism and subsequently affect fetal and ultimately infant iron metabolism both in humans and animals (Hossain et al, 2011). Zn also easily penetrates the placenta and blood-brain barriers, and among the different organs, the brain of developing animals is most sensitive to the neurotoxic effects of Zn and other heavy metals (Nowak et al, 2006).
• The submitted in vitro experiments using rat embryos in culture and pyrithione or its principal metabolite exposure is not sufficient to disregard the additive or synergistic effects of the whole molecule as zinc pyrithione (details at CLH page 81) to the developing embryos.
• As also explained in the present CLH report (page 51) in the context of carcinogenicity evaluation, zinc status can influence various physiological processes. Accordingly, chronic studies with sodium pyrithione were also not considered sufficient to evaluate this endpoint for zinc pyrithione.
• Taking the importance of dietary zinc into account, the studies with other molecules (i.e. sodium pyrithione) should be given limited weight only in this case.
• The proposed mechanism of zinc pyrithione toxicity via inhibition of oxidative metabolism (in vitro studies –IUCLID comments from applicant) affects the Kreb's cycle energy metabolic pathway. It is true that gastrulating and neurulating embryos metabolism is dependent mostly on the glycolysis, but metabolic pathways may be different in different regions of the embryo even during early stages of morphogenesis that predate differentiation of definitive organ systems (Sadler et al., 1993). Therefore, the ATP synthesis inhibition and impact on fetuses' subsequent development cannot be completely disregarded.
• In summary, there is sufficient evidence of foetal toxicity (increased incidences of post implantation losses, resorptions and skeletal malformations) in two different species tested in guideline studies with zinc pyrithione. As there is no evidence from human studies or mechanistic information about the relevance of the effects for humans, the evidence from experimental animals is sufficient to classify zinc pyrithione as Repr. 1B.
• The labelling as "suspected human reproductive or developmental toxicants" is also part of the current classification of New Zealand (HSNO CCID) and Japan (GHS-J – Repr. 2).

Other references: Hossain et al. 2011. Maternal Iron and Zinc Supplementation during Pregnancy Affects Body Weight and Iron Status in Rat Pups at Weaning. American Society for Nutrition, doi:10.3945/jn.110.135681. Nowak et al., 2006. Effects of Pre- and Postnatal Zinc Exposure on Adult Rat Brain Dopamine Activity and Behavior. Polish J. of Environ. Stud. Vol. 15, No. 4 (2006), 565-572. Sadler et al., 1993. Effects of altered maternal metabolism during gastrulation and neurulation stages of embryogenesis.Ann N Y Acad Sci. 1993 Mar 15;678:48-61. IUCLID 2016. CLH_Comments_ZnPt_Consortium.pdf. Summary comments from applicant during zinc pyrithione CLH public consultation.
Dossier Submitter's Response
Thank you for your support and for highlighting several of the key points.
RAC's response
Agreed. All these comments can be supported.

Date	Country	Organisation	Type of Organisation	Comment number
22.06.2017	Netherlands		Individual	34
Comment received				
It looks like industry with approval of the authorities have used ant-dandruff shampoo for controlling the birthrate in Europe.				
Dossier Submitter's Response				
Comment irrelevant to classification and labelling of ZnPT.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
21.06.2017	Belgium	CEPE	Industry or trade association	35
Comment received				
Our input to the public consultation aims at raising awareness on the importance to take into account all scientific arguments because this substance is important to our business ECHA note – An attachment was submitted with the comment above. Refer to public attachment CEPE ZnPT Public Consultation final 20170621.pdf				
Dossier Submitter's Response				
Thank you for your comment. In the attachment you wanted to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium		MemberState	36
Comment received				

Considering the consistency across the majority of the available studies on the same kind of effects occurring sometimes at very low doses (1.5 mg/kg bw/d): increase in post-implantation loss, increase in skeletal/total/soft tissues malformations, decrease in fetuses viability and/or whole litter resorption, BECA agrees that reproductive toxicity is a very relevant endpoint that warrants a classification. BECA considers there is sufficient information to classify as Repr. 1B particularly because effects observed are severe, they may occur at very low doses, and dose-response relationship was reported in some studies.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany	GEHOLIT+WIEMER Lack- und Kunststoff-Chemie GmbH	Company-Downstream user	37

Comment received
We are part of the coatings industry formulating mainly protective coatings for industrial and commercial users. In the past years legislation has strained to reduce emissions of volatile organics to prevent global warming and improve air quality. Hence producers made an effort in the development of water based paints that have to be provided with algae and fungi protection. These improvements are now severly endangered by the current classification intentions and the biocidal review program. Zinkpyrithione is the one of the very few substances to be used in in-can or dry film preservation against algae and fungi. Alternative substances not only have to be effective but also compatible with coatings ingredients and production processes. Currently there are none available in the market, that would match these requirements. It has to be feared, that any substance with biocidal effect will sooner or later be classified for some hazard that leads to their ban. Classification of ZnPT as reprotox 1B would have eventually much more severe effects than the well controlled risk of its uses as biocide.
Dossier Submitter's Response
Thank you for your comment. You want to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation, however, these are not relevant to the current CLH process.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	38

Comment received
We agree with the Repr.1B classification for reproductive toxicity
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Finland	Tikkurila.Oyj	Company-Downstream user	39
Comment received				
As downstream user our input to the public consultation aims at raising awareness on the importance to take into account all scientific arguments because this substance is important to our business.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Japan	Mitsubishi Chemical Corporation	Company-Manufacturer	40
Comment received				
Our concerns over the proposed classification as Repro. 1B are included in the attached position statement. The studies included in the CLH report that support classification as a Category 1B reprotoxicant all appear to have a test substance purity value of <99.9 %. With this substance it is often observed that heavy metals can potentially be present including Lead and Cadmium (amongst others) and accordingly the effects in the detailed studies may be as a result of the presence of such heavy metals which have well known and observed reproductive effects. The presence or absence of such impurities is lacking in the experimental details and if present, may have contributed to the noted effects. For older data, there is also the possibility that the trace amounts of impurities are unknown, and that some commercial suspensions contained preservatives and dispersants which were considered to be CBI, with unknown reportoxicity details available. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment MCC ZnPT Public Consultaion July 2017.pdf				
Dossier Submitter's Response				
Thank you for your comment and the attachment. Please see our response under the comment number 10.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Japan	<confidential>	Company-Downstream user	41
Comment received				
Data may not show hazard of pyrithione zinc. In active pharmaceutical ingredient field, test for reproductive toxicity cannot be recognized when substances includes impurity more than 0,1 percent. In general, pyrithion zinc is available 95percent purity or 48-52 percent sunspension. Therefore data				

from these low purity material cannot be used in such important change for classification.
Dossier Submitter's Response
Please see our response under the comment number 10.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Japan		Individual	42
Comment received				
Our company has been dealing this chemical as a commercial trader for almost 20 years but we have never heard serious problem from any of our customers.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium	Cosmetics Europe	Industry or trade association	43
Comment received				

Cosmetics Europe welcomes the opportunity to contribute to the Public Consultation on the CLH Proposal for Zinc Pyrithione (ZnPT, CAS # 13463-41-7) as Reproductive toxicant 1B, H360D. In this section, we will provide specific comments on the endpoint of Reproductive Toxicity relating to the CLH proposal and dossier. It has been proposed by the dossier submitter (DS) that ZnPT should be classified as a reproductive toxicant Category 1B. We do not agree with this proposed classification and labelling of ZnPT which is in our view not warranted considering the CLP Classification criteria. To determine Classification as Reproductive Toxicant per CLP it is necessary to assess:
(1) absence of other toxic effects (e.g. excessive maternal toxicity),
(2) lack of intrinsic property to produce adverse effects on development, and
(3) mode of action, i.e., secondary non-specific toxicity

Moreover, the complete data set of pyrithione salts, i.e., ZnPT, NaPT and CuPT, need to be considered in a weight of evidence approach to fully understand toxicity and draw suitable conclusions (we refer to our general comments for details).
Given the format of this public consultation and the web interface requirements, our submission is accompanied by 2 Attachments which contain more details as well as references and therefore need to be viewed together with the comments submitted here (1 – General Comments, 2 – Comments on the Reprotoxicity Endpoint).

(1) ZnPT does not have intrinsic properties to produce developmental effects

The CLP states in Annex I, Section 3.7.2.2.1 that: "Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects".

To study whether there is an intrinsic property of the pyrithione (PT) moiety to produce developmental effects, the validated ECVAM isolated rodent whole embryo culture (WEC) method was used. Rodent whole embryo culture is a method that has been developed to distinguish whether a chemical is directly embryotoxic, or not, independent of extrinsic factors such as maternal toxicity. Rodent whole embryo culture is an in vitro technique in which intact embryos are maintained in culture, outside the uterus, for up to two days during organogenesis. The method has significant value since it can be used to determine the direct effects of a chemical on the embryo in the absence of maternal factors. If a chemical is directly embryotoxic at concentrations that are also maternally toxic, then the outcome of the embryo culture study would be embryotoxicity at the same concentrations that were maternally and developmentally toxic in vivo. However, if the effects on the embryo are secondary to maternal toxicity, then the results of the embryo culture study would be no embryotoxicity at the same (or even higher) concentrations that are maternally toxic in vivo.
Pages 81/82: Summary of Rat Whole Embryo Culture Studies (rWEC) with ZnPT
The DS cites the results of rWEC studies conducted with PT, however states that "the results of these rWEC assays are not completely relevant to conclude that zinc pyrithione is not directly embryotoxic as the toxicological significance of Zn2+ in synergy with the pyrithione is not addressed in these assays". We disagree with this statement. As the SCCS (1512/13) notes in Chapter 3.4.9.2., General information on Metabolism and Toxicokinetics of ZPT (page 58), "Studies performed with 65ZnP indicate that following oral administration, zinc pyrithione disassociates to liberate Zn and the pyrithione moiety which are then absorbed independently.". The same metabolic scheme is described for NaPT, which was used in rWEC studies (as ZnPT is insoluble in culture medium whereas NaPT is soluble). Plasma, as well as culture medium used in the rWEC experiments, contains free zinc; as such, dissolved PT is free to interact with free zinc, in either case, i.e., in vivo or in vitro.

Moreover the DS states that the "predictability and applicability domains are not yet sufficiently defined for regulatory implementation". We disagree with this interpretation; while the rWEC results should not replace relevant in vivo findings, they are to be used in the course of classification in a Weight-of-Evidence (WoE) approach. It has been evaluated and validated by ECVAM as a relevant assay for embryotoxicity (Spielmann et al., 2006) and was designated as "ready to be considered for regulatory purposes" (ECVAM ESAC, June 2002). Importantly, ECHA has stated in their guidance on "Information Requirements" (page 474), that this study may be used as supporting information along with other more reliable data to predict developmental toxicity. As the CLP requires to conduct "an integrated assessment of all available data and their interrelationship using a weight of evidence approach", Cosmetics Europe wants to stress the importance of the WEC studies in a WoE approach.
We want to point out that alternative methods are especially relevant for the cosmetics industry which is subject to animal testing ban on cosmetic ingredients. In fact, the SCCS acknowledges in their most recent Notes of Guidance the use of the WEC assay: "Since the field of reproductive toxicity is very complex, it is expected that the various stages cannot be mimicked using one alternative method and that a battery of tests is needed. Three alternative methods, restricted to the embryotoxicity area, have been developed: (i) The Whole Embryo Culture test (WEC); (ii) The MicroMass test (MM); (iii) The Embryonic Stem cell Test (EST)" (page 37). Although the SCCS also notes that these assays cannot be used for quantitative risk assessment, it is noted that they can be "useful in the CMR strategy for screening out embryotoxic substances".
The rWEC study provides valuable insight in this respect as it clearly shows that the pyrithione moiety has no intrinsic, specific property to produce an adverse effect directly on the embryo.

The results from this validated in vitro study of direct acting embryotoxicity indicate no effects on growth or morphological development for PT or MSP (Olavarria, 2016a, Olavarria, 2016b). There was a non-dose-responsive increase in dysmorphology at the 0.46 µM concentration of PT, but this was not treatment related. These results clearly indicate that neither PT nor its primary plasma metabolite MSP have embryotoxic potential, even at concentrations that were equivalent to plasma concentrations of PT at excessively maternally toxic dose levels (0.46, 0.92, and 2.3 µM).

(2) Influence of Maternal Toxicity

Section 3.7.2.2.2. of Annex I to the CLP requires that, when evaluating potential developmental effects of a substance, consideration to the possible influence of maternal toxicity should be taken into account (see also Section 3.7.2.4 of Annex I to the CLP). In case of excessive maternal toxicity or if the substance is so toxic, developmental effects should be discounted and should normally not lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate." (CLP, Annex I, Sections 3.7.2.3.5.; 3.7.2.4.3.; 3.7.2.4.4. and 3.7.2.5.8.).
The CLP does not provide defined criteria for excessive maternal toxicity (except for mortality) but states in Annex I: 3.7.2.4.4. that inter alia „Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available". Contrary to evaluation of other clinical signs (which might be subjective), measurement of maternal body weight is objective and not based on observation.
In their opinion 1512/13, the SCCS (page 55, in context of Study E23, Schardein et al 1993a) defines maternal toxicity as "significant inhibition of weight gain during treatment and a higher incidence of increased salivation post-dose relative to the control group". We also want to point to the conclusions of an ILSI workshop of 2009 (published in 2011 by Beyer et al.) which is also referenced in the CLH report (page 84) where "a decrease in body weight gain of 20% was considered excessive".
Taken together, (adjusted) maternal body weight gain is a critical, yet objective parameter to assess magnitude of maternal toxicity; a decrease of >20% is generally accepted as "excessive". Unfortunately, the CLH report does not assess whether the developmental effects observed in various studies were observed in presence of excessive maternal toxicity (decrease in body weight gain of >20%).
Pages 82ff: Influence of Maternal Toxicity and Summary of Developmental and Reproductive Toxicity Studies on ZnPT
Several developmental and reproductive toxicity studies have been conducted with ZnPT. These studies are summarized in and discussed in detail in Attachment 2 to this submission. In summary, developmental toxicity in the ZnPT studies is observed only at doses that produce excessive maternal toxicity. As stated in our general comments and following the SCCS´s opinion (2014), studies conducted with related salts i.e. NaPT and CuPT, are relevant and necessary to conclude on classification of ZnPT as a reproductive toxicant. In summary, there are multiple developmental reproductive toxicity studies with related salts, i.e., NaPT and CuPT, and they are remarkably consistent in that maternal toxicity is excessive before any developmental abnormalities are produced.
(3) Mode of Action, i.e., secondary non-specific toxicity
The Guidance on CLP (Section 3.7.2.2.1) provides that, in order to warrant a classification as reproductive toxicant, "Developmental effects must be observed in the absence of other toxic effects, or if occurring together with other toxic effects, substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects".

As stated above, in none of the in vivo studies was there evidence of developmental effects without significant maternal toxicity. The relationship between maternal toxicity and developmental toxicity has been written about extensively. It is recognized that some perturbations of maternal physiology can produce malformations (Beyer et al., 2011). Some of these include changes in acid-base balance, chemically-induced maternal nutritional deficiencies, maternal diabetes (Carney, 1997, Taubeneck, 1994), and maternal perturbations that result in embryonic hypoxia, including maternal anemia, maternal cardiovascular insufficiency, or uterine blood vessel constriction (reviewed by (Danielsson, 2013).
Page 84: The DS notes that maternal toxicity was noted in all developmental studies and concludes that this was "not likely the cause of the effect on foetal viability" by referencing feed restriction studies (Cappon, 2005). Feed restriction studies were commissioned to study a direct link between (low) caloric intake, decreased weight gain and developmental effects, and were intended to provide a basis for interpreting developmental toxicity studies on drugs that induce anorexia. In studies with chemical agents that cause developmental toxicity there are many possible mechanisms of action, depending on the metabolic pathway of the chemical in question. This may lead inter alia to decreased maternal weight gain, however, it is in our view unjustified to interpret these studies in a way where decrease in maternal weight gain is a necessary step on the course to development toxicity. While the presence of a weight gain decrement can occur concomitantly with maternally-mediated developmental effects, and is an indicator of excessive maternal toxicity, it is not in the causal chain of events between maternal toxicity and adverse developmental effects.
Consequently, we challenge the conclusions in the CLH Report based on the feed restriction studies.
Page 84: ZnPT affects embryonic development indirectly, via excessive maternal toxicity. The mechanism by which the pyrithione salt act is a disruption of iron-sulfur (Fe-S) clusters within the mitochondria and inhibition of oxidative metabolism, which causes profound effects on maternal metabolism that indirectly affect the embryo. The DS does not address this mechanism of action in the reasoning for classification of ZnPT. Cosmetics Europe notes that it is important to consider the mechanism of action in more detail to understand the indirect effect on development. It is noted that also the SCCS has considered such under the "special investigations" section of their 2014 Opinion (pp 74ff).
Pyrithione acts as an ionophore, carrying metals across the mitochondrial membrane where it inhibits aconitase, a Krebs cycle enzyme that has an Fe-S group on the surface of the molecule and is therefore susceptible to inhibition by divalent metals. This inhibition leads to extreme metabolic disturbances in the adult animal, as mitochondrial metabolism is the most important source of energy for the animal. Importantly, this is not the case for the embryo, which is much more dependent on glycolysis during critical stages of organogenesis. The effects of aconitase inhibition on the intact animal is expected to have a spectrum of biochemical and metabolic effects. These can be discerned from an examination of the data from subchronic and chronic studies for pyrithiones (i.e., weight of evidence including NaPT and CuPT studies). These effects include a short-term increase in serum glucose (because of negative feedback from lactate accumulation, as pyruvate cannot enter the inhibited Krebs cycle); and a significant decrease in food conversion efficiency (the ratio of body weight gain to food intake). While the repeat-dose studies are not ideally designed to investigate these effects, effects on glucose and feed efficiency are consistent across multiple studies on Zn and Na PT.
The steep dose-response curve is also consistent with an effect on oxidative metabolism, including the rapid progression from seeming normality to death. An indication of effect on organs with high metabolic demands is a consistent increase in relative heart weight at

high doses in subchronic studies.
The non-specific secondary effects of aconitase inhibition are developmentally adverse. The short -term hyperglycemia, the metabolic acidosis from lactate and citrate accumulation, and possibly hemodynamic insufficiency, are all developmentally adverse from an indirect mechanism.
Another consistent observation across studies is a decrease in hemoglobin and or red blood cell number, along with an increase in relative spleen weight. The molecular mechanism of pyrithione, carrying of metals across membranes and disruption of Fe-S clusters, is expected to interfer with haem synthesis and the amount of hemoglobin. This produces a mild hypoxia, which would serve to exacerbate the maternally-caused developmental toxicity.
The embryo culture experiments show unequivocally that pyrithione is not intrinsically embryotoxic. The inhibition of aconitase with Zn PT and NaPT demonstrate unequivocally that aconitase inhibition is complete at concentrations that are equivalent to those measured at excessively toxic dose levels in vivo. Analysis of the repeat-dose toxicity studies on Zn PT and other pyrithiones provide consistent results on food conversion efficiency and other endpoints, e.g., hyperglycemia, that are consequences of aconitase inhibition, and reduction in hemoglobin by disrupting synthesis of haem, offer conclusive support of this mechanism. While it is not possible, short of conducting a series of dedicated mechanistic toxicology studies, to demonstrate every step from aconitase inhibition to indirect embryotoxicity, we have demonstrated the key events in this mode of action.

Summary: Based on the CLP classification criteria a classification of ZnPT as Repr 1B is not warranted
As we stated above, we believe that classification of ZnPT as Reproductive Toxicant is unwarranted because:
1. there is no evidence of developmental abnormalities for ZnPT in the absence of maternal toxicity; Pyrithione inactivates aconitase which blocks the Krebs cycle (i.e. inhibition of oxidative metabolism) and this inhibition leads to metabolic disruption in the pregnant female which are non-specific and indirectly lead to adverse developmental effects. This is a non-specific secondary effect and as such should not lead to classification of ZnPT, in accordance with the CLP criteria.

2. the dose-response for ZnPT toxicity in the adult rat and rabbit is extraordinarily steep. As such, the higher doses used in the developmental studies at which the reproductive toxicity effects were observed, produced excessive maternal toxicity in the form of severely impaired maternal weight gain (or even weight loss in some instances) and significant clinical signs. In accordance with CLP, OECD guidelines and best toxicological practice those dose levels which produce excessive maternal toxicity (> 20% maternal weight gain decrement) should be discounted and should not lead to classification of ZnPT.

3. the developmental effects observed in the in vivo ZnPT studies are not caused by intrinsic toxicity of ZnPT to the embryo. A rat whole embryo culture experiment confirms that neither pyrithione nor its principal plasma metabolite, MSP, has the potential to cause embryotoxicity even at plasma concentrations associated with severe maternal toxicity in vivo. As stated above, according to CLP, classification pertains only to substances that have intrinsic specific property to produce an adverse effect on reproduction i.e. not to ZnPT.

4. A consistent pattern of malformations, and underlying variations, which would be expected of a true, primary developmental toxicant, is not seen across and within studies.

Instead, observed effects appear more random, as would be expected of changes that occur through maternal toxicity. ECHA note – An attachment was submitted with the comment above. Refer to public attachment CE ZnPT Public Consultation.zip
Dossier Submitter's Response
Thank you for your comment and the attachment. All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
All the metabolic arguments leading to toxicity in the dams/does are valid for the embryo/foetus as well. The same disturbance to oxidative metabolism could also work during organogenesis leading to excessive intrauterine death. It would be very difficult to determine the primary target organism – the maternal unit or the foetal unit?

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	United States	Personal Care Products Council	Industry or trade association	44
Comment received				
Taking all of the data into consideration, maternal toxicity observed at or above 3 mg/kg/day is considered excessive, and below this threshold, there is no evidence of developmental effects. Additional comments in the attached document. ECHA note – An attachment was submitted with the comment above. Refer to public attachment PCPC Comments ECHA consultation Zinc Pyrithione.pdf				
Dossier Submitter's Response				
Thank you for your comment and the attachment. All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Germany		Individual	45
Comment received				
As a toxicologist, I disagree with the classification of Zinc pyrithione as reprotoxic ingredient, because the provided rationale is not following the criteria of the CLP regulation and is scientifically not justified. It has been laid out in detail in other comments that the substance has no intrinsic embryotoxic properties, any developmental effects noticed were unspecific and secondary toxic effects and were only seen in the presence of maternal toxicity. What I find unacceptable is the justification why the whole embryo culture assay that clearly demonstrates the absence of embryotoxic effects of pyrithione was not considered for the classification. How will we ever make progress in replacing animal testing if European authorities are not willing to apply Weight of Evidence approaches including validated alternative test methods?				
Dossier Submitter's Response				
Please see our response under the comment number 56.				

RAC's response
Noted and understand your exasperation. Understand that the proposal is based upon the most relevant data for the substance under review.

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	United Kingdom	AkzoNobel	Company-Downstream user	46

Comment received

AkzoNobel manufactures liquid paints and coatings globally for professional and consumer uses. Zinc Pyrithione (ZnPT) provides broad spectrum fungal, bactericidal & algal control in paints and coatings. It is used for dry film preservation and anti-fouling, Product Types 7 and 21 under the Biocides Products Regulation.Use as an in can preservative (Product Type 6) for water based paints is increasing due to substitution of other in can preservatives such as methylisothiazolinone and formaldehyde donors which are facing regulatory restrictions.

ZnPT is seen as a key active for paints and coatings in future product development due to a very limited choice of approved biocides for these Products Types and lack of new actives coming to the market. The decreasing choice of effective biocides for use in paints and coatings is a key threat to this sector of the market as highlighted by comments submitted by our National and European trade associations.

AkzoNobel has participated in the ZnPT industry consortium organized by Lonza, P&G and Janssen and fully supports the comments made in response to the CLH dossier with respect to the classification for aquatic toxicity, repeated dose toxicity and reproductive toxicity. In particular, on the proposed reprotoxic classification we urge due attention is given to new information presented on the mode of action. Furthermore, the use of a weight of evidence determination on ZnPT pre-natal and sub-chronic studies, and data on the chemically related substances (Sodium Pyrithione [NaPT] and Copper Pyrithione [CuPT]) needs to be taken into account. Based on this we conclude:

• Data Developmental effects are observed only in the presence of excessive maternal toxicity.

• ZnPT does not have an intrinsic property to produce adverse effects on development. A consistent pattern of malformations, and underlying variations, which would be expected of a true, primary developmental toxicant, is not seen across and within studies. Instead, observed effects appear more random, as would be expected of changes that occur through maternal toxicity.

• The mode of action of ZnPT provides evidence that this substance produces developmental effects through a non-specific secondary consequence of maternal toxicity.

When taking all evidence into account we do not consider the classification of reprotoxic category 1B is warranted.

We also support the consortium conclusions that:

• A STOT-RE 1 classification is warranted by exposure via the inhalation route only.

• The ecotoxicity data support an environmental classification of Aquatic Acute 1, with an

M factor 100 and Aquatic Chronic 1 with an M factor 1.
Dossier Submitter's Response
We note that you have participated in the ZnPT Industry CLH Consortium. Please see our response under the comment numbers 56, 75 and 79.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Germany	<confidential>	Company-Downstream user	47
Comment received				
see general comment				
Dossier Submitter's Response				
Please see our response to your general comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Sweden		Individual	48
Comment received				

I'm a MD, MSc, PhD and former Professor in Toxicology who has long experience (> 35 years) in assessing the impact of maternal toxicity in embryofetal development studies of chemicals and drugs (both as regulator and working in industry). I have participated in arranging ILSI workshops at SOT, American and European Teratology Societies on the complex issue of Maternal Toxicity in order to get a better understanding of how to interpret developmental toxicity studies. I was co-author to a publication summarizing conclusions and recommendations from these workshops (Beyer et al 2011, Birth Defects Research (Part B) 92:36–51, 2011) and have written chapter on the topic Maternal toxicity in a book on teratogenicty testing.

I do not agree with the proposed classification and labelling of Zinc Pyrithione (ZnPT) as reproductive toxicant Cat 1B; this is not warranted. The CHL report does not follow the CLP regulation (CLP). Furthermore, the report has not properly addressed a number important aspects in relation to excessive maternal toxicity (e.g. other manifestions of the maternal toxicity than effects on maternal weight) and neglected findings showing that ZnPT lack the potential to directly affect embryonic development. The intention of CLP is that all relevant available data information should be examined by the use of a weight of evidence approach (WoE). Unfortunately, the report has not considered any data beyond ZnPT prenatal in vivo studies alone.

A proper CLP evaluation should have taken all relevant information available in consideration, including relevant results in:

- general toxicology and kinetic studies with ZnPT
- reproductive and general toxicity studies as well as kinetic studies with other pyrithione salts (NaPT and CuPT)
- mode of action for ZnPT induced toxicity in the adult organism
- whole rat embryo culture studies (WEC) in vitro.

The neglect of assessing relevant information has resulted in incorrect interpretations I disagree with; two of these are of high importance for CLP.

A) The first interpretation I strongly disagree with is related to the absence of direct toxicity of ZnPT (section 10.10.6., pages 83 and 84) in the CLH report:

”the results of these rWEC assays are not completely relevant to conclude that zinc pyrithione is not directly embryotoxic as the toxicological significance of Zn in synergy with the pyrithione is not addressed in these assays. Moreover, it should be noted that even though the WEC assay is validated by ECVAM, the predictability and applicability domains are not yet sufficiently defined for regulatory implementation”.

There are a number of arguments indicating that the results in the WEC assay are highly relevant and should be considered in the CLP process. I below list a number important aspects which not were taken into consideration in the CLH report, but are discussed in the document entitled ”Supportive document to the ZnPT industry CLH consortium comments on reproductive toxicity”.

1) Kinetic and absorbtion studies show that all pyrithione salts disassociate to liberate Zn (or Cu or Na) and the pyrithione moiety in the gastrointestinal tract, which are then absorbed and distributed in the circulation independently. Zn and pyrithione are transferred to the embryo independently and not as a Zn-pyrithione complex; it’s thus not relevant to test the ZnPT complex in WEC.

2) The culture media (rat serum) in the WEC study contains relatively high concentrations of Zn (approximately 20 µM). The highest tested concentration of pyrithione in the WEC study was 2,3 µM. Therefore, there was almost a tenfold excess of Zn, compared to pyrithione, in the culture media, more than enough to test any possibly synergy between Zn and pyrithione.

3) The rat whole embryo culture (WEC) study clearly shows that neither pyrithione nor its principal serum metabolite have an intrinsic specific property to produce adverse effects on development at concentrations causing maternal toxicity in the vivo developmental toxicity studies.

4) I also disagree on the comment about regulatory status of the WEC assay (“not yet sufficiently defined for regulatory implementation”). ECHA actually encourages and explicitly allows use of nonstandard methods for the classification assessment in a weight–of-evidence (WoE) approach. Importantly, the WEC was not used as a replacement for in vivo studies, but rather as an adjunct to aid understanding within the context of WoE involving expert judgment.

