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Ecotoxicological Assessment of Antifouling Biocides and Nonbiocidal Antifouling Paints

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Preface

This report was prepared in continuation of the project "Preliminary assessment of mechanical cleaning as an alternative to biocide-containing marine bottom paints and assessment of biocide-containing antifoulants with presumed reduced environmental impact". The project was funded by the Council for recycling and cleaner technology and was carried out as a collaboration between the Danish Sailing Association, Hempel's Marine Paints A/S (hereinafter called Hempel) and VKI. Eva Bie Kjær, Hempel, was the project manager.

The present report was prepared by VKI. The objective of this part of the project was to make:

- Ecotoxicological assessments of the biocides, copper, Sea-Nine and zinc pyrithione on the basis of existing data and new laboratory tests and

- Ecotoxicological assessments of leachates from panels applied with the non-biocidal paints used in the project.

The project was followed by a steering committee, which held eight meetings during the project period. The steering committee was composed of the following members:

Frank Jensen (chairman)	Danish EPA
Eva Bie Kjær (secretary)	Hempel's Marine Paints A/S
Torben Madsen	VKI
Steen Wintlev-Jensen	Danish Sailing Association
Carl Gerstrøm	Danish Sailing Association
Anders Stubkjær	National Association of Local Authorities in Denmark
Christian A. Jensen	Association of County Councils in Denmark
Jens A. Jacobsen	National Environmental Research Institute (NERI)
Jan B. Nielsen	Agency for Environmental Protection (AEP)
G. Høpner Petersen	The Zoological Museum, University of Copenhagen
Peter Györkös	Danish EPA
Alf Aagaard	Danish EPA
Tim Blangsted	Hempel's Marine Paints A/S
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Johs. Egede Olsen	Hempel's Marine Paints A/S
Susanne Holm Faarbæk	Hempel's Marine Paints A/S

Furthermore, Pia Ølgaard Nielsen, Danish EPA, participated in the finalization phase of the project.

We thank the members of the steering committee for a constructive co-operation during the project.

In connection with the preparation of this report, Nordox (copper), Rohm and Haas (Sea-Nine) and Arch Chemicals (zinc pyrithione) were contacted. We would like to thank these companies for their co-operation and a constructive dialogue.

The English translation was made by Tove Krogsbøll Holt, VKI.

Hørsholm, 1 November, 1999,
Torben Madsen, VKI

Summary

The objective of this investigation was to assess the environmental hazards of the active substances, copper, 4,5-dichloro-2-n-octyl-4-isothiazolin-3-on (DCOI) and zinc pyrithione and of substances leaching from non-biocidal antifouling paints.

The bioavailability of copper is the key parameter for the assessment of the toxicity of the metal in the aquatic environment. Sequestration of copper to organic substances normally reduces their bioavailability, however, this sequestration is apparently dependent on the composition of the organic matter. The bioavailability of copper in aquatic sediments depends on the speciation of the metal, on the sediment and on the physiology and food selection of the exposed organisms. It has been demonstrated that metals sequestrated to easily digested food are absorbed more easily by aquatic organisms than metals sequestrated to indigestible food. Bioavailable copper is very toxic to aquatic organisms. A permanent immobilization of copper may occur only by sequestration to undisturbed, anoxic sediments. Harbour sediments are usually anoxic and have a high content of sulfides that sequestrate to copper. Therefore, the bioavailability of copper in harbour sediments is expected to be low. Copper may be released by disposal of sediment and, on the Danish dumping sites, the sediment is usually scattered by water current and waves. As an element, copper is not degradable. The potential toxic effect of copper on the aquatic environment is reduced by sequestration to organic compounds and sediments, which means that the actual bioavailability of copper is low. Disturbances of the sediment, and the consequent changes in the oxygen conditions, may remobilize sequestrated copper, and such changes may cause effects on sensitive organisms in the vicinity of harbour areas and dumping sites.

