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Analysis, Fate and Toxicity of Zinc- and Copper Pyrithione in the Marine Environment

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Environmental co-operation is aimed at contributing to the improvement of the environment and forestall problems in the Nordic countries as well as on the international scene. The co-operation is conducted by the Nordic Committee of Senior Officials for Environmental Affairs. The co-operation endeavours to advance joint aims for Action Plans and joint projects, exchange of information and assistance, e.g. to Eastern Europe, through the Nordic Environmental Finance Corporation (NEFCO).

Nordic co-operation

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Contents

Preface

The project Analysis, fate and toxicity of zinc- and copper pyrithione in the marine environment, was funded by the Nordic Council of Ministers in 2001 and stretched over a two-year period. The aims were to develop an analytical method that simultaneously could measure zinc- and copper pyrithione (ZPT/CPT) in seawater, investigate fate and toxicity, and finally use the results to assess the toxicity of ZPT and CPT in the marine environment. The outcome of this project can be used for evaluation of anti-fouling additives in marine anti-fouling products by the respective Nordic countries.

During this project a strong co-operation between the partners involved has been the pre-requisite for the success, resulting in three already published peer-review publications, another three submitted manuscripts, and with an additional three manuscripts under preparation, several poster presented at international conferences, as well as three oral presentations. The results presented here also include work from projects not founded by the NMR, but where the NMR-project has been the instigation for further research. A number of master and PhD-students, both Danish and Swedish have been involved in the project, strengthening Nordic co-operation also at the educational level.

Summary

The aims of this project were to:

- Develop an analytical method for the simultaneous determination of zinc- and copper pyrithione (ZPT/CPT)
- Investigate the fate of ZPT/CPT in seawater
- Determine the toxicity of ZPT/CPT for marine pelagic and benthic communities
- Perform a risk assessment based on the results from the project.

A method that combines solid-phase extraction (SPE) with HPLC-UV/VIS detection was developed for the simultaneous determination analysis of ZPT and CPT, with a detection limit of ~2 nM in seawater for both compounds. The fate of ZPT was shown to be dependent on the concentration of copper and ligands present in the water. Up to 50% of added nominal concentrations of ZPT was tranchelated to CPT at ambient seawater copper concentrations, and CPT was shown to be a stable complex. Both compounds are photodegradable, with half-lives between 7 to 45 minutes depending on light intensities. Under in-situ reduced light conditions and in the dark, the compounds are stable for >48 hours.

The toxicity of ZPT and CPT given as EC_{50}-values varied between 1.6-60 nM for pelagic bacteria, algae and zooplankton communities. The effect of a three-pulse addition of 5 nM ZPT affected the function of the pelagic community for the duration of a six-day mesocosm experiment. ZPT and CPT affected the benthic community nutrient cycling at concentrations over 0.001 nmol/g dry sediment. The most pronounced effect was on the cycling of nitrogen, which was also evident in a 38-day mesocosm experiment.

A Predicted No Effect Concentration (PNEC) for ZPT in the pelagic environment could be calculated to 0.2 nM, using a species sensitivity distribution (SSD) with a 95% confidence level. The same calculation could not be performed for CPT in the pelagic environment, or for ZPT and CPT in the benthic environment due to a too small data set. PNECs were therefore calculated using the Lowest Observed Effect Concentrations (LOECs), with an appropriate application factor. PNEC for CPT in the pelagic environment was found to be 0.001nM. PNEC for ZPT and CPT in the benthic environment was found to be 10 fmol and 1 pmol/g dry sediment respectively.

It can be concluded that ZPT present in boat-paint will be tranchelated to CPT when released into the marine environment. CPT is a more stable, and for the pelagic community, a more toxic compound than ZPT.

Furthermore photodegradation is not as efficient as earlier reported. The presence of ZPT and CPT in the marine environment can affect vital functions such as primary and secondary production, as well as nutrient cycling at concentrations far below the only so far reported measurement from the marine environment.

1. Introduction

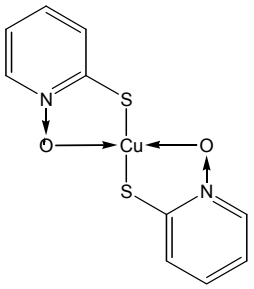
1.1 Background

The ban on Tri-butyl-tin (TBT) in anti-fouling paints for pleasure boats in all the Nordic countries has resulted in the use of other booster biocides such as Irgarol 1050, Sea-nine 211 and zinc pyrithione (ZPT). Most of these booster biocides are used together with copper in the paints. The Nordic countries have chosen different strategies with respect to which booster compounds that are allowed, where for example Denmark has banned Diuron and Irgarol but allowed ZPT, and Sweden has banned all booster biocides except Irgarol, and in Norway only TBT is forbidden. In Finland products containing TBT, Irgarol and Diuron are not expected to be on the market, as approval of these substances has not been sought by paint-manufactures. ZPT is currently used in some of the products available in Finland. The abbreviations ZPT and CPT will mainly be used throughout this report, since they are most commonly used in the toxicity literature. However, for the analytical-chemical part of this report, the chemical nomenclature $ZnPT_2$ and $CuPT_2$, which indicates the complex structure, will be used.

1.2 Chemical Properties of $ZnPT_2$ and $CuPT_2$

$ZnPT_2$ and $CuPT_2$ are metal complexes where two pyrithione ligands bind to a central metal ion (Fig. 1). The metal ion can be exchanged, leading to other metal complexes with two or three pyrithione ligands ($MePT_{2/3}$), but also to single pyrithione complexes such as NaPT and $CuPT^+$ (Sun et al. 1964). The speciation of complexes is determined by the complex constants for each complex, as well as the concentration of pyrithione and the metals. In the marine environment, the presence of other ligands, both organic and inorganic, will influence the distribution of complexes. The constants are only determined for some of the complexes, but the order of complex strength is thought to be: Na<Fe<Mn<Zn<Cu. Chemical properties for $ZnPT_2$ are summarised in Table 1.