B) The second interpretation I strongly disagree with in the CLH report is the use of results in feed restriction studies in rat and rabbit (e.g. Cappon et al. 2005, Fleeman et 2005) to conclude that maternal toxicity is not the cause of observed developmental effects after administration of high doses of ZnPT (section10.10.6, pages 83 and 84). There are a several reasons to disagree with this interpretation:

1) The feed restriction studies were conducted as aid to for interpreting results in developmental toxicology studies with pharmaceuticals which decrease appetite and

cause weight loss. The purpose of the feed restriction studies was to isolate the effects of feed restriction and resulting maternal weight gain decrements from other types toxicity caused by drug/chemical exposure which may occur in developmental toxicology studies. In these studies, significant decreases in maternal body weight were associated with adverse embryofetal effects, e.g. decreased fetal weights and retardation in skeletal development, but not malformations. It's incorrect to conclude (as done in the CLH report) that maternal weight gain decrement is the only aspect of maternal toxicity that could lead to adverse developmental effects.

2) Chemicals and drugs can cause severe disturbances in maternal homeostasis (= "maternal toxicity") by various mechanisms, which may result in severe effects on maternal hemodynamics and/or metabolism. Such maternally mediated toxicity can indirectly cause developmental toxic effects, including embryonic death and malformations. For examples it's well established that severe alterations in maternal hemodynamics can result in various malformations due to temporary interruption of oxygen supply to the embryo.

3) Alterations of maternal homeostasis by chemicals/drugs often results in decreased maternal body weight gain. This is an important reason why the endpoint "decrease in maternal body weight gain" is used as an indicator for different types of maternal toxicity and why excessive decreases in maternal body weight gain should be avoided in reproductive toxicology studies. However, the CLH report incorrectly interprets weight gain decrement as the causal link between maternal toxicity and developmental toxicity.

4) Developmental effects of ZnPT were only observed in the presence of excessive maternal toxicity, including severe decrements in maternal body weights at higher doses. This is evident from a review of all available data in a weight of evidence approach. CLP requires that, when evaluating potential developmental effects of a substance, consideration to the possible influence of maternal toxicity should be taken into account. In case of excessive maternal toxicity or if the substance is so toxic, developmental effects should be discounted and should normally not lead to classification.

5) In the rat, the only species used in both general repeated dose toxicity studies for 90 days and in developmental toxicity studies (dosing during organogenesis GD 6-16 = 11 days) with ZnPT, the high dose in the oral teratology study was 15 mg/kg. This dose level was 3 times higher than the dose level (5 mg/kg) initially used in the 90 day oral repeated dose study. However, the dose level 5 mg/kg in the 90 days study, resulted in maternal deaths already after 17-18 days of dosing and had to be decreased to 2,5 mg/kg. These data show that the high dose in the oral teratology (15 mg/kg) – which was the only dose which was associated with significant teratogenicity – can be considered to be a lethal dose of ZnPT and "excessive toxic" for the adult rat.

6) ZnPT at "excessive toxic" doses causes severe disturbances in maternal homeostasis (= "maternal toxicity") by several mechanisms. The main mechanism for ZnPT maternal toxicity is related to dose dependent aconitase inhibition. However, the downstream effects of aconitase inhibition are not only affecting maternal homeostasis, but has the potential to cause developmental toxicity (including malformations) due to maternally induced acidosis, maternal hyperglycemia and hypoxia. The acidosis is a consequence of citrate and lactate accumulation; hyperglycemia is the result of negative feedback on glycolysis from lactate and citrate accumulation and hypoxia from decreased haemoglobin in maternal blood. Each of these maternal conditions has been associated with embryofetal adverse effects.

* In conclusion, ZnPT does not have an intrinsic property to produce adverse effects on development. The mode of action of ZnPT provides evidence that this substance produces developmental effects through a non-specific secondary consequence of maternal toxicity. In such cases, according to CLP, the substance should not be classified since "classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects".
Dossier Submitter's Response
All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
06.07.2017	Norway	Jotun A/S	Company-Downstream user	49
Comment received				
Jotun supports the comments by the ZnPT Industry Consortium stating that the proposed reprotox classification and the assignment of M-factors are not in line with the CLP criteria.				
Dossier Submitter's Response				
Please see our response under the comment numbers 56 and 79.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	United Kingdom		Individual	50
Comment received				
The issue of foetal abnormalities in laboratory animals and whether they are caused directly by the chemical itself(or a metabolite) or they are consequencies of maternal toxicity, is a very long standing one(Tyl, 2012; Danielsson 2012). I believe we need to identify transparent criteria for both scenarios(i) and (ii) as applied to zinc pyrithione. (i) Evidence for direct effect on the foetus of the chemical and/or its metabolites: 1. The foetal effects in vivo are specific, often reflecting the identified mode of action. There was an increase in resorptions in the rat reprotoxicity studies at both 3 mg and 15 mg/kg/day, although this was not statistically significant in the 3 mg/kg/day group and within the range of historic controls. In the 15 mg/kg/day group foetal weight was decreased with malformation in all litters. An increase in malformed fetuses were also identified in the 3 mg/kg/day group, compared with controls, although the incidence appears to be within the historic background levels. The only significant skeltal malformations occurred at the highest dose levels(15/kg/day). With the exception of fused and wavt ribs, changes in the 3 mg/kg/day groupd varied widely. The nature and severity of such changes does not comply with any recognisable pattern for a direct acting embryotoxin. They do not reflect the mode of action of zinc pyrithione. The profile				

of changes is similar to that in historic controls and is unlike the type of profile to be expected of a direct acting embryotoxin.
2. Adverse change(s) occur in vitro in suitable tests. To identify whether or not zinc pyrithione has a direct effect on the fetus during the critical window for organ developement(including skeletal developement), the test system should reflect the key stages. For skeletal development in rat it is day 9/10 to day 11/12. To reflect this stage the most robust in vitro test for this purpose is the whole embryo test. It has been in use for over twenty years and was validated for reprotoxicity studies in 2002. It seems strange that CLH Report discounts in vitro tests, which is in conflict with ECHA policy. Exposing the rat fetus during the critical window to pyrithione or its principla metabolite at the highest level in vivo does not result in any embyotoxic effects.
3. The effect occurs in vivo at doses that are without/or minimal adverse effect on the mother. The effect(s) increase in the number of fetuse affected and/or the severity of the effect with increasing dose level. It is evident that foetal effects only occur at doses at which there is unquestionable maternal toxicity.
4. Chemicals with the same mode of action cause similar and specific adverse effects. CHL Report ignores read across. It should have considered fluoroacetate, which also does not have a direct effect on the fetus and is an aconitase inhibitor.
(ii) Evidence for indirect effect(s) on the fetus due to maternal toxicity:
1. Foetal effects only occur at doses significantly above those causing maternal toxicity. The parameters representing maternal toxicity nee to reflect: significant loss of maternal body weight or failure to increase body weight, gross/histopathology findings resulting in altered organ structure and/or functions, maternal deaths. DLH Report only focusses on food consumption, which is inappropriate.
2. Maternal toxicity has to be clearly demonstrated. The main physiologically apparent effect was paralysis of the hind limbs. Biochemical changes include a reduction in circulating haemoglobin and an increase in blood glucose levels.DLH Report hardly refers to this matter.
3. Mode of action in the mother is unlikely to be relevant in the developing fetus. DLH Report has not considered the mode of action. A primary mode of action in the inhibition of the mitochondrial enzyme aconitase, which plays a key role in the Krebs cycle - the main source of energy generation by oxidative metabolism for the mother. This provides a rationale for the very steep dose-response relationship observed, for example, in the 90 day studies. In the developing fetus the energy is provided to a very large extent by glycolysis(Villee, 1984; Kuwata et al, 2017). Aconitase plays no part in glycolysis Inhibition of the Krebs cycle has serious consequences for the mother, since it causes the disruption of many aspects of normal metabolism, resulting in weight loss, reduced mobility and increased reliance of other means of generating ATP.
4.Foetal effects are scattered/non-specific and may not reflect dose-response trends. DLH Report has not paid any attention to the fact that the lesions are scattered, non-specific and do not follow dose-response relationship.
Overall conclusions:
According to weight of evidence methodology it can be concluded that maternal toxicity is the reason that the foetal effects occur in rats and rabbits and that the hypothesis that ZPT and/or its metabolites cause a direct effect on the fetus should be discounted. Consequently, the assighnment by CLH of a Reprotox Category of 1B is totally unjustified.

Dossier Submitter's Response
All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
05.07.2017	United Kingdom		Individual	51

Comment received

There is a significant amount of data that support that the effects are secondary to the maternal toxicity and moreover not a direct effect on the foetus including data on the mode of action. The classification proposal has not considered all the available data, including data from other pyrithiones (Cu, Na) and hence not used a weight of evidence approach that indicates that the reproductive effects observed are seen only at maternally toxic doses and moreover, that these effects are secondary to maternal toxicity and not a direct effect on the foetus. With regards to the other pyrithiones, there is very good read across information that supports read across (a category approach) among the various pyrithiones (Cu, Na & Zn). Data on the other pyrithiones add weight to the case that reproductive effects observed are seen only at maternally toxic doses and are secondary to maternal toxicity and are key in considering the mode of action. This must be considered.

Extensive comments have been submitted by the ZnPT Industry CLH Consortium which covers all these aspects and details the mode of action and the data on the other pyrithiones which add to the weight of evidence that the reproductive effects observed are seen only at maternally toxic doses and moreover, that these effects are secondary to maternal toxicity and not a direct effect on the foetus.

The consideration that the effects as secondary effects non-specific consequence of other toxic effects, mode of action and using weight of evidence is explicit in the CLP Regulation.
More specifically consideration of the secondary effects is stated in Annex I: 3.7.2.1.1. Cat 1 A criteria state that 'Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.' Also, Cat 2 criteria state that 'Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects'.
Moreover Annex 3.7.2.4 considers Maternal Toxicity specifically. In this section Annex 1: 3.7.2.4.2. states that 'Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects' and Annex 1: 3.7.2.4.3. specifically mentions that a mode of action can downgrade a classification.

Dossier Submitter's Response

All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number

03.07.2017	Belgium	Procter & Gamble Manufacturing Belgium N.V.	Company-Downstream user	52

Comment received

As indicated in our general comments, we believe that the evaluation of the potential for reproductive toxicity of ZnPT was incompletely addressed in the CLH Report and we would like herewith to provide our perspective on the influence of maternal toxicity in the prenatal studies assessed in the proposal and we would appreciate your consideration of these comments.

Indeed, upon on our review of the CLH Report, we noted that maternal toxicity parameters were inconsistently reported across the prenatal studies described in the proposal, which does not reflect the actual degree of maternal toxicity in these studies. Further, the CLH report has not evaluated whether the developmental effects retained as the basis for the classification proposal were observed in presence or absence of maternal toxicity and particularly, whether the level of maternal intoxication in those studies could be responsible for the observed developmental effects, as required by the CLP.
Our assessment of maternal toxicity data in available studies reveals that developmental effects are only observed in the presence of excessive maternal toxicity. This is evident from a review of all available data in a weight of evidence approach :
Section 3.7.2.2.2. of Annex I to the CLP requires that, when evaluating potential developmental effects of a substance, consideration to the possible influence of maternal toxicity should be taken into account (see also Section 3.7.2.4 of Annex I to the CLP). In case of excessive maternal toxicity or if the substance is so toxic, developmental effects should be discounted and should normally not lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate." (CLP, Annex I, Sections 3.7.2.3.5.; 3.7.2.4.3.; 3.7.2.4.4. and 3.7.2.5.8.).

If all the available data are taken into account and fully considered in a weight of evidence approach as requested by the CLP (Annex I, Part 1 and Part 3 (Sections 3.7.2.2.1. and 3.7.2.3.1)), the conclusion should be that the developmental effects of ZnPT are only associated with excessive maternal toxicity.

Indeed, the data summarized below show that in those studies where developmental effects were reported, these effects were observed only in presence of excessive maternal toxicity. And developmental effects are not observed when maternal toxicity is absent. The maternal toxicity data for ZnPT is consistent with the definition of excessive maternal toxicity in the CLP legislation and with expert opinion. One of the complicating factors with ZnPT is that the dose-response curve for maternal toxicity is extremely steep, such that even a small increase in dosage produces a disproportionate increase in the severity of the maternal response. As such, dose selection by investigators conducting these studies was, in some cases, too high and there is even justification that whole dose groups should be completely discounted from any evaluation because of this. The rules for interpretation of developmental toxicity studies provide the basis for excluding dose(s) at which excessive maternal toxicity occurred.

Although the CLH report refers to excessive maternal toxicity criteria in section 10.10.6 (page 84), as indicated in point 1 below, it has not fully assessed this parameter and it has considered that maternal toxicity is not a reliable explanation for developmental toxicity.

1) Criteria for excessive maternal toxicity:

Maternal toxicity is typically assessed as changes in weight gain, food consumption, observation of clinical signs, and mortality, and guidance on how much maternal toxicity is acceptable depends on the extent and severity of the effects.

Indeed, the CLP provides some examples of excessive maternal toxicity but there are no quantitative thresholds linked to excessive maternal toxicity for most indicators. Guidance on excessive toxicity is more qualitative, including behavioral or neurological symptoms that are severe enough to hamper basic functions like mobility and feeding, or significant decrements in food consumption to suggest inappetence or inanition. Only for mortality is there a defined ("bright line") level of 10% that is used as the definition for excessive maternal toxicity in the CLP regulation (Annex 1, Section 3.7.2.4.4) and elsewhere (e.g., US EPA, 1991).

Further, while the test guidelines for developmental toxicity call for clinical observations, these observations tend to be highly variable among laboratories in the amount of information that is recorded, and probably the amount of time spent in evaluating behavior.

The CLP regulation (Annex 1, Section 3.7.2.4.4) requires consideration of maternal body weight change and indicates that adjusted maternal weight (overall gestational weight gain minus the uterine content) be provided as an index of maternal toxicity. We regard this as the minimum information needed to assess maternal growth during this period. We have considered maternal body weight gain during dosing period, as an objective indicator of maternal toxicity (in addition to mortality), for the following reasons:

a) maternal body weight gain is a key factor to assess maternal toxicity (based on CLP Annex I, Section 3.7.2.4.4.);

b) it is assessed in an objective and consistent way in all laboratories (based on measurement and not observation);

c) it is influenced very little by the weight of the litter - at least for studies performed under earlier versions of testing guidelines, when dosing was ended at the end of the embryonic period (gestation day 15 or 16 in the rat, gestation day 19 in the rabbit), as the vast majority (>90%) of foetal growth occurs in the last several days of gestation;

d) there are scientifically agreed criteria to characterise excessive maternal toxicity on the basis of maternal body weight changes:

- For weight gain during treatment, there is guidance from consensus workshops in Europe and the US, e.g., ILSI workshop of 2009; conclusions published in 2011 (Beyer et al., 2011), that maternal weight gain decrements up to 10% are well tolerated and not of concern for producing secondary developmental effects. However, experts agreed to put an upper limit value of a 10-20% decrease at or above which the developmental data become uninterpretable.

- Notably, some participants in the workshop noted that even a 5% decrement in weight gain would be considered adverse in a general toxicity study and that a 10% decrement in weight gain is considered "toxicologically meaningful". Finally, the consensus was that a 20% weight gain decrement should be considered excessive.

- This is also supported by other scientific articles (e.g. Wise et al. 2009; Giavini & Menegola, 2012) and OECD Guidelines 426 (Developmental Neurotoxicity Study, 2007) which provides a limit of 10% decrement in weight gain above which maternal toxicity would be considered excessive ("the highest dose level should be chosen with the aim to induce some maternal toxicity (e.g., clinical signs, decreased body weight gain (not more than 10%) and/or evidence of dose-limiting toxicity in a target organ").

- Therefore, a 20% decrease or more in maternal weight gain should be considered as excessive maternal toxicity.
It is also important to measure maternal weight gain over the period of dosing, as this is the time when the test agent is present and the measurement is most sensitive to toxicity.

The CLH report referred to the ILSI workshop scientific consensus that "a decrease in body weight of 20% was considered excessive" (in Section 10.10.6.; page 84) but it has not assessed whether the developmental effects reported in ZnPT prenatal studies were observed in presence of excessive maternal toxicity.

2) A complete analysis of the data on maternal toxicity in the prenatal ZnPT studies shows the magnitude of maternal toxicity exhibited :

While some data during the dosing period (e.g., food consumption) were reported in tables of the CLH Report, the reporting did not include the most relevant and well documented measures of maternal toxicity.

The maternal toxicity of several prenatal rat (gavage and dermal) studies (CAR Doc IIIA A6.8.1/02 and 03 described in Sections 10.10.4 and 10.10.5 of the CLH report) is in fact much more severe than what has been reported in the CLH report. These data also show that maternal toxicity was excessive at doses at which developmental effects were observed as explained in point 3 below.

Maternal body weight is typically measured at three-day intervals, starting on the first day of dosing (gestation day 6 in rats and rabbits). Maternal body weight gain, a key factor to assess maternal toxicity (based on CLP Annex I Section 3.7.2.4.4.), should normally be considered at every measurement interval, especially over the dosing period and not just at the end of gestation. In the CLH report, for several prenatal studies, the maternal body weight gain reported relates to measurements taken several days after the last dose (when many animals recover body weight). Indeed, structural malformations and embryonic death take place during the dosing periods (gestation days 6-15 in the rat and days 6-19 in rabbit). Therefore, the maternal toxicity measured during this period has the highest relevance for interpreting developmental effects rather at the end of the gestation.

In addition, the CLP indicates that it is necessary to verify whether developmental effects are observed in the presence of maternal toxicity as this can have an important impact on classification (Annex 1, Sections 3.7.2.3.5.; 3.7.2.4.3.; 3.7.2.4.4. and 3.7.2.5.8.).

2.1) CAR Doc IIIA A6.8.1/02 (Prenatal; Rat; gavage, 1993):

Maternal weight gain was decreased during the dosing period (gestation days 6-16) by more than 20% at the mid-dose (3.0 mg/kg bw/day), and 67% in the high dose (15 mg/kg bw/day) (see values in Tables 56b, page 64 of the CLH Report). However, the CLH

document:

- does not report the maternal weight gain for the 3 mg/kg bw/day dose in Table 53 (page 54) and in Table 56a (page 63) of the CLH Report; but the maternal effects described in Table 56b (page 64) show a statistical decrease in body weight gain of 22% at that dose during the dosing period (gestation days 6-16).
- only reports a decrease of maternal weight gain of 38% at 15 mg/kg bw/day measured at the end of gestation period i.e day 20 (see Table 53 (page 54); Table 56a (page 62); Table 56b (page 64) of the CLH Report).

In addition, the food consumption decrease at 15 mg/kg bw/day of gestation days 6-16 should be 25% (see Table 56b) and not 48% as indicated in Table 53 (page54) and Table 56 (page 62) of the CLH Report.

2.2) CAR Doc IIIA A6.8.1/03 (Prenatal; Rat; dermal, 2005):

Maternal weight gain was decreased during the dosing period (gestation days 6-16) by 23% at the mid-dose (30 mg/kg) and 70% in the high dose (60 mg/kg) while the CLH document reports a decrease of maternal weight gain of 12% at 30 mg/kg and 31% at 60 mg/kg (see Table 53 (page 58) and Table 61a (pages 71 and 72) of the CLH Report).

2.3) Thor GmbH Art 95 (Prenatal, Rabbit; oral gavage, 2015):

Excessive maternal toxicity was clearly evident in the high dose group in this study. It was also evident in the mid-dose level (1.5 mg/kg/day) in six rabbits, and virtually all of the developmental effects were observed in the litters of these six. These data should be set aside as uninterpretable.

This has been recognized in the CLH report in Section 10.10.5. and Table 63c (page 80), but has not been mentioned in Table 53 (page 61).

Figure 1 in page 11 of the document "1. Supportive Document on Reproduction Toxicity of the ZnPT Industry CLH consortium - June 30 2017 - Final.pdf" (provided as a public attachment to the comments of the ZnPT Industry CLH Consortium, hereafter mentioned as the "ZICC Supportive Reprotox Document") illustrates the extent of maternal toxicity in the mid-dose group (1.5 mg/kg/day). Each graph represents the body weight gain for one animal in that group (N=21) during the dosing period (gestation days 0-29), with the solid black line in each graph representing the control mean. It is clear from this figure that six of the animals (# 46; 58; 62; 54; 55; 49) had profound effects on weight gain during the study. When the litters of these animals are removed from the data analysis, there is no difference in the resorption rate or number of live foetuses per litter in the mid-dose vs. control. Furthermore, the two instances of omphalocele were in litters of these highly intoxicated does.

3) A weight of evidence determination demonstrates that all developmental effects were observed only in the presence of excessive maternal toxicity :

As described above, excessive maternal toxicity, expressed as significant maternal body weight gain reductions (>20%), has not been judged relevant for the evaluation of the developmental effects from the prenatal studies in the CLH report. However, this is because each ZnPT prenatal study has been evaluated in isolation and other ZnPT data (e.g. sub-chronic data) or data from chemically-related substances as additional

supporting evidence have not been addressed ; even though the Dossier Submitter (DS) "acknowledges that zinc pyrithione shows some structural similarity to sodium pyrithione (EC 223-296-5) and copper pyrithione (238-984-0), in that they share the common organic moiety i.e. pyrithione " (CLH report, Section 6 ; page 7). The CLP supports the use of analogue substances in a WoE approach (See Annex I, Section 1.1.1.3 of the CLP, and in particular also for reproductive toxicity under Annex I, Sections 3.7.2.3.1 and 3.7.2.5.4. of the CLP). In addition, a full analysis based on the ECHA RAAF guidance has shown that ZnPT, NaPT and CuPT constitute a "Category" (this detailed analysis is provided in Annex 1 of the ZICC Supportive Reprotox Document).

Use of data on analogue substances is particularly indicated in this case because findings from the ZnPT prenatal studies alone are not sufficient to fully assess reproductive toxicity criteria, as indicated in Section I "Preliminary Remarks" of the ZICC Supportive Reprotox Document.

If the CLH report had assessed in a weight of evidence determination the influence of maternal toxicity on developmental effects, it would have concluded that all the reported effects of toxicological relevance have occurred concomitantly to excessive maternal toxicity.

Indeed, a weight of evidence approach based on ZnPT and NaPT maternal weight decrement analysis and maternal toxicity in the prenatal studies, and mortality assessment in subacute and subchronic toxicity tests, has shown that maternal toxicity observed at or above 3 mg/kg/day in the rat or at or above 1.5 mg/kg/day in the rabbit is considered excessive. This is based on the threshold values for maternal weight gain decrement (i.e. >20%) as defined in the consensus ILSI workshop (Beyer & al. 2011) and the limit for mortality as described in the CLP (Annex 1, Section 3.7.2.4.4.) and detailed above in point 1. Developmental effects were only observed at those specific doses or above, i.e., in the presence of excessive maternal toxicity. Below these thresholds, there is no evidence of any effect on development.

A summary of each NaPT study used in this weight of evidence approach is provided in Annex 2 of the ZICC Supportive Reprotox Document.

3.1. Prenatal oral and dermal studies in rat and rabbit

Table 1 in page 10 of the ZICC Supportive Reprotox Document provides a summary of all the ZnPT prenatal studies available i.e. oral and dermal studies in rat and rabbit, including the dose levels at which maternal toxicity was observed (in bold/underlined type in "dose level" column). This Table also provides the studies which show statistically significant effects on foetal viability or malformations (based on CLH Report) together with the doses at which these statistically significant effects were observed. These data show that developmental effects only occur in presence of excessive maternal toxicity as supported by the data provided on rat and rabbit prenatal studies (see Figures 2 and 3 in pages 14 and 15 of the ZICC Supportive Reprotox Document, respectively).

In addition, there were no adverse effects on reproduction in the two-generation reproductive toxicity studies carried out on ZnPT (Thor GmbH, Art. 95 dossier year 2015; rat, gavage; OECD 416) and other pyrithione salts.

Moreover, there are also several prenatal studies conducted with NaPT and CuPT which are summarized in Annex 2 of the ZICC Supportive Reprotox Document. When developmental effects are observed in those studies, they only occur in presence of

excessive maternal toxicity, consistent with studies with ZnPT.

The CLH report states in page 82 that "Effects on foetal viability were observed in four of the seven studies available", and in pages 83 and 84 respectively, that "Malformations were seen mainly in three oral studies of high reliability, one in rats and two in rabbits" and…"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)". However, considering all the prenatal studies available as reported in the Table 1 of the ZICC Supportive Reprotox Document, statistically significant effects on foetal viability or malformations were however only observed in four studies out of the 10 studies available.

- Rat, oral studies:

In a first assessment, we have put ZnPT and NaPT prenatal oral rat data together in a dose-response relationship for maternal toxicity as recommended in CLP Annex 1, Section 3.7.2.4.4. (see Figure 2 of ZICC Supportive Reprotox Document, graph plot maternal weight gain data vs dose from all the oral gavage rat Segment 2 studies as % of control).

The graph shows that maternal toxicity becomes increasingly severe at a dose level of around 3 mg/kg/day, to an extent that is inappropriate and confounding to interpretation of the relevance of developmental effects.

Indeed, maternal weight gain was decreased by more than 20 % in the 3 mg/kg/day ZnPT group (i.e. beyond the acceptable level of 20% as defined in the ILSI workshop; see points 1 and 2. above), and the severity of the effect increased in a dose-responsive manner at higher dosages (weight gain decrement of 40% at 4 mg/kg/day and of 67% at 15 mg/kg/day).

The extent of the maternal toxicity is excessive at the dose levels of 3 mg/kg/day and above, to the point that interpretation is confounded.

The results from the rat prenatal dietary study (rat, oral diet; Thor GmbH art 95 dossier; 2015b), even though not provided in this Figure 2, are comparable, with the apparent threshold for maternal toxicity between 1.18 and 1.68 mg/kg/day, with a steep dose-response. Importantly, developmental effects are negligible at doses that have no or tolerable maternal toxicity.

Rabbit, oral studies:

The same conclusion about an excessive decrement in maternal weight gain can be made for the rabbit prenatal oral studies (see Figure 3 of the ZICC Supportive Reprotox Document). Weight gain over the treatment period was decreased by 41% vs. controls in the 1.5 mg/kg bw/day group in the rabbit, oral gavage study (CAR Doc IIIA A6.8.1/01; Schardein, 1993). In rabbit, oral gavage (Thor GmbH Art.95 dossier, 2015a) study, while the average weight gain decrement in the mid-dose (1.5 mg/kg bw/day) group was not excessive, 6 of the 21 rabbits in that group had severe weight gain decrements: more than 70% decrement over the first two weeks of dosing, and more than 40% over the entire dosing period. Almost all of the developmental toxicity in that group was restricted to those six litters (See point 2.3 above and Figure 1 of the ZICC Supportive Reprotox Document for the details). This has been recognized in the CLH report in Section 10.10.5. and Table 63 c (page 80).

The 3 mg/kg bw/day group (Rabbit, oral gavage ; CAR Doc IIIA A6.8.1/01; Schardein,

1993b) and 4 mg/kg/day group (Thor GmbH Art.95 dossier; Thor 2015a) produced a large decrement in weight gain, including weight loss over extended periods during treatment, and more than 50% over the entire period from onset of treatment to sacrifice. These are both excessive levels according to the scientific agreed threshold.

Similarly the Nolen and Dierckman (1979) rabbit oral study shows an important decrement in weight gain which already exceeds 20 % at the lowest dose i.e. 1 mg/kg bw/day and goes up to -154% at 10 mg/kg bw/day (see Figure 3 of the ZICC Supportive Reprotox Document).

3.2. Supporting evidence from ZnPT, NaPT sub-acute and sub-chronic studies :

Both the CLP and the Test Method Regulations are explicit in that a mortality rate of more than 10% is considered excessive (CLP Annex 1 Section3.7.2.4.4 : "Maternal mortality greater than 10% is considered excessive and the data for that dose level shall not normally be considered for further evaluation." And EU Test Method Regulation, (EC) No 440/2008; B.31 Prenatal developmental toxicity study : "Maternal mortality does not necessarily invalidate the study providing it does not exceed approximately 10 %").

Analysis of slightly longer ZnPT oral (gavage) sub-chronic studies in rats indicate that dose levels greater than approximately 2.5 mg/kg/day produced excessive toxicity.

In a 90-day oral rat study on ZnPT (ZnPT CAR Doc IIIA A6.4.1./03), the top dose of 5 mg/kg/day had to be reduced to 2.5 mg/kg/day during the course of the study because it was not tolerated by the animals and they were in danger of dying. However, 3 high dosed females were killed in extremis on Day 16 and Day 19 (see Annex 4 of the ZICC Supportive Reprotox Document for details).

In a 90-day study (rat, oral) on NaPT, half the females in the top dose group of 8 mg/kg/day were sacrificed in extremis, most between 4 and 9 weeks into the dosing period. These data support a dose level of 2.5 mg/kg/day as a maximum tolerated dose (see Annex 5 of the ZICC Supportive Reprotox Document for details).

The same analysis can be made for rabbit studies. While there are no rabbit sub-chronic studies, a range-finding study for developmental rabbit oral study demonstrated that a 4 mg/kg/day dose level was lethal (Thor GmbH Art.95 dossier; Thor 2015a).

In conclusion, we believe that the CLH Report has not fully addressed the influence of maternal toxicity on the observed developmental effects. Our evaluation of maternal toxicity data in a weight of evidence determination reveals that developmental effects are only observed in the presence of excessive maternal toxicity.

Note: Citations can found in the Section " References" of the ZICC Supportive Reprotox Document (pages 33-36).