DCOI is rapidly transformed into metabolites in seawater, where half-lives of 11 and 14 hours were found. The transformation of DCOI is very much quicker in aquatic sediment as half-lives of less than 1 hour have been found. The biodegradation of DCOI was examined in two Danish marine sediments with different textures. The mineralization into CO_2 in a clayey and sandy sediment represented 13% and 24%, respectively, of the added ^{14}C during an aerobic incubation of 42 days at a temperature of 15°C. The mineralization under anaerobic, sulfate-reducing conditions was examined in the clayey sediment and represented 14% of the added ^{14}C after an incubation of 56 days at a temperature of 15°C. DCOI is very toxic to aquatic organisms as the lowest effect concentrations (EC/LC50) are lower than 10 µg/L. The aquatic toxicity of the stable metabolite, N-(n-octyl) malonamic acid, is several orders of magnitude lower as the lowest effect concentrations (LC50) are estimated to be between 90 and 160 mg/L. Laboratory tests performed with seawater and sediment containing DCOI showed that degradation and sorption eliminated the acute aquatic toxicity of water samples in less than one day. On the basis of available data regarding effects on aquatic organisms, Predicted No-Effect Concentrations (PNEC) were estimated at 0.06 µg/L for DCOI and 90 µg/L for N-(n-octyl) malonamic acid. PNEC for N-(octyl) malonamic

acid is considered to be representative of the other metabolites from the transformation of DCOI. In order to calculate exposure concentrations (Predicted Environmental Concentration, PEC), a model was set up on the basis of principles recommended by the EU "Technical Guidance Document" for risk assessment. The model used was not validated as regards concentrations in harbours and navigation routes. The basis of the calculation of PEC was defined by way of realistic worst-case scenarios, which means that, in practice, the calculated PEC values are seldom exceeded. The highest calculated exposure concentrations for DCOI were PEC (water), which was 0.52 µg/L in a pleasure craft harbour and 0.006 µg/L in a busy navigation route outside the harbour. As for the metabolites, PEC (water) was 2.2 µg/L in the pleasure craft harbour and 0.047 µg/L in the navigation route outside the harbour. On the basis of the values for PNEC and PEC, the risk quotients (PEC/PNEC) for DCOI were calculated at 8.7 for the pleasure craft harbour and at 0.1 for the navigation route outside the harbour. The calculated risk quotients of the total amount of metabolites from the transformation of DCOI were 0.02 in the pleasure craft harbour and 0.0005 in the navigation route outside the harbour. Because of the short half-life in water and sediment, DCOI will most likely be rapidly eliminated as soon as the pleasure craft are taken out of the water at the end of the sailing season.

By photolysis and biodegradation, zinc pyrithione is transformed very rapidly. Analyses of the degradation of zinc pyrithione in two Danish sediments showed that mineralization into CO_2 in a clayey and a sandy sediment represented 2.8 and 5%, respectively, of the added ^{14}C under aerobic conditions. The mineralization under anaerobic, sulfate-reducing conditions represented 3.5% of the added ^{14}C in the clayey sediment. Like DCOI, zinc pyrithione is very toxic to aquatic organisms as the lowest effect concentrations (EC/LC50) are less than 10 µg/L. The toxicity of the stable metabolites, omadine sulfonic acid and pyridine sulfonic acid, is several orders of magnitude lower as the lowest effect concentrations (LC50) of these compounds are 36 and 29 mg/L, respectively. Laboratory tests performed with seawater and sediment containing zinc pyrithione showed that degradation and sorption eliminated the acute aquatic toxicity of water samples in less than one day. The available data regarding effects on aquatic organisms form the basis of an estimation of PNEC values at 0.1 µg/L for zinc pyrithione and 30 µg/L for stable metabolites represented by pyridine sulfonic acid. By using the same realistic worst-case scenarios as for DCOI, the highest exposure concentrations (PEC, water) of zinc pyrithione were calculated to be between 0.56 and 1.7 µg/L for the pleasure craft harbour and between 0.0053 and 0.022 µg/L for the navigation route outside the harbour. For the total amount of metabolites, PEC (sediment, pore water) was between 1.6 and 2.7 µg/L in the pleasure craft harbour and between 0.037 and 0.042 µg/L in the navigation route. On the basis of the values for PNEC and PEC, the risk quotients (PEC/PNEC) for zinc pyrithione were calculated to be between 5.6 and 17 for the pleasure craft harbour and between 0.05 and 0.22 for the navigation route. The risk quotients for the total amount of metabolites from the transformation of zinc pyrithione were 0.05-0.09 for the pleasure craft harbour and 0.0012-0.0014 for the navigation route. The lowest risk quotients are based on PEC values, for which transformation of zinc pyrithione by photolysis is included in the calculations. The high-

est risk quotients are, however, based on PEC values in which transformation by photolysis is not taken into account. Like DCOI, zinc pyrithione will most likely be rapidly eliminated as soon as the pleasure craft are taken out of the water at the end of the sailing season, in consequence of the short half-life in water and sediment.

