SUPPORTIVE DOCUMENT TO THE ZnPT INDUSTRY CLH CONSORTIUM COMMENTS ON:

The targeted consultation on harmonised classification and labelling of zinc pyrithione: assessment of the two new algal toxicity studies conducted in *Skeletonema costatum*

The proposed acute and chronic environmental classification for zinc pyrithione (ZnPT) is based on the Ward and Boeri (2004) study in *Skeletonema costatum*, which is currently considered the key study for the aquatic classification of ZnPT. As was raised during the Public Consultation and in comments on the RAC Opinion (ODD), the consortium considers the Ward and Boeri (2004) study to be fundamentally flawed and, thus, not adequate for classification purposes. The reliability of the study is compromised by:

- the presence of copper contamination in culture and test medium at 5 times above the reliable NOEC in the copper REACH dossier for *S. costatum*; this alone renders the study unreliable;
- the use of photolysis data to estimate the TWA exposure in an algal study is inappropriate because the experimental conditions in photolysis studies and algal toxicity studies are different and not comparable;
- the lack of intermediate and final analytical determinations makes any estimated TWA effect concentrations highly uncertain;
- the statistical analysis in the Ward and Boeri study is not reproducible;
- the high variability in growth and growth rate between control replicates are indicative of questionable study quality and reliability; and,
- the 24h light regime is not appropriate for a light-sensitive compound nor recommended for the test species *S. costatum*.

An additional study on *S. costatum* with ZnPT (Rebstock, 2010) had been submitted, previously, for evaluation and had been considered reliable (RI=2) in the BPR PT21 evaluation of CuPT, but was not included in the CLH report on ZnPT. To confirm the reliability of the Rebstock study and to further address any uncertainties about the relative reliability of both studies, the consortium conducted two additional studies in *S. costatum* with ZnPT. The two new studies (report numbers 86820 and 86821) were conducted by different study directors and run at different times using different batches of ZnPT.

The Rebstock study and the two new studies were designed, technically, to remove the original flaws and improve on the Ward and Boeri study. ZnPT, due to rapid photolysis, is exceptionally difficult to test in algal toxicity studies, which are conducted under static conditions with light exposure. The Rebstock, 86820 and 86821 studies were conducted using study conditions appropriate for *S. costatum*, included daily analytical determinations, and produced robust statistical results with low variability, suggesting optimal algal growth conditions. All three studies are valid, reliable, and give results that are remarkably similar, indicating that the results are reproducible. Therefore, the Ward and Boeri study should be excluded from further consideration in the classification process and the endpoints from the remaining three reliable studies should be used for the classification of ZnPT.

Because of the rapid degradation of ZnPT in the test system, the 48 h results from the *S. costatum* studies with ZnPT are most appropriate for classification, as this timepoint best balances the

statistical considerations for growth rate with the decreasing certainty in the results as ZnPT degrades. Especially for an acute evaluation of a rapidly degrading (photolytically unstable) substance, results based on peak exposures make the most sense, scientifically. Considering the weight of the evidence provided by the three reliable studies on *S. costatum*, the appropriate aquatic classification for this rapidly degradable substance, ZnPT, should be as follows:

Aquatic Acute 1 with an M-factor of 100 (0.001 mg/L < ErC50 <= 0.01 mg/L)

Aquatic Chronic 1 with an M-factor of 10 (0.0001 mg/L < ErC10 <= 0.001 mg/L)

The relevant guidance, the adequacy of all four studies for classification purposes, an overview of independent evaluations of the Ward and Boeri and Rebstock studies, and conclusions on classification are discussed in detail below.

1 RELEVANT GUIDANCE

According to CLP guidance (p. 556 of v5.0), 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 relevant to *S. costatum* can be found in internationally accepted guidance documents, including ASTM E1218-04 and ISO 10253, as well as in US EPA OCSPP 850.5400 and its predecessor OPPTS 850.5400. While the taxon is mentioned, as well as some indications on preferred culture conditions, these other non-OECD test guidelines are not as specific as OECD 201. In addition, the preferred endpoints of interest in OECD 201 (intrinsic rate of growth) are not at all mentioned in the US EPA technical guidance. This is not a criticism of the USEPA Test Guidelines, but highlight that many relevant facets of the use and interpretation of OECD 201 are not taken into account in the use of *S. costatum* in those guidelines.