Dossier Submitter's Response
All your comments are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Germany	German Paint and Printing Ink Association (VdL)	Industry or trade association	53
Comment received				
The proposed classification as reprotoxic category 1B will most likely result in ZnPT being no longer available as an important bicode active (see general comments).				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.06.2017	United Kingdom	British Coatings Federation	Industry or trade association	54
Comment received				
We have detailed our concerns over the proposed classification in the attached position statement The statement refers to the specific arguments against the proposed classification, mentioning the assessments made by other global regulatory authorities and that ZnPT does not have an intrinsic property to produce adverse reprotoxic effects. It also details the use of ZnPT in the paints, coatings and inks industry, and reiterates the major concerns that our industry has regarding the progressive loss of active substances from the paint formulators toolbox, citing the **NINE** industry papers that have already been submitted on this subject over the past 3 years. ECHA note – An attachment was submitted with the comment above. Refer to public attachment BCF ZnPT - Public Consultation response June 2017 Final.pdf				
Dossier Submitter's Response				
Thank you for your comment. In the attachment you wanted to draw attention to the implications of the proposed classification of ZnPT as Repr. 1B in the context of the BPR Regulation while you do realise that these are not relevant to the current CLH process. We do note that in your attachment, you support comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
26.06.2017	United Kingdom	Farrow & Ball	Company-Manufacturer	55
Comment received				
See above ECHA note - *[see comment No. 25]*				
Dossier Submitter's Response				
Please see our response under the comment number 25.				

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.07.2017	Belgium	The ZnPT Industry CLH Consortium	Industry or trade association	56

Comment received

The ZnPT Industry CLH Consortium does not agree with the proposed classification and labelling of Zinc Pyrithione (ZnPT) as reproductive toxicant Cat 1B (H360D), which we believe is not warranted, according to the CLP Regulation (the "CLP") criteria. The review of all relevant data shows that ZnPT is not a reproductive toxicant.

There is a large data set on the reproductive toxicity of ZnPT, which consistently demonstrates that this compound (as well as the Na and Cu salts of pyrithione which make up a "Category" with ZnPT based on the ECHA RAAF (Read-Across Assessment Framework) Guidance) is associated with developmental effects only at dose levels that produce excessive maternal toxicity. Considering the weight of evidence (WoE) from prenatal developmental toxicity studies (referred hereafter as "prenatal studies") and repeat-dose toxicity studies with ZnPT, NaPT and/or CuPT together with scientific literature related to pyrithiones, it is possible to make the following conclusions:

• Developmental effects are observed only in the presence of excessive maternal toxicity. Conversely, developmental effects are not observed when excessive maternal toxicity is absent;
• ZnPT does not have an intrinsic property to produce adverse effects on development; and
• A consistent pattern of malformations, and underlying variations, which would be expected of a true, primary developmental toxicant, is not seen across and within studies. Instead, observed effects appear more random, as would be expected of changes that occur through maternal toxicity; and
• The mode of action of ZnPT provides evidence that this substance produces developmental effects through a non-specific secondary consequence of maternal toxicity.

ZnPT has an extremely steep dose-response curve, which led to the selection of dose levels in prenatal studies that were, in retrospect, too high. These dose levels produced maternal toxicity in excess of what is prescribed in the CLP. There were no developmental effects below 3 mg/kg bw/day for rat and below 1.5 mg/kg bw/day for rabbit. Higher dose levels, at which developmental effects were observed in some studies, lead to excessive maternal toxicity. The mechanism of toxicity of ZnPT, i.e. the inhibition of oxidative metabolism, produces adverse effects on the adult animal but not on the embryo, as the latter is less dependent on oxidative metabolism for its energy needs during the most critical stage of development. The metabolic disturbance in the pregnant animal is, however, adverse to the embryo by indirect means i.e. developmental effects are non-specific secondary consequences of maternal toxicity. Experiments using intact rat embryos in culture, away from maternal influences, demonstrate that neither pyrithione nor its principal plasma metabolite, have the ability to directly affect embryonic development showing that ZnPT has no intrinsic property to produce adverse effects on the development. Therefore, ZnPT is not a reproductive toxicant.

The CLH report does not reach the same conclusion as it has only considered ZnPT prenatal studies, and not other relevant available data (including information on the Mode

of Action submitted in the supportive document attached i.e. "1. Supportive Document on Reproduction Toxicity of the ZnPT Industry CLH consortium - June 30 2017 - Final.pdf") and not addressed in the CLH Report). Those data provide new and further critical information to assess reproductive toxicity classification of ZnPT and therefore, these should be considered in a WoE approach as required by the CLP.

Due to the specific format of comments in the webform, we provide below a summary of our comments for the reproductive toxicity endpoint. However, more detailed information on these comments are provided in a supportive document as a "Public attachment" (see document "1. Supportive Document on Reproduction Toxicity of the ZnPT Industry CLH consortium - June 30 2017 - Final.pdf". Please review the attachment in conjunction with these comments. All citations made in these comments are provided in the supportive document.

Section I. - Preliminary Remarks
1. There are several parameters which have a direct impact on the reproductive toxicity classification and which need to be assessed when allocating such a classification to a substance. We have listed below the most relevant ones to assess ZnPT Reproductive Toxicity Classification:

a) Excessive maternal toxicity: The CLP states that in case of excessive maternal toxicity or if the substance is so toxic, developmental effects should be discounted and should normally not lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. (CLP, Annex I, Sections 3.7.2.3.5.; 3.7.2.4.3.; 3.7.2.4.4. and 3.7.2.5.8.)

b) Intrinsic property: "Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction". (CLP, Annex I, Section 3.7.2.2.1 and Table 3.7.1.(a). Also supported by Annex I, Sections 3.7.2.1.; 3.7.2.3.5.; 3.7.2.4.1.; 3.7.2.4.2. and 3.7.2.4.3).

c) Non-specific secondary consequence of other toxic effects: "Developmental effects must be observed in the absence of other toxic effects, or if occurring together with other toxic effects, substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects". (CLP, Annex I, Section 3.7.2.2.1 and Table 3.7.1.(a). Also supported by Annex I, Sections 3.7.2.3.5.;3.7.2.4.2. and 3.7.2.3.4.)

2. As described in the comments below, relevant data available not only on ZnPT but also on NaPT (EC 223-296-5) and CuPT (EC 238-984-0) are needed to address these parameters i.e. excessive maternal toxicity, intrinsic property and non-specific secondary consequence of other toxic effects, and to assess the reproductive toxicity classification of the substance i.e. :

a) ZnPT, NaPT and CuPT prenatal studies and ZnPT and NaPT repeat-dose toxicity studies are needed to assess if developmental effects are observed in presence of excessive maternal toxicity

b) ZnPT prenatal studies and embryotoxicity data on NaPT and the principle serum metabolite of pyrithione, 2-methylsulfonylpyridine (MSP), are needed to assess if ZnPT has an intrinsic property to produce adverse effects on reproduction

c) ZnPT, NaPT and CuPT prenatal studies; ZnPT and NaPT repeat-dose toxicity studies and published literature on ZnPT and pyrithione mode of action are needed to assess if developmental effects are produced solely as a non-specific secondary consequence of other toxic effects or not

3. The CLH report has unfortunately not considered any data beyond the ZnPT prenatal studies, based on the argument that ZnPT has a "complete data set" and, as such,a read across/category approach is not necessary. We disagree with this statement. Indeed, ZnPT meets the CLP criteria where WoE approach needs to be used.

Specifically:
• Article 5 of the CLP requires to examine "available information" which is not limited to the data on the substance itself.

• The CLP also states in Article 9.3. that "Where the criteria cannot be applied directly to available identified information, manufacturers, importers and downstream users shall carry out an evaluation by applying a weight of evidence determination, using expert judgement in accordance with section 1.1.1. of Annex 1…".

As indicated above and in more details below, findings from the ZnPT prenatal studies alone are not sufficient to fully assess reproductive toxicity classification criteria and to define if the development effects are linked to maternal toxicity or not. Moreover, the use of data beyond ZnPT pre-natal studies provides more certainty. Additional studies are better for determining replicability; better for characterizing dose-response; better for elucidating the full range of toxicity; and better for understanding mode of action.

• In addition the CLP requires to use the weight of evidence approach (WoE) in assessing classification i.e. this is needed for all the endpoints (Annex I , Part 1 (1.1.1.)) and this is also specifically required for toxicity for reproduction: "Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1)…". (See also Annex 3, Part 3 (Reproductive Toxicity); 3.7.2.2.1.; 3.7.2.3.1.; 3.7.2.5.2.; 3.7.2.5.3.; 3.7.2.5.6.).

• Moreover, the CLP supports the use of structurally-similar substances in a WoE approach (See Annex I, Section 1.1.1.3 of the CLP, and in particular also for reproductive toxicity under Annex I, Sections 3.7.2.3.1 and 3.7.2.5.4. of the CLP).

4. NaPT and CuPT toxicity studies can be used in the WoE approach to assess ZnPT classification as they form a Category with ZnPT based on the ECHA RAAF Guidance.

Indeed, ZnPT, NaPT and CuPT are toxicologically indistinguishable because the common organic moiety, pyrithione (PT) is responsible for the effects of these salts following oral administration. The pharmacokinetic profiles, i.e., absorption, distribution, metabolism and excretion of ZnPT, NaPT and CuPT following oral administration are comparable and therefore these salts should be considered equivalent. In this regard, the structural similarity of these salts has been proven using the ECHA RAAF Guidance as prepared by the ZnPT Industry CLH Consortium and shared with the Dossier Submitter and ECHA . A full analysis based on the ECHA RAAF Guidance has shown that ZnPT, NaPT and CuPT constitute a "Category". (See Section I. point 4 and Annex 1 of the supportive document attached).
This has also been implicitly acknowledged by the Dossier Submitter in Section 6, page 7 who has made reference to NaPT and CuPT data for other endpoints.

5. Also, importantly, the zinc ion from ZnPT, alone or in synergy with pyrithione, could not be responsible for adverse reproductive effects.
Indeed, the study from Klaassen (1976) showed that ZnPT dissociates into Zn++ and PT- portions following different routes of administration to rabbits. Importantly following oral administration, absorption of 65Zn++ was limited if not negligible (Klaassen, 1976).

In addition, Zinc is an essential nutrient and is present naturally in the diet. A rat consumes 4 – 8.5 mg zinc/kg bw/day and a rabbit 2.5 – 3.5 mg zinc/kg bw/day based on typical amounts of zinc in standard rats and rabbits laboratory chow. This dietary intake is more than the amount of zinc administered in the highest dose used in the oral rat prenatal study of ZnPT (3 mg /kg bw/day of zinc in 15 mg/kg bw/day of ZnPT). Furthermore, homeostatic mechanisms are present in the organism that allow it to maintain circulating zinc levels within a narrow range, largely through the induction of a zinc-binding protein in the liver. Even much higher supplementation of zinc (diets containing 3 to 12 x higher levels of zinc) does not have adverse developmental consequences (Bui et al., 1998; Taubeneck et al., 1995).

Section II - Scientific rationale to demonstrate absence of reproductive toxicity potential of ZnPT

In this Section, we demonstrate that ZnPT is not a reproductive toxicant when all available data are employed in a weight of evidence assessment.

1. Developmental effects are only observed in the presence of excessive maternal toxicity. This is evident from a review of all available data in a weight of evidence approach.

Section 3.7.2.2.2. of Annex I to the CLP requires that, when evaluating potential developmental effects of a substance, consideration to the possible influence of maternal toxicity should be taken into account (see also Section 3.7.2.4 of Annex I to the CLP). In case of excessive maternal toxicity or if the substance is so toxic, developmental effects should be discounted and should normally not lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate." (CLP, Annex I, Sections 3.7.2.3.5.; 3.7.2.4.3.; 3.7.2.4.4. and 3.7.2.5.8.).
If all the available data are taken into account and fully considered in a weight of evidence approach as requested by the CLP (Annex I, Part 1 and Part 3 (Sections 3.7.2.2.1. and 3.7.2.3.1)), the conclusion should be that the developmental effects of ZnPT are only associated with excessive maternal toxicity.

Indeed, the data summarized below show that in those studies where developmental effects were reported, such effects were observed only in the presence of excessive maternal toxicity. And developmental effects are not observed when maternal toxicity is absent. The maternal toxicity data for ZnPT is consistent with the definition of excessive maternal toxicity in the CLP legislation and with expert opinion. One of the complicating factors with ZnPT is that the dose-response curve for maternal toxicity is extremely steep, such that even a small increase in dosage produces a disproportionate increase in the severity of the maternal response. As such, dose selection by investigators conducting these studies was, in some cases, too high and there is even justification that whole dose groups should be completely discounted from any evaluation because of this. The rules for interpretation of developmental toxicity studies provide the basis for excluding dose(s) at which excessive maternal toxicity occurred.

Although the CLH report refers to excessive maternal toxicity criteria in section 10.10.6 (page 84), as indicated in Section 1.1. below, it has not fully assessed this parameter and it has considered that maternal toxicity is not a reliable explanation for developmental toxicity, as indicated in Section 1.4. below.

1.1. Criteria for excessive maternal toxicity was not considered in the CLH report

Except for mortality, there are only qualitative criteria with no specific threshold parameters provided in the CLP Regulation or the ECHA Guidance to determine excessive maternal toxicity.

We have considered maternal body weight gain during dosing period as an objective indicator of maternal toxicity (in addition to mortality) for the following reasons:

a) maternal body weight gain is a key factor to assess maternal toxicity (based on CLP Annex I, Section 3.7.2.4.4.);

b) it is assessed in an objective and consistent way in all laboratories (based on measurement and not observation);

c) it is influenced very little by the weight of the litter - at least for studies performed under earlier versions of testing guidelines, when dosing was completed at the end of the embryonic period, as the vast majority (>90%) of foetal growth occurs in the last several days of gestation in the rat; and

d) there is guidance from consensus workshops in Europe and US [International Life Sciences Institute (ILSI) workshop of 2009; conclusions published in 2011 (Beyer & al., 2011)] that at > 10-20% decrease in maternal weight gain, the developmental data become un-interpretable. This is also supported by other scientific articles (e.g. Wise et al. 2009; Giavini & Menegola, 2012).), and by the OECD Guidelines 426 (Developmental Neurotoxicity Study, 2007) which provide a limit of 10% decrement in weight gain above which maternal toxicity would be considered excessive. Therefore, a 20% decrease or more in maternal weight gain should be considered as excessive maternal toxicity.

However, the CLH report did not follow this well-established scientific consensus even though the ILSI conclusion stating that "a decrease in body weight gain of 20% was considered excessive" was actually quoted in the CLH Report (section 10.10.6., page 84) – although not acted upon.

1.2. A complete analysis of the data on maternal toxicity in the prenatal ZnPT studies shows the magnitude of maternal toxicity exhibited

While some data during the dosing period (e.g., food consumption) were reported in tables of the CLH Report, the reporting did not include the most relevant and well documented measures of maternal toxicity.

Maternal body weight gain should be considered at every measurement interval, especially over the dosing period and not just at the end of gestation, as reported in the CLH report for two prenatal studies (rat and rabbit, oral or dermal; CAR Doc IIIA A6.8.1/02 and 03), which in those studies was several days after the last dose. Indeed, structural malformations and embryonic death take place during the dosing periods (gestation days 6-15 in the rat and days 6-19 in rabbit). Therefore, the maternal toxicity

measured during this period has the highest relevance for interpreting developmental effects rather than at the end of the gestation.

If maternal body weight gain measurements had been reported during the dosing period of these two prenatal studies, the maternal toxicity would have been even more severe and in any case excessive (>20% maternal weight gain decrement observed) at doses at which developmental effects are observed.

Further details on the assessment of maternal weight gain for the two prenatal studies, and also for the study "Thor GmbH Art 95 (Prenatal, Rabbit; oral gavage, 2015)" are provided in Section II, point 1.2 of the supportive document.

1.3. A weight of evidence determination demonstrates that all developmental effects were observed only in the presence of excessive maternal toxicity
If the CLH report had assessed in a weight of evidence determination the influence of maternal toxicity on developmental effects (as is required by the CLP for classification purpose), it would have concluded that all the reported effects of toxicological relevance have occurred concomitantly to excessive maternal toxicity.

Indeed, a weight of evidence approach based on ZnPT and NaPT maternal weight gain decrement analysis and maternal toxicity in the prenatal studies, and mortality assessment in subacute and subchronic toxicity tests, has shown that maternal toxicity observed at or above 3 mg/kg bw/day in the rat or at or above 1.5 mg/kg bw/day in the rabbit is considered excessive. This is based on the threshold values for maternal weight gain decrement (i.e. >20%) as defined in the consensus ILSI workshop (Beyer & al. 2011) and the limit for mortality as described in the CLP (Annex 1, Section 3.7.2.4.4.) and detailed above in Section 1.1.

Developmental effects were only observed at those specific doses or above, i.e., in the presence of excessive maternal toxicity. Below these thresholds, there is no evidence of any effect on development.

Analysis of slightly longer ZnPT oral (gavage) sub-chronic studies in rats indicates that dose levels greater than approximately 2.5 mg/kg bw/day produced excessive toxicity. In a 90-day oral rat study on ZnPT (ZnPT CAR Doc IIIA A6.4.1./03), the top dose of 5 mg/kg bw/day had to be reduced to 2.5 mg/kg bw/day during the course of the study because it was not tolerated by the animals and they were in danger of dying. In a 90-day study (rat, oral) on NaPT, half the females in the top dose group of 8 mg/kg bw/day were sacrificed in extremis, most between 4 and 9 weeks into the dosing period. These data support a dose level of 2.5 mg/kg bw/day as a maximum tolerated dose . The same analysis can be made for rabbit studies. While there are no rabbit sub-chronic studies, a range-finding study for developmental rabbit oral study demonstrated that a 4 mg/kg bw/day dose level was lethal (Thor GmbH Art.95 dossier; Thor (2015a)).

The CLH report has not assessed if the developmental effects occurred in presence of excessive maternal toxicity or not, and it has only evaluated each ZnPT prenatal study in isolation and has not considered other ZnPT data (e.g. sub-chronic data) or data from chemically-related substances as additional supporting evidence, even though the CLH Report " acknowledges that zinc pyrithione shows some structural similarity to sodium pyrithione (EC 223-296-5) and copper pyrithione (238-984-0), in that they share the common organic moiety i.e. pyrithione " (Section 6; page 7).

As explained in our preliminary remarks (Section I) above, we reiterate the conclusion

that the available data on NaPT and CuPT are fully relevant and consequently need to be taken into account in a weight-of-evidence approach.

In Section II., point 1.3. of our supportive document, we provide all the details (including detailed Tables and Figures) which support the above discussion.

1.4. Disagreement with the interpretation made in the CLH report about statements from the ILSI Workshop on maternal toxicity and from prenatal feed restriction studies to justify that maternal toxicity is not the cause of developmental effects and to propose classification of ZnPT as Reproductive Toxicant Category 1B.

1.4.1. The statement from the ILSI workshop on maternal toxicity (i.e., Beyer et al., 2011) presented on Section 10.10.6, page 84 of the CLH report is taken out of context and may a priori suppose that maternal toxicity would not be the cause of developmental effects observed which would potentially justify classification of ZnPT as Reproductive Toxicant Cat 1B.

According to the CLH Report (page 84), "In the zinc pyrithione developmental toxicity review paper (June 2016) submitted to the DS by the zinc pyrithione task force, reference is made to the maternal toxicity workshops in 2009 (Beyer et al.,2011) wherein a decrease in body weight gain of 20% was considered excessive. The participants of the workshops recommended that all relevant information should be considered for good dose selection for developmental and reproductive toxicology studies with an intent to avoid marked maternal toxicity leading to mortality or decreased body weight gains of greater than 20% for prolonged periods".

The CLH Report goes on by quoting a statement from the workshop, which is used to support the proposed Reproductive Toxicant Cat 1B classification: "However, several participants considered maternal toxicity to be an indicator to stop dose escalation, but generally do not consider maternal toxicity as a reliable explanation for developmental toxicity."

We disagree with this interpretation because it is not in line with the common understanding on maternal toxicity as explained above, nor with the CLP provisions and Guidance which highlight the importance of addressing maternal toxicity as this might influence the classification of a substance (Sections 3.7.2.3.5.; 3.7.2.4.3.; 3.7.2.4.4. and 3.7.2.5.8. of Annex I to CLP). Rather, on the contrary, the ILSI workshop statements confirm that a 20% decrement in body weight gain is the expression of excessive maternal toxicity as outlined in Section 1.1. above.

1.4.2. The CLH Report has used the feed restriction studies in rat and rabbit (i.e., Cappon & al. 2005) discussed in Section 10.10.6., pages 83 and 84 of the CLH report to conclude that maternal toxicity is not the cause of observed developmental effects because in these studies, significant weight gain decrements were observed without statistically significant increase in pre- or post-implementation loss or in number of viable foetuses or in malformations. The CLH report concludes that decrements in maternal weight gain cannot affect foetal development and therefore ZnPT developmental effects observed are not due to maternal toxicity. We disagree with this interpretation of these studies. The studies in question were conducted as an aid for interpreting studies on candidate pharmaceuticals intended for appetite suppression and weight loss. They are not related to ZnPT and their purpose was to isolate feed restriction and the resulting weight gain decrement from other aspects of maternal toxicity caused by chemical/drug exposure.

Importantly, it cannot be concluded from these studies that maternal weight gain decrement is the only aspect of maternal toxicity that could lead to adverse developmental effects. In the feed restriction studies, there is a direct connection between decreased caloric intake and decreased weight gain. In studies with chemical agents that cause toxicity, there are many possible mechanisms of action, affecting multiple biochemical and metabolic pathways.

While one of the manifestations of effects on these pathways may be maternal weight gain decrement, the effects on maternal physiology are varied, and many of these effects can be detrimental to development.

However, the CLH report interprets those studies as though the weight gain decrement was a necessary step in the causal chain between maternal toxicity and developmental toxicity. This reasoning does not reflect the actual knowledge on biology.

So, while the presence of a weight gain decrement can occur concomitantly with maternally-mediated developmental effects, and is an indicator of excessive maternal toxicity, it is not in the causal chain of events between maternal toxicity and adverse developmental effects.

Consequently, the CLH Report cannot deny the impact of maternal toxicity to foetal development on the basis of the feed restriction studies.

2. The CLH Report did not consider data showing that ZnPT has no intrinsic specific property to produce an adverse effect directly on foetal development and that the mode of action of ZnPT provides evidence that developmental effects are the result of non-specific secondary consequence of maternal toxicity.

Based on the CLP criteria for reproductive toxicity, it is essential to differentiate between substances which have an intrinsic, specific property for producing an adverse effect on foetus and those which produce developmental effects as a secondary consequence of maternal toxicity, as this will have an impact on classification (CLP Regulation (Annex 1, Sections 3.7.2.1.; 3.7.2.2.1.; 3.7.2.3.5.; 3.7.2.4.1.; 3.7.2.4.2.; 3.7.2.4.3.). Indeed, the CLP states explicitely in Annex 1, Section 3.7.2.2.1 that "classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects."

2.1. Results from a rat whole embryo culture (rWEC) study provide unequivocable evidence that neither pyrithione nor its principal serum metabolite 2-(methylsulfonyl)pyridine produce toxicity to the embryo at concentrations that are associated with maternal toxicity in vivo thereby demonstrating that ZnPT does not have an intrinsic specific property to produce adverse effects on development.

The rWEC method has been used extensively to distinguish whether a chemical is directly embryo-toxic, at concentrations that are associated with maternal toxicity in vivo. Indeed, it has value in that it is possible to determine the effects of a chemical on the embryo in the absence of maternal factors. If a chemical is directly embryo-toxic at concentrations that are also maternally toxic, then the outcome of the embryo culture study would be embryo-toxicity at the same concentrations that were maternally and developmentally toxic in vivo. However, if the effects on the embryo are secondary to maternal toxicity, then the results of the embryo culture study would be no embryo-

toxicity at the same (or even higher) concentrations that are maternally toxic in vivo.

Sodium pyrithione was used as the test agent because it is soluble in the culture medium at physiological pH (rat serum) (this is not the case of ZnPT) and because salts of pyrithione, e.g., ZnPT and NaPT dissociate in the gastric lumen and are absorbed as pyrithione. The dosages involved were comparable to the maximum concentration in maternal plasma that would be achieved after a dose of 15 mg/kg bw/day ZnPT, the highest dose level tested in any of the oral GLP prenatal studies. The results clearly show a lack of intrinsic developmental toxicity potential of pyrithione, leaving the only plausible conclusion that the effect seen in vivo is indirect and maternally-mediated. Similar results were obtained using rWEC in the evaluation of 2-(methylsulfonyl)pyridine (MSP), the primary serum metabolite of pyrithione.

More details on the subject are provided in Section II., point 2.1 and Annex 3 of the supportive document.

These data are an additional key element to consider in a weight of evidence assessment. However, these results have been inappropriately put aside in the CLH report (see Section 10.10.5 pages 81-82).

Two reasons are given in the CLH report to put aside the rWEC method:

a) First, because "Moreover, it should be noted that even though the rWEC assay is validated by ECVAM, the predictability and applicability domains are not yet sufficiently defined for regulatory implementation (Adler et al., 2011)."

We disagree with this statement. Indeed, Adler et al. reviewed alternative methods within the context of the pending 2013 prohibition of animal testing for cosmetics for 5 endpoints (including reproductive toxicity). The participating experts were asked to analyse the status and prospects of the available alternative methods and to provide a scientifically sound estimate of the time necessary to achieve full replacement of animal testing.
Even though this method has not yet been approved as a standard test method under the Test Methods Regulation, it has been evaluated and validated by ECVAM as a relevant assay for embryo-toxicity (Spielmann et al., 2006) and was designated as "ready to be considered for regulatory purposes" (ECVAM ESAC, June 2002). Further, this method is mentioned in the latest ECHA Guidance on "Information Requirements and Chemical Safety Assessment", Chapter R.7.a which states, in the chapter on alternative methods for reproductive toxicity (page 474), that this study may be used as supporting information along with other more reliable data to predict developmental toxicity. Further, this method is already in use to determine embryo-toxicity potential per se and the scientific literature is replete with whole embryo culture experiments showing that the results are highly credible.

Moreover, the ECHA's FAQ 6.4 on CLP explicitly allows for the use of nonstandard methods for classification assessment in a weight of evidence approach, when it states that "by reference to Annex XI of the REACH Regulation, CLP encourages the use of alternative testing and non-testing methods. As the results of alternative testing and non-testing methods may not directly correspond to the classification criteria, they should be evaluated in the context of a weight of evidence approach involving expert judgment".

Importantly, the rWEC study discussed here was not proposed as a replacement for established in vivo assays, but rather as an adjunct to aid understanding within the

context of the required weight of evidence approach to classification. Considering the above, results of the rWEC study, showing that the pyrithione moiety does not have the potential to be embryo-toxic, are considered supportive of a nonspecific, secondary maternal toxicity mechanism mediating the developmental effects presented in the CLH report.

Unfortunately, the CLH report failed to make this point.
To not consider this data is contrary to the requirements of Annex I, Section 3.7.2.3.4 of the CLP, which requires that " if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of generalised adverse effects shall be assessed to the extent possible".

b) Second, because "the results of these rWEC assays are not completely relevant to conclude that zinc pyrithione is not directly embryotoxic as the toxicological significance of Zn2+ in synergy with the pyrithione is not addressed in these assays."

This comment must be challenged in that pyrithione was tested as the sodium salt in this assay because the zinc salt is not soluble at physiological pH in the culture media. However, once dissolved, pyrithione is free to interact with metals in the media, including zinc. The media used in this study was 100% rat serum. Rat serum contains about 130 µg Zn/dl, approximately 20 µM (Hurley et al., 1982). The highest concentration of pyrithione used in the embryo culture study was 2.3 µM, corresponding to the plasma concentration after a 15 mg/kg bw/day oral dose of ZnPT (ZnPT CAR Doc IIIA A6.8.1/02; Schardein 1993a). Therefore, there was almost a tenfold excess of zinc, compared to pyrithione, in the culture media, more than enough to implicitly test any possible synergy between the two.

It is worth noting that ZnPT dissociates into pyrithione and zinc at the pH of the stomach, and the chemical is almost certainly absorbed as pyrithione. Therefore, one would expect the same interactions between pyrithione and plasma zinc in vivo, where the absorbed pyrithione is initially unbound to zinc, as in the in vitro experiment.

The rWEC study provides valuable insight in this respect as it clearly shows that the pyrithione moiety has no intrinsic, specific property to produce an adverse effect directly on the embryo.

2.2. Additional supportive evidence showing that ZnPT does not have an intrinsic property to produce an adverse effect on development:

2.2.1. Absence of consistency in malformations observed:

When reviewing the ZnPT prenatal studies with “statistically significant effect(s) on foetal viability or malformations”, we observed that the different types of malformations across various dose-ranges do not indicate any specificity in organs/tissues affected i.e. these malformations do not conform to any recognizable syndrome.

2.2.2. Dose response relationship: foetal weight and maternal weight gain:

In the attached supportive document, we provide an analysis whereby we have compared graphs with relationship between dose and maternal weight gain versus dose and foetal weight (see Figure 4 in supportive document) in rat oral, prenatal studies on ZnPT or NaPT (as % of control). Foetal weight was selected because it is measured in all studies and because it has been shown to be a sensitive determinant of developmental response.