Figure 1. Chemical structure of $CuPT_2$. The co-ordination of the complex is thought to be planar, whereas the co-ordination of $ZnPT_2$ is unknown.



The pyrithione complexes are neutral and hydrophobic, with a low solubility in water, resulting in a high affinity for organic matter. The pyrithione molecule can be photolysed at wavelengths between 320-355 nm (Neihof et al, 1979) or degraded by chemical or biological oxidation (Turley et al, 2000). Reported half-lives of $ZnPT_2$ and $CuPT_2$ vary between minutes to hours in water, whereas no studies have been made in sediments. However, the complexes are thought to be stored as stable manganese or iron complexes (Galvin et al 1998).

Table 1. Physicochemical properties of $ZnPT_2$.

			References
CAS nr.	13463-41-7		Madsen *et al*, 1999
Empirical formula	$C_{10}H_8N_2O_2S_2Zn$		Madsen *et al*, 1999
Molar weight	317.68 g/mol		Madsen *et al*, 1999
Melting point	262 °C		Robinson, 1964
Water solubility, S_w	6 mg / l 8 mg / l		Madsen *et al*, 1999 Thomas, 2001
Vapour pressure, P_v	Not volatile	(25 ºC)	Madsen *et al*, 1999
Octanol-water partition coefficient (log K_{ow})	0.97		Madsen *et al*, 1999
Organic material-water partition coefficient (log K_{oc})	2.9-4.0 0.7	 (calc)	Madsen *et al*, 1999 Thomas, 2001
Stability constant log K_1	5.9	(20 ºC)	Albert *et al.*, 1956
Stability constant log K_2	5.4	(20 ºC)	Albert *et al.*, 1956

Synonyms: Bis(1-hydroxy-2[1H]-pyridinethionato-O-S)-(T4)zinc, Zinc Omadine, zinc salt of 2-mercaptopyridine-N-oxide, zinc salt of 1-hydroxy 2-pyridinethione.

1.3 Mode of Action of ZPT and CPT

The mode of action for ZPT and CPT includes disruption of cell membranes, disruption of pH-gradients, and complex binding with metals and proteins (Chandler et al. 1978, Dinning et al. 1998). These mechanisms lead to disruption of ATP-synthesis and transport through membranes, as well as starvation of metals and other cations in the cell.

1.4 Toxicity of ZPT and CPT

Toxicity tests have so far been performed as single species test using, fungi, bacteria, algae, yeast, fish, oysters, sea urchin and crustacea, most of them fresh water species. The range of lowest effect concentrations earlier reported spans concentrations of fg/l for sea urchin egg development (Kobayashi et al., 2002), to 80 mg/l for *Pseudomonas aerigunosa* (Khattar et al. 1988). Previous toxicity data relevant for the marine environment are collected in Table 2.

Table 2. Acute toxicity of ZPT

Test organism	Toxic effect	Duration	Value	Source
Suspension-cultured fish cell CHSE-sp	EC_{50}	24 hours	0.18 mg/l (≈ 570 nM)	Okamura *et al*., 2002
Juvenile rainbow trout *(Oncorhynchus mykiss)*	LC_{50}	28 days	0.0046 mg/l (≈ 14 nM)	Okamura *et al*., 2002
Zebra Fish *(Brachydanio rerio)*	EC_{50}	7 days	0.009 mg/l (≈ 30 nM)	Goka, 1999
Japanese Medaka *(Oryzias latipes)*	EC_{50}	7 days	0.005 mg/l (≈ 20 nM)	Goka, 1999
Fish *(Cyprinodon variegates)*	LC_{50}	96 hours	0.4 mg/l (≈ 1000 nM)	Madsen *et al*., 1999
Freshwater microalga *(Selenastrum capricornutum)*	EC_{50}	3 days	15 µg/l (≈ 47 nM)	Okamura *et al*., 2003
Macroalga *(Ceramium tenuicorne)*	EC_{50}	7 days	3.3-6.4 µg/l (≈ 10-20 nM)	Karlsson and Eklund, 2004
Crustacean *(Nitocra spinipes)*	LC_{50}	96 hours	178-343g/l (≈ 560-1080 nM)	Karlsson and Eklund, 2004
Crustacean *(Mysidopsis bahia)*	LC_{50}	96 hours	0.0063 mg/l (≈ 20 nM)	Madsen *et al*., 1999
Oyster *(Crassostrea virginica)*	LC_{50}	96 hours	0.0016 mg/l (≈ 5.0 nM)	Madsen *et al*., 1999
Leukemia rat cells (IPC-81)	EC_{50}	48 hours	0.40 µM (≈ 400 nM)	Doose *et al*., 2004

2. Aims and Approach

The aim of this project was four-fold:

- to develop an analytical method for $ZnPT_2$ and $CuPT_2$ in seawater
- to analyse the fate of $ZnPT_2$ and $CuPT_2$ in seawater
- to assess toxicity of ZPT and CPT for marine organisms and communities
- to use the results in a risk assessment

The approach used throughout this project was designed to collect data valid for the marine environment. The approach includes analysis of the pyrithione complexes in seawater, fate studies in environmentally realistic set-ups, toxicity test using marine communities for direct effects, as well as two mesocosm studies where both direct and indirect effects were studied.

Pelagic communities including bacterial, algal and zooplankton communities, as well as benthic bacterial communities have been used. Both the pelagic and benthic system constitute the base for the marine food webs, including primary and secondary producers, as well as degraders that are important in the cycling of nutrients in the marine environment. One mesocosm experiment was pelagic including bacteria, algae and zoo plankton, and one focused on a sun-lit sediment and included benthic micro algae, bacteria and meiofauna.

The approach to work with effects on community and system level has two main advantages. Firstly, the effects observed are closer to those that can be expected to occur in the marine environment, compared to when using single-species test, as both direct and indirect effects are taken into account. Secondly, studying effects on central processes such as primary and secondary production and cycling of nutrients, impact beyond community level can be assessed.