2 SUMMARIES OF FOUR AVAILABLE STUDIES ON *SKELETONEMA COSTATUM*

2.1 Ward and Boeri (2004)

This 120 h static algal study on *S. costatum*, was performed in accordance with US EPA-FIFRA, Guideline 123-2 (a predecessor of current guidance). 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 test was conducted using an 8 day old culture and initial cell density was about 10,000 cells/mL. A 24 h light photoperiod was used. Cell density in pooled controls was about 4x, 38x and 84x above nominal and 24, 48 and 72 h, respectively. The effect concentrations in the study report are based on nominal concentrations only.

The culture conditions used in this study are not consistent with those described in relevant guidance for this taxon (ASTM E1218-04, OPPTS or OCSPP 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

(40 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 presence of copper contamination in culture and test medium was 5 times above the reliable NOEC in the copper REACH dossier for *S. costatum (*ECHA). The use of a 24 h light period is not standard for marine species including *S. costatum* and is not advisable for light sensitive substances such as ZnPT.

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 Dossier Submitter has applied photolysis rates from the outdoor low concentration photolysis study to predict a single first order photolytic degradation rate in this study and estimate a TWA exposure concentration. Factors such as light intensity and spectrum, attenuation by glassware and (growing) algae, influences of TOC and suspended matter, and test vessel dimensions are not reflected in such an estimate, leading to a highly uncertain estimate of ZnPT loss and correspondingly uncertain estimate of ZnPT toxicity. Furthermore, the concentration in the dark control sample decreased by about 80% over 120 h, suggesting that photolysis was not the only removal process in the system.

The cell density in the controls were highly variable in this study, which may be due to the culturing conditions described above. The growth in the controls was faster than is usually observed in *S. costatum*, which is not considered a fast-growing species; this is probably in part due to the 24 h light cycle. 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 variability. For example, the cell density in the controls at 48 h ranges from 194,000 to 510,000 cells/ml with a CV of 30%. The ErC50s provided in the study report could not be independently reproduced using the statistical model cited in the study report.

Consequently, with all the evidence available on the conduct of the study, the reliability of the study is significantly compromised and it should not be considered for any regulatory decision- making purposes.

2.2 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. The test medium was prepared by the addition of appropriate reagent grade salts to filtered synthetic sweater. The base water used to prepare the saltwater algal nutrient medium was shown to be free from contaminants that could adversely affect the study.

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/MS/MS. 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* and appropriate for light-sensitive substances.

The number of cells in the pooled controls showed a 3x, 8x, 13x, 18x and 22x increase by 24, 48, 72, 96 hours and 120 h, respectively. The growth rate in controls 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 10^6 cells/mL by 96 h). The growth in controls slowed after 48h, which may have been due to nutrient depletion.

The variability of the cell density and the growth rates in the control replicates were low. The reported CVs in Tables 2 and 4 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 and time points without the additional uncertainty imposed by modelling and extrapolating photolytic losses under different assay conditions as applied in the Submitter's interpretation of the Ward and Boeri (2004) study. The mean measured concentrations were 61 to 93% of nominal at 24 h, 48 to 71% of nominal at 48 h, 39 to 59% of nominal at 72 h, 35 to 48% of nominal at 96 h, and 30 to 38% of nominal at 120 h (Table 1 of study report).

Growth rate inhibition at each time point, based on nominal concentrations, are reported in Table 4 of the study report. 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, the 24 h ErC50 in the study report is the only ErC50 that provides a bounded estimate and a 95% confidence interval.

The study report additionally provides the growth rate NOECs at each time point. However, ErC10s are more statistically meaningful and are now considered 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 range of <10% to 11% inhibition and consistent with the preferred statistic of ErC10s at each time point. The ErC10s and confidence intervals from this study have been derived using the cell density data and analytical results provided in the study report (see Section 5 of this document or the De Schamphalaere expert report).