The foetal weight graph indicates that even with substantial maternal toxicity there is a shift of the curve to the right in the dose-response contrary to what is observed in the maternal weight gain curve (i.e., foetal effects only start at doses that are already maternally toxic). Significant effects are only observed when there is excessive maternal toxicity. These results demonstrate that the intrinsic property of pyrithione to produce adverse effects in the adult animal does not appear to have the same activity in the foetus, suggesting an indirect mechanism on the foetus that is related to the maternal toxicity.

2.2.3. Mode of Action of ZnPT providing evidence that this substance produces developmental effects by non-specific secondary consequences of maternal toxicity.

In this section, we provide additional information on the mechanism of toxicity of ZnPT. Please note that this ZnPT Mode of Action is a new information and is not addressed in the CLH Report. The detailed description of ZnPT mode of action is provided in the supportive document in Section II, point 2.2.3.

The mechanism by which the pyrithiones act is an inhibition of oxidative metabolism which causes profound effects on maternal metabolism that indirectly affect development of the embryo.

Pyrithione acts as an ionophore, carrying metals across cellular membranes, such as the mitochondrial membrane where it inhibits aconitase, a Krebs cycle enzyme that has an iron sulfide (Fe-S) group on the surface of the molecule and is therefore susceptible to inhibition by divalent metals. This inhibition leads to profound effects on the adult animal, as mitochondrial metabolism is the most important source of energy for the animal. Importantly, this is not the case for the embryo, which is much more dependent on glycolysis.

The effects of aconitase inhibition on the intact animal will have a spectrum of biochemical and metabolic effects. These can be discerned from an examination of the data from subchronic and chronic studies for pyrithiones. These would include a short-term increase in serum glucose (as a consequence of negative feedback from lactate accumulation, as pyruvate cannot enter the inhibited Krebs cycle); and a significant decrease in food conversion efficiency (the ratio of body weight gain to food intake). While the repeat-dose studies are not ideally designed to investigate these effects, effects on glucose and feed efficiency are consistent across multiple studies on ZnPT and NaPT. The steep dose-response curve is also consistent with an effect on oxidative metabolism, including the rapid progression from seeming normality to death. An indication of effect on organs with high metabolic demands is a consistent increase in relative heart weight at high doses in subchronic studies.

The non-specific secondary effects of aconitase inhibition are developmentally adverse. The short-term hyperglycemia, the metabolic acidosis from lactate and citrate accumulation, and possibly hemodynamic insufficiency, are all developmentally adverse from an indirect mechanism.

Another consistent observation across studies is a decrease in haemoglobin and or red blood cells number, along with an increase in relative spleen weight. The molecular mechanism of pyrithione, carrying of metals across membranes with subsequent interaction with metalloproteins, is expected to affect haemoglobin. This produces a mild hypoxia, which would serve to exacerbate the maternally-caused developmental toxicity.

*Concluding Remarks:

The aconitase inhibition experiments with ZnPT and NaPT demonstrate unequivocally that aconitase inhibition is complete at concentrations that are equivalent to those measured at excessively toxic dose levels in vivo. Analysis of the repeat-dose toxicity studies on ZnPT and other pyrithiones provide consistent results on food conversion efficiency and other endpoints that are consequences of aconitase inhibition, offering conclusive support of this mechanism. While it is not possible, short of conducting a series of dedicated mechanistic toxicology studies, to demonstrate every step from aconitase inhibition to indirect embryotoxicity, we have demonstrated the key events in this mode of action.

The downstream effects of aconitase inhibition not only profoundly perturb maternal homeostasis, but these homeostatic changes are developmentally adverse. Section 3.7.2.3.5. of Annex I to CLP regards homeostasis disruption as a non-specific secondary mechanism of toxicity that may affect development: « if appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. » Accordingly, it can be concluded that ZnPT does not have an intrinsic property to adversely affect foetal development but that observed developmental effects are the consequence of the non-specific secondary effect of ZnPT in the dams. As discussed above, the CLP (Annex I, Section 3.7.2.2.1) requires to differentiate between substances which have an intrinsic, specific property for producing an adverse effect on foetus and those which produce developmental effects as a secondary consequence of maternal toxicity, as this will have an impact on classification.

3. Consideration of the extensive information on historical controls for two key reproductive studies (rat and rabbit gavage prenatal studies) shows that the effects seen are consistent with a historical background rate in laboratory rats.

Extensive information and links to large historical control databases that were matched at the time when the two studies were conducted have been provided to the Dossier Submitter. These databases are necessary to assess whether a certain effect is treatment related or whether it is within the range of spontaneous rates of malformation recorded in historical controls. However, only the historical data from the individual laboratory was considered in the CLH report in interpreting these studies.
If the extensive data from these comprehensive control databases is considered, one would have concluded that the overall rate of malformations, or that of any of the individual malformations observed in the rat prenatal gavage study (ZnPT Doc IIIA /A.6.8.1./02; Schardein, 1993a) at mid-dose of 3 mg/kg bw/day, are consistent with a historical background rate in laboratory rats. In this study, the prevalence of malformed foetuses was increased in the 3 mg/kg bw/day group (7 foetuses of the 345 examined (2%), vs. 1 of the 392 examined in the control group, 6/25 litters with a foetus affected, vs. 1/27 in controls). The study report indicates that this was a statistically signifcant effect. However, the historical control malformation prevalence for this species and strain is in the range of 1.5-3%, a rate that has not changed and is consistent with large scale historical control data collected contemporaneously with the Schardein study (MARTA, 1993). In other words, the expected background rate of malformations in 392 foetuses would be 5-10, comparable to the rate in the 3 mg/kg bw/day dose group, i.e., 7. Further, some apparent skeletal abnormalities reported in the ZnPT Doc IIIA /A.6.8.1./02; Schardein (1993a) study at 3 mg/kg bw/day, and considered as malformations by the author and in the CLH report, can actually be explained by a delay in ossification of the examined foetuses. The only statistically significant finding in this

study concerns skeletal malformations in the high-dose group (15 mg/kg bw/day) in conjunction with maternal toxicity.

The same conclusions can be taken on the rabbit oral pre-natal study (Thor GmbH Art. 95 Dossier; Thor, 2015a) where the type of malformations reported (omphalocele) in the mid and high dose groups have an incidence that is comparable to that of the historical control range for ventral wall defects (Posobiec et al., 2016).

Further details on the rate of malformations observed for each of the two studies are presented in Section II. Point 3 of our supportive document.

4. Conclusions made by other official bodies on the reproductive toxicity of ZnPT and notably by the SCCS, suggest that the effects seen are attributable to maternal toxicity.

The CLH report addresses the SCCS opinion only in relation to its uses in cosmetic applications but it does not discuss the position of the SCCS, in its revised opinion of 2014, which supports the conclusions from MAK (2012) and the HSE (2003) that developmental effects are most likely attributable to maternal toxicity.

Conclusion on Reproductive Toxicity:

Based on the above information and an assessment of all available data in a weight-of-evidence determination, ZnPT should not be considered as a reproductive toxicant since the evidence available shows that ZnPT has no intrinsic property to produce adverse effects on reproduction. The mode of action of ZnPT provides evidence that this substance produces developmental effects through a non-specific secondary consequence of maternal toxicity. And developmental effects are observed only in presence of excessive maternal toxicity.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT CLH Consortium Comments 4 attachments -June 30, 2017.zip

Dossier Submitter's Response

Thank you for your extensive comment and the attachment "Supportive document to the ZnPT Industry CLH Consortium comments on reproductive toxicity", however, most of the information in your comments comes from the data already submitted to the DS by the zinc pyrithione task force in the read-across position paper (May 2016) and in the developmental toxicity review paper (June 2016). We acknowledge that the CLH report did not include any discussion on the Mode of Action (MoA) of ZnPT and we thank you in particular for comments on the MoA of the pyrithiones.

Weight of evidence:

Your critical comments include that the data from other pyrithiones (i.e. NaPT and CuPT) is not considered in the CLH report. In page 7 of the CLH report the DS has stated with references to legal text why it is not mandatory to consider data from NaPT and CuPT: "*for zinc pyrithione there is reliable and adequate substance-specific information (i.e. a complete dataset[1]) precluding the necessity to consider a grouping and/or read-across (see sections 1.1.1.1 and 1.1.1.3 of Annex I to the CLP Regulation). Therefore, the DS submitted a CLH report on zinc pyrithione without including data from the other*

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

pyrithiones. In other words, the DS does not use grouping and/or read-across in the CLH proposal for zinc pyrithione."

However, your disagreement with our statement seems to stem from an incomplete interpretation of Article 5 of the CLP Regulation. You state in your comments that "*Article 5 of the CLP requires to examine "available information" which is not limited to the data on the substance itself"*.

According to Article 5 of the CLP Regulation (copied below), the identified information on a substance even "*shall relate to the forms or physical states in which the substance is placed on the market […]"*. Article 5(c), by giving reference to the REACH Regulation allows the use of grouping of substances and read-across approach which is meant to **predict** properties of a substance from data for reference substance(s) (see Annex XI, Section 1.5 of the REACH Regulation).

Article 5

Identification and examination of available information on substances

1. Manufacturers, importers and downstream users of a substance shall identify the relevant available information for the purposes of determining whether the substance entails a physical, health or environmental hazard as set out in Annex I, and, in particular, the following:

(a) data generated in accordance with any of the methods referred to in Article 8(3);

(b) epidemiological data and experience on the effects on humans, such as occupational data and data from accident databases;

(c) any other information generated in accordance with section 1 of Annex XI to Regulation (EC) No 1907/2006;

(d) any new scientific information;

(e) any other information generated under internationally recognised chemical programmes.

The information shall relate to the forms or physical states in which the substance is placed on the market and in which it can reasonably be expected to be used.

More specifically, Annex I, Section 3.7.2.3.1 of the CLP Regulation (which is regarding the use of weight of evidence under the Reproductive toxicity section) states that "*Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is **scarce**."* [emphasis added]

Concerning reproductive toxicity endpoint, there are reliable and relevant experimental studies performed with ZnPT to apply the CLP criteria. Therefore, there is no need for **prediction** as information on ZnPT for this endpoint is neither lacking nor **scarce** (see Table 53 in the CLH report which contains summaries of seven animal studies on adverse effects on development performed with ZnPT).

Nevertheless, the DS has considered and commented here on the data on NaPT and CuPT.

Please see our response under the comment number 58 concerning your "category" designation for NaPT, CuPT and ZnPT based on ECHA RAAF (Read-Across Asessment Framework).

The maternal toxicity and developmental toxicity observed in the pre-natal developmental toxicity and two-generation reproductive toxicity studies with NaPT and CuPT are summarised below

	Maternal toxicity	**Foetal viability**	**Malformations**
Copper pyrithione			
Rat: Oral OECD 414, GLP Reliability factor: 1 Purity: >95% Doses: 0, 0.5, 1.5 and 3.0 mg/kg bw	↓ food consumption days 6-8 (3.0 mg/kg bw)	No effects observed.	One foetus with agnathia and microstomia
Rat: Oral Fulfils the earlier version of OECD 416. Reliability factor: 2, since sperm parameters were not investigated. (This is required by the amended guideline.) Deviations from the guideline: Weighing of the following organs was not performed: liver, kidneys, thyroid and adrenal glands. Sperm parameters were not investigated. The highest dose did not produce toxicity in the parental animals (F0). Purity: >95% Doses: 0, 0.05, 0.2, 1.0 mg/kg bw	<u>F0 – 1.0 mg/kg bw:</u> ↓ mean body weight change last week of the pre-mating period (♂: -44%, p<0.001) <u>F1 adults – 1.0 mg/kg bw:</u> ↓ body weight during the 2nd half of the pre-mating period (only ♂, -5%, p<0.05) ↓ uterus weight relative to terminal body weight (-26%, p<0.05) ↓ ovary weights relative to terminal body weight (-12%, p<0.05) <u>F1 adults – 0.2 mg/kg bw:</u> ↓ body weight during the 2nd half of the pre-mating period (only ♂, -5%, p<0.05) ↓ ovary weights relative to terminal body weight (-10%, p<0.05).	<u>F2 pups – 1.0 mg/kg bw:</u> ↓ thymus weight relative to terminal body weight (♂: -24% compared to F1 control, p<0.001, ♀: -6% compared to F1 control, p<0.01)	
Sodium pyrithione			

Rat: Oral OECD 414 Reliability factor: 1 Purity: 40.8% aq suspension. Purity of the active ingredient prior to suspension in water not specified Doses: 0, 1, 2, 4 mg/kg bw	<u>Dams – 4.0 mg/kg/day:</u> ↓ food consumption compared to controls (-20-28%, p<0.01) ↓ adjusted body weight compared to controls (-19%, p<0.01) ↓ mean gravid uterus weights compared to controls (-19%, p<0.01) ↑ number of animals having difficulty in movement (21 compared to 0 in controls) ↑ number of animals with impairment of hind limbs (7 compared to 0 in controls) ↑ number of animals with hunched posture (3 compared to 0 in controls) ↑ number of animals with emaciation (2 compared to 0 in controls)	No effect on foetal viability was observed.	<u>Foetuses – 4.0 mg/kg/day:</u> ↓ foetal weights compared to controls (-15%, p<0.05) ↑ number of small (<2.7g) foetuses (11.3% compared to 0.3% in controls) ↑ incidence of foetuses with incomplete ossifications: • 6th sternebrae (11.2% compared to 4.4% in controls) • metacarpals, no ossification (51% compared to 17.6% in controls) • metatarsals, no ossification (7.7% compared to 2.2% in controls)
Rabbit: Dermal US EPA FIFRA Guideline 83-3 similar to OECD 414 GLP Reliability factor: 1 Purity: 43.76% aq suspension. Purity of the active ingredient prior to suspension in water not specified Doses: 0, 1.0, 2.5, and 5.0 mg/kg/day	<u>Dams – 5.0 mg/kg/day:</u> ↓ body weight gain compared to controls (-32-52%, not statistically significant)	<u>Foetuses:</u> No developmental toxicity or effects on foetal viability or foetal body weights were observed in any dose group	<u>Foetuses:</u> No developmental toxicity or effects on foetal viability or foetal body weights were observed in any dose group

Rat: Dermal Exposure through GD 6 to 15 Reliability factor: 2, no guideline or GLP Purity: >90% Doses: 0, 0.5, 1.5, 3.0, and 7.0 mg/kg/day	<u>Dams – 7 mg/kg/day:</u> ↓ body weight gain (p<0.05) ↑ deaths (5 animals) ↓ thymus weight ↑ forelimb and hindlimb weakness (>50% of animals	No effects on foetal viability were observed.	<u>Foetuses – 7 mg/kg dose group:</u> ↓ foetus bodyweights (71% of control) Delayed ossification. Malformations observed included bent ribs (54 compared to 4 in control group) and limbs (19 compared to 0 in control group).
Rat: Oral EPA FIFRA guideline 83-4 (equivalent to OECD 416 with the following exceptions: the oestrus cycle was not monitored in parental animals during the three weeks before the mating period; no sperm characterisation; no organ weights). Reliability factor: 1: Deviations from guideline: Spinal cord and sciatic nerve (previously identified target organs) were not subjected to histopathology. Purity: 41.2% aq suspension. Purity of the active ingredient prior to suspension in water not specified.	<u>F0 – 3.5 mg/kg bw/day:</u> ↓ body weight (♀ and ♂) compared to control from week 3 throughout most of the study, including during gestation and lactation (ca -4-9%, p<0.01) ↑ atrophy of skeletal muscle fibres from the upper hindlimb with related hind limb paralysis/impairment of movement (♂:8; ♀: 19) ↓ number of animals mating (70.8 % of number paired, p<0.05) ↓ percentage of matings resulting in pregnancy (70.6 % of number mated, p<0.05) ↑ time taken to mate, not statistically significant <u>F1 adults – 3.5 mg/kg bw/day:</u> ↑ mortality, 2 ♀ killed *in extremis* ↓ body weight gain, (♀: -9-11 %, p<0.0001, throughout gestation and lactation; ♂: -5%, p<0.05 but not statistically significant when compared individually ↓ food consumption at the pre-mating period (♀: -8%, p<0.01) ↑ atrophy of skeletal muscle fibres from the upper hindlimb with related hind limb paralysis/impairment of movement (9 ♂ and 20 ♀)	<u>F1 pups – 3.5 mg/kg bw/day:</u> ↑ mortality (1♂ + 1♀) shortly after weaning, may have been due to gavage trauma ↓ pup body weights towards the end of lactation, particularly for females reaching statistical significance (p<0.05) from day 14 when compared on group basis but not individually. Compared to controls day 21: females -5%, males -1%. No difference was seen at birth. ↓ number of pups with startle response on day 15 (-10%, p<0.01) <u>F2 pups – 3.5 mg/kg bw/day:</u> ↓ pup body weights towards the end of lactation compared to controls (-5% in ♂ and -10% in ♀, not statistically significant)	

Doses: 0, 0.5, 1.5, 3.5 (4.5 during the first three weeks) mg/kg bw	F1 adults – 1.5 mg/kg bw/day: ↑ atrophy of skeletal muscle fibres from the upper hindlimb with related hind limb paralysis/impairment of movement (3 ♀)	
Rat: Oral EPA OPPTS 870.3800 of 1998 and OECD 416 of 1983 Reliability factor: 1: Deviations from the guideline: Only 10 randomly selected P and F1adults were selected for histopathology; according to the OECD guideline, all animals of the high dose and control group should be selected. Furthermore, the previously identified target organs, skeletal muscle, sciatic nerve and spinal cord, were not investigated. Purity: 40.8% aq suspension. Purity of the active ingredient prior to suspension in water not specified. Doses: 0, 0.7, 1.4, 2.8 mg/kg bw	F0 – 2.8 mg/kg bw: ↑ emaciation during weeks 1-17 (16 ♀) ↓ food consumption (♂: -6%, p<0.01; ♀: -8%, p<0.05, on gestation day 7-11-19%, p<0.01, post-partum days 14 and 21) ↓ body weights (♂: -5%, p<0.01, ♀: -9%, p<0.01, during pre-mating, gestation and post-partum periods from day 22) ↓ terminal body weights (♂+♀: -12%, p<0.01) F0 – all dose groups: ↓ fertility index (♂+♀): 87.5% in low-dose, 79.2% in mid-dose and 83.9% in high-dose F1 adults – 2.8 mg/kg bw: ↑ number of days taken for preputial separation (mean 45.38 compared to mean 44.17 in control, p<0.05) ↑ emaciation during nominal weeks 5-17 (5 ♀), ↓ body weights (-8-11% in ♂ on nominal days 28-42 (p<0.01), and -6% in ♀ (p<0.01) on nominal days 77-91) ↓ food consumption on nominal days 35 and 42, gestation days 7 and 14 and post-partum day 21 (- 6-12% in ♀, p<0.01)	F1 pups – 2.8 and 1.4 mg/kg bw: ↓ mean pup weight before/at weaning compared to controls (post-partum day 21 for mid-dose (-12%, p<0.05) and days 14 and 21 for high-dose (- 13-18%, p<0.05)) ↑ number of small pups (determined clinically) before weaning (15 (7.0%) in mid-dose and 24 (8.6%) in high-dose compared to 1 (0.4%) in controls) ↑ number of pups with testis not descended at weaning (5 in mid-dose and 6 in high-dose compared to 0 in control group) F2 pups – 2.8 mg/kg bw: ↓ pup weight post-partum days 7-21 (-10% day 7 and -24% day 21, p<0.05) ↑ testis not descended at weaning (10 compared to 3 in the control group) F2 pups – 1.4 mg/kg bw: ↑ pup loss post-partum day 4 (-13%, p<0.05). No effects observed in high dose. ↓ litter weight compared to controls post-partum day 21 (-10%, p<0.05)

Maternal toxicity vs developmental toxicity:

You state in your comments that "*a weight of evidence approach based on ZnPT and NaPT maternal weight gain decrement analysis and maternal toxicity in the prenatal studies, and mortality assessment in subacute and subchronic toxicity tests, has shown that maternal toxicity observed at or above 3 mg/kg bw/day in the rat or at or above 1.5 mg/kg bw/day in the rabbit is considered excessive*". Consequently, in your view, any developmental effects observed at or above these dose levels should be discounted. The DS disagrees with this and highlights some of the reasons below.

Examples for the level of maternal toxicity to be considered excessive for the developmental effects to be discounted are given in the CLP Regulation in

- Annex I, Section 3.7.2.3.5: "*In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.*"
- Annex I, Section 3.7.2.4.4: "*Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.*"

Such excessive maternal toxicity was however not observed at doses approximate to 3 mg/kg bw/day in the rat or at 1.5 mg/kg bw/day in the rabbit in the below studies with pyrithiones via oral route

- In the 28-day repeated dose toxicity studies in rats, 3.2 mg/kg bw/day was the NOAEL for NaPT and 2.5 mg/kg bw/day was the NOAEL for CuPT (References: Oral 28-Day Range-Finding Study, NaPT. 1987 and 28-Day Oral Tox Study with 14-Day Recovery Period, CuPT, 1995; These references are from 'Appendix II Repeat dose oral and dermal studies PT salts' of the Read-Across document based on ECHA RAAF prepared by the ZnPT Industry CLH Consortium and available as public attachment to this RCOM under the comment number 58.)
- In a two-generation study with NaPT in rats (summarised in the table above), F1 females in the 3.5 mg/kg bw/day dose group had decreased body weight gain of 9-11% throughout gestation and lactation.
- In the pre-natal developmental toxicity with CuPT in rats (summarised in the table above), the only maternal toxicity in the 3 mg/kg bw/day dose group was decreased food consumption during days 6 to 8.
- In the two-generation study with ZnPT in rats (Thor GmbH Art. 95 dossier, 2015), in 2.5 mg/kg bw/day dose group, F0 females had decreased body gain of 10 to 20% from days 22-64, and F1 females had statistically significant lower absolute body weights (but not body weight gains) on GD 4-20 and LD 1 compared to controls.
- In the pre-natal developmental toxicity study with ZnPT in rats (ZnPT CAR Doc IIIA A6.8.1/02, 1993), the only maternal toxicity in the 3 mg/kg bw/day dose group was increased salivation in 8 of the 30 females.
- In the pre-natal developmental toxicity study with ZnPT in rabbits (Thor GmbH Art. 95 dossier, 2015), females in the mid-dose (1.5 mg/kg bw/day) group level had no statistically significant changes compared to controls in body weights (gain) or (relative) food consumption.

You disagree with the DS's interpretation of the statements from the ILSI/HESI workshop on maternal toxicity (Beyer et al., 2011) mentioned in the CLH report (page 84): "*In the zinc pyrithione developmental toxicity review paper (June 2016) submitted to the DS by*

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

You state in your comments that the CLH report did not follow this (20% decrease in maternal body weight gain to be considered as excessive) "*well-established scientific consensus".* The DS does not consider such 20% upper limit value as a well-established scientific consensus to be followed by regulatory authorities in their evaluation. The Beyer et al. (2011) paper merely summarised the presentations and discussions from workshops on maternal toxicity. The opinions expressed therein are of individual presenters. The context of the 20% value was that it was one of the recommendations based on the outcome of the discussions at the workshops

"*A comprehensive evaluation of all available data from general toxicity studies, range-finding DART studies, class effects, structure–activity relationships, exposure studies, etc. is essential for good dose selection for definitive DART studies. The intent is to avoid marked maternal toxicity leading to mortality or decreased body weight gains of >20% for prolonged periods*."

In addition to the earlier quote in the CLH report "*several participants considered maternal toxicity to be an indicator to stop dose escalation, but generally do not consider maternal toxicity as a reliable explanation for developmental toxicity*", the DS quotes below a paragraph (also from the Beyer et al. (2011) paper) which followed after a paragraph containing even examples of substances for which maternal effects have been implicated in developmental toxicity

"*From a regulatory perspective (US EPA, 1991; US FDA, 2000; OECD, 2007), it is difficult to distinguish between those effects on in utero development that are attributable to direct fetal exposure to the toxicant versus those effects that are due to, or exacerbated by, maternal toxicity. Therefore, adverse effects on the developing organism are considered by most regulators to be toxic manifestations of treatment, regardless of the cause. For that reason, evidence of maternal toxicity does not automatically negate the observation of fetal toxicity at a similar dose level*."

Also, "*a decrease in body weight gain of 20% was considered excessive for <u>most test articles/test materials</u> (compounds designed to produce weight loss would be an exception)"* [emphasis added] by the workshop participants. There is no data in the Beyer et al. (2011) paper which suggests that the pyrithiones should be included among those undefined most test materials.

More importantly, the CLP Regulation states

- Annex I, Section 3.7.2.3.4: *"Discounting developmental effects that are observed at maternally toxic doses can only be done on a case- by-case basis when a causal relationship is established or refuted."*
- Annex I, Section 3.7.2.4.2: "[…] *the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by- case basis that the developmental*

effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies."

You also disagree with the DS's use of the feed restriction studies (Cappon et al., 2005 and Fleeman et al., 2005) to support why the developmental toxicity seen in the ZnPT pre-natal development toxicity studies are not considered secondary to the maternal toxicity in terms of decreased body weight (gain). Unlike, the Beyer et al. (2011) paper which is a summary of ILSI/HESI maternal toxicity workshop without any presentation of actual data, the outcome of the feed restriction studies have been published in detail. In addition the interpretation of these feed restriction studies have been referred to in the OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (OECD, 2008) which has a broad regulatory consensus.

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

Adverse effects on foetal morphology were observed in both mid- and high-dose groups. External malformations of omphalocele were observed in two foetuses from two litters in the high-dose group and also in two foetuses from two litters in the mid-dose group. Two foetuses (one each from mid and high-dose group) among the four affected foetuses also had an absent tail. <u>These external malformations were not found in controls and in only one historical control foetus (out of more than 2000 fetuses from 15 studies)</u>. **The study report stated that these malformations were treatment related**. The foetus in the high-dose group with omphalocele and absent tail also had several urinary tract malformations/variations (absent right kidney and ureter, dilated left ureter and absent urine bladder). These were not observed in controls and only one historical control foetus had absent urine bladder. The malformations of omphalocele in the mid-dose group were

observed only in the litters of six does that had statistically significant decrease in body weight gain (see Table below).

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

Dose (mg/kg/day)	0	1.5 (15 does without significant effects on maternal weight gain)	1.5 (6 does with significant effects on maternal weight gain)
Maternal weight gain, (g/animal) gestation day 7-20	143	156	26*
Maternal weight gain (g/animal) gestation day 7-29	359	365	208*
% of corrected (for uterus) body weight gain range	**+5 to -10.4**	**+2.3 to -8.9**	**+7.7 to -6**
Post-implantation loss (%)	7.9	7.7	55.8*
Fetuses with malformations (total fetuses)	2 (146)	3 (106)	2 (21)*

* Statistically different from control (P<0.05)

It is important to note that although the maternal weight gain decrease was high in the 6 does, 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 is due to the high incidence of resorptions in this group (similar as in the high-dose group). The fact that the corrected body weight gain was unaffected in these 6 does of the mid-dose group (1.5 mg/kg bw/day) is not mentioned in the CLH report and in consequence, the ZnPT Industry CLH Consortium incorrectly concludes that the developmental toxicity seen in the mid-dose group should be set aside as uninterpretable because of the effects on body weight gain.

Historical control databases:
You state that the effects on historical controls for two key rat and rabbit gavage prenatal studies shows that the effects seen are consistent with a historical background rate in laboratory rats. Please note that in the rabbit gavage study (Thor GmbH Art. 95 dossier, 2015) the historical control data (2008-2012) on rabbits is provided and has been considered (see above the summary of this study). For more information on the consideration of concurrent and historical control data please see paragraph 67 in the OECD Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment (OECD, 2008).

Evaluation by other official bodies:
You also refer to the conclusions made by other official bodies, SCCS (2014), MAK (2012) and HSE (2003), suggesting that the effects are most likely attributable to maternal toxicity. Please note that the relevant newly performed studies with ZnPT (one two-generation study in rats, and two pre-natal developmental toxicity studies - one each in

rats and rabbits) were available only in 2015 in the Thor GmbH Art. 95 dossier and obviously, these were not considered by those official bodies.

Mode of Action:
Concerning mode of action of the pyrithiones, it is stated under section 2.2.3 of your comments "*The mechanism by which the pyrithiones act is an inhibition of oxidative metabolism which causes profound effects on maternal metabolism that indirectly affect development of the embryo.*

Pyrithione acts as an ionophore, carrying metals across cellular membranes, such as the mitochondrial membrane where it inhibits aconitase, a Krebs cycle enzyme that has an iron sulfide (Fe-S) group on the surface of the molecule and is therefore susceptible to inhibition by divalent metals. This inhibition leads to profound effects on the adult animal, as mitochondrial metabolism is the most important source of energy for the animal. Importantly, this is not the case for the embryo, which is much more dependent on glycolysis."

You refer to also another substance (fluoroacetate) which is a Kreb's cycle inhibitor and has much less effect on embryonic mitochondria than adults, and is not teratogenic in the Spielmann et al. (1973) study. However, in this study only a single dose of fluoroacetate was administered to pregnant rats at day 9 or 10.