2.1 Development of the Analytical Method

An HPLC- approach with absorbance detection was chosen as the analytical method, combined with a solid-phase extraction (SPE) procedure. An HPLC-MS approach was also developed but was not used for the fate studies, since the detection limit was too high. It was however used for confirmation of the chromatographic method with regard to the speciation of $ZnPT_2$ and $CuPT_2$. Several extraction and chromatographic protocols were tested and are reported in two master theses.

2.2 Fate experiments

A series of fate experiments including both degradation and transformation experiments were performed during the project.

2.2.1 Photodegradation

Before the analytical method was developed an indirect approach according to Callow and Finlay (1995), was used to determine the breakdown of $ZnPT_2$ and $CuPT_2$ under light and dark conditions. The photodegradation experiments were repeated once the analytical method was in place in order to confirm the results from the indirect measurements, and to assess the effect of light intensities. A degradation experiment was also performed in the water column at Isefjorden, Denmark.

2.2.2 Transchelation of ZPT

During the development of the analytical method it became clear that the recovery of $ZnPT_2$ was low when added to seawater. In order to elucidate whether this was due to poor sample handling/analytical method, or simply a result of transformation of $ZnPT_2$, a series of recovery experiments were performed. Among the factors tested were influence of salinity and concentration of copper.

2.2.3 Leaching Experiments

The fate of $ZnPT_2$ from two paints were tested; 1) Alu-Safe 7120 (Hempels Marine Paints, Lyngby, Denmark), a copper free ZPT paint for all underwater surfaces including aluminium hulls, and 2) Mille Ocean 7110 (Hempels Marine Paints, Lyngby, Denmark), a two-component self polishing paint containing ZPT and Cu_2O as active ingredients.

2.2.4 Fate of ZPT in a Mesocosm Experiment

A mesocosm experiment was set up in the shallow waters of Isefjorden, Denmark. The fate of $ZnPT_2$ in the highest of the additions, 50nM was investigated in the three replicated enclosures, during the three days of $ZnPT_2$ addition which occurred after sunset.

2.3 Effects on ZPT and CPT on Marine Communities

2.3.1 Direct Effects

Short-term tests were used for determining the direct toxicity of ZPT and CPT using pelagic bacterial, algal, and zooplankton communities, as well as on benthic bacteria. The functional endpoints used in the short-term test are summarised in Table 3.

Table 3. Overview of end-points for the different communities

Community	End-point	Method	Compound tested
Phytoplankton	Growth	$H^{14}CO_3^-$- incorporation	ZPT, CPT, Zn, Cu
Pelagic bacteria	Growth	^{14}C-Leucine incorporation	ZPT, CPT
Zooplankton	Grazing potential	^{14}C-labelled prey	ZPT
Benthic bacteria	Nutrient cycling, growth	Flux-incubations	ZPT, CPT

2.3.2 Effect of ZPT and CPT on communities in mesocosms

Two mesocosm experiments were performed, one on the pelagic and one on the benthic community. The pelagic mesocosm included three trophic levels, bacteria, algae and zooplankton, where both functional and structural variables were measured. The benthic mesocosm also included three trophic levels, benthic algae, bacteria and meiofauna. The experimental design for the benthic mesocosm included the combined effect of CPT and increased eutrophication, where inorganic nutrients had been added to half of the sediment cores three weeks before the addition of CPT. Functional and structural variables were measured during 38 days to assess direct and indirect effects as well as the resilience of the benthic community, depending on the degree of eutrophication. To assess the impact on the function of the total system for the two food webs, the individual functional endpoints were combined using the Bray-Curtis similarity index. Effects of the pyrithiones on the system function including all three trophic levels could thereby be compared to the control systems.

2.4 Risk Assessment

Risk assessment can be performed in a multitude of ways. Here we have chosen to use a species sensitivity test (SSD) for the tested communities in this project that includes 95% confidence intervals of the predicted no effect concentration for a limited data set according to Aldenburg and Slob 1993.

A Predicted No Effect Concentration (PNEC) for ZPT was calculated using the SSD for the pelagic community. For CPT there is not enough

trophic levels available why a SSD was not constructed. Instead a PNEC using the NOEC for the most sensitive end-point was used. For risk assessment of the benthic system NOEC for the most sensitive end-point was also used. The PNEC values, together with an evaluation of the fate of ZPT and CPT concludes the risk assessment.

3. Results

This section is a compilation of the results from the different experiments to provide an overview of the work performed. More detailed presentation and discussion of the results are given in the articles published, and in the manuscripts under preparation. In the following section, references to articles and manuscripts produced in this project are given with Roman numbers.

3.1 Analytical Method (I)

One litre of seawater is spiked with the internal standard (I.S.), Xylene cyanol, to a concentration that provides a peak height similar to the peak height of $ZnPT_2$/$CuPT_2$ at the median concentration in the standard curve used. The analytes and internal standard is extracted on a Strata X SPE at a flow rate of ~15 ml/min. The compounds are then extracted from the column using an acetonitrile- methanol-water mix of 70:20:10, and is blow down to ~1 ml before injecting 40 µl into the HPLC. The gradient conditions for the HPLC-analysis are given in Table 4.

Table 4. HPLC-gradient conditions

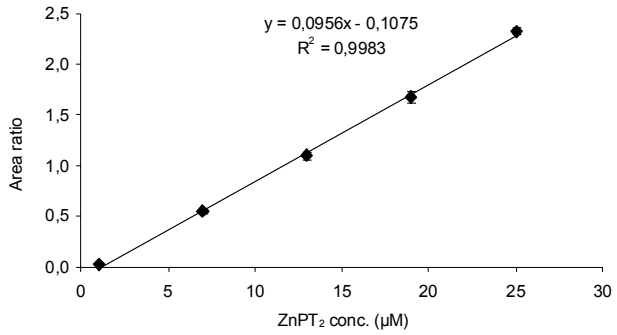
Time (min)	% solvent A	% solvent B
0	100	0
7	100	0
10	65	35
25	0	100
30	0	100

Solvent A consists of 0.4 % (w/v) $Zn(NO_3)_2$ in 20:80 methanol:water, and solvent B consists of 0.4 % (w/v) $Zn(NO_3)_2$ in 77:23 methanol:water. The flow rate is kept at 1 ml/min, and column temperature at 26°C. $ZnPT_2$ is detected at 270 nm, $CuPT_2$ at 320 nm and the internal standard at 616 nm.