2.3 Study report 86820 (2018)

This study reports a 120 h static test with the marine diatom *S. costatum* using ZnPT as test substance. The study was conducted according to US EPA Guideline OCSPP 850.5400. The test medium was prepared by the addition of appropriate reagent grade salts to filtered synthetic seawater. The base water used to prepare the saltwater algal nutrient medium was shown to be free from contaminants that could adversely affect the study. Seven test concentrations (nominally 0.20, 0.40, 0.80, 1.6, 3.2, 6.4 and 12.8 µg ZnPT/L) were prepared using DMF as solvent and tested in triplicate. The test concentrations were analysed for ZnPT at 0, 24, 48, 72, 96, and 120 h exposure by HPLC/MS/MS. The test was conducted using a 3-day old algal culture at test initiation with an initial algal cell concentration of about 10,000 cells/mL. The test was maintained at 20°C in a 14 h light: 10 h dark photoperiod as is standard for *S. costatum* and appropriate for light-sensitive substances.

The test concentrations declined over the duration of the study. The mean measured concentrations were to 35 to 52% of nominal at 24 hours, 21 to 41% of nominal at 48 hours, 15 to 35% of nominal at 72 hours, 13 to 30% at 96 hours and 11 to 30% of nominal at 120 hours.

The number of cells increased in the controls by 10x initial cell density at 48h and 25x initial cell density at 72h. The growth rate in the controls were consistent over the test duration. The reported CVs for cell density and growth rate in the pooled controls ranged from 4 to 12% and 1 to 2%, respectively (Tables 2 and 3 of study report). The growth rate inhibition showed a steep dose response curve, with low level inhibition at the nominal dose levels of 3.2 µg/L and nearly complete inhibition at higher dose levels. The growth rate at the 3.2 µg/L dose level recovered after 24h of exposure (Figure 9 of study report).

2.4 Study report 86821 (2018)

This 120h static test was conducted with the marine diatom *S. costatum* using ZnPT as test substance. The study was conducted according to US EPA Guideline OCSPP 850.5400. The test medium was prepared by the addition of appropriate reagent grade salts to filtered synthetic seawater. The base water used to prepare the saltwater algal nutrient medium was shown to be free from contaminants that could adversely affect the study. Seven test concentrations (nominally 0.20, 0.40, 0.80, 1.6, 3.2, 6.4 and 12.8 µg ZnPT/L) were prepared using DMF as solvent and tested in triplicate. The test concentrations were analysed for ZnPT after 0, 24, 48, 72, 96, and 120 h exposure by HPLC/MS/MS. The test was conducted using a 3-day old algal culture at test initiation with an initial algal cell concentration of about 10,000 cells/mL. The test was maintained at 20°C in a 14 h light: 10 h dark photoperiod as is standard for *S. costatum* and appropriate for light-sensitive substances.

The test concentrations declined over the duration of the study. The mean measured concentrations were 45 to 69% of nominal at 24 hours, 30 to 57% of nominal at 48 hours, 24 to 48% of nominal at 72 hours, 19 to 43% at 96 hours and 16 to 37% of nominal at 120 hours.

The number of cells increased in the controls by 13x initial cell density at 48h and 47x initial cell density at 72h. The reported CVs for cell density and growth rate in the pooled controls ranged from 6 to 10% and 1 to 2%, respectively (Tables 2 and 3 of study report). The growth rate in the controls were consistent over the test duration, with somewhat slower growth after 72h. The growth rate inhibition showed a steep dose response curve, with low level inhibition at the nominal dose levels of 3.2 µg/L and complete or nearly complete inhibition at higher dose levels. The growth rate at the 3.2 µg/L dose level starts to recover after 48h of exposure (Figure 9 of study report).

3 INDEPENDENT EVALUATIONS OF THE WARD AND BOERI (2004) AND REBSTOCK (2010) STUDIES

Two independent algal ecotoxicology experts (Prof. Dr. ir. Karel De Schamphelaere, Ghent University and Dr Gertie Arts, University Wageningen) reviewed both studies (Ward and Boeri [2004] and Rebstock [2010]) and prepared expert reports, which were submitted to the ECHA RAC in May 2018 by the Stakeholder CEFIC.

3.1 Independent evaluation of Ward and Boeri

Both experts confirm that the Ward and Boeri (2004) study should not be considered reliable and, therefore, not adequate for classification purposes (De Schamphelaere, 2018; Arts, 2018).