Baker and Ebert (2013) in their review article have convincingly highlighted the crucial importance of oxidative metabolism during the embryonic and foetal development; "*while glycolysis remains the primary generator of 3-carbon substrates for the Kreb's Cycle throughout the prenatal period, the embryo and subsequent foetus cannot survive without activation of mitochondrial function and oxidative phosphorylation during the embryonic period.*"

Authors of another review article (Wilding et al., 2009), while presenting the relationship between mitochondrial activity and human embryo development, suggested that oxidative metabolism and glycolysis "*work in synchrony to guarantee the level of ATP required for embryo development, implantation and birth".* It is also suggested that the interaction between glycolysis and oxidative metabolism "*to provide optimal ATP level for development explains why embryos can develop in a wide range of culture systems"* – which could perhaps partly explain the lack of embryotoxicity in the rWEC assay with NaPT.

rWEC assays cannot address if embryotoxicity (the increased resorptions) observed in the ZnPT prenatal developmental toxicity studies is direct or indirect.
We note that you acknowledge that you have not provided dedicated mechanistic toxicology studies[2], to demonstrate the link between aconitase inhibition to indirect embryotoxicity that would be needed to fulfil the requirement of Annex I, Section 3.7.2.4.2 of the CLP Regulation in order to disregard developmental effects that are observed in the presence of maternal toxicity.

Therefore, the mode of action of the pyrithiones (inhibition of oxidative metabolism) suggests that the developmental effects of ZnPT seen in the prenatal developmental

[2] Page 29 of the 'Supportive document to the ZnPT Industry CLH Consortium comments on reproductive toxicity'.

toxicity studies cannot be considered to be produced solely as a non-specific secondary consequence of maternal toxicity. (see sections 3.7.2.2.1 and 3.7.2.3.5 of Annex I to the CLP Regulation)

Also, the Member State Germany in the comment number 33 states: "*It is true that gastrulating and neurulating embryos metabolism is dependent mostly on the glycolysis, but metabolic pathways may be different in different regions of the embryo even during early stages of morphogenesis that predate differentiation of definitive organ systems (Sadler et al., 1993). Therefore, the ATP synthesis inhibition and impact on fetuses' subsequent development cannot be completely disregarded.*"

Conclusion

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

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

Therefore, classification in Category 1B is proposed for ZnPT in a weight of evidence assessment based on the clear evidence of malformations and post-implantation losses seen in three reliable independent guideline studies in two different species.

References:

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RAC's response

Comprehensive and detailed. Well thought out by all parties.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Belgium	Procter & Gamble Co.	Company-Downstream user	57

Comment received

The CLH Report did not consider data showing that ZnPT has no intrinsic specific property to producing an adverse effect directly on foetal development and that the mode of action of ZnPT provides evidence that this substance produces developmental effects through non-specific secondary consequence of maternal toxicity

Based on the CLP criteria for reproductive toxicity, it is essential to differentiate between substances which have an intrinsic, specific property for producing an adverse effect on foetus and those which produce developmental effects as a secondary consequence of maternal toxicity, as this will have an impact on classification (CLP Regulation (Annex 1, Sections 3.7.2.1.; 3.7.2.2.1.; 3.7.2.3.5.; 3.7.2.4.1.; 3.7.2.4.2.; 3.7.2.4.3.).

Indeed, the CLP states explicitly in Annex 1, Section 3.7.2.2.1 that "classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects."

By considering all the data provided below, together in a weight-of-evidence approach, it can be observed that:

☐ the results from the rat whole embryo culture (rWEC) study show clear evidence that pyrithione is not a direct acting developmental toxicant i.e. it has no intrinsic, specific property to produce an adverse effect on embryo

☐ ZnPT mode of action shows that pyrithione inactivates aconitase which blocks the Krebs' cycle. This inhibition leads to metabolic disruption in the pregnant female which are non-specific and indirectly lead to adverse developmental effects.

Therefore, the data show that developmental effects of ZnPT are a non-specific secondary consequence of maternal toxicity.

This conclusion is contrary to the statement made in the CLH report (page 84): " With the available information, it cannot be unequivocally demonstrated that the developmental effects of zinc pyrithione are secondary to maternal toxicity" which is the basis to the proposed classification as Reproductive Toxicant Category 1B.

When all the available and relevant data are considered together in a weight of evidence approach, it can be demonstrated that ZnPT does not have an intrinsic property to produce developmental effects and that the developmental effects observed are non-specific secondary consequences of maternal toxicity.
Results from a rat whole embryo culture (rWEC) study provide unequivocable evidence that neither pyrithione nor its principal serum metabolite 2-(methylsulfonyl)pyridine produce toxicity to the embryo at concentrations that are associated with maternal toxicity in vivo thereby demonstrating that ZnPT does not have an intrinsic specific property to produce adverse effects on development
The results of an embryo-toxicity study (rat Whole Embryo Culture (rWEC) concluded that the pyrithione moeity (PT) and its principal serum metabolite 2-(methylsulfonyl) pyridine are not embryo-toxic (for details about rWEC study see Annex 3).
Rodent whole embryo culture is a method that has been used extensively to distinguish whether a chemical is directly embryo-toxic, at concentrations that are associated with maternal toxicity in vivo. Indeed, it has value in that it is possible to determine the effects of a chemical on the embryo in the absence of maternal factors. If a chemical is directly embryo-toxic at concentrations that are also maternally toxic, then the outcome of the embryo culture study would be embryo-toxicity at the same concentrations that were maternally and developmentally toxic in vivo.
However, if the effects on the embryo are secondary to maternal toxicity, then the results of the embryo culture study would be no embryo-toxicity at the same (or even higher) concentrations that are maternally toxic in vivo.
In order to determine whether pyrithione is a direct embryo-toxicant, we evaluated it in rat whole embryo culture. Sodium pyrithione was used as the test agent because it is soluble in the culture medium at physiological pH (rat serum) (this is not the case of ZnPT) and because salts of pyrithione, e.g., ZnPT and NaPT dissociate in the gastric lumen and are absorbed as pyrithione.
The dosages involved were comparable to the maximum concentration in maternal plasma that would be achieved after a dose of 15 mg/kg ZnPT, the highest dose level tested in any of the oral GLP prenatal studies conducted.
Rat embryos were cultured from gestation days 9.5-11.5. This period of development covers the critical periods of the malformations observed in the various pyrithione studies at maternally toxic dose levels. These results provide strong support for the relevance of the embryo culture protocol to address questions about the direct embryo-toxicity of pyrithione.
The results clearly show a lack of intrinsic developmental toxicity potential of pyrithione, leaving the only plausible conclusion that the effect seen in vivo is indirect and maternally-mediated. Similar results were obtained using rWEC in the evaluation of 2-(methylsulfonyl)pyridine (MSP), the primary plasma metabolite of pyrithione.
These data are an additional key element to consider in a weight of evidence assessment. However, while the CLH report acknowledges the study results, it dismisses it for two reasons (see Section 10.10.5 pages 81-82):
a) First, because "the results of these rWEC assays are not completely relevant to conclude that zinc pyrithione is not directly embryotoxic as the toxicological significance of Zn2+ in synergy with the pyrithione is not addressed in these assays."
This comment must be challenged in that pyrithione was tested as the sodium salt in this assay because the zinc salt is not soluble at physiological pH in the culture media. However, once dissolved, pyrithione is free to interact with metals in the media, including zinc. The media used in this study was 100% rat serum. Rat serum contains about 130 µg Zn/dl, approximately 20 µM (Hurley et al., 1982). The highest concentration of pyrithione used in the embryo culture study was 2.3 µM, corresponding to the plasma concentration after a 15 mg/kg oral dose of ZnPT (ZnPT CAR Doc IIIA A6.8.1/02; Schardein 1993a). Therefore, there was almost a tenfold excess of zinc, compared to

pyrithione, in the culture media, more than enough to implicitly test any possible synergy between the two.
It is worth noting that ZnPT dissociates into pyrithione and zinc at the pH of the stomach, and the chemical is almost certainly absorbed as pyrithione. Therefore, one would expect the same interactions between pyrithione and plasma zinc in vivo, where the absorbed pyrithione is initially unbound to zinc, as in the in vitro experiment.
b) Second, because "Moreover, it should be noted that even though the rWEC assay is validated by ECVAM, the predictability and applicability domains are not yet sufficiently defined for regulatory implementation (Adler et al., 2011)."
We disagree with this statement. Indeed, Adler et al. reviewed alternative methods within the context of the pending 2013 prohibition of animal testing for cosmetics for 5 endpoints (including reproductive toxicity). The participating experts were asked to analyse the status and prospects of the available alternative methods and to provide a scientifically sound estimate of the time necessary to achieve full replacement of animal testing.
Even though this method has not yet been approved as a standard test method under the Test Method Regulation, it has been evaluated and validated by ECVAM as a relevant assay for embryo-toxicity (Spielmann et al., 2006) and was designated as "ready to be considered for regulatory purposes" (ECVAM ESAC, June 2002). Further, this method is mentioned in the latest ECHA Guidance on "Information Requirements " which states, in the chapter on alternative methods for reproductive toxicity (page 474), that this study may be used as supporting information along with other more reliable data to predict developmental toxicity. Further, this method is already in use to determine embryo-toxicity potential per se and the scientific literature is replete with whole embryo culture experiments showing that the results are highly credible.
Moreover, the ECHA's FAQ 6.4 on CLP explicitly allows for the use of nonstandard methods for classification assessment in a weight of evidence approach, when it states that "by reference to Annex XI of the REACH Regulation, CLP encourages the use of alternative testing and non-testing methods. As the results of alternative testing and non-testing methods may not directly correspond to the classification criteria, they should be evaluated in the context of a weight of evidence approach involving expert judgment".
Importantly, the rWEC study discussed here was not proposed as a replacement for established in vivo assays, but rather as an adjunct to aid understanding within the context of the required weight of evidence approach to classification.
Considering the above, results of the rWEC study, showing that the pyrithione moiety does not have the potential to be embryo-toxic, are considered supportive of a nonspecific, secondary maternal toxicity mechanism mediating the developmental effects presented in the CLH report. Unfortunately, the CLH report failed to make this point.
To not consider this data is contrary to the requirements of Annex I, Section 3.7.2.3.4 of the CLP, which requires that " if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of generalised adverse effects shall be assessed to the extent possible".
The rWEC study provides valuable insight in this respect as it clearly shows that the pyrithione moiety has no intrinsic, specific property to produce an adverse effect directly on the embryo.
Mode of Action of ZnPT providing evidence that this substance produces developmental effects by non-specific secondary consequences of maternal toxicity
The CLP Regulation (Annex 1, Sections 3.7.2.1.; 3.7.2.2.1.; 3.7.2.3.5.; 3.7.2.4.1.; 3.7.2.4.2.; 3.7.2.4.3.) requires to differentiate between substances which have an intrinsic, specific property for producing an adverse effect on foetus and those which produce developmental effects as a secondary consequence of maternal toxicity, as this will have an impact on classification.
Developmental effects must be observed in the absence of other toxic effects, or if

occurring together with other toxic effects, "substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects". (See Annex I, Section 3.7.2.2.1 and Table 3.7.1.(a). Also supported by Annex I, Sections 3.7.2.1.; 3.7.2.3.5.; 3.7.2.4.1.; 3.7.2.4.2. and 3.7.2.4.3 of the CLP).
Based on ZnPT Mode of Action provided below, it can be concluded that ZnPT does not have an intrinsic property to adversely affect foetal development but that observed developmental effects are the consequence of the non-specific toxic effect of ZnPT in the dams.
ZnPT Mode of Action
Summary
ZnPT affects embryonic development indirectly, via excessive maternal toxicity. We have demonstrated unequivocally that this is the case by using rat whole embryo culture to show that pyrithione does not affect development even at concentrations that are highly toxic to the pregnant animal. The mechanism by which the pyrithiones act is an inhibition of oxidative metabolism which causes profound effects on maternal metabolism that indirectly affect development of the embryo.
Pyrithione acts as an ionophore, carrying metals across the mitochondrial membrane where it inhibits aconitase, a Krebs cycle enzyme that has an iron sulfide (Fe-S) group on the surface of the molecule and is therefore susceptible to inhibition by divalent metals. This inhibition leads to profound effects on the adult animal, as mitochondrial metabolism is the most important source of energy for the animal. Importantly, this is not the case for the embryo, which is much more dependent on glycolysis.
The effects of aconitase inhibition on the intact animal will have a spectrum of biochemical and metabolic effects. These can be discerned from an examination of the data from subchronic and chronic studies for pyrithiones. These would include a short-term increase in serum glucose (as a consequence of negative feedback from lactate accumulation, as pyruvate cannot enter the inhibited Krebs cycle); and a significant decrease in food conversion efficiency (the ratio of body weight gain to food intake). While the repeat-dose studies are not ideally designed to investigate these effects, effects on glucose and feed efficiency are consistent across multiple studies on Zn and Na PT. The steep dose-response curve is also consistent with an effect on oxidative metabolism, including the rapid progression from seeming normality to death. An indication of effect on organs with high metabolic demands is a consistent increase in relative heart weight at high doses in subchronic studies.
The non-specific secondary effects of aconitase inhibition are developmentally adverse. The short -term hyperglycemia, the metabolic acidosis from lactate and citrate accumulation, and possibly hemodynamic insufficiency, are all developmentally adverse from an indirect mechanism.
Another consistent observation across studies is a decrease in haemoglobin and or red blood cell number, along with an increase in relative spleen weight. The molecular mechanism of pyrithione, carrying of metals across membranes with subsequent interaction with metalloproteins, is expected to affect haemoglobin. This produces a mild hypoxia, which would serve to exacerbate the maternally-caused developmental toxicity.
The embryo culture experiments show unequivocally that pyrithione is not intrinsically embryotoxic. The aconitase inhibition experiments with ZnPT and NaPT demonstrate unequivocally that aconitase inhibition is complete at concentrations that are equivalent to those measured at excessively toxic dose levels in vivo. Analysis of the repeat-dose toxicity studies on ZnPT and other pyrithiones provide consistent results on food conversion efficiency and other endpoints that are consequences of aconitase inhibition, offering conclusive support of this mechanism. While it is not possible, short of conducting a series of dedicated mechanistic toxicology studies, to demonstrate every step from aconitase inhibition to indirect embryotoxicity, we have demonstrated the key events in this mode of action.

Introduction to this section
The relationship between maternal toxicity and developmental toxicity has been written about and discussed extensively (Beyer et al. 2011, Giavini & Menegola 2012). It is clearly recognized that some perturbations of maternal physiology can produce malformations. Some of these include changes in acid-base balance, chemically induced maternal nutritional deficiencies, maternal diabetes (Daston, 1994; Carney, 1997), and maternal perturbations that result in embryonic hypoxia, including maternal anemia, maternal cardiovascular insufficiency, or uterine blood vessel constriction (reviewed by Danielsson, 2013).
One of the tools that has been used many times to determine whether a developmental effect is direct or indirect is rodent whole embryo culture. Rodent whole embryo culture has been used for more than forty years. The procedure has been evaluated by ECVAM and found to be a valid method for assessing the direct effects of chemicals on mammalian embryonic development (https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/publication//Embryotoxicity_statements). Whole embryo culture has been used many times to understand whether chemicals that produce developmental toxicity only in the presence of maternal toxicity are directly toxic to the embryo (e.g., Carney et al., 1996; Daston et al. 1989; Fantel et al., 2001). The results of experiments on pyrithione and its principal metabolite, 2-MSP, have been summarized in Section 2.1. of this report. Neither has the potential to affect embryonic development, even at concentrations that are equivalent to the in vivo concentrations that produce severe maternal toxicity in vivo. The remainder of this section explains the mechanism of action of pyrithione in the adult animal, and how this indirectly affects development by interfering with maternal homeostasis.
Mechanism of action at a molecular level
Pyrithione is an ionophore; i.e., it has the ability to carry metals across membranes, including the mitochondrial membrane. The distinction between chelation and ionophoretic properties relates to the solubility/dissociation constants for ZnPT and CuPT. In aqueous neutral environments, ZnPT and CuPT are insoluble. At acidic pH, ZnPT is soluble liberating zinc and pyrithione. CuPT appears to be less readily soluble although measures of [14C] PT following oral administration of CuPT show near complete absorption. Moreover, the counter ions, Zn2+ or Cu2+, like other divalent transition metals such as nickel (Borg-Neczak and Tjalve, 1994, Jasim and Tjälve, 1986) and iron (Kontoghiorghes et al., 1986), are transported across biological membranes the effects of which are enhanced when excess metal is present.
PT carries metal ions across membranes, increasing the intracellular concentration of copper which inactivates Fe-S proteins such as aconitase (Carraway and Dobner, 2012, Reeder et al., 2011, Yasokawa et al., 2010). The metals carried by pyrithione interact with iron-sulfide groups that are present on certain proteins, notably aconitase. Some of the pyrithione exchanges zinc for copper and transports copper across the plasma membrane and intracellular membranes. The Fe-S protein assembly is damaged, leading to loss of Acol and Leu1 activity.

We have subsequently determined that pyrithione also inhibits aconitase in mammalian cells (further details on the method used to measure aconitase activity in the presence of pyrithione is provided in Annex 6 to the document submitted by the ZNPT industry consortium). Cells derived from rats (RAT-1), rabbits (SIRC) and humans (A549) were exposed to sodium or zinc pyrithione. At concentrations that are comparable to in vivo concentrations that produced toxicity, pyrithione effectively inhibited aconitase activity in all three cell types (figure provided in the attached pdf). Aconitase is a key enzyme in the Krebs cycle. Inhibition of aconitase effectively blocks oxidative metabolism, by far the

major source of ATP synthesis (and therefore cellular energy) in the body. This mechanism explains effects reported in the literature that ZnPT affects ATP synthesis in organisms as diverse as bacteria (Dunning et al., 1998) and rat (Bragadin et al., 2003).

Why then, is the embryo not directly affected by this same mechanism? The reason is that the embryo, until relatively late in gestation, is much less dependent on Kreb's cycle metabolism than the adult. Its primary source of energy is glycolytic (Neubert et al., 1971; Hunter 1997). Kreb's cycle inhibitors such as fluoroacetate have much less effect on embryonic mitochondria than adults, and are not teratogenic (Spielmann et al., 1973). It has been suggested that the shift to glycolytic metabolism, while much less efficient in producing ATP, is a protective mechanism for the embryo as oxidative phosphorylation can produce oxygen free radicals, which could be damaging to the developing foetus.
Effects of aconitase inhibition at an organismal level
Inhibition of aconitase, and by extension, of oxidative metabolism, have many downstream effects that are detectable at an organismal level by several of the measures that are made as part of repeat-dose toxicity studies. We evaluated these studies in order to determine whether they support the conclusion that aconitase inhibition is the mode of action for pyrithiones.
Results from studies on sodium fluoroacetate were used to generate a list of endpoints of toxicity that are responsive to aconitase inhibition. Fluoroacetate is a prototypical aconitase inhibitor. Effects observed acutely after exposure to fluoroacetate are an increase in citrate (the normal substrate for aconitase) and lactate (as a result of increased reliance on glycolysis for energy), followed by hyperglycemia, due to a feedback inhibition of glycolysis as a result of high lactate and citrate concentrations (Proudfoot et al., 2006; Bosakowski and Levin, 1986). Glucose concentration in serum is often measured in repeat-dose toxicity studies. Because the effects of pyrithione on aconitase are reversible, one would expect these effects to be most pronounced within the first few hours after dosing, after which the pyrithione has largely been eliminated. Still, there may be residual effects observed even after 24 hours. In reviewing the studies on ZnPT and NaPT, we find only two repeat-dose studies, both on NaPT (rat, oral (gavage)), that used dose levels above the 3 mg/kg bw/day level that appears to be the threshold for excessive toxicity in rats: a 91-day study (Toxikol Lab, 1988) and a chronic study (RTC) (described in REACH Registration dossier of NaPT , in "Repeated toxicity dose: oral"; 91 -day study is "003- Supporting/Experimental result" and Chronic study is "006- Supporting/Experimental result" https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/11645/7/6/2). At the earliest time point evaluated, after 5 weeks of treatment in the 91-day study, there was a consistent increase of serum glucose in both sexes of rat, at dose levels of 2 and 8 mg/kg bw/day. The increase was approximately 20-25% above control levels.
The effect was no longer detectable at 13 weeks. In the chronic study, there was a decrease in serum glucose after long-term exposure. While there are no chronic studies on fluoroacetate with which to compare this result, it would be expected that after a long period of exposure that glycogen stores would be depleted due to the continuing dependence on glycolysis for energy. Consistent with this notion, the serum glucose measurements at the 1.5 and 2 year timepoints in this study were all reduced 10-20% compared to control.
Food conversion efficiency is another metric of inhibited energy production. Food conversion is the number of grams of food required to produce one gram of body mass. This measure has the advantage that it can be calculated in every study in which food consumption and body weight gain are measured, including developmental toxicity studies. Furthermore, it is a more stable measure than serum chemistry measurements and therefore not dependent on the elimination kinetics of the test compound. The expected result for an aconitase inhibitor is an increase in the number of grams of food

ingested to increase body mass by one gram. The effects that we see with analysis of the repeat-dose and prenatal studies on pyrithiones is consistent, dose-related, and at dose levels where there is excessive toxicity, profound. In three subchronic studies (Toxicol Lab (1988) rat, oral (gavage) study on NaPT (dose levels of 0, 0.5, 2 and 8 mg/kg bw/day), the Thor study on ZnPT (dose levels of 0, 0.2, 0.5, and 2.5 mg/kg bw/day), and the Thor study on NaPT (dose levels of 0, 0.5, 2.5 and 5 mg/kg bw/day)) feed conversion efficiency decreased in a dose-related manner, with more than a two-fold decrease in efficiency at th ehighest dose level tested (see attached pdf for a table with values). Similar effects were observed in the prenatal studies. Again, tables with actual values are included in the pdf attachment.

As noted in the description of the prenatal studies, there was considerable variability in the 1.5 mg/kg bw/day group in the ZnPT prenatal oral (gavage) rabbit study (Thor GmbH Art.95 dossier). Six rabbits in this group were excessively intoxicated, with all adverse developmental effects occurring in their litters. An analysis of individual feed conversion efficiency indicates that some animals were also affected disproportionately, with values over the dosing period of up to 9.33, vs 2.56 in control.

In addition to these effects, there were also increases in relative heart weight in 91-day studies: a 3% increase in the high dose group (2.5 mg/kg bw/day) females of the Thor ZnPT study and a 9% increase in the high dose group (5 mg/kg bw/day) females of the Thor NaPT study. This effect would also be expected from an agent that interferes with energy metabolism. Finally, the steepness of the dose-response curve is also consistent with fluoroacetate.

Another consistent observation in the pyrithione repeat-dose toxicity studies is a decrease in haemoglobin and/or hematocrit. While these were not always statistically significant, they were always in the same direction. Some examples from the available data (all in female, all in high dose):

- Thor 91-day study, NaPT: 7% decrease in hematocrit, 7% decrease in red blood cell concentration, 2% decrease in haemoglobin concentration
- Thor 91-day study, ZnPT: 4% decrease in hematocrit, 4% decrease in red blood cell concentration, 2% decrease in haemoglobin concentration
- Toxicol 91-day study, NaPT: 8% decrease in hematocrit, 9% decrease in red blood cell concentration, 10% decrease in haemoglobin concentration

This effect may be due either to the downstream effects of Krebs cycle inhibition, or to effects on enzymes in the heme synthesis pathway. Heme synthesis is dependent on succinyl coA, a Krebs cycle intermediate that is downstream of aconitase (Hunter et al., 2011). Furthermore, ferrochelatase, an enzyme in the heme synthesis pathway, also has a superficial iron sulfide group (Wu et al. 2001) so it may also be a target for pyrithione. Regardless of whether the effect is a downstream effect of aconitase inhibition or a direct effect on heme synthesis, it is consistent and reproducible. While the ensuing hypoxia from this level of effect is probably not sufficient by itself to affect indirectly development, it may exacerbate the developmental effects stemming from the downstream consequences of aconitase inhibition in the pregnant animal.

How are the maternal perturbations affecting development?

The downstream effects of aconitase inhibition not only profoundly perturb maternal homeostasis, but these homeostatic changes are developmentally adverse. The perturbations include acidosis as a consequence of citrate and lactate accumulation; transient hyperglycemia resulting from negative feedback on glycolysis from lactate and citrate accumulation; competition between embryo and mother for remaining energy sources, including amino acids that can enter Krebs cycle downstream of aconitase; and hypoxia from decreased haemoglobin in maternal blood. Each of these has been shown to be developmentally adverse (see, for example Carney, 2010, for a review on maternal toxicity).

Acidosis has mainly been studied in the context of understanding whether this condition

exacerbates the teratogenicity of direct-acting toxicants. In those cases where the acidosis has been experimentally corrected (e.g., for salicylic acid (Khera, 1991); and for glycolic acid (Carney et al., 1999)), it has been shown that acidosis exacerbates the developmental toxicity. In addition, acidotic conditions have been shown to have a deleterious effect on rat embryonic heart function (Hall, 1955; Andrews et al., 1995). It is noteworthy that the agents that produce acidosis also cause skeletal malformations. Maternal diabetes in rats has also been shown to cause skeletal defects (Eriksson et al., 1983).
In addition, the effects on oxygen carrying ability of red cells is likely to lead to a mild hypoxia. By itself, this may not have adverse effects but is likely to exacerbate the other responses to perturbed maternal homeostasis. There are reports in the literature that demonstrate that maternal anemia, induced by various means, causes malformations of the type seen at highly maternally toxic dose levels in the ZnPT studies. Grote (1969) produced a high rate of axial skeletal malformations in rabbits by inducing a hemorrhagic anemia on gestation day 9. Diflunisal, a non-steroidal anti-inflammatory, produced hemolytic anemia in pregnant rabbits and an increase in malformations in the foetuses after treatment on gestation day 5, with the compound having been eliminated days before the critical period for axial skeletal defects identified by Grote (Clark et al., 1984). This finding shows that maternal anemia and not the test chemical was responsible for the developmental toxicity. The majority of the defects, whether by hemorrhagic or hemolytic anemia, were vertebral and rib anomalies similar to what was seen in the high-dose ZnPT group. Data in rats with agents that induce embryonic hypoxia, such as uterine vessel clamping, also induce a spectrum of defects (reviewed by Harris, 1997). Danielsson (2013) provides additional examples of agents that induce malformations in laboratory animals by producing severe cardiovascular compromise in the dam, affecting maternal oxygenation of the embryo. These effects also produce mainly axial skeletal defects, with some visceral malformations. Decreased haemoglobin is clearly a form of maternal toxicity, but is not measured in developmental studies. However, this form of maternal toxicity can affect the developing embryo indirectly.
Also Section 3.7.2.3.5. of Annex I to CLP regards homeostasis disruption as a non-specific secondary mechanism of toxicity that may affect development : « if appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. »
Finally, the one guideline developmental toxicity study on fluoroacetate reports skeletal anomalies at maternally toxic dose levels (Turck et al., 1998).
ZnPT Mode of Action - Concluding remarks
The embryo culture experiments show unequivocally that pyrithione is not intrinsically embryotoxic. The aconitase inhibition experiments with ZnPT and NaPT demonstrate unequivocally that aconitase inhibition is complete at concentrations that are equivalent to those measured at excessively toxic dose levels in vivo. Analysis of the repeat-dose toxicity studies on ZnPT and other pyrithiones provide consistent results on food conversion efficiency and other endpoints that are consequences of aconitase inhibition, offering conclusive support of this mechanism. While it is not possible, short of conducting a series of dedicated mechanistic toxicology studies, to demonstrate every step from aconitase inhibition to indirect embryotoxicity, we have demonstrated the key events in this mode of action.
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ECHA note – An attachment was submitted with the comment above. Refer to public attachment zptmoaforpdf.pdf

Dossier Submitter's Response
Your comments including those in the attachment are covered by the comments made by the ZnPT Industry CLH Consortium. Please see our response under the comment number 56.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	United Kingdom	Lonza Cologne GmbH	Company-Manufacturer	58
Comment received				
We are concerned that the CLH Report, as prepared by the Dossier Submitter, concludes that ZnPT is a Cat. 1B toxic to reproduction substance based solely on the evaluation of ZnPT pre-natal studies and has, unfortunately, not taken into account reproductive and repeat dose toxicity studies on ZnPT and comparable data with Copper and Sodium				

Pyrithione, which were all made available to the Dossier Submitter. In response, the Dossier Submitter has merely claimed that "for zinc pyrithione there is reliable and adequate substance-specific information (i.e. a complete dataset) precluding the necessity to consider a grouping and/or read- across (see sections 1.1.1.1 and 1.1.1.3 of Annex I to the CLP Regulation). Therefore, the DS submitted a CLH report on zinc pyrithione without including data from the other pyrithiones. In other words, the DS does not use grouping and/or read-across in the CLH proposal for zinc pyrithione."

We disagree with this statement. Indeed, ZnPT meets the CLP criteria where a Weight of Evidence (WoE) approach needs to be used. As such, in this specific case, based on the CLP, all the relevant available data should be considered together in a WoE approach.

The CLH report has, unfortunately, not considered any data beyond the ZnPT prenatal studies, based on the argument that ZnPT has a "complete data set" and, thus, that a read across/category approach is not necessary.

We contest that the CLP regulation requires differently, specifically:

• The CLP (Article 5) requires to examine "relevant available information"; there is no limitation on what is "relevant available data" (compared with Art. 6).

• The CLP also states in Article 9.3. that "Where the criteria cannot be applied directly to available identified information, manufacturers, importers and downstream users shall carry out an evaluation by applying a weight of evidence determination, using expert judgement in accordance with section 1.1.1. of Annex 1…".