This analytical method has a detection limit of 2 nM $ZnPT_2$/ $CuPT_2$ in seawater using a pre-concentration factor of 1000. The detection limit can be lowered either by extracting more water, by pre-concentrating the SPE-extract further, or by injecting a larger sample. However, the present detection limit covers most of the NOECs/LOECs observed in this report.

The linear range of the method spans an order of magnitude (Fig 2), and can be extended by injecting a smaller sample volume or diluting the samples. The standard curves are prepared for $ZnPT_2$ and $CuPT_2$ individually, since a surplus of copper in the $CuPT_2$-powder affects the stability of $ZnPT_2$ if a two-component standard is made.

Figure 2. Calibration curve for $ZnPT_2$



Recovery of $ZnPT_2$ from seawater was 0% at 5nM and 85 ± 4% at 250 nM. The recovery for $CuPT_2$ was 81 ± 3 % at 5 nM and 95 ± 5% at 25 nM. A further discussion on the low recovery of $ZnPT_2$ is given in the Fate section of this report.

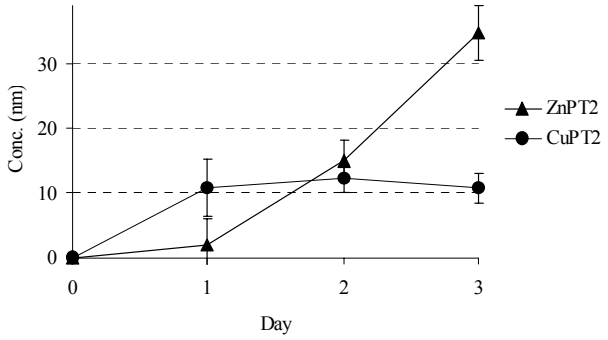
3.2 Fate (II)

3.2.1 Transchelation

The low recovery of $ZnPT_2$ in seawater observed in the development of the analytical method can be explained by transchelation of $ZnPT_2$. In seawater at low concentrations, 48 ± 3 % of the added $ZnPT_2$ was recovered as $CuPT_2$. Further experiments showed that when ~ equimolar concentrations of Cu to $ZnPT_2$ was added to seawater, ~100% was transchelated to $CuPT_2$. However, when a larger concentration of copper was added,only ~50% was transchelated into $CuPT_2$. These results suggest the formation of single Cu-PT^+ complexes that are not detected by the present analytical method. The fate of the remaining $ZnPT_2$ is unknown, but could be caused by other cations present at high concentrations such as Na, Mg and Ca, or the presence of other ligands such as phosphate, or organic molecules that complex binds with Zn. When Cu is added to $CuPT_2$ in seawater there is no change in $CuPT_2$ concentration which shows that $CuPT_2$ is a strong complex, where substitution of Cu or the pyrithione ligand is unlikely.

The same pattern for transchelation of $ZnPT_2$ was also observed in the mesocosm experiment. Here $ZnPT_2$ at a nominal concentration of 50 nM was added at four occasions over three days. One hour after the first addition, 20% of the added $ZnPT_2$ was recovered as $CuPT_2$. The same fraction was recovered as $CuPT_2$ following the second and third addition, whereas an increasing amount of $ZnPT_2$ was found and at day three ~85% of the addition was found with $ZnPT_2$ accounting for 65% and for $CuPT_2$ 20%.

Figure 3. $ZnPT_2$ and $CuPT_2$ concentration in a mesocosm bag with a nominal concentration of 50 nM $ZnPT_2$ (n=4).

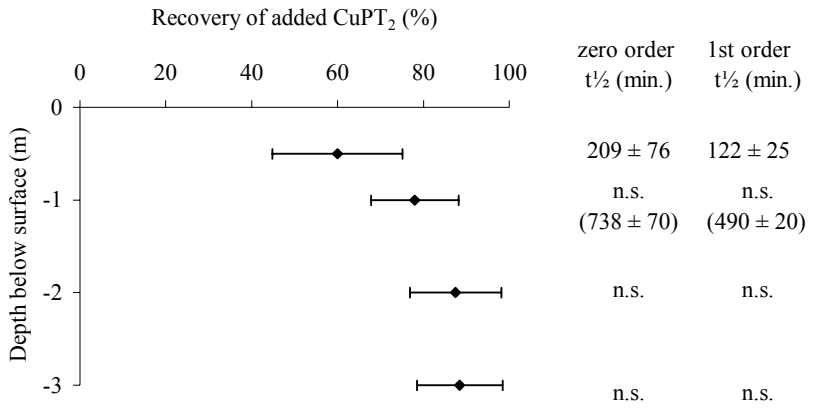


3.2.2 Degradation

The indirect estimation of degradation gave half-lives for $ZnPT_2$ and $CuPT_2$ of 7-8 minutes when sterile seawater solutions were exposed to sunlight (III). However, when both compounds were kept in the dark, a half-life could not be determined during the 48-hours of the experiment. When seawater containing bacteria and algae was used, no increase in degradation time was observed suggesting no biological degradation.

The degradation experiments were repeated using chemical analysis, where $CuPT_2$ was used to avoid transchelation. The results were the same as in the indirect estimation, with degradation times of a few minutes in direct sunlight. However, when exposed to the light in the laboratory, no degradation was observed. When a degradation experiment was performed at different depths in Isefjorden, the half-life was between 120 and 210 min at 0.5 meters (Fig 4), and with no significant degradation below 2m.

Figure 4. Recovery of $CuPT_2$ at different depths in Isejorden.



3.3 Direct effects on marine communities

3.3.1 Pelagic algae and bacteria (IV)

The effect of ZPT and CPT was determined over a few months using natural pelagic communities from Roskilde fjord. EC_{50} for ZPT and CPT varied between 2 and 60 nM and 4 and 25 nM, respectively (Fig 5 and 6).