The experts concluded that the reliability of the study is compromised by:

- the presence of copper contamination in culture and test medium at 5 times above the reliable NOEC in the copper REACH dossier for *S. costatum*; this alone renders the study unreliable;
- the use of photolysis data to estimate the TWA exposure in an algal study is inappropriate because the experimental conditions in photolysis studies and algal growth inhibition studies are different and not comparable;
- the lack of intermediate and final analytical determinations makes any estimated TWA effect concentrations highly uncertain;
- the statistical analysis in the Ward and Boeri study is not reproducible;
- the high variability in growth and growth rate between control replicates are indicative of questionable study quality and reliability; and,
- the 24 h light regime is not appropriate for a light-sensitive compound.

3.2 Independent evaluation of Rebstock

The two independent scientific experts have now reconfirmed the reliability of this study, which is considered more reliable mainly due to:

- use of appropriate culturing conditions, particularly with regards to contaminants in the media;
- availability of intermediate and final analytical determinations allowing for reliable estimates of TWA exposure;
- low variability and acceptable growth rate in control replicates;
- appropriate light regime for a light-sensitive compound.

Both experts concluded that the EC10 values are preferred over the NOEC, as they are derived from regression analysis of the full dose response curve and are independent of dose selection. Dr. DeSchamphelaere did a detailed re-analysis of the data from the Rebstock study and proposed that the 48h ErC10 and ErC50 give the most reliable estimates of ZnPT toxicity in this study, as the growth rate is sufficiently high and exponential in the first 48h. The recalculated values, which Dr. DeSchamphelaere concluded to be reliable without restriction (p. 6 of report), were:

48 h ErC10 = 1.393 µg/L

48 h ErC50 = 6.036 µg/L

4 COMPARISON AND RELIABILITY ASSESSMENT OF THE FOUR STUDIES

4.1 Comparison of four studies

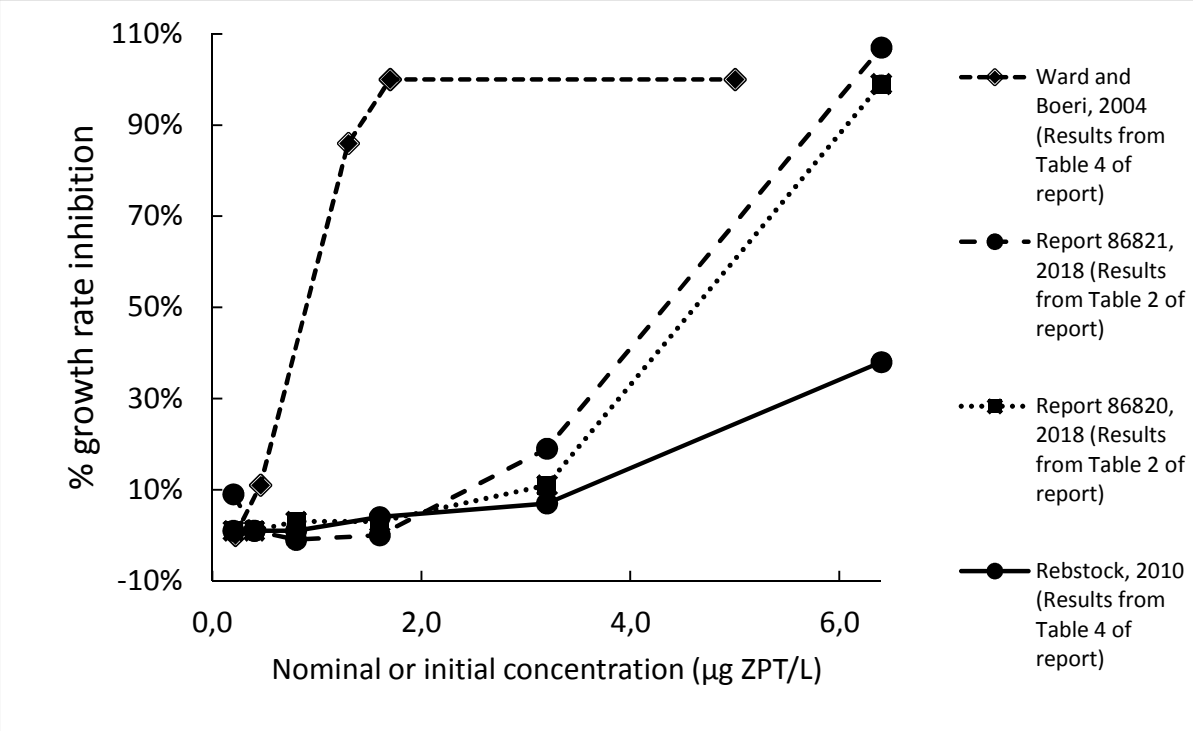
A comparison of the key design features of the four studies, including the culturing conditions, availability of analytical data and incorporation of exposure concentrations into the reported results, are shown below (Table 1).