As indicated in the second point, findings from the ZnPT prenatal studies alone are not sufficient to fully assess reproductive toxicity criteria and to define if the development effects are linked to maternal toxicity or not. Additional data on ZnPT and information/studies on other pyrithione salts (NaPT and CuPT), e.g. embryotoxicity study (rWEC), mode of action etc…, are required to assess reproductive toxicity classification of ZnPT.

Moreover, the use of data beyond ZnPT pre-natal studies provides more certainty. Additional studies are better for determining replicability; better for characterizing dose-response; better for elucidating the full range of toxicity; and better for understanding mode of action.

• The CLP requires to use the weight of evidence approach (WoE) in assessing classification (this is required for all the endpoints (Annex I , Part 1 (1.1.1.). This is also specifically required for toxicity for reproduction: "Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1)…"
(See also Annex 3, Part 3 (Reproductive Toxicity); 3.7.2.2.1.; 3.7.2.3.1.; 3.7.2.5.2.; 3.7.2.5.3.; 3.7.2.5.6.)

• Moreover, the CLP supports the use of structurally-similar substances in a WoE approach.(See Annex I, Section 1.1.1.3 of the CLP, and in particular also for reproductive toxicity under Annex I, Sections 3.7.2.3.1 and 3.7.2.5.4. of the CLP).

NaPT and CuPT toxicity studies can be used in the WoE approach to assess ZnPT classification as they form a Category with ZnPT based on the ECHA RAAF Guidance. Notably:

• ZnPT, NaPT and CuPT are toxicologically indistinguishable because the common organic moiety, pyrithione (PT) is responsible for the effects of these salts following oral administration. Repeat dose studies following oral administration of NaPT and CuPT produce comparable toxicodynamic response patterns as ZnPT with the same no adverse effect level and dose-response pattern.

• The structural similarity of these salts has been proven using the ECHA RAAF Guidance (Read-Across Assessment Framework) as prepared by the ZnPT Industry CLH Consortium and shared with the Dossier Submitter and ECHA (full details are provided in the Public Attachment). A full analysis based on the ECHA RAAF Guidance has shown that ZnPT, NaPT and CuPT constitute a "Category".

• The absorption, distribution, metabolism and excretion of NaPT and CuPT following oral administration are indistinguishable from ZnPT and these salts should be considered together when administered orally (gavage or diet). Specifically, oral administration (gavage, diet) of radio-labelled NaPT, CuPT or ZnPT to rats results in near complete absorption of 14C-PT. Pyrithione, as 14C-PT is uniformly distributed throughout the body with highest concentrations in red blood cells, liver, kidney, and spleen. The metabolism of pyrithione salts is by two pathways: (1) glucuronidation, 2 major products, and (2) formation of a terminal metabolite 2-methylsulfonylpyridine with a long half-life. Finally, excretion is primarily in the urine (> 60%) as water-soluble glucuronidation products. These data support the view that the toxicokinetic profiles of PT salts are qualitatively similar based on route of exposure. Therefore, it is reasonable to consider the data from these salts together to determine the consistency of data across multiple studies.

The "category" designation of NaPT, ZnPT and CuPT has also been recognized by the Dossier Submitter in the CLH Report (Section 6, page 7) " The Dossier submitter acknowledges that zinc pyrithione shows some structural similarity to sodium pyrithione (EC 223-296-5) and copper pyrithione (238-984-0), in that they share the common organic moiety i.e. pyrithione ". The CLH Report has made reference to NaPT and CuPT data for other endpoints (carcinogenicity and aquatic toxicity) but not for toxicity for reproduction. However, ZnPT pre-natal studies alone (and reviewed in isolation) are not sufficient to assess the above parameters. Indeed, further available evidence on ZnPT (e.g. sub-chronic data) but also data on NaPT, CuPT, pyrithione such as embryotoxicity on pyrithione moiety, and additional scientific literature on pyrithione salts are available and needed to assess these parameters and to conclude on the reproductive toxicity classification of ZnPT.

Therefore, we consider that for the RAC to review and make a decision on reproductive classification based only on the incomplete data in the CLH Report is not a proper basis for the classification of ZnPT and its listing in Annex VI to the CLP. As such, we request that all the available data on the pyrithiones is reviewed and considered during the process of making any final assessment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT CLH response_Read across documents_June 2017.zip

Dossier Submitter's Response

Please see our response also under the comment number 56, in particular under the sub-headings 'Weight of evidence' and 'Maternal toxicity vs developmental toxicity'.

Here, we comment on your attachment [hereinafter referred to as 'the read-across paper'] concerning your "category" designation for NaPT, CuPT and ZnPT based on ECHA RAAF (Read-Across Asessment Framework).

You (being part of the ZnPT Industry CLH Consortium) propose that NaPT, CuPT and ZnPT represent a "category" for purposes of classification and labelling based on the RAAF.

- A pre-condition that has to be considered before the scientific assessment of the read-across under the RAAF is that the substance identity is well characterised. "*Chemical composition, including structural information should be well defined. In addition, other constituents of a substance (e.g. impurities) can have a significant impact on the hazard or fate of a substance. Unambiguous substance identity for both the target and the source substances is therefore a prerequisite for read-across assessment."* (section 4.2 of the RAAF). This has not been considered in your read-across paper and in several of the experimental studies with NaPT and ZnPT the purity of the substance is not specified under the test material identity.

- As also quoted by you under the scenario selection of the read-across paper, the RAAF states that

  "*Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar **or** follow a regular pattern as a result of structural similarity may be considered as a group, or 'category' of substances*". [emphasis added]

  Your application of the RAAF in the read-across paper seem incorrect which is clear from the statement (on page 4 of the read-across paper) "*As presented herein, the data that exists for NaPT, CuPT and ZnPT are directly applicable to this category definition as these substances are structurally analogous with similar toxicological properties that follow a regular and predictable pattern.*" [emphasis added]. As a result of the structural similarity, the toxicological properties of the substances in a group should either all be similar or follow a regular pattern; but you propose that the toxicological properties of the three pyrithiones are similar that follow a regular and predictable pattern.

- You have identified "(bio)transformation to common compound(s)" as the basis of your read-across hypothesis. Scenarios 3 and 5 of the RAAF fall under such category hypothesis.

  "*Note: Scenarios 3 and 5 are based on the same category hypothesis, i.e. (bio)transformation to common compound(s), but differ in the way the predicted property is related quantitatively to the properties described for the source substances. The regular pattern observed for the source substances, which is used for the prediction, may be based on variations in the properties (Scenario 3) or in the absence of such variations (Scenario 5). […]"* (section 4.3.1 of the RAAF)

  You have selected Scenario 5 for your category and state in page 5 of your read-across paper
  "*In support of this conclusion, the RAAF provides an example of Scenario 5 directly applicable to NaPT, CuPT and ZnPT salts (pgs. 27 – 28):*
    - *Scenario 5 - Qualitatively and quantitatively similar effects are caused by a common compound, which is formed from all category members. Substances AZ, BZ, CZ and DZ are different inorganic salts of a common acid. They dissociate rapidly in the test organism to the common anion Z*

and to their different counter ions. ***The counter ions do not influence the solubility*** *and the toxicity of the category members.* ***In the repeated-dose toxicity studies,*** *the exposure to AZ, BZ, and DZ causes* ***similar type of effects both qualitatively and quantitatively, i.e. the same severity/degree of the effects is observed at similar doses.*** *The effects of the target substance CZ are predicted to be equal to the effects of the source substances AZ, BZ and DZ for the property under consideration."* [emphasis added]

For the Scenario 5 to hold, the counter ions should not influence the solubility of the category members. But, as you have correctly stated in page 8 of the read-across paper "*The most notable differences among these substances are: (1) water solubility, (2) octanol/water partition coefficient (Po/w or Log Po/w), and (3) dissociation constants. NaPT has a greater water solubility compared to CuPT and ZnPT."* NaPT is a common salt that is very soluble in water whereas CuPT and ZnPT are chelates that are barely soluble in water.

In the table below the 90-day oral repeated dose toxicity studies with NaPT, CuPT and ZnPT are presented (which are copied from the Appendix II of your read-across paper). It is apparent from the table that the LOAELs for neurotoxic effects are different for the three pyrithiones (considering their steep dose-response curve), and the liver and haematological effects observed with NaPT are not reported for CuPT and ZnPT.

	NaPT	**ZnPT**	**CuPT**
Study Type	90-Day Oral Toxicity Study		
Reference	90-Day Oral Tox with Neurotx Eval + FOB in the Rat. NaPT 1997	13-Week Dietary Tox Study in the Rat with ZPT. Sterling Winthrop 1973	90-Day Oral Neurotoxicity Study in Rats with CuPT. 1997
Doses	0.1, 0.5, 2.5 mg/kg day	5, 25, & 125 ppm Dietary Levels 0.35, 1.75, & 10.04 - males 0.35, 2.13, & 10.26 - females	0.5, 1.25, or 2.25 mg/kg day
NOAEL	0.5 mg/kg day	1.75/2.13 mg/kg day	0.5 mg/kg day
LOAEL	2.5 - minor adaptave changes observed in the liver, but no degenerative changes observed via histopath in the liver at any [doses tested]	10.04 mg/kg day	1.25 mg/kg day - increase in salivation was observed during the dosage period
Toxic endpoints	Salivation observed before and after dosing. Heamotological changes observed in males and females at the high dose only; increase in hemaglobin, and increases in MCH and MCV and were statitically increased over control animals but witin historical control ranges. Increase incidence of hepatocellular hypertofpy in both males and females at	hindlimb weaknes mild to severe, death, blood disgracies	2.25 mg/kg day - treatment releated decrease in muscle mass in both male and female rats and a highly significant decrease in the average maximum amplitude from electrophysiolocial measurments of the sensory sural nerves. Treatment-related microscopic of the sensory sural nerves

	all doses tested.		were observed but limited to the sections of the skeletal muscle from rats at 2.25 mg/kg/day, with definitive muscle fiber atrophy being observed in one (minimal) of the five male rats and three (mild to moderate) of the four female rats that had skeletal muscle retained at necropsy
Study Type	90-Day Oral Toxicity Study in the rat		
Reference	90-day Oral toxicity Study in Rat. 1988	90-Day Oral Tox with Neurotx Eval + FOB, Safepharm 1996	[NA]
Doses	0.5, 2.0, or 8.0 mg/kg day	0.2, 1.0, 5.0; note 5.0 reduced to 2.5 starting on day 17 - males and day 18 females	
NOAEL	0.5 mg/kg day	0.2	
LOAEL	2.0 - animals minimal atrophy of the hindlimb muscles in 1/20 females and slight atrophy in 5/20 male and female animals. haemoglobin concentration and packed cells volumes were reduced and while significantly different than controls within historical control values. NOTE: On day one study authors noted piloerection and/or slight hypoactivity observed in 11/20 males nd 11/20 females between 2.5 and 6 hours after dosing on day one...ONLY not observed in these animals or any other animal through the rest of the study	1.0 - due to signs of irritation in gut NOTE - authors claim neurophathy consistent with ZPT administration observed at 1.0 also, however, very mild if non-existent, also 2 females observed with hunched posture; TRUE LOAEL - 5.0/2.5	
Toxic endpoints	Death was observed in the high dosed animals as well as a decrease in growth and food consumption, varying degrees of skeletal muscle atrophy which were mostly confined to the high dosed animals with minimal to slight changes in the skeletal muscles being observed at 2.0	Death - females only, hindlimb weakness only observed at 5.0 but not at the reduced dose of 2.5, increase salivation - before and after dosing; decrease in bodyweight, increase in neutrophils & eosinophils. NOTE: Neurotox and FOB were considered normal for all animals…although FOB was not conducted in high dosed animals until day 27 well after the reduction of	

		dose and the improved condition of animals	

Owing to the above mentioned critical deficiencies in your read-across paper, the DS does not accept your presentation of NaPT, CuPT and ZnPT as a "category" for the purposes of classification and labelling for systemic toxicological properties.

RAC's response

RAC supports the DS comment.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Netherlands		MemberState	59

Comment received

We agree with the proposed classification of pyrithione zinc for reproductive toxicity for effects on development (Repro. 1B; H360D, May damage the unborn child). The available data show that there is clear evidence of an adverse effect on development, seen in several independent studies both in rat and rabbit. Serious developmental effects included reduced foetal viability and increased number of malformations. These effects are considered relevant for humans. Although maternal toxicity was also noticed, which might explain some of the observed foetal effects (for example the retarded foetal growth), this maternal toxicity cannot explain all the observed developmental effects. Moreover, the CLP-guidance 3.7.2.2.1.2 states that "In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.". Further, the developmental effects were not only observed at the highest dose level in the toxicity studies, but also at the mid dose level there were indications of developmental effects. Indications for maternal toxicity at the mid dose in at least one of the rat studies are only minimal. There are no indications for treatment-related adverse effects on reproductive parameters.
Altogether, the available data warrant classification in category 1B for developmental effects.

Dossier Submitter's Response

Thank you for your support.

RAC's response

Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Germany	Procter and Gamble Manufacturing GmbH	Company-Downstream user	60

Comment received

We do not have specific comment about acute toxicity but just want to provide a general comment about natural presence of pyrithione in food. Please see the comment provided in the general comment box.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Natural presence of pyrithione in food-Attachment-July 1,2017.pdf

Dossier Submitter's Response

Your comment (with the attachment) and the conclusion therein "Pyrithione, including zinc pyrithione, has been reported to occur in plants where human consumption is documented" is noted.
RAC's response
Thank you very much for your comment. However, RAC notes that the presence of the substance in food is not relevant for classification purposes.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	61
Comment received				
We agree with the acute Tox.3 classification for acute oral toxicity. We agree with the acute Tox.2 classification for acute inhalation toxicity.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Finland		MemberState	62
Comment received				
FI CA supports the proposed classification of Acute Tox. 3; Toxic if swallowed for pyrithione zinc. FI CA supports the proposed classification of Acute Tox. 2; Fatal if inhaled for pyrithione zinc.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany		MemberState	63
Comment received				
The DS should propose harmonised ATE values for the acute toxicity classes for which he proposes classification to ensure consistent classification of mixtures containing pyrithione zinc.				
Dossier Submitter's Response				
Thank you for your comment. The DS proposes Oral ATE = 221 mg/kg (based on the LD50 = 221 mg/kg in rats in the OECD 401 study). For inhalation route, ATE value is unclear as the Acute Tox. 2 H330 classification is proposed based on LC50 ranging 0.05 - 0.5 mg/L in rats in the OECD 403 study.				
RAC's response				
RAC agrees with the DS. The final ATE should be set in Plenary session.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium		MemberState	64
Comment received				
Acute tox 3 - H301 : BECA supports the proposal to classify zinc pyrithione as Acute Tox. 3 (H301) considering a LD50 in rats of 221 mg/kg according to the CLP criteria (50 < ATE ≤ 300). Actue Tox 2 - H330 : BECA agrees to classify zinc pyrithione as Acute Tox. 2 (H330) according to a LD50 between 0.05 and 0.5mg/l (Thor GmBH in 2014, Klimisch score 1).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany		MemberState	65
Comment received				
Page 30: It is agreed that Zinc pyrithione does not fulfil the criteria for skin irritation because of low scores for erythema and oedema and lack of inflammatory signs from the animal studies. However, in the SCCS study report (2014) human data is also available and could be included in the present CLH report. Nevertheless, the slight irritation observed in these studies still does not warrant classification.				
Dossier Submitter's Response				
Thank you for your support on the skin irritation conclusion. The human data included in the SCCS, 2014 report (SCCS/1512/13) on page 13 is: "*A study evaluated the effect of ZPT in a marketed shampoo base on human skin pigmentation at sub-irritating levels. Product was applied daily at 0.2, 0.4, and 2.0% under non-occluded dressings to each of eight Caucasians and eight black males for 64 consecutive days. Under the experimental conditions used, the cream and lotion shampoos did not produce any skin irritation, nor did they change the skin pigmentation level in Caucasian or black skin.*" "*A case report described a reaction by a patient to a shampoo containing 2% ZPT. The patient had had a similar reaction after using a hair cream with a lower level seven years before. Another report described a case of eczema of the scalp and face after using a shampoo containing 2% ZPT for a short period.*"				
RAC's response				
Thank you very much for your comment. The human data will be included in the opinion but RAC agrees that it does not support classification for this hazard.				

Date	Country	Organisation	Type of Organisation	Comment number

07.07.2017	Japan	<confidential>	Company-Downstream user	66
Comment received				
SCCS data shows this substances can be used for hair/hair skin.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Thank you very much for your comment. However, RAC notes that the use of the substance is not relevant for setting classification.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	Finland		MemberState	67
Comment received				
FI CA supports the proposed classification of Eye Irrit. 1; Causes serious eye damage for pyrithione zinc.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	68
Comment received				
We agree with the Eye Dam.1 classification for eye damage/eye irritation.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium		MemberState	69
Comment received				
BE CA supports the proposal to classify as Eye Dam. (H318) considering the ZnTP study (OECD 405, in 2001) where severe lesions were observed and lead to the sacrifice of the animal: a corneal opacity score of 4 was seen after 24h and was not expected to fully reverse in 21 days. The score for iritis could not be determined due to the severe corneal alteration and the chemosis. For all these reason, we agree with the DS proposal.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	70
Comment received				
We agree that no classification could be proposed for respiratory sensitisation due to lack of data				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Denmark		Individual	71
Comment received				
See above ECHA note - *[see comment No. 26]*				
Dossier Submitter's Response				
Please see our response under the comment number 26.				
RAC's response				
RAC support's the DS response.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany		MemberState	72
Comment received				
Page 33: Even though zinc pyrithione is not sensitising in animal studies, in the SCCS study report (2014) there was human test data on cosmetic formulations for at least the pyrithione moiety. These studies confirm pyrithione's low potential to induce contact hypersensitivity and the DS decision to not classify zinc pyrithione for skin sensitization is supported. However, the human test data on zinc pyrithione as such or as part of a cosmetic formulation could be included in the present CLH report.				
Dossier Submitter's Response				
Thank you for your support on the skin sensitisation conclusion. We agree that the human data on pages 17 to 22 in the SCCS, 2014 report (SCCS/1512/13) can be included.				
RAC's response				
Thank you very much for your comment. The human data will be included in the opinion. However, RAC notes that these data do not provide enough robustness for supporting a classification of the substance.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	73

Comment received
We agree with STOT RE1 classification for STOT RE
Dossier Submitter's Response
Thank you for your support.
RAC's response
Thank you very much for your comment. Noted.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Netherlands		MemberState	74
Comment received				
We agree with the proposed classification of pyrithione zinc for specific target organ toxicity - repeated exposure (STOT RE 1; H372). Relevant effects for STOT RE observed in the various repeated dose studies at dose levels below the (extrapolated) guidance values for category 1 include haematological effects (>20% Hb-reduction in monkey oral 28-d study), mortality (rat oral 90-d studies, rat inhalation studies) and neurological effects (in rat dermal developmental study, and rat oral 90-d studies). We agree that the observed mortality in the repeated dose studies should not be considered as an acute effect, and should therefore be included in the classification for STOT-RE. Altogether, this warrants classification in category 1 (without specification of target route and target organ).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.07.2017	Belgium	The ZnPT Industry CLH Consortium	Industry or trade association	75
Comment received				
(1) Proposed STOT-RE Classification by the Dossier Submitter (Sweden) The Dossier submitter of the CLH document on zinc pyrithione proposes STOT RE Cat 1 classification based on: - Haemolytic anaemia in monkeys at 22 mg/kg bw/day after oral exposure for 28 days. - Neurotoxicity and mortalities in rats at 5 and 10 mg/kg bw/day after oral exposure for 90 days. - Neurotoxicity in rats at 60 mg/kg bw/day after dermal exposure for 21 days. - Mortalities and a single case of neurotoxicity in rats at 0.0135 and 0.005 mg/L after 21 and 28 days, respectively, after inhalation exposure. According to the dossier submitter, each of these effects justify classification in STOT RE 1 (with adjusted guidance values for shorter exposure times). As the dose-response curve for pyrithione toxicity is steep, mortalities were seen in rats at comparable dose levels as the other effects. Instead of listing all target organs, the DS suggests to only state mortality in the hazard statement as this effect is more severe and makes the other				

effects of less concern.

It is 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.

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

(1.1) ZnPT Industry CLH Consortium response to the Proposed Classification by Dossier Submitter.

The ZnPT Industry CLH Consortium challenges this proposal and, for the reasons outlined below, it is proposed that a STOT-RE 1 classification is warranted for zinc pyrithione by exposure via the inhalation route only.

Therefore, we propose the following classification and wording:

Classification in STOT RE 1 (hazard statement H372 – Causes damage through prolonged or repeated exposure via the inhalation route).

(2)Rationale :

(2.1) Pyrithione Exposure: Hind limb Weakness

Repeated exposures to rats for at least 10 consecutive days, regardless of the route of administration, has resulted in the observation of decreases in weight gain and skeletal muscle atrophy (characterized by generalized muscle weakness), generally referred to as hind limb weakness, that has been shown to be fully, and completely, reversible following cessation of treatment. The finding of hind limb weakness has only been observed in rats and occasionally in rabbits, but has never been observed in mice following repeated administration that includes an 18-month dermal carcinogenicity study.

In addition, no observation of skeletal muscle atrophy (hind limb weakness) has been observed in non-human primates following 28-days, 90-days, or up to one year of repeated oral administration of zinc-, sodium-, and copper pyrithione at doses from 11 to 75 mg/kg/day. The clear NOAEL for the pyrithiones from primate studies is 11 mg/kg bw/day [1] (Lonza, 1992) with no signs of neurological deficit even at doses 75 to 100 times greater than those shown to cause such effects in rats. Moreover, the NOAEL is over 20 times greater than the corresponding dose for rodents (0.5 mg/kg bw/day). These data bring into question the likelihood that pyrithione exposure produces neurotoxicity in humans.

It is considered by the ZnPT Industry CLH consortium that, for the purposes of classification and labelling, primates are the more appropriate surrogate for humans and data on primates should be used to derive the classification if the data already exist. The Consortium would, therefore, welcome the opportunity to discuss this at the RAC meeting and to hear the views of the experts at RAC as we consider it pivotal that all available data are taken into account when classifying a relatively data rich substance like zinc pyrithione.

In the available primate studies, neurotoxic effects are not observed at the highest dose rate tested, 22 mg/kg bw/day [1](Lonza, 1992) and therefore it is proposed that a STOT-

RE based on neurotoxicity and, therefore, the central nervous system as the target organ is not warranted.

(2.2) Pyrithione exposure: Haemolytic anaemia.

(2.2.1) CLH proposal

The dossier submitter reports the finding of haemolytic anaemia in primates at 22mg/kg bw/day after oral exposure for 28 days [1] (Lonza, 1992). This study was performed in accordance with GLP and EC Guideline B.7, animals were exposed to 0, 5.5, 11 and 22 mg/kg bw/day for 28 days followed by a 2-week recovery period. One high-dose animal died in the study, but this animal vomited prior to dosing on day 1 and it is possible that it was unhealthy at the onset of the study. Animals showed decreased activity and reduced food intake. The main effects observed were gastrointestinal effects (vomiting and diarrhoea) and effects on blood parameters. The effects on blood parameters reported by the dossier submitter consisted of reductions in Hb (-22%), RBC (-29%) and Hct (-16%) accompanied by an increase in MCV (18%) at 22.0 mg/kg bw/day compared to control values. The control values were higher than normal published data for Macaca fascicularis, while the values detected in the 22.0 mg/kg bw/day dose group were within the published normal range except for Hb which were lower. The changes were statistically significant in the highest dose group.

The dossier submitter states that, according to Annex 1: 3.9.2.9.5, for a 28-day study the guidance values are increased by a factor of 3, i.e. to 30 mg/kg bw/day, in accordance with Haber's rule and that, as the effect occurred at 22 mg/kg bw/day, classification in STOT RE 1 is thus warranted.

(2.2.2) ZnPT Industry CLH consortium response: 28-day study evaluation.

The consortium has further evaluated the Hb data from the 28-day oral toxicity study in cynomolgus monkeys, with particular attention to the high dose group (22 mg/kg bw/day) in which the reduction in Hb (-22%) is reported by the dossier submitter. The Hb data for all animals in the Control and high dose groups are presented in Table 1 of the "Public Attachment" related to these comments (Document named "2. ZnPT CLH Consortium_RESPONSE TO THE PROPOSED STOT CLASSIFICATION OF ZINC PYRITHIONE_Attachment June 2017.pdf").

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, reduction in Hb levels are calculated as: Female (-22.1%), Male (-21.8%), Female/Male combined (-21.9%). However, when the comparison of the high dose group data (wk4 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%).

According to Guidance on the Application of the CLP Criteria (v. 4.1), Section 3.9.2.5.2, any consistent and significant adverse effect in clinical biochemistry, haematology or urinalysis parameters, such as a reduction in Hb at > 20%, is sufficient for classification with STOT RE. The Consortium considers that the comparison between the same animals pre-dose and after 4 weeks represents the true decrease in Hb levels during the exposure period and that, therefore, classification as STOT-RE may warrant reconsideration.

In addition, the CLP Guidance, in Annex I, Section 3.9.2.8.1 indicates that certain effects

seen in humans/animals do not justify classification, such as 'small changes in clinical biochemistry, haematology or urinalysis parameters and/or transient effects, when such changes or effects are of doubtful or minimal toxicological importance' and provides as an example 'Significant decrease in Hb without any other significant indicators of haemolytic anaemia'.

Since the dossier submitter indicates that there were no other indications of haemolytic anaemia, and the effects were seen to be fully reversible after the 2-week recovery period (data in Table 1 of the Attachment), the Consortium considers that classification as STOT RE may be reconsidered.

(2.3) Evaluation of further studies.

To further support the reconsideration of the STOT RE classification, the Consortium has also evaluated two further studies on monkeys, where haematological criteria were measured – a non-GLP 90-day oral toxicity study in rhesus monkeys with zinc pyrithione [2](Lonza 1973), and a GLP 1-year oral toxicity study in cynomolgus monkeys with sodium pyrithione [3](Lonza, 1989). The 1-year study was submitted in support of the BPR approval of sodium pyrithione, but was not submitted in support of zinc pyrithione.

The dossier submitter has reviewed the 90-day study in the CLH dossier, and concluded it to be of low reliability, and unsuitable for classification purposes due to the following:

- the purity of the test substance was not given,
- only few animals were used,
- blood parameters were affected in control animals,
- animals from the lowest dose group were not necropsied,
- no significant difference between treated and control animals,
- values in all dose groups were also higher than published normal values at the onset of the study.

The Consortium agrees with this evaluation and also considers the study unsuitable for classification purposes.

In the GLP 1-year study, groups of five male and five female monkeys were dosed daily by oral gavage with sodium pyrithione for one year, at doses of 5, 25 and 75 mg/kg bw/day. Haematological parameters were measured at 1, 3, 6 and 12 months.

Since this is a longer-term study than the standard 90-days on which the CLP guidance values for STOT RE classification are based, the values may be adjusted, as the dossier submitter has for the 28-day study, by Haber's rule. CLP Annex 1, Section 3.9.2.5 confirms that Haber's rule may be used as a basis to extrapolate equivalent guidance values for toxicity studies of greater or lesser duration. In the case of a 1-year study, the guidance values for a 90-day oral study of < 10 mg/kg bw/d and < 100 mg/kg bw/d for STOT RE 1 and STOT RE 2, respectively, may be extrapolated to < 2.5 mg/kg bw/d and < 25 mg/kg bw/d for STOT RE 1 and STOT RE 2, respectively.

Considering these guidance values, only the results from the low-dose group (5 mg/kg bw/d) are considered relevant for purposes of STOT RE classification. However, the Hb data for all animals in the Control, low-, mid- and high-dose groups are presented in Tables 2,3 and 4 of the attachment to our response.

Mean data, after 1, 3, 6 and 12 months' exposure, for both male and female groups

separately, and male/female groups combined are compared to the equivalent Control group data, and the comparison is also made with the same animals at the start of the study (pre-dose). In all groups, and at all dose levels, reduction in Hb levels are significantly less than 20%.

The Consortium considers that this longer-term study with sodium pyrithione is a key study for the pyrithiones and may be considered, together with the 28-day study, to provide no basis to classify zinc pyrithione as STOT RE for haemolytic anaemia.

(2.3.1) Comparative toxicity of rat and primate.

The Consortium considers that an allometric scaling factor may also be appropriate to account for the differences in toxicity between rat and primate, since the thresholds for classification for STOT RE given in Annex 1 Sections 3.9.2.9.6 and 3.9.2.9.7 are based, specifically, on data from the rat.

REACH guidance indicates that the allometric scaling factor from rat to primate should be set at 2, meaning that effects in a primate study should be seen in a 28-day oral study at or below 15 mg/kg bw/day to justify classification in STOT RE 1 and STOT RE 2 would be justified where the effects occur between 15 and 150 mg/kg bw/day.

Similarly, in a 1-yr oral study, effects in a primate study should be seen at < 1.25 mg/kg bw/day to justify classification in STOT RE 1 and STOT RE 2 would be justified where the effects occur between 1.25 and 12.5 mg/kg bw/day.

(2.3.2) Conclusion.

The Consortium considers that the overall evaluation of the studies discussed above provides no basis to classify zinc pyrithione as STOT RE for haemolytic anaemia.