Figure 5. ZnPT2 EC50-values for phytoplankton communities in Roskilde fjord.

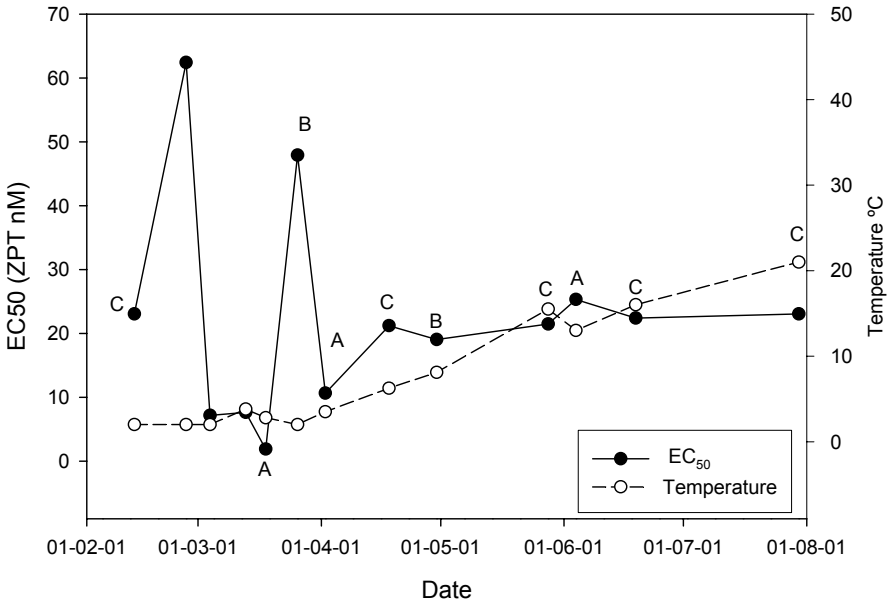
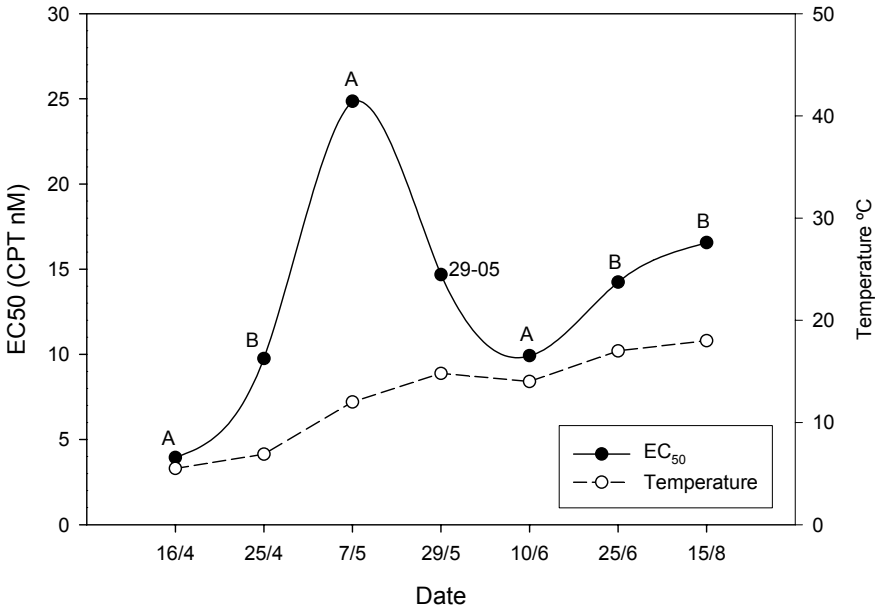


Figure 6. CPT EC_{50}-values for phytoplankton communities in Roskilde fjord.



Changes in sensitivity throughout the season were related to changes in phytoplankton community composition and density, and to nutrient levels. It was found that the variation in sensitivity of ZPT and CPT was related to abundance of the groups of Cryptophyceae, Bacillariophycaea and Dinophyceae when they were dominating the community, where higher densities give a lower toxicity. Furthermore, the sensitivity to ZPT was increased at low concentrations of phosphate per cell (<0.2 nmol/cell). For CPT the toxicity was the lowest at higher concentration of phosphate in the water. Consequently, in aquatic environments where phytoplankton is phosphate limited the effect of ZPT and CPT may be enhanced due to reduced interaction with phosphate.

On the two occasions that the toxicity of ZPT and CPT was tested on the same community under the same abiotic conditions, CPT was more toxic at the same nominal additions (Table 5). This can of course in part be explained by the transchelation of ZPT in seawater.

Table 5. Comparison of toxicity between ZPT and CPT as EC_{50}-values

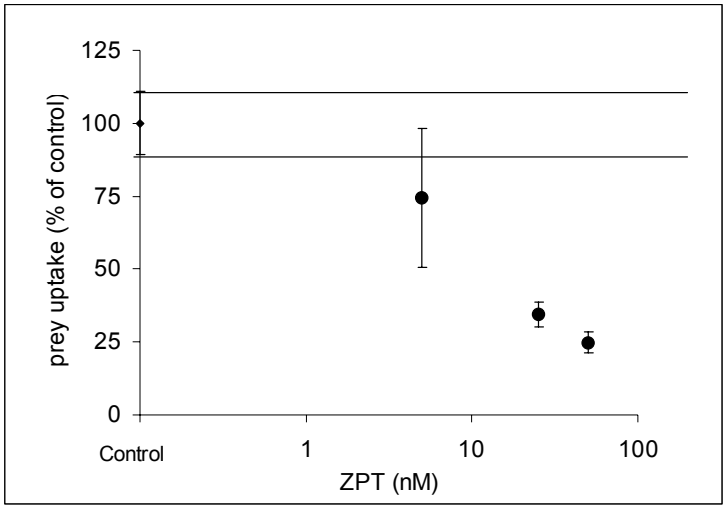
Date	ZPT (nM)	CPT (nM)
16-04 & 18-04	21	15
28-05 & 29-05	21	4

The toxicity of ZPT and CPT measured as EC_{50} for pelagic bacteria varied between 3 and 8 nM and there was no significant difference between ZPT and CPT.