Table 1. Test conditions and reporting in the four studies on *S. costatum*

	Initial cell density	Test medium	Contaminant levels	Light:dark cycle	Analytical determinations	Results in nominal or mean measured concentrations?
Ward and Boeri (2004)	10,000 cells/mL (estimated)	Unfiltered sea water	Particulate: 36 mg/L Cu: 40 µg/L Pb: 19.4 µg/L	24:0	0 and 120 h	Nominal
Rebstock (2010)	77,000 cells/mL (estimated)	Reconstituted marine medium	Particulate removed by filtration Cu: <10 µg/L Pb: <2 µg/L	14:10	0, 24, 48, 72, 96 and 120 h	Mean measured
86820 (2018)	9,260 cells/mL (measured)	Reconstituted marine medium	Particulate removed by filtration Cu: <5 µg/L Pb: <1 µg/L	14:10	0, 24, 48, 72, 96 and 120 h	Mean measured
86821 (2018)	8,890 cells/mL (measured)	Reconstituted marine medium	Particulate removed by filtration Cu: <5 µg/L Pb: <1 µg/L	14:10	0, 24, 48, 72, 96 and 120 h	Mean measured

A comparison of the growth rate inhibition results at 72 hours across the four studies, based on nominal concentrations at 72 hours, was submitted in response to the ODD in May 2018 and is reproduced below (Figure 1). This comparison was prepared before the analytical data were available for the two new studies, but a comparison of all four studies based on mean measured concentrations is not possible, since such measurements were not done in the Ward and Boeri study. Even this simple representation of the data indicates that the Ward and Boeri results are not reproducible.

Figure 1. 72-hour growth rate inhibition results in four *S. costatum* studies, comparing the originally submitted studies from Ward and Boeri (2004) and Rebstock (2010) with two new GLP studies (86820 and 86821)



4.2 Conclusions regarding the reliability of the four studies available on S. *costatum*

The overall results of the four studies available to characterize the toxicity of ZnPT to *S. costatum* unambiguously confirm that the results from the Ward and Boeri study are neither reliable nor reproducible. This is not surprising, as the Ward and Boeri study employed test conditions that are detrimental to *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, and should not be considered further.

5 EVALUATION OF RELIABLE DATA ON *S. COSTATUM* FOR PURPOSES OF CLASSIFICATION

Zinc pyrithione is an exceptionally difficult substance to test in algae. Algae studies are by necessity conducted under static conditions and with light, whereas ZnPT undergoes rapid photolytic degradation. Stable concentrations of ZnPT cannot be achieved in an algal toxicity study. Furthermore, as concentrations decrease, the growth rate of the algae recovers. The average specific growth rates and geometric mean concentrations used for statistical modelling in algal toxicity studies can be insensitive to recovery. Therefore the results must be evaluated carefully and the specific characteristics of ZnPT taken into consideration when interpreting the results.

The studies described here on *S. costatum* were conducted for 120 h, which was the duration used in the original *S. costatum* tests according to US guidance. Algal toxicity tests according to OECD 201 are typically 72 hours, and it is the 72h results from such studies that are usually considered in

classification. Test durations of 72, 96, or even 120 h are all reasonable for stable substances, so long as algal growth is maintained; unlimited exponential growth is necessary for study validity and statistical uncertainty can be high at early timepoints in the study.

There are cases where the use of even shorter study durations (i.e., 48 h) is scientifically valid. One is when the exponential growth rate in the controls is not maintained beyond 48 h. Another is when the test substance is rapidly degrading under test conditions. For rapidly degrading substances, effect concentrations calculated at timepoints beyond those at which the substance has an inhibitory effect are misleading (i.e., overestimate toxicity). This is because of the use of averages in effect estimation. For example, if at a certain dose level, a substance causes significant growth rate inhibition in the first 24 h of exposure, followed by growth rates equivalent to the controls at all subsequent time intervals, the average growth rate for this dose level will be lower than controls at all time points. Then in the statistical analysis, effects at this dose level, which in reality are only associated with the initial exposure concentration (as recovery occurred after 24 h) are then associated with increasingly lower geometric mean concentrations at e.g., 72 h, 96 h or 120 h, if these study durations are used to estimate effect concentrations.