Table 1: Haematology in cynomolgus monkeys – 28d study (Lonza, 1992). Full details submitted in attached STOT RE Supportive Document i.e. "2. ZnPT CLH Consortium_RESPONSE TO THE PROPOSED STOT CLASSIFICATION OF ZINC PYRITHIONE_Attachment June 2017"

Table 2: Haematology in cynomolgus monkeys – 1 yr study, 5.0 mg/kg bw/day Sodium pyrithione (Lonza, 1989). Full details submitted in attached STOT RE Supportive Document

Table 3: Haematology in cynomolgus monkeys – 1 yr study, 25.0 mg/kg bw/day Sodium pyrithione (Lonza, 1989) Full details submitted in attached STOT RE Supportive Document

Table 4: Haematology in cynomolgus monkeys – 1 yr study, 75.0 mg/kg bw/day Sodium pyrithione (Lonza, 1989) Full details submitted in attached STOT RE Supportive Document

(2.4) Pyrithione exposure: Mortality

As stated above by the Consortium, for the purposes of classification and labelling, primates are the more appropriate surrogate for humans and, therefore, data on primates should be used to derive the classification if the data already exist.

No substance related mortalities have been observed in primates treated with pyrithiones by the oral route at much higher dose levels in comparison to the rat. Therefore,

mortality is not considered to be an appropriate endpoint for the determination of a STOT-RE by this route of administration.

As stated by the Dossier Submitter, no deaths have been observed in the repeat dermal studies in the rat and although the Dossier Submitter hypothesizes that deaths would occur at higher dose levels if tested, there is no direct evidence from the available studies on zinc pyrithione to indicate the need to classify zinc pyrithione on this basis.

However as reported by the Dossier Submitter, mortalities and a single case of neurotoxicity in rats at 0.0135 and 0.005 mg/L after 21 and 28 days, respectively, has been reported in repeat dose inhalation studies. The Applicant therefore agrees that this does warrant consideration for classification by the inhalation route as no data are available in primates.

(2.5) Proposed STOT-RE Classification by the Consortium :

It is proposed by the Consortium that a STOT-RE 1 classification is warranted for zinc pyrithione by exposure via the inhalation route. Therefore, we propose the following classification and wording:

Classification in STOT RE 1 (hazard statement H372 – Causes damage through prolonged or repeated exposure via the inhalation route).

(2.6) References

[1.] Lonza (1992), A Repeated Dose Toxicity Study of Zinc Omadine Powder to Cynomolgus Monkeys for 28 Days Followed by a 2-Week Recovery Period

[2.] Lonza (1973), Oral Administration of Zinc Omadine (WIN 9546) to Rhesus Monkeys for Three Months

[3.] Lonza (1989), A One Year Oral Toxicity Study in Cynomolgus Monkeys with Sodium Omadine

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT CLH Consortium Comments 4 attachments -June 30, 2017.zip

Dossier Submitter's Response

Hind limb weakness and mortality

For classification, generally the studies giving the most severe classification are used in a weight of evidence evaluation. With ZnPT, neurotoxicity and mortalities observed in rats gives the most severe classification. We note that you propose to have a discussion at the RAC meeting on your considerations that "*[…] for the purposes of classification and labelling, primates are the more appropriate surrogate for humans and data on primates should be used to derive the classification if the data already exist.*"

Haemolytic anaemia

Please note that according to Guidance on the Application of the CLP criteria section 3.9.2.5.2 (version 4.1 and also the recent version 5.0) reduction in Hb at **greater than or equal** to 20% is given as an example of haematological effects warranting classification as STOT-RE. In the 28-day 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%.

Concerning your comment on comparative toxicity of rat and primate: To the DS's knowledge, RAC doesn't apply allometric scaling for classification and labelling purposes.
RAC's response
Thank you very much for your comment. Noted. Your considerations will be included in the final opinion.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany	Thor GmbH	Company-Manufacturer	76
Comment received				
Thor GmbH disagrees with the DS's proposal for classification of ZnPT in STOT RE 1. No consistent specific target organ toxicity was observed in different species after oral application (rat, monkey). The primary effect is organ-independent (see mode of action below). CLP Annex 1, table 3.9.1 – Note: "(…) One shall carefully evaluate the data and, where possible, not include secondary effects (a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal systems)." Evaluation of repeated dose studies with pyrithione compounds in a weight of evidence approach: The DS failed to adequately address and consider two repeated dose studies submitted by Thor GmbH as part of an Article 95 Dossier under the Biocidal Products Regulation (BPR, EU No. 528/2012). The two studies of interest are: - Zinc pyrithione: 90-Day rat oral toxicity study combined with a neurotoxicity study (OECD 408 +424) (Thor GmbH Art.95 dossier; 2015) - Sodium pyrithione: 90-Day rat oral toxicity study (OECD 408) (Thor GmbH Art.95 dossier; 2015) Toxicologically relevant effects in the rat sub-chronic studies with ZnPT and Sodium pyrithione (NaPT) were evident only at the high dose (2.5 mg ZnPT/kg bw/d and 5 mg NaPT/kg bw/d) and were characterised by clinical signs, lower body weights/weight gains, and effects on the hind limb skeletal muscle including functional deficits, muscle atrophy, fat replacement and axonal degeneration. For the sub-chronic neurotoxicity study with ZnPT, effects were found partially reversible during the treatment-free period. Importantly, no treatment-related changes were noted in any of the remaining elements of the neurotoxicity battery, OECD 424 (ZnPT only). The proposed mode of action of pyrithione compounds (elaborated in the "Supportive Document to the ZnPT Industry CLH Consortium comments on Reproductive Toxicity", July 2017) 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. Since food consumption and body weight gain are routinely measured during sub-chronic toxicity studies, we evaluated these measures in the sub-chronic toxicity studies to support the mode of action for pyrithiones. Here, figures 1 and 2 (cf. figures in the attached 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, does not change during the treatment with ZnPT (2.5 mg/kg bw/d). However, at the same time there is a decrease in BWG in males (16% decrease) and females (36% decrease)				

during the last two weeks of ZnPT treatment (Figure 1, cf. figure in the attached document). For NaPT (5 mg/kg bw/d), the FC in male and female rats is again constant over the complete treatment duration. Notably, while the BWG of male rats does not differ over time, female rats show a BWG decrement of 74% compared to the corresponding control group during the last two weeks of NaPT treatment (Figure 2, cf. figure in the attached document).
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. Such effects are also evident in reproduction toxicity studies in the rat (OECD 416/414). Notabene and as delineated above, hind limb weakness was observed solely in female rats, i.e. the sex that showed a biologically significant decrement in BWG after repeated exposure to ZnPT and NaPT.

Taken together, these observations support the mode of action of pyrithiones. 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, i.e. no specific target organ toxicity (STOT).

For classification, the relevance of secondary effects observed in rats (axonal degeneration of peripheral nervous system, atrophy of skeletal muscle) must be questioned in general. Notably, the predominant effects observed in primates (Cynomolgus monkey) were local effects on gastrointestinal mucosa and effects on haematology (haemolytic anaemia in monkeys at 22 mg/kg bw/d after 28-day oral exposure).
The underlying common mode of action is comparable in the different species. However, existing data show that affected organs are inconsistent across species. Furthermore, the LOAEL/NOAEL in monkeys is 22 / 11 mg/kg bw/day and thus, factor 9 (LOAEL) or 22 (NOAEL) higher compared to rat (2.5 / 0.5 mg ZnPT/kg bw/day; 90 day toxicity study, Thor GmbH Art.95 dossier; 2015). The distinct target organs (secondary effect) as well as the different toxicity threshold values (e.g. NOAEL) in rats and monkeys indicate a species-specific dose-response relationship of ZnPT.

In sum, it can be concluded that Specific target organ toxicity is no intrinsic property of ZnPT, but a secondary effect of its primary mode of action.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2017 07 07 Thor GmbH comments to ZnPT CLH report.pdf

Dossier Submitter's Response

For the studies that you state that the DS did not adequately address and consider, please see table 15b in the CLH report (for the 90-day study with ZnPT) and our response under the comment numbers 56 and 58 (for the 90-day study with NaPT).

You state in your comments that "*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*." And "*[…] 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, i.e. no specific target organ toxicity (STOT)"*

Your conclusion is that "*[…] Specific target organ toxicity is no intrinsic property of ZnPT, but a secondary effect of its primary mode of action.*"

For STOT-RE classification it's the secondary effects observed in other organs that should be carefully evaluated and where possible, not considered (for eg. a hepatotoxicant can produce secondary effects in the nervous or gastro-intestinal systems).

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.

RAC's response
Thank you very much for your comment. Noted. Your considerations will be included in the final opinion.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Germany		MemberState	77
Comment received				
Page 101, section 10.12.3 Conclusion on classification and labelling for STOT RE: A proposal of Classification as STOT RE 1 is supported; however, as neurotoxicity in rats was evident in many animal studies and via different routes of exposure, it is therefore appropriate to designate the nervous system as damaged by the substance on prolonged or repeated exposure and without route specification. Therefore, the hazard statement is suggested as: "H372 – Causes damage to nervous system through prolonged or repeated exposure".				
Dossier Submitter's Response				
Thank you for your support on the Conclusion. Since mortalities were also observed in rats via oral and inhalation exposure and haemolytic anaemia was observed in monkeys via oral exposure, we have not specified 'nervous system' in the hazard statement. Subject to the discussion at RAC as proposed by the ZnPT Industry CLH Consortium, the hazard statement can be reconsidered.				
RAC's response				
Thank you very much for your comment. Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium		MemberState	78
Comment received				
BE CA agrees to classify zinc pyrithione as STOT RE 1 (H372) considering the neurotoxicity effects caused by the substance. We agree not to specify the route of exposure.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you very much for your comment. Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Note: Important Updates

There was a further targeted Public Consultation on additional data submitted following the first RAC plenary discussion on environment. An additional Response to Comments Document followed and is found as an Annex to this document. The responses to the initial comments in this document have now been superseded. New studies were analysed and the results suggested a new proposal for environmental endpoints and classification was in order. The results from the two new studies indicated that *Skeletonema costatum* was 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 reasons outlined in the opinion, address all previous concerns with the two older studies in the *Skeletonema costatum* toxicity database. Alone, these two new studies (both 2018), were sufficient for RAC to propose a revised classification with associated M-factors.

Summary and background to the proposed classification:

(1) Zinc Pyrithione was rapidly degradable.
(2) Skeletonema costatum may be considered the most sensitive species for aquatic toxicity to zinc pyrithione.
(3) The 72h ErC50 of 0.00088 mg/L for the marine diatom Skeletonema costatum (*Goudie, 2018*) supports Aquatic Acute 1; H400 with an acute M-factor of 1000.
(4) The 72h ErC10 of 0.00068 mg/L for the marine diatom Skeletonema costatum (*Goudie, 2018*) supports Aquatic Chronic 1; H410 with a chronic M-factor of 10
(5) These results are supported by the second new study (*Hoover, 2018*)

Date	Country	Organisation	Type of Organisation	Comment number
01.07.2017	Belgium	The ZnPT Industry CLH Consortium	Industry or trade association	79

Comment received

Due to the specific format of comments in the webform, we provide below a summary of our text comments for the environmental endpoints. However, more detailed information on these comments, including detailed tables and figures, are provided in «Public attachment» in the document called «3. ZnPT Industry CLH Consortia_Environmental Classification_Supportive Attachment June 2017.pdf» . Therefore, for full detail, please review the attachment in conjunction with these comments.

1 INTRODUCTION

The Dossier Submitter of the CLH document on zinc pyrithione proposes the following environmental classification (p. 128 of the CLH dossier):

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

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

Hazard statement codes: Hazardous to the aquatic environment
Aquatic Acute 1; H 400, M factor 1000
Aquatic Chronic 1; H410, M factor 10

The ZnPT Industry CLH Consortium does not agree with the acute and chronic environmental classifications proposed by the Dossier Submitter and, for the reasons outlined below, believes M factors of 100 for acute and 1 for chronic are appropriate.

The acute aquatic classification is based on the Ward and Boeri (2004) algal toxicity study on the marine diatom Skeletonema costatum, which the Dossier Submitter considered the key study. Although an additional study on S. costatum with ZnPT (Rebstock, 2010) was submitted for evaluation and has been considered reliable (RI=2) in the BPR PT21 evaluation of CuPT, it has not been included in the CLH report. The study conditions in the Ward and Boeri study were not appropriate for S. costatum, the growth in controls was highly variable, there were too few analytical determinations for a rapidly degrading substance and the statistical evaluation is neither detailed nor reproducible. Therefore, the Ward and Boeri study is considered not adequate for classification purposes. The Rebstock study, in contrast, was conducted using study conditions specific to S. costatum, included daily analytical determination, and produced robust statistical results with extremely low variability, suggesting optimal algal growth conditions. The Rebstock study is valid, reliable, and, based on the study conditions, considered adequate for classification. Therefore, the Rebstock study should be considered the key study and the endpoints from this study used in the classification of ZnPT. Taking this into account, the acute classification of Category Acute 1 is retained but the M-Factor is now reduced to M= 100 (based on an LC50 of 0.0026 mg/L).

An M factor of 10 has been proposed for the Chronic Aquatic 1 classification, based on the substance not being considered rapidly biodegradable. The OECD 301B ready biodegradation study on ZnPT that the Dossier Submitter considered reliable (RI=1) was conducted at a test concentration that is well above both the solubility limit of ZnPT and the EC50 for activated sludge microorganisms. A new study following OECD 301B Ready Biodegradability test guidelines has been conducted using radiolabelled ZnPT at concentrations of 100, 210 and 520 µg/L, which are below the activated sludge respiration inhibition EC50 and the solubility limit; at study completion biodegradation reached 64.9%, 65.7% and 72.4%, respectively, and the 10 d window criterion was met in each case. The study demonstrates that ZnPT is readily biodegradable. Therefore, ZnPT can be considered rapidly degradable and an M-factor of 1 applied to the Aquatic Chronic 1 classification.
In addition, errors noted in the CLH dossier and requiring correction are listed in Appendix 1 of the submitted environmental attachment i.e. document called «3. ZnPT Industry CLH Consortia_Environmental Classification_Supportive Attachment June 2017.pdf»

2 TOXICITY TO SKELETONEMA COSTATUM

Two algal toxicity studies with ZnPT on the marine diatom Skeletonema costatum have been submitted for the BPR evaluation of ZnPT. Only one of these, the Ward and Boeri (2004) study, has been included in the CLH dossier. The Rebstock (2010) study was conducted specifically to address the deficiencies in the Ward and Boeri study and the

omission of this study is a significant oversight in the CLH report.

The relevant guidance, the adequacy of both studies for classification purposes, and an overview of previous regulatory evaluations of these studies are discussed in detail below.

2.1 Relevant guidance

According to CLP guidance (p. 556 of v4.1), an algal toxicity study 'consistent with OECD Test Guideline 201 should be used'. OECD 201 is a guideline for freshwater algae and the culture conditions for S. costatum, a marine diatom, are not among those provided in this guideline.

However, OECD 201 allows modification of test conditions so long as sufficient growth is achieved. Culture conditions specific to S. costatum can be found in internationally accepted guidance documents, including ASTM E1218-04.

2.2 Ward and Boeri (2004)

This 120 h static algal study on Skeletonema costatum, was performed in accordance with US EPA-FIFRA, Guideline 123-2 (a predecessor of the current OPPTS 850.5400). The test was conducted at 20°C and used triplicate nominal concentrations of 0.3, 0.6, 1.2, 2.4 and 4.8 µg ZnPT/L, prepared with unfiltered natural seawater. Analytical determination of ZnPT with HPLC was performed at the beginning and end of the test. The initial cell density was 10’000 cells/mL and a 24 h light photoperiod was used.

The culture conditions used in this study are not consistent with those described in relevant guidance for this taxon (ASTM E1218-04, OPPTS 850.5400) and may have affected cell growth. For example, S. costatum should be cultured in filtered artificial seawater. In this study, the culture media was prepared with unfiltered seawater containing levels of particulate matter (36 mg/L), Cu (12 µg/L), and Pb (19.4 µg/L) sufficient to influence growth rate (Bilotta GS and Brazier RE, 2008; ECI, 2008; SCHER, 2009, respectively). The use of a 24 h light period is used for freshwater organisms but is not standard for marine species including S. costatum.

The control cultures demonstrated exponential growth during the first 48 h but growth rate slowed between 48 and 72 h and approaches a plateau between 72 and 120 h. The Dossier Submitter suggested use of the 48 h EC50 for classification purposes. Although using growth data from only 48 h is accepted according to OECD 201, the exposure period is significantly shorter than recommendations for S. costatum (e.g., 96 - 120 h according to ASTM E1218-04).

Analytical determinations were only made at the start and at the end (120 h) of the test; for every concentration tested, the final ZnPT concentration measured was below the LOQ. The calculation of a time weighted average (TWA) concentration using results below the LOQ is highly uncertain and according to the OECD guidance 23 on difficult substances, the validity of a study in such cases requires confirmation.

The cell density in the controls were highly variable in this study, which may be due to the use of culturing conditions that were inappropriate for S. costatum. The study report does not include measures of variability in growth rate estimates; however, visual inspection of the cell densities in the control replicates suggest significant differences in growth between the replicates.

For example, the density in the control cells, which were used to estimate growth inhibition, were 66,000; 28,000; and 20,000 cells/mL at 24 h (CoV = 65%) and 388,000; 194,000; and 396,000 at 48 h (CoV = 35%). The ErC50s provided in the study report could not be independently reproduced using the statistical model cited in the study report.

2.3 Rebstock (2010)

This study reports a 120 h static test with the marine diatom S. costatum using ZnPT as test substance. While the study conditions were according to OPPTS 850.5400 (i.e., specific to S. costatum), the data collection was conducted to meet the reporting requirements of OECD 201. Six nominal test concentrations (0.20, 0.40, 0.80, 1.6, 3.2, and 6.4 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 120 h exposure by HPLC. The test was conducted using 6 days old algal culture at test initiation with an initial algal cell concentration of 77,000 cells/mL. The test was maintained at 20°C in a 14 h light: 10 h dark photoperiod as is standard for S. costatum.
The number of cells in the control showed a 13-fold increase by 72 h and an 18-fold increase by 96 h. This growth rate was considered by the author sufficient to verify logarithmic phase and meets the criteria for growth in both the OECD 201 (16-fold increase during exposure duration) and OPPTS guidelines (about 1.5 x 106 cells/mL by 96 h).

The variability of the cell density and the growth rates in the control replicates were low. The reported CoVs in Table 2 and 3 of the study report are 0 to 7% for cell density and 0 to 3% for average specific growth rate.

Pyrithione is light sensitive and undergoes rapid photolysis. The analytical determinations at every 24 h make it possible to calculate mean measured exposure concentrations at all dose levels time points. The mean measured concentrations range from 78.2% of nominal at 24 h to 34.4% of nominal at 120 h (Refer to Table 1 in the supportive attachment).

Growth inhibition at each time point, based on nominal concentrations, are reported in Table 2 (as in the supportive attachment). At 24 h, greater than 50% inhibition was achieved, but this was not seen at later time points, for which the average exposure concentration was significantly lower than nominal due to degradation. As a result, only the 24 h ErC50 provides a bounded estimate (Table 3; as in the supportive attachment) and a 95% confidence interval.

The study report additionally provides the growth rate NOECs at each time point, which are relevant for the evaluation of chronic toxicity. However, ErC10s are more statistically meaningful and are the preferred endpoint for evaluation of chronic toxicity in algal toxicity studies (OECD 201). In this study, the NOErCs are extremely sensitive due to very low statistical variability at each exposure concentrations, with growth rate inhibition of about 1 to 4% at the reported NOErCs. The LOErCs are in the same range of <10% to 11% inhibition and consistent with the preferred statistic of ErC10's at each time point. The ErC10 at 72 h has been calculated to be 1.94 µg/L (1.55-2.43), which is the preferred time point in OECD guidance.

2.4 Previous evaluations of both studies

Both studies were evaluated in the BPR PT21 evaluation of CuPT, for which read across between Na/Zn/CuPT was relied upon. A brief summary of the previous conclusions is

provided below.

2.4.1 Previous evaluation of Ward and Boeri (2004)

The evaluator rated this study with RI=1-2. A general description of the study is provided, and no deficiencies are noted. The eCA notes that inhibition lasted for 48 h, after which recovery was observed. Therefore, the 48 h growth data was used to estimate endpoints, with TWA concentrations estimated using SFO kinetics. Critically, the use of degradation rates derived from studies in different systems to estimate TWA concentrations is highly uncertain and is only used in cases where measured concentrations are not available.

2.4.2 Previous evaluation of Rebstock (2010)

The evaluator rated this study with RI=2, noting that the following deviations from OECD 201 may have influenced test results (p. 260 of Doc IIA):

a) Cell density was 10x higher than general recommendation in OECD 201

b) Exponential growth was not maintained through test period or first 72 h

c) Test concentrations did not remain within 80% of nominal

d) Light regimes follows EPA guideline for Skeletonema.

The evaluator proposed a NOErC of 0.31 µg/L based on the TWA concentration at 72 h in the 0.8 µg/L nominal dose level.

The points a) to d) described above are neither deviations from OECD 201 nor do they affect the reliability of the test. Furthermore, the study report does not claim to have followed OECD 201 when setting study conditions. Point by point responses by the consortium follow;

a) OECD 201 provides recommendations for culturing specific freshwater algae. Skeletonema is not among the organisms listed in Annex II of this guidance and other recognized guidelines provide study conditions for culturing this organism. Nonetheless, OECD 201 is flexible regarding culturing conditions so long as sufficient growth in controls is achieved.

b) OECD 201 specifies 16-fold increase in biomass in 72 h test period. However, it also says that this criterion may not be met with slower growing species of which S. costatum is known to be one. In this case the test period should be extended to obtain 16-fold growth. The biomass increase in Rebstock (2010) was 13-fold at 72 h and 18-fold at 96 h and met criteria specific for S. costatum growth (e.g. ASTM E1218-04).

c) Concentrations in this static test on a rapidly degrading substance would not be expected to remain above 80% nominal. The OECD 201 Test Guideline specifies that in these situations effects should be interpreted based on measured concentrations as done in Rebstock (2010). For this reason, analytical determinations were made daily.

d) Using culture conditions appropriate for the test organism is not excluded in OECD 201. OECD 201 Test Guideline only specifically addresses conditions for the ubiquitous freshwater algae, Desmodesmus subspicatus and Pseudokirchneriella subcapitata with

minor culture adaptations for other freshwater species. OECD 201 does not employ sufficient silica in a saltwater environment as needed by the marine diatom Skeletonema.

Additionally, the NOErC proposed by the eCA is associated with 0.8% inhibition. This is far below the 10% level that is generally recommended especially for algal toxicity studies (OECD 201, ASTM E128-04). As reported above, the ErC10 of 1.94 µg/L is a more appropriate chronic endpoint from this study.

2.5 Conclusions regarding toxicity to S. costatum

The Ward and Boeri study employed test conditions that are not appropriate for S. costatum, did not provide sufficient analytical determinations, had high variability in untreated control replicates, and provided ErC50 estimates that cannot be independently reproduced. The study is therefore considered not reliable and not adequate for classification purposes.

The Rebstock study employed culture conditions consistent with accepted guidelines for S. costatum, included daily analytical determinations at an improved level of quantitative precision and detection, had low variability in control replicates, and provided reproducible and robust statistical analysis. This study is reliable and adequate and should be considered as the key study for assessing toxicity of ZnPT to S. costatum.

From the data in Table 3 (as in the supportive attachment), the bounded 24 h ErC50 (5.12 µg/L) reported in this study, based on mean measured concentrations, should be use for classification purposes. (The unbounded 72 h ErC50 >3.80 µg/L would give the same classification result). The most sensitive acute endpoint for ZnPT, according to all of the available high quality data (reliability 1-2), is now derived from the freshwater fish Pimephales promelas at 0.0026 mg ZnPT/L (Table 4; as in the supportive attachment).

Therefore, the acute classification of Category Acute 1 is retained but the M-Factor is now reduced to M= 100 (based on an LC50 of 0.0026 mg/L).

3 RAPID DEGRADATION IN THE ENVIRONMENT

The dossier submitter has proposed an Aquatic Chronic 1 classification with an M-factor of 10, based on the NOEC in the most sensitive species (P. promelas) and ZnPT not meeting the criteria for rapid degradability. The applicant agrees with the dossier submitter on the NOEC on which the endpoint is based, but the conclusions on rapid degradability and the corresponding M-Factor do not take into account all recently available information and are not correct.

3.1 Ready biodegradability

According to paragraphs 4.1.2.9.2 and 4.1.2.9.5 of EU Regulation (EC) No 1272/2008, the primary test method to assess rapid degradability is the 28 d ready biodegradation study. If a substance passes the strict criteria of the ready biodegradability test, then the substance is considered rapidly degradable and no additional testing or evidence is necessary. The conservatism of this criteria is emphasized by the situation described in Section 4.1.3.2.4.3 of the CLP guidance document, on weight of evidence for degradation, where even in cases of conflicting results in ready biodegradability tests, a pass in even one reliable ready biodegradability test is considered sufficient to consider a substance rapidly biodegradable. A summary of the relevant ready biodegradation tests are provided in Table 5 (as in the supportive attachment) and discussed in more detail in below.

3.1.1 Background on pyrithione toxicity to activated sludge microorganisms

For biocidal substances, the toxicity of the substance to activated sludge microorganisms is an important consideration when designing and interpreting ready biodegradation tests. As noted in Annex II of the OECD 301 Ready Biodegradability test guidelines, which addresses the evaluation of substances suspected to be toxic to the inoculum, test concentrations in the ready biodegradation test should be less than 1/10th the EC50 (or less than the EC20) value derived from an activated sludge respiration inhibition test. Further, if the EC50 is less than 20 mg/L, radiolabelled test chemicals should be employed in the ready biodegradation test.

The applicant has submitted two GLP zinc pyrithione OECD 209 activated sludge respiration inhibition studies. The Mead (2001) study reported a 3 h EC50 of 2.4 mg/L and a NOEC of 0.10 mg/L; this study was considered the key study by the applicant and is included in the CLH report

[Footnote: the text description of the Mead study (first paragraph of Section 11.1.4 of CLH report) incorrectly reports the endpoints. The activated sludge respiration inhibition EC50 and NOEC at 3 hrs were 2.4 and 0.10 mg/L, respectively].

An additional OECD 209 study submitted by the applicant, but not included in the CLH report, showed that ZnPT inhibited respiration at the lowest test concentration of 0.69 mg/L (27% inhibition) and reported an EC50 of 1.84 mg/L (Weniger, 2002). Overall, the EC50s for activated sludge respiration inhibition are consistent between the two studies and show significant inhibition at concentrations in the range typically used for ready biodegradation studies.

3.1.2 Menzies (2017) OECD 301B on ZnPT

This recently completed new study followed OECD 301B Ready Biodegradability test guidelines and was conducted using radiolabelled ZnPT at test concentrations of 100, 210 and 520 µg/L. The test concentrations were selected per OECD 301 test guidelines to be 1/10th of the EC50 for activated sludge respiration inhibition in order to negate issues with inocula inhibition and are well below the solubility limit for ZnPT (4.93 mg/L; Wenighofer, 2002).

Mineralization was evaluated by trapping the [14]CO2 generated during the study in base traps and using liquid scintillation counting to quantify percent of theoretical CO2 evolution.

At all test concentrations, ZnPT was ready biodegradable. 100 µg/L test systems reached 64.9 ± 0.4% CO2 production in 28 d and met the 10 d window (Figure 1; as in the supportive attachment);
210 µg/L test systems reached 65.7 ± 0.6% CO2 production in 28 d and met the 10 d window (Figure 2; as in the supportive attachment);

520 µg/L test systems reached 72.4 ± 2.1% CO2 production in 28 d and met the 10 d window (Figure 3; as in the supportive attachment).

This test is considered fully reliable (RI=1) and demonstrates that ZnPT meets the CLP criteria for being considered rapidly biodegradable.

A detailed study report is provided as an attachment to these comments i.e. the document called "4. ZnPT Industry CLH Consortia_Environmental Classification - OECD 301B Final Report.pdf".

3.1.3 Clarke (2002) OECD 301B on ZnPT

In this OECD 301B study using non-radiolabelled test material, identified by the Dossier Submitter as the key ready biodegradation study, ZnPT showed significant mineralization reaching 49% CO2 evolution after 28 d (Clarke, 2002).
[Footnote: biodegradation reached 49% of theoretical CO2 evolution at study completion once dissolved phase CO2 was accounted for, which is in accordance with the OECD 301B method; the value was incorrectly reported as 39% in the study summary and final conclusions.]

There are two critical deficiencies in this study:

(1) the poor solubility of the test item is not adequately considered and

(2) the study does not take the suspected toxicity to the inoculum into account.

At the dose concentration of 13.2 mg/L, the concentration of ZnPT is well above the solubility limit of 4.93 mg/L which would lead to slower biodegradation rates, as the chemical will not be well dispersed in the system and interaction with the inocula will take longer. Notably, the CuPT CAR PT21 states that "the results from the ready test thereby has very little relevance for estimation of biodegradation under environmental conditions (where low/soluble concentrations are expected), except for cases dealing with major spills of highly concentrated preparations" (p. 143 of Doc IIA).