3.3.2 Zooplankton (V)

The direct effect on a zooplankton community was assessed on Day 1 in the mesocosm experiment, by adjusting the total grazing potential with number of individuals (Fig 7), giving an EC_{50} of 16 nM.

Figure 7. Effect of ZPT on grazing potential of zooplankton



3.3.3 Benthic microbial communities

The effect of ZPT and CPT was assessed using functional endpoints, such as fluxes of nutrients and protein synthesis. Molecular fingerprinting methods (PCR-DGGE) were used to describe the bacterial community structure. The lowest observed effect concentration (LOEC) for ZPT was found at 0.001 nmol/g dry sediment for the phosphate flux (data not shown) and total DNA content (Fig. 8). The LOEC for CPT was 0.1 nmol/g dry sediment observed in the nitrate flux (Fig. 9), and total DNA content (Fig. 8). Nitrate fluxes increased significantly following additions of both ZPT and CPT (Fig. 9, 10), while the ammonium flux decreased significantly after ZPT addition (Fig. 10), suggesting changes in both nitrification and denitrification processes. The total DNA content decreased significantly following addition of both ZPT and CPT, but at the highest addition of ZPT (10 nmol ZPT/g dry sediment) an increase in total DNA content was found. Increased protein synthesis and bacterial diversity was also observed at this concentration of ZPT, which suggests growth of a tolerant opportunistic species.

Figure 8. Effect of ZPT, light bars, and CPT, dark bars, on total DNA content

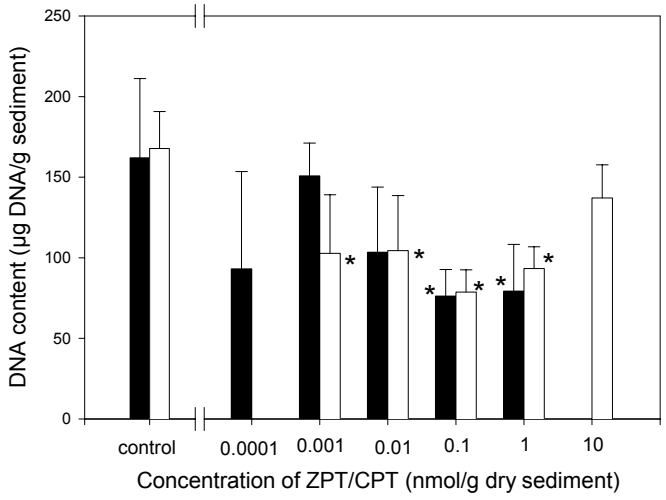


Figure 9. Effect of CPT on nitrate (▲), and ammonium flux (■).

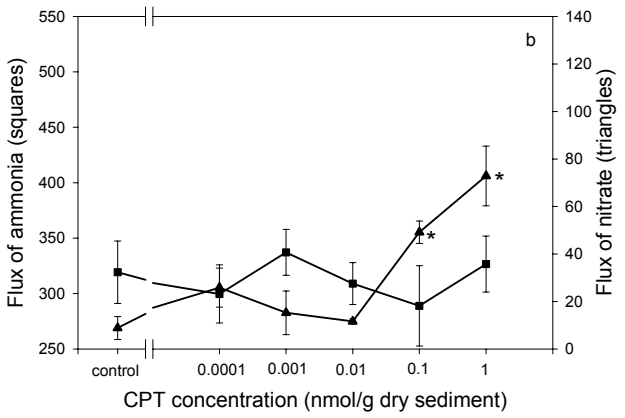
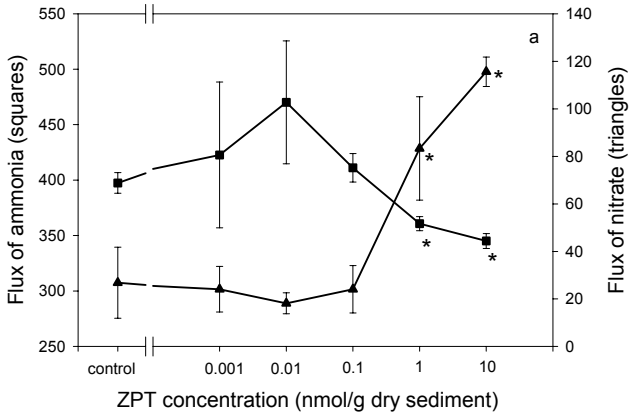


Figure 10. Effect of ZPT on nitrate (▲), and ammonium flux (■).



3.4 Effect of ZPT and CPT on food webs (VII-IX)

Two mesocosm experiments were performed during the project. The effect of a concentration range of ZPT (5-50 nM) was studied in the pelagic system. A single CPT concentration was used to study the simultaneous impact of nutrient addition and CPT on a shallow-water sediment system including benthic algae. The scenario for both mesocosm experiments was a quiet bay during a long-weekend where pleasure boats sail in and anchor up for three-four consecutive nights. The effect of the released ZPT from the boats was then measured during six days in the pelagic system, for CPT during 38 days in the benthic system. In this report, results of the integrated functional response of the two systems are presented, and the more detailed analyses of results are compiled in three articles in preparation