A comparison of the 24, 48 and 72 h ErC10s and ErC50s for ZnPT and *S. costatum* are provided in Table 2. There is generally a preference among algal toxicologists to use the ECx compared to the NOEC, including among both independent experts described previously (DeSchamphelaere, 2018; Arts, 2018). OECD 201 (para. 58) also indicates that regression based effect estimates such as ErC10s and ErC20s are preferred because regression based estimates are less influenced by dose selection and make full use of the dose response curve. The ErC10s in all cases are bounded by concentrations that are both below and above the 10% effect level providing the best opportunity to estimate the ErC10s with high confidence. This is reflected in their relatively narrow 95% confidence intervals. In these cases, modern test guidelines for all aquatic taxa, including algae, recommend use of the EC10 as the statistic above the NOEC as originally recommended by OECD in 1998 (OECD Monograph 10, 1998).

The effect concentrations in Table 2 are remarkably similar between the three studies, especially considering the unique challenges of ZnPT. The ErC50s and ErC10s decrease considerably from 24 to 72h in the two new studies. From these numbers alone, it is not clear if this decrease is due to the decrease in concentration alone, or also because of prolonged growth rate inhibition. However, careful investigation of the growth curves indicates that the dose corresponding to low level growth rate inhibition (nominally 3.2 µg/L) starts to recover in all three studies by 24 to 48 h (Figure 6 of Rebstock report; Figure 9 of report 86820; Figure 9 of report 86821). Differences in rate of recovery and concentration decline may explain why the Rebstock ErC50 is closer to the other values at 24 h than at later time points.

Table 2. Summarized results from the three reliable *Skeletonema costatum* studies with ZnPT

	24 h results (µg/L) (95% CL)		48 h results (µg/L) (95% CL)		72 h results (µg/L) (95% CL)	
	Acute (ErC50)	Chronic (ErC10)	Acute (ErC50)	Chronic (ErC10)	Acute (ErC50)	Chronic (ErC10)
Rebstock [a, b]	5.12 (4.36 – 5.88)	NR *(NOErC = 2.66)*	6.036 (4.822 – 7.557)	1.393 (0.919 – 2.113)	>3.80 (NR)	1.88 (1.47 – 2.47)
86820 [a]	4.33 (0 -12.6)	3.41 (0 -13.4)	1.68 (1.14 – 2.22)	1.35 (0.912 – 1.78)	0.881 (0-2.39)	0.680 (0 -1.71)
86821 [a]	4.19 (3.43 – 4.94)	1.13 (0.528 – 1.73)	1.23 (0.987 – 1.48)	0.991 (0.793 – 1.19)	0.969 (0.441 – 1.50)	0.778 (0.354 – 1.20)

a. Results based on geometric mean measured concentrations.

b. 24h results from Rebstock taken from report, ErC10 was not reported (NR). 48h results from Rebstock based on DeSchamphelaere recalculation and ErC50 includes extrapolation above highest dose tested. 72h ErC50 is taken from study report; 72h ErC10 based on consortium internal recalculation.

Since ZnPT is rapidly degrading in the test system and recovery occurs, the precision of these algal tox studies declines with increasing exposure duration. The timepoint that best reflects the true toxicity is that which balances the statistical uncertainty surrounding the growth and growth rate at the beginning of the study and the unreliability of later timepoints due to degradation of test item and corresponding recovery. For ZnPT, 48 h is the timepoint which best achieves this balance. It is clear that sufficient growth in controls has occurred to allow the 48 h time point to be statistically meaningful. By 48 h, control yield and growth rates showed low variability (2.0-8.5%); this translates to being able to detect small changes in growth rate. By 48 h it is possible to identify a LOEC (statistically significant reduction) at 4.4-7.2% of growth rate inhibition across the three studies. Clearly, in these studies, the 48 h time point can be considered suitable for assessing growth rate inhibition, a conclusion also reached by the algal experts identified earlier in this summary and by KemI, when they proposed the 48 h timepoint for *S. costatum* in the CLH dossier.