Further, at 13.2 mg/L, ZnPT would have significantly inhibited the microorganisms present in the study. When comparing the data from the sodium benzoate positive control to the toxicity control, the positive control immediately began degrading, reaching 28% CO2 evolution in 3 days, while the toxicity control showed a lag during that period and did not show significant degradation until day 6. In addition, the ZnPT treatments produced up to 20% less CO2 than the inoculum controls in the first week of the study, indicating that the activated sludge respiration was being inhibited in the ZnPT test treatments.

Further, in the Weniger (2002) activated sludge respiration inhibition study, 85% inhibition was observed at a 10.7 mg/L, a concentration comparable to that used in this OECD 301B study.

These data indicate that at the OECD 301B test concentrations of 13.2 mg/L microbial inhibition would have been significant. Therefore, it is likely that ZnPT failed to meet the OECD 301B Ready Biodegradation test criteria (60% CO2 generation in 28 d and meeting the 10 d window) due to a combination of solubility limitations and inocula inhibition.

For classification purposes the adequacy of the study should be questioned and the study rather considered a supporting study instead of key study.

3.1.4 Clarke (2002) OECD 301B on NaPT

ZnPT is predicted to dissociate into Zn2+ and PT- at environmentally relevant concentrations, which will further speciate as a function of pyrithione concentration and characteristics of the environmental compartment. A comprehensive review of pyrithione

speciation models and experimental data is provided in the CuPT PT 21 CAR, which concludes that "in waters where the total pyrithione concentration is low, for instance in seawater, the pattern of pyrithione ions is quite likely dominated by the free pyrithione ion, PT– " (p. 139 of CuPT CAR Doc IIA).

Using a weight-of-evidence approach, as described in CLP Annex 1, section 1.1.1.3, the OECD 301B ready biodegradation study using NaPT supports the conclusion of ready biodegradability for ZnPT.

At test concentrations used in the study, NaPT is fully dissociated into Na+ and PT-, and PT- is considered both the toxicologically and environmentally relevant species.

This 28 d OECD 301B study was conducted using non-adapted activated sludge and 12.4 mg NaPT/L. Degradation was 0% until day 14 but proceeded rapidly thereafter, reaching 9% CO2 on day 18, 24% CO2 evolution on day 20 and 79% CO2 evolution on day 28. The study demonstrated NaPT to be ready biodegradable and meeting the 10-day window. Like the Clarke (2002) study on ZnPT, the concentration tested is high relative to the EC50 for activated sludge respiration inhibition, which may explain the initial lag time in the degradation curve. However, complete solubility of the test item was achieved in this study. This study is considered fully reliable.

The relevant metabolite of pyrithione, PSA, is also considered readily biodegradable in a reliable OECD 301B test (Clarke, 2002).

The Copper Pyrithione Competent Authority Report includes essentially the same reasoning (p. 42):

"When testing the pyrithiones in the Ready biodegradability test, OECD 301, copper pyrithione and zinc pyrithione were "not readily biodegradable". This is due to that the suspensions of solid substances were tested, and the soluble (degradable) concentrations were too low to generate the requested amount of inorganic carbon within the time limits. The salts simply dissolve to slow. When sodium pyrithione was tested in comparable concentrations, but administered in the form of a liquid solution (feasible because this salt has much higher water solubility), the test result was "readily biodegradable". Hence, the pyrithione moiety is readily biodegradable. The relevant metabolite PSA, was also readily biodegradable in the OECD test, with 73% mineralised after 28 days."

3.2 Conclusions on rapid biodegradation

Ready biodegradation is the preferred criterion for assessing whether a substance can be considered rapidly degradable for classification purposes. A reliable ready biodegradation test on 14C-ZnPT, conducted at test concentrations that are readily soluble and not associated with significant toxicity to activated sludge microorganisms, demonstrates that ZnPT meets the criteria for being considered ready biodegradable and thus rapidly degradable. The results of this study are supported by ready biodegradation studies on NaPT and PSA (metabolite of pyrithione), both of which also meet the criteria for ready biodegradability.

Thus, for the purposes of classification, ZnPT is rapidly degradable and should be classified Aquatic Chronic 1 with an M Factor of 1.

4 CONCLUSIONS ON THE CLASSIFICATION OF ZNPT

Zinc pyrithione can be classified Aquatic Acute 1, with an M factor 100 (0.001<LC50 <0. 01 mg/L) based on acute toxicity of ZnPT to freshwater fish Pimephales promelas LC50 = 0.0026 mg/L (2.6 µg/L).

Zinc pyrithione can be classified with Aquatic Chronic 1 with an M factor 1 (0.001<NOEC< = 0.01 mg/L) based on fish with the most sensitive species Pimephales promelas 32 d NOEC = 0.00122 mg/L and that the substance is rapidly degradable.

Hazard statement codes: Hazardous to the aquatic environment

Aquatic acute 1; H 400, M factor 100

Aquatic chronic 1; H410, M factor 1

Table 1. Geometric mean measured concentrations of ZnPT (µg/L) [including % of nominal concentration] as reported over the over the 120h exposure period for Skeletonema costatum (Rebstock, 2010). Full detail provided in the Supportive Environmental Attachment i.e. the document called «3. ZnPT Industry CLH Consortia_Environmental Classification_Supportive Attachment June 2017.pdf».

Table 2. Growth rate inhibition for S. costatum over a 120h exposure period to ZnPT (Rebstock, 2010). Full detail provided in the Supportive Environmental Attachment.

Table 3. Summary of EC50 data for growth rate for S. costatum exposed to ZnPT based on geometric mean measured data (Rebstock, 2010). Full detail provided in the Supportive Environmental Attachment.

Table 4. Key acute toxicity studies for ZnPT to fish, invertebrates and algae. Full detail provided in the Supportive Environmental Attachment.

Table 5. Summary of relevant OECD 301 ready biodegradation studies. Full detail provided in the Supportive Environmental Attachment.

Figure 1. Biodegradation of 100 µg/L 14C- Zinc Pyrithione and Reference Material (Menzies, 2017). Full detail provided in the Supportive Environmental Attachment.

Figure 2. Biodegradation of 210 µg/L 14C-Zinc Pyrithione and Reference Material (Menzies, 2017). Full detail provided in the Supportive Environmental Attachment.

Figure 3. Biodegradation of 520 µg/L 14C-Zinc Pyrithione and Reference Material (Menzies, 2017). Full detail provided in the Supportive Environmental Attachment.

5 REFERENCES

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APPENDIX 1
Errors or corrections needed in the CLH report. Full detail provided in the Supportive

Environmental Attachment.

APPENDIX 2
Study report: Menzies J. 2017. 14-C Zinc Pyrithione: Ready Biodegradability: CO2 Evolution Test, The Procter and Gamble Company Environmental Stewardship and Sustainability Laboratory, Mason, OH, Study: 232619-98551 (unpublished). A full copy is provided as a Supportive Attachment.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment ZnPT CLH Consortium Comments 4 attachments -June 30, 2017.zip

Dossier Submitter's Response

Thank you for your comment. In general we have choosen the most reliable studies and stringent classification for the classification proposal of zinc pyrithione.

TOXICITY TO SKELETONEMA COSTATUM

The ZnPT Industry Consortium is critical to the choice of acute toxicity with the algae *Skeletonema costatum* as a key study (Ward and Boeri, 2004, reliability 1-2) in the CLH report of ZnPT and would instead choose the acute toxicity study with the same species of algae, carried out by Rebstock (2010, reliability 2).

The reason for why we chose the study carried out by Ward and Boeri, with the algae *Skeletonema costatum* as a key study, is that in the Guidance of the application of the CLP criteria 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 element, it can be read "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 algae study and the Rebstock algae study have about the same quality data on their studies (reliability 1-2). The most sensitive EC_{50} and NOEC is the study with the diatom *Skeletonema costatum* by Ward and Boeri with 48 h $^{initial}EC_{50}$=0.00060 mg/L (^{twa} EC50=0.000 108 mg/L) and ^{twa}NOEC= 0.000040-0.000080 mg/L.

A typing error could be found in table 75 in the CLH report of ZnPT under section "11.1 Acute Aquatic hazard" and the endpoint for acute toxicity with *Skeletonema costatum* it should be NOEC=0.00004-0.00008 mg/L (TWA) instead of NOEC=0.00004 mg/L (initial). The initial concentration should instead be NOEC=0.220 µg/L. Also the reliability should be 1-2 and not only 2. Another typing error was found under section "11.1.3 Acute (short-term) toxicity to algae and other aquatic plants "it should be 0.000040-0.000080 mg/L instead of µg/L.

We agree with France, the Netherlands and the United Kingdom (comments 80, 81 and 82) that the chronic toxicity should be based on the 48 NOEC of algae *Skeletonema costatum* NOEC=0.00004-0.000080 mg/L instead of the chronic study of fish *Pimehales promales*. The acute toxicity study on algae with NOEC, does represent chronic toxicity.

In conclusion the algae group diatoms seem to be most sensitive for both acute and chronic toxicity of ZnPT.

The new proposal on chronic toxicity would be that ZnPT still be classified as Aquatic Chronic 1, but the M-factor should be changed to 100 (0.00001<NOEC<=0.0001 mg/L)

based on diatom algae *Skeletonema costatum* as the most sensitive species and that the substance is rapidly degradable.

In conclusion we still think that acute algae toxicity study carried out by Ward and Boeri (2014) is the most relevant study for the classification of both the acute and chronic toxicity for algae.

RAPID DEGRADATION IN THE ENVIRONMENT

The ZnPT Industry Consortium sent in a new ready biodegradable test according to OECD guidelines 301 B, the 28th of June, 2017.

The test has been carried out with radiolabelled ZnPT over a 28 day period and with lower doses (as noted in Annex II of the OECD-guideline 301 B) because of ZnPT moderate to high toxicity towards microorgansisms, and its low solubility.

The new study is of good quality and follows the OECD-301B Ready Biodegradability: CO_2 Evolution Test Guideline and shows that ZnPT is rapidly degradable. The Annex II of the OECD 301 test guideline is used in reasoning to reduce the dose of ZnPT 1/10 of the activated sludge respiration inhibition EC50 to pevent ZnPT toxic effect to microorganisms and to use a concentration below the water solubility. A radiolabelled test material is used to overcome the issues with analytical detection.

We agree with the ZnPT Industry Consortium that this new ready biodegradable test according to OECD-guideline 301 B, fulfills the criteria of rapid degradability according to the decision scheme (see Section II.4 in the Guidance on the Application of the CLP criteria, Version 4.0, November 2013).

ZnPT is demonstrated to be readily biodegradable in a 28-d test for ready biodegradability. The pass level of the test (60% theoretical oxygen demand) was achieved within 10 days from the onset of biodegradation.

At all test concentrations, ZnPT was ready biodegradable. At the concentration 100 µg/L test systems reached 64.9 ± 0.4% CO2 production in 28 d and met the 10 d window and concentration 210 µg/L test systems reached 65.7 ± 0.6% CO2 production in 28 d and met the 10 d window and at the concentration of 520 µg/L test systems reached 72.4 ± 2.1% CO2 production in 28 d and met the 10 d window.

CONCLUSION

Dossier submitter still proposes that zinc pyrithione can be classified as Aquatic Acute 1, with a M-factor 1000 (0.0001<LC50<0.001 mg/L) based on the acute toxicity of algae *Skeletonema costatum* 48 h EC50=0.0006 mg/L (initial concentration). The same conclusion would be reached if we based it on ^{twa}EC50=0.000 108 mg/L.

However the M-factor for the chronic aquatic toxicity has to be changed considering that ZnPT now must be considered as rapidly degradable, according to ZnPT Industry Consortium's new biodegradability study (OECD 301B, 28th of June, 2017).

Also the comments of the Netherlands, France and the United Kingdom are relevant on the choice of the M-factor of chronic toxicity, which should be based on the 48 h ^{twa}NOEC

of algae *Skeletonema costatum* 0.00004-0.00008 mg/L which is the algae group (diatoms) that is most sensitive to ZnPT, instead of the proposed chronic fish study with *Pimephales promales.*

The new proposal on chronic toxicity would be that ZnPT still be classified as Aquatic Chronic 1, but the M-factor should be changed to 100 (0.00001<NOEC<=0.0001 mg/L) based on diatom algae *Skeletonema costatum* as the most sensitive species and that the substance is rapidly degradable.

The dossier submitter's new proposed classification and labelling of ZnPT would be:

Classification
Hazard Class and Category codes: Aquatic Acute 1
Aquatic Chronic 1

Hazard Statement codes: H400
H410

Labelling
Hazard statement Codes: H410

Specific Concentration Limits (M factor)

M-factor=1000
(acute)

M-factor=100
(chronic)

RAC's response

RAC supports the comments by the DS. Agree with the rapid degradability.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	France		MemberState	80

Comment received

p 116-124 We agree that, based on available data, the substance should not be considered as rapidly biodegradable. We have nevertheless minor comment on this part. Although the Guidance on the Application on the CLP Criteria is not really clear on this issue, the temperature at which the DT50 have been determined should be added. Moreover, the relevance of the metabolites could have been more explained. It is indeed not clear why only the PSA is taken into account whereas 3 other metabolites >10% have been identified in the aerobic seawater/sediment in test tubes (p 118-119).

Depending of the previous comment regarding the relevant metabolites, more data on the aquatic toxicity of the relevant metabolites should be provided. Nevertheless, as available data tend to indicate that metabolite are less toxic than the parent compound (p 108), QSAR data could be sufficient to assess the toxicity of relevant metabolites.

For a better interpretation, it could have been indicated in the table 75 and 76 when the endpoints are expressed as nominal or measured concentrations.

We agree with the acute aquatic classification.

Could you please clarify why the NOEC from the study on Skelotonema costanum (NOEC

48h = 0.00004 – 0.00008 mg/L) has not been taken into account for the chronic aquatic classification. Please note that if the validity criteria are fulfilled at 48h, the NOEC from this study could be considered as relevant for chronic classification, leading to a M factor of 1000.

Dossier Submitter's Response

We agree that the temperature on which the DT50 should have been reported for degradation studies but they were not the key studies and would not affect the classification outcome.
We also agree that there is a data gap or unreliable studies, when it comes to the identified metabolites PSA, OMSA, OMSiA and OTS especially aquatic toxicity studies on all trophic levels (see section 11.1 Acute aquatic hazard-metabolites). However, it seems that under relevant concentration of total pyrithione (< µg/L water) the metabolite PSA forms at the highest percent. In water-sediment microcosms under such condition this metabolite is slightly persistent. The metabolite is however not a P-substance since it passes the ready degradation tests (CuPT CAR, 2014).

According to the Guidance on the Application of the CLP criteria, preferably data shall be derived using the standardised test methods. We agree that QSAR data would be helpful to indicate the toxicity for the metabolites but the standardized test according to OECD-guidelines or other standardised test method would be preferable.

It probably would have been better to interpret the data if the endpoints would have been expressed as measured concentration, but it would not have affected the outcome of the classification proposal.

We agree with France, the Netherlands and the United Kingdom that the M-factor for chronic toxicity should be changed and also the reasoning why it should be changed.

The chronic toxicity should be based on the 48 h ^{twa} NOEC of algae *Skeletonema costatum* NOEC=0.00004-0.000080 mg/L, instead of the chronic study of fish *Pimehales promales*.

The acute toxicity study on algae with NOEC, does represent chronic toxicity.

In conclusion the algae group diatoms seem to be most sensitive both for the acute and chronic toxicity of ZnPT.

This choice of NOEC from acute toxicity data instead of NOEC from chronic toxicity data is also based on the reasoning in the Guidance on the Application of the CLP criteria in section 4.1.3.3 Classification categories and criteria, where it can be read "when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor".

Therefore, the M-factor of chronic toxicity should be changed from M-factor 10 based on the fish study to 100 based on the algae study and that the substance is not rapidly biodegradable according to the ZnPT Industry Consortium's new study.

RAC's response

Presumably a typing mistake by the DS, RAC considers zinc pyrithione as rapidly degradable. RAC supports the revised M-factor for aquatic chronic toxicity. RAC supports the use of the acute study with *Skeletonema* as a source for a chronic NOEC.

Date	Country	Organisation	Type of Organisation	Comment number
30.06.2017	Netherlands		MemberState	81

Comment received

Endpoints considered: Biodegradation and aquatic toxicity.

-The NOEC of 0.00004 mg/L (initial) for Skeletonema costatum presented in table 75 is not in agreement with section 11.1.3 where a NOEC initial of 0.220 µg/L is presented. The Dossier Submitter is requested to indicate the actual endpoints that should have been presented in table 75.

-In table 75 and section 11.1.3, endpoints for Selenastrum capricornutum and Pseudokirchneriella subcapitata are presented as if two different species are considered. Please note that these are the same species currently named Raphidocelis subcapitata. The Dossier Submitter is requests to confirm this and to consider if the endpoints from these studies could be meaned provided that they are based on similar test set-up and exposure period.

-NL agrees with the proposed classification as Aquatic Acute 1 and proposed acute M-factor of 1000 for acute aquatic toxicity based on the EC50 of 0.0006 mg/L for Skeletonema costatum.

-NL agrees with the proposed classification as Aquatic Chronic 1 but does not agree with the proposed chronic M-factor of 10. In the consideration of the long-term aquatic hazard (section 11.6.1) for aquatic plants, only Lemna gibba is mentioned and the proposed classification and chronic M-factor as based on a NOEC for fish. It should however be noted that according to the Guidance on the Application of the CLP Criteria, chronic data on algae should also be considered for the classification. In the guidance is also indicated that NOEC and EC10 values from the short term algae tests are accepted as chronic endpoints. Algae also cover diatoms and therefore, endpoints on Raphidocelis subcapitata as well as endpoints on Skeletonema costatum should have been considered for the chronic classification. Following this, the key study for the chronic classification should be the study on the marine diatom Skeletonema costatum that was also the key study for the acute classification. The NOEC from this study as indicated in section 11.1.3 is in the range of 0.000040 – 0.000080 mg/L based on time-weighted-average measured concentrations. This NOEC, with the fact that the substance is considered as not rapidly degradable, leads to an M-factor of 1000 (0.00001 < NOEC < 0.0001 mg/L). Therefore, NL is in the opinion that the chronic M-factor must be 1000.

-NL agrees with the conclusion that zinc pyrithione should be considered as a not rapidly degradable substance. Nevertheless, for the assessment of the biodegradability of pyrithione zinc, only the organic part of the substance is considered in the report. It should be noted that with the degradation of pyrithione zinc also zinc is released to the environment. The Guidance on the Application of the CLP Criteria states in Annex IV on metals that "the concept that a substance, or a toxic metabolite/reaction product may not be rapidly lost from the environment and/or may bioaccumulate, are as applicable to metals and metal compounds as they are to organic substances." Furthermore, in section 4.1.3.2.3.2 of the guidance is indicated that a substance is considered to be not rapidly degradable when a degradation product does fulfil classification as hazardous to the environment. Considering that Zn2+ and several salts of zinc like ZnO and ZnCl are classified as aquatic acute 1 and aquatic chronic 1, the release of zinc as degradation product is on itself a reason to consider zinc pyrithione as not rapidly degradable. This fact including the reasoning should have been be included in the section on degradability

of the report and be part of the conclusions on the biodegradability of the substance. Therefore, NL agrees with the conclusion that the substances should be considered as not rapidly degradable but not only because of the arguments given in the report but also considering the fact that zinc is released as a result of the degradation.

-To the knowledge of NL, the actual active substance is pyrithione, the metal ion only determines the release rate of the pyrithione. The use of sodium as cation would increase the release rate of pyrithione and copper would reduce the release rate. On the basis of this, endpoints for data on endpoints for copper-, sodium- and zinc pyrithone have been considered in the CAR for copper pyrithione. Also, in the draft CAR for zinc pyrithione read-across with copper- and sodium- pyrithone seems to be applied. In the current CLH proposal only ecotoxicity data for zinc pyrithione is considered. It seems inconsistent that in the CAR for copper pyrithione and potentially also the CAR for zinc pyrithione a read-across between the three species is accepted but that for classification of zinc pyrithione only data on the zinc species is considered. Could the Dossier Submitter reflect on this different approach?

Dossier Submitter's Response

We agree that the NOEC of 0.00004 mg/L of the alge *Skeletonema costatum* presented in table 75 should be replaced by the NOEC initial 0.220 µg/L and the NOEC of NOEC=0.000080 mg/L (TWA) in table 75 should be replaced with NOEC=0.00004-0.000080 mg/L (TWA). We also found two typing errors, one in table 75 under *Skeletonema costatum* the reliability should be 1-2 instead of only two (however, it is correct in the section 11.1.3 Acute (short term) toxicity to algae or other aquatic plants) and the other typing error in the text of section 11.1.3 Acute (short term) toxicity to algae or other aquatic plants it says "The TWA for 48 h NOEC is thereby 0.000040-0.000080 µg/L" and it should be "0.000040-0.000080 mg/L".

The NOEC of 0.220 µg/L was based on initial concentration. The NOEC 0.000040-0.000080 mg/L was based on TWA which was determined in the ZnPT CAR Doc IIIA A.4.3.1/04.

We agree that in table 75 and section 11.1.3 the *Selenastrum capricornutum* and *Pseudokirchneriella* are the same species and are currently named *Raphidocelis subcapitata.* However, there are two different studies conducted with the same species carried out under different conditions and therefore have different endpoints. This will not have an influence on the final classification proposal.

We agree with the reasoning of the Netherlands, France and the United Kingdom that the chronic toxicity should be based on the algae *Skeletonema costatum* 48 ^{twa} NOEC= 0.00004-0.000080 mg/L, instead of the chronic study of fish *Pimehales promales*.

The acute toxicity study on algae with NOEC, does also represent chronic toxicity.

In conclusion, the algae group diatoms seem to be most sensitive both for both the acute and chronic toxicity of ZnPT.

Since the new study of the ZnPT Industry Consortium showed that ZnPT is rapidly degradable and combined with chronic toxicity of the algae between 0.00001<NOEC<=0.0001 (mg/L), the M-factor would be 100 instead of 10.

This classification is also based on the reasoning in the Guidance on the Application of the CLP criteria in section 4.1.3.3 Classification categories and criteria, where it can be read

"when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor"

We agree that in a risk assessment one should also consider that zinc ions are released into the environment by degradation of zinc pyrithione, however we are classifying the metal-organic substances and not the zinc metals. Zinc pyrithione degrades, although the zinc ion persist.

As stated in Section 6 of the CLH report, the DS did not use 'grouping' approach for classification and labelling of ZnPT. Nevertheless, the information from other pyrithiones can be considered for risk assessment.

RAC's response

The new study by Menzies (2017), provides sufficient data to consider zinc pyrithione rapidly degradable.
RAC supports the revised chronic M-factors as proposed by several MSCAs.
RAC supports the conclusions of the DS.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2017	United Kingdom		MemberState	82

Comment received

We agree that the substance should be classified as Aquatic Acute 1 and Aquatic Chronic 1. However, we feel further detail is required to agree the M-factors.

Aquatic acute classification:
The acute M-factor key endpoint is an algal non-standard duration 48h ErC50 of 0.0006 mg/l for S. costatum based on initial measured 0h concentrations. We feel justification is required to base the endpoint on i) 48 hour duration, and ii) 0 hour concentrations when >20% losses were observed over the study. It would be useful to present standard 72, 96, 120 hour endpoints and endpoints based on actual concentrations for comparison.

Aquatic chronic classification:
Regarding the chronic M-factor, the basis of all chronic endpoints (i.e. duration and verified nominal, mean measured etc) is required.

Algae are the most acutely sensitive species. Chronic endpoint data is available for this trophic group although not considered in the current M-factor proposal. Please can you consider the chronic M-factor based on standard duration algal NOECs or EC10 endpoints. This should include justification if nominal or initial measured concentrations are used.

Dossier Submitter's Response

We understand the concern that we have choosen the endpoint NOEC with the 48 hour instead of the standardized duration of 72 h, 96 h and 120 h. The reasoning for this is described in 11.1.3 Acute (short-term)toxicity to algae or other aquatic plants in the CLH report.

The growth inhibition tests on algae are performed under static system. The growth curves from some tests indicate that the inhibition lasts until around 48 h after initiation

of the test. After this time point, the culture start to recover, and the inhibition is no longer seen. Nonetheless, the inhibition was real during the first 48 h. The DS has therefore considered the first 48 h of the test and taken that as a reliable endpoint of the short-term toxicity for algae and other plants.

We agree with the United Kingdom, the Netherlands and France that the chronic toxicity should be based on the algae *Skeletonema costatum* 48 h ^{twa} NOEC= 0.00004-0.000080 mg/L instead of the chronic study of fish *Pimehales promales*.

The acute toxicity study on algae with NOEC, does represent chronic toxicity.

In conclusion the algae group diatoms seem to be most sensitive both for the acute and chronic toxicity of ZnPT.

Since the new study of the ZnPT Industry Consortium showed that ZnPT is rapidly degradable and combined with chronic toxicity of the algae between 0.00001<NOEC<=0.0001 (mg/L), the M-factor would be 100 instead of 10.

This classification is also based on the reasoning in the Guidance on the Application of the CLP criteria in section 4.1.3.3 Classification categories and criteria, where it can be read ”when assessing the adequacy there may be some cases (such as data poor substances) where the chronic data do not represent the species that is considered the most sensitive in available short-term tests. In such cases the classification should be based on the data (acute or chronic) that gives the most strict classification and M-factor”.

We agree that it would be more informative to have more detailed information like the duration, verified normal, mean of the chronic studies, but since reliability is given and more detailed information can be found in the final CAR Copper pyrithione (Doc IIA, and Doc IIIA;) and in the draft ZnPT CAR Doc IIIA and in Thor GmbH Art.95 dossier (2015), in our view, it would not contribute to the outcome of the classification proposal.

RAC’s response

RAC supports the comments of the DS.

Date	Country	Organisation	Type of Organisation	Comment number
07.07.2017	Belgium		MemberState	83

Comment received

Although no overview is given of all available studies, BE CA supports the proposed classification for the environment by SE CA :
Aquatic Acute 1, H400, M-factor=1000
Aquatic Chronic 1, H410, M-factor=10

Studies reported in the draft CAR and new available studies were evaluated by the dossier submitter. Those studies, on the substance itself, considered reliable and relevant for classification purpose were taken into account.

Dossier Submitter’s Response

After comments from the ZnPT Industry CLH Consortium (comment 79), France, the Netherlands and the United Kingdom (comments 80, 81 and 82), we have come to the following conclusion:

CONCLUSION

Dossier submitter still proposes that zinc pyrithione can be classified as Aquatic Acute 1, with a M-factor 1000 (0.0001<LC50<0.001 mg/L) based on the acute toxicity of algae *Skeletonema costatum* 48 h EC50=0.0006 mg/L (initial concentration). The same conclusion would be reached if we based it on ^{twa} EC50=0.000 108 mg/L.

However the M-factor for the chronic aquatic toxicity has to be changed considering that ZnPT now must be considered as rapidly degradable, according to the ZnPT Industry Consortium new biodegradability study (OECD 301B, 28th of June, 2017)

Also the comments of the Netherlands, France and the United Kingdom are relevant on the choice of the M-factor of chronic toxicity, which should be based on the 48 h ^{twa}NOEC of algae *Skeletonema costatum* 0.00004-0.00008 mg/L which is the algae group (diatoms) that is most sensitive to ZnPT instead of the proposed chronic fish study with *Pimephales promales.*

The new proposal on chronic toxicity would be that ZnPT still be classified as Aquatic Chronic 1, but the M-factor should be changed to 100 (0.00001<NOEC<=0.0001 mg/L) based on diatom algae *Skeletonema costatum* as the most sensitive species and that the substance is rapidly degradable.

The dossier submitter's new proposed classification and labelling of ZnPT would be:

Classification

Hazard Class and Category codes: Aquatic Acute 1
Aquatic Chronic 1

Hazard Statement codes: H400
H410

Labelling

Hazard statement Codes:H410

Specific Concentration Limits (M factor)

M-factor=1000
(acute)

M-factor=100
(chronic)

RAC's response
agreed.

PUBLIC ATTACHMENTS
1. EPDLA-Comments on CLH of zinc pyrithione.pdf [Please refer to comment No. 3]
2. 2017 07 07 Thor GmbH comments to ZnPT CLH report.pdf [Please refer to comment No. 4, 32, 76]

3. CE ZnPT Public Consultation.zip [Please refer to comment No. 12, 43]
4. PCPC Comments ECHA consultation Zinc Pyrithione.pdf [Please refer to comment No. 44]
5. Natural presence of pyrithione in food-Attachment-July 1,2017.pdf [Please refer to comment No. 21, 60]
6. ZnPT CLH Consortium Comments 4 attachments -June 30, 2017.zip [Please refer to comment No. 22, 56, 75, 79]
7. zptmoaforpdf.pdf [Please refer to comment No. 23, 57]
8. ZnPT CLH response_Read across documents_June 2017.zip [Please refer to comment No. 24, 58]
9. ZnPT comments Jochen Buschmann.pdf [Please refer to comment No. 1, 30]
10. CEPE ZnPT Public Consultation final 20170621.pdf [Please refer to comment No. 7, 35]
11. BCF ZnPT - Public Consultation response June 2017 Final.pdf [Please refer to comment No. 8, 54]

CONFIDENTIAL ATTACHMENTS
1. MCC ZnPT Public Consultaion July 2017.pdf [Please refer to comment No. 10, 40]
2. Comment ZPT-ECHA.pdf [Please refer to comment No. 2, 31]