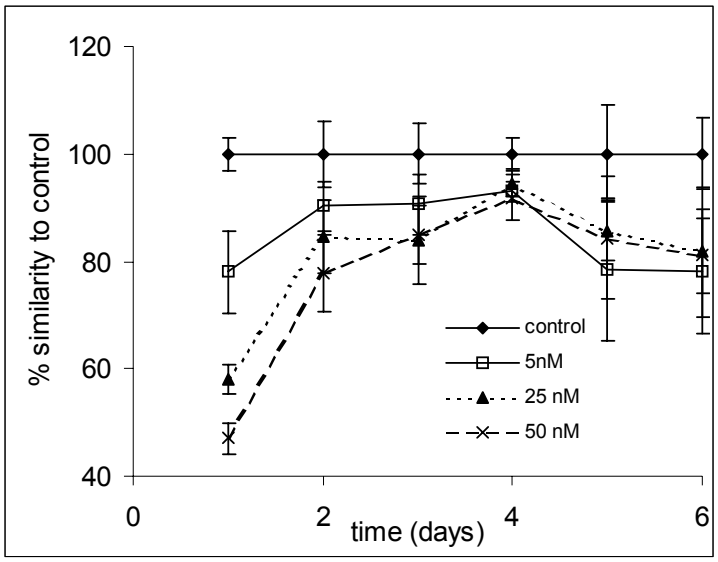
3.4.1 Effects on pelagic foodwebs (VII)

Three functional variables, algal and bacterial production, and zooplankton grazing where combined using the Bray-Curtis index, and the likeness of the ZPT contaminated bags were compared to the controls. There were three replicate bags of all treatments. Significant differences compared to control were analysed using Analysis of Variance with a Student-Newman-Kuels post-hoc test.

ZPT affected the integrated function of the pelagic system (Fig 11). On day 1 there was a significant difference also between the individual ZPT-treatments, which suggests that direct effects were still present. This was also evident when considering primary production and grazing separately. During the following days the dose-response pattern became less clear and disappeared on day four. However, at the end of the experiment, the ZPT treated communities started to diverge significantly from the

control communities. This is most likely due to indirect effects through changes in community structure leading to a different pelagic community compared to the control.

Figure 11. Effect of nominal additions of ZPT on the function of the pelagic food web.



3.4.2 Benthic system (XIII, IX)

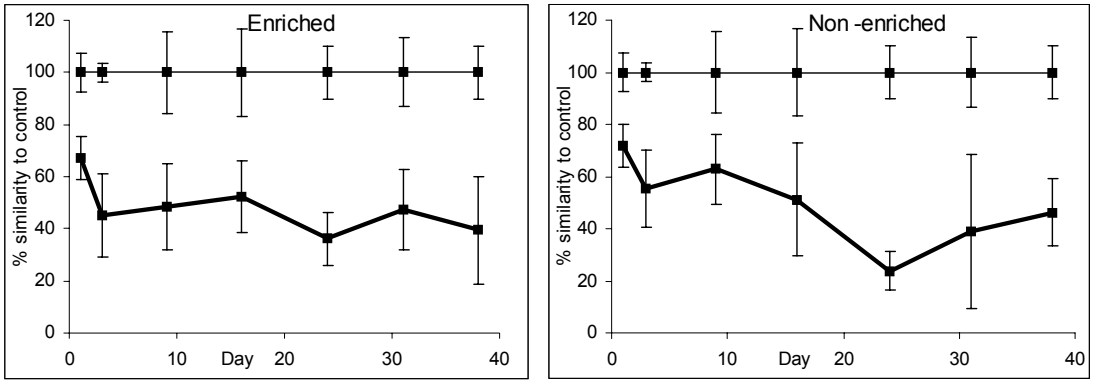
The same integrative approach of functional variables was used in the benthic mesocosm experiment (VIII, IX). The variables used were primary production, community respiration, and nutrient fluxes.

Both in the enriched and the non-enriched sediment the function of the CPT contaminated sediment was significantly different on all days (Fig 12), except day one in the non-enriched. In the non-enriched system the similarity to control was smaller, (down to 20%) than in the enriched (38%). On the other hand, there is an indication of a return towards the control system, which is not evident in the enriched system.

The most remarkable effect in the enriched system was an increase in ammonium flux from the CPT-treated sediment.

Both the mesocosm experiments show that pulse contamination of ZPT can affect the function of the marine ecosystem over a longer period of time.

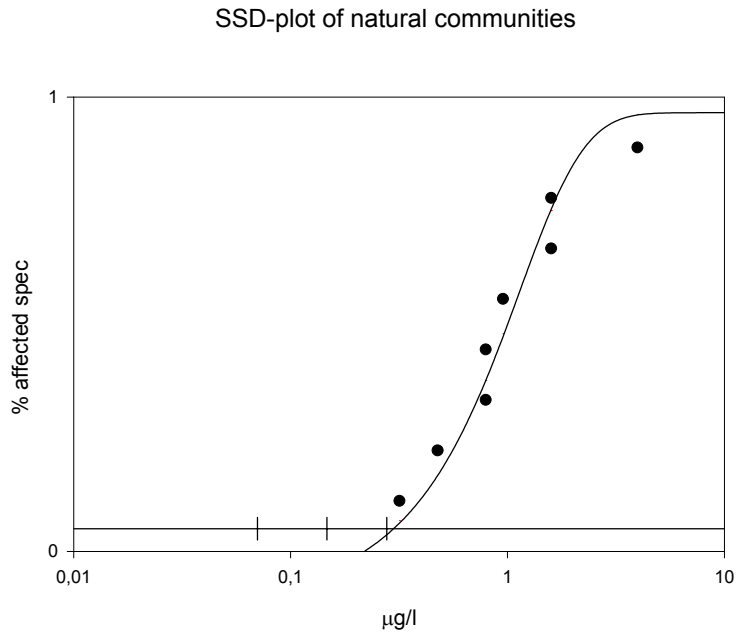
Figure 12. Effect of CPT on the integrated community function in the enriched and non-enriched system.



3.5 Risk assessment

An SSD-curve for ZPT was constructed using eight of the NOECs and LOECs determined in this project for the pelagic system (Fig 13). The NOECs/LOECs represent effects on three trophic levels, bacteria, algae and zooplankton, and are based on nominal concentrations.

Figure 13. SSD-plot using community EC_{50}-values obtained in this project.



The vertical lines represent 95% protection level (n=∞) right, including 50% (middle) and 95% (left) confidence limit for n=8.