6 CONCLUSIONS REGARDING AQUATIC CLASSIFICATION OF ZNPT

The two new algal toxicity studies provide robust and reliable characterisations of ZnPT toxicity to *S. costatum*. The culture medium was free from contamination. The growth rates in the control were consistent and exponential. The cell counts in the controls showed low CoVs. Growth media was collected at 24h intervals for all treatment levels (including controls) and analysed for ZnPT using a fully validated analytical method. All reported results are based on geometric mean measured concentrations. Both studies are considered fully reliable (Klimisch 1) and clarify any remaining uncertainty about sensitivity of *S. costatum* to ZnPT.

According to CLP guidance, the lowest results from acceptable tests are used for classification in cases where there are less than four reliable studies. (This approach, unfortunately, ignores the increased scientific certainty brought by additional reliable studies in the same species). The 48 h results from the *S. costatum* studies with ZnPT are most appropriate for classification, as this timepoint best balances the statistical considerations for growth rate with the decreasing certainty in

the results as ZnPT degrades. Especially for an acute evaluation of a rapidly degrading (photolytically unstable) substance, results based on peak exposures make the most sense, scientifically.

Based on the 48h results of the three reliable Skeletonema studies, the lowest ErC50 is 1.23 µg/L and the lowest ErC10 is 0.991 µg/L, as reported in study number 86821. These results are slightly lower than the next most sensitive aquatic species, *Pimephales promelas*, which has an LC50 of 2.6 µg/L and chronic NOEC of 1.22 µg/L.

Species	**Acute (ErC50 or LC50) (µg/L)**	**Chronic (ErC10 or NOEC) (µg/L)**
Skeletonema costatum (marine diatom algae)	1.23	0.991
Pimephales promelas (fathead minnow)	2.6	1.22

Considering the weight of the evidence provided by the three reliable studies on *S. costatum*, the appropriate aquatic classification for this rapidly degradable substance should be as follows:

Aquatic Acute 1 with an M-factor of 100 (0.001 mg/L < ErC50 <= 0.01 mg/L)
Aquatic Chronic 1 with an M-factor of 10 (0.0001 mg/L < ErC10 <= 0.001 mg/L)

7 REFERENCES

86820. Zinc pyrithione: static growth inhibition test with the marine diatom, *Skeletonema costatum*. 2 July 2018. Report number 86820.

86821. Zinc pyrithione: static growth inhibition test with the marine diatom, *Skeletonema costatum*. 11 July 2018. Report number 86821.

Arts, Gertie. Summary and evaluation of two algal inhibition studies investigating the toxicity of zinc pyrithione to the marine diatom *Skeletonema costatum*. 7 May 2018.

Bilotta GS and Brazier RE. 2008. Understanding the influence of suspended solids on water quality and aquatic biota. Water Research. 42: 2849-2861.

De Schamphelaere, Karel. Comparative evaluation of two toxicity studies of zinc-pyrithione to *Skeletonema costatum* for classification purposes. 4 May 2018.

European Copper Institute (ECI). 2008. European Union Risk Assessment Report; Voluntary risk assessment of copper, copper II sulphate pentahydrate, copper(I)oxide, copper(II)oxide, dicopper chloride trihydroxide. European Copper Institute (ECI). Reviewed by; Italy.

European Chemicals Agency (ECHA). Registration dossier for copper (EC No. 231-159-6). https://echa.europa.eu/registration-dossier/-/registered-dossier/15562/6/2/6/?documentUUID=08f77cc9-a2ee-4ce0-937b-de5595b99b84

European Chemicals Agency (ECHA). Guidance on the application of the CLP criteria. Version 5.0. July 2017.

OECD Series on Testing and Assessment Number 23. Guidance document on aquatic toxicity testing of difficult substances and mixtures (15 Dec 2000).

OECD Guidelines for The Testing of Chemicals No. 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test (Adopted: 23 March 2006, Annex 5 corrected: 28 July 2011).

Rebstock, M. 2010. Zinc pyrithione: Static Growth Inhibition Test with the Marine Diatom, *Skeletonema costatum*, ABC Laboratories, Inc. Study No. 66218 (unpublished).

SCHER. 2009. Voluntary risk assessment report on lead and its compounds. Environmental part.

Ward TJ, Boeri RL. 2004. Zinc pyrithione: Influence on growth and growth rate of the marine diatom, *Skeletonema cost*atum, T.R Wilbury Laboratories, Inc., Report No. 2652-AR (unpublished).