The concentration where 95% of the species are protected is 0.9 nM ZPT (0.3 µg/l). Applying the 95% confidence interval that takes number of tests, and thereby the uncertainty into account, the 95% protection level equals 0.2 nM ZPT (0.07 µg/l).

PNEC for CPT in the pelagic system was calculated to 1 pM, using the NOEC for bacteria and algae of 1nM and an application factor of 1000, that is, 200 times lower than for ZPT. It is reasonable that the PNEC for CPT should be considerably lower than for ZPT, since the effects measured in experiments using nominal concentrations of ZPT are effects of substantially lower concentrations of CPT and ZPT due to the transchelation. Effects of a nominal concentration of 3 X 5 nM ZPT in the pelagic mesocosm experiment were still evident after 6 days. Again, the measured concentrations in the highest treatment also show a large tranchelation of ZPT, which means that the effects observed were due to a 5-times lower concentration of CPT.

PNEC for ZPT and CPT in the benthic system was calculated to be 10 fmol/g dry sediment and 1 pmol/g dry sediment respectively, using an application factor of 100.

The uncertainty of the PNECs for the benthic environment is largely due to lack of data representing multiple trophic levels. However, as shown in the mesocosm experiment, a nominal addition of 1 nmol CPT/ g dry sediment, repeated 4 times, gave functional effects that could still be detected after 38 days.

The fate of ZPT and CPT in seawater is highly dependent on light intensity. The short half-lives earlier reported are only valid when the light intensity is high and longer half-lives are to be expected at low light regimes. In a recent article by Mackie et al (2004), no degradation was observed in samples left in ambient light for 10 days. Concentrations of the pyrithione ligand of up to 105 nM were also detected in a marina by the same authors, a concentration much higher than most EC_{50}-values reported here, even when considering that it takes two ligands to form a complex.

There is no evidence of biodegradation in the fate experiments performed within this project, and the most likely loss of ZPT and CPT from the water mass is through adsorbtion to particles and sedimentation. So far there is no method to measure ZPT/CPT in sediments, which makes it hard to verify transport, fate and levels of ZPT and CPT in sediments.

3.6 Conclusions

ZPT present as anti-fouling boost biocide in boat paints is rapidly transchelated into CPT, also when Cu is not present in the paint. Therefore a regulation on the use of ZPT should also include effects of CPT.

ZPT and CPT are quickly broken down at high light intensities, but persist for days in light conditions typical for estuarine and coastal areas.

ZPT and CPT affect bacteria, algae and zooplankton, both through direct effects, but also through indirect effects due to initial changes in community function and structure. These changes lead to changes in system functions such as primary and secondary production as well as changes in nutrient turnover.

The estimated nominal 95% protection level for ZPT in the pelagic environment is 0.2 nM.

It is recommended that CPT should be included in future risk assessment of ZPT.

Resumé

Formålene med dette projekt var:

- At udvikle en analytisk metode til samtidig bestemmelse af zink- og kobber pyrithion (ZPT/CPT)
- At undersøge skæbnen for ZPT/CPT i havvand
- At bestemme toksiciteten af ZPT/CPT overfor marine pelagiske og bentiske samfund
- At udføre en risikovurdering baseret på resultater opnået i dette projekt.

En metode der kombinerer fast-fase ekstraktion (SPE) med HPLC-UV/VIS detektion blev udviklet til samtidig bestemmelse af både ZPT og CPT med en detektionsgrænse på 2 nM for begge stoffer.

Det blev vist at skæbnen for ZPT i havvand i høj grad er afhængig af koncentrationen af kobber og tilstedeværelsen af ligander. Ved naturligt forekommende kobber koncentrationer i havvand vil op ~50% af det tilsatte ZPT (nominelle koncentrationer) blive transcheleret til CPT. Endvidere blev det dokumenteret, at CPT er et stabilt kompleks. Begge stoffer nedbrydes af lys med halveringstider på 7 til 45 minutter afhængigt at lysintensiteten. I svagere belysning og i mørke er stofferne stabile i mere end 48 timer.

Toksiciteten af ZPT og CPT varierer med EC_{50} værdier mellem 1.6-60 nM for pelagiske bakterier, alger og zooplankton samfund. I et 6 dages mesokosmos forsøg blev det vist, at 3 tilsætninger af 5 nM ZPT ved forsøgets start påvirkede funktionen af det pelagiske samfund igennem hele perioden. ZPT og CPT påvirkede næringsomsætningen i et bentisk samfund ved koncentrationer over 0.001 nmol/g tørt sediment. Den mest udtalte effekt blev set på nitrogen omsætningen, hvilket også blev dokumenteret i et 38-dages mesokosmos eksperiment.

Ved brug af en fordeling af følsomme arter (SSD) med 95 % konfidensinterval, kunne en forventet koncentration uden effekt (PNEC) på 0.2 nM, beregnes for ZPT i det pelagiske miljø. Den samme beregning kunne ikke foretages for CPT i det pelagiske miljø, eller for ZPT og CPT i det bentiske miljø pga. et for lille datasæt. PNEC's blev derfor beregnet ved brug af de laveste koncentrationer, hvor der var observerede effekter (LOEC) multipliceret med en passende applikations faktor. PNEC for CPT i det pelagiske miljø blev beregnet til at være 0.001 nM. For ZPT og CPT i det bentiske miljø blev PNEC beregnet til henholdsvis 10 fmol og 1 pmol/g tørt sediment.

Det kan konkluderes, at ZPT fra bådmaling vil blive transcheleret til CPT når det frigives til det marine miljø. CPT er et mere stabilt stof og mere toksisk for det pelagiske samfund, og ydermere er det ikke så nedbrydeligt i lys som tidligere rapporteret. Tilstedeværelsen af ZPT og CPT i det marine miljø kan påvirke vitale funktioner som primær- og sekundær produktionen, såvel som cirkulationen af næringssalte ved meget lavere koncentrationer end den hidtil eneste rapporterede koncentration i det marine miljø.

Det anbefales at inkludere CPT i fremtidige risikoanalyser af ZPT.

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