Effects on aquatic organisms of water samples from leaching tests with non-biocidal paints, the epoxy-based High Protect 35651 and the experimental silicone-containing 86330 paint, were tested on the marine green alga, *Skeletonema costatum*, and on the marine crustacean, *Acartia tonsa*. A similar test was performed with an organotin-based antifouling paint, Hempel's Antifouling Nautic 76800. Water samples from the leaching test with High Protect 35651 caused no inhibition of growth of *S. costatum*, and chronic effects on *A. tonsa* were observed only in undiluted leachate (No-Effect Concentration, NOEC = 100 mL/L). Water samples from the leaching test with the experimental 86330 paint showed toxicity to *S. costatum* and in acute and chronic tests with *A. tonsa* (NOEC, acute <100 mL/L; NOEC, chronic <10 mL/L). However, some factors seem to indicate that variations in production and in application may have an effect on the leaching of substances from this type of paint. These indications should be investigated further before a final assessment of the environmental properties of the paint is made. The leachates of both non-biocidal paints showed a significantly lower effect than water samples from similar tests with the organotin-based paint, Hempel's Antifouling Nautic 76800. Leachates from the paints, High Protect 35651 and the experimental 86330, caused chronic NOEC values for *A. tonsa*, which were at least 1,000 and 100 times higher, respectively, than the corresponding NOEC values for leachates from the organotin-based paint.

1 Introduction

The present study includes ecotoxicological properties and risk assessment with relation to active substances in antifouling paints and to chemical compounds leaching from non-biocidal paints. Recently, the properties of a number of active substances in marine bottom paints for pleasure craft and larger vessels with regard to health and the environment were assessed in the report "Survey and assessment of antifouling products for pleasure craft in Denmark" (Madsen *et al*. 1998) prepared by CETOX (Centre for Integrated Environment and Toxicology) and the National Environmental Research Institute (NERI). These assessments (Madsen *et al*. 1998) were completed in three months, which did not allow a more detailed examination of the available information on the active substances. On the basis of the recommendations in the "Survey and assessment of antifouling products for pleasure craft in Denmark", the active substances, copper, 4,5-dichloro-2-n-octyl-4-isothiazolin-3-on (DCOI) and zinc pyrithione were selected for a more careful assessment of their environmental hazard.

The assessment of copper is based on a study of available literature focusing the relations between the speciation, bioavailability and ecotoxicity of copper.

The very scanty literature published on DCOI and zinc pyrithione has necessitated the inclusion of investigations carried out by the manufacturers, Rohm and Haas and Arch Chemicals. This material was supplemented with new investigations of the biodegradability of the two substances in Danish coastal sediments under aerobic and anaerobic conditions. Furthermore, the effect of degradation and sorption to sediment on the aquatic toxicity of the two biocides was illustrated in laboratory tests with the marine crustacean *Acartia tonsa*. The information on the degradation, distribution and toxicity of the biocides in the marine environment was used for a risk assessment based on the following two scenarios: a Danish pleasure craft harbour and a busy navigation route. The two scenarios are defined in such a way that the estimated exposure concentrations (Predicted Environmental Concentration, PEC) are expected to be realistically conservative resulting in the estimated PEC values only seldom being exceeded in practice (see Appendix 1 for a more detailed description of the calculation of PEC).

The ecotoxicological properties of an epoxy-based and a silicone-based paint without biocides were examined in studies of the toxicity of water samples from leaching tests. In these tests, the ratio of painted area to liquid volume was 13-14 times higher than this ratio is expected to be in a harbour with a large amount of pleasure craft. The ecotoxicological studies included tests with the marine green alga *Skeletonema costatum* and tests for acute and chronic toxicity to *A. tonsa*.

2 Copper

2.1 Copper concentrations measured in the vicinity of pleasure craft harbours

Denmark
In the Egå Marina at Århus Bay, the copper content in the harbour sediment was 150-600 mg/kg dry weight at a distance of 5-10 m from discharge from consolidated areas, decreasing to 53-120 mg/kg dry weight at 30 m from discharge (Jensen and Heslop 1997a). By way of comparison, the copper content in sediment in Århus Bay was 25-50 mg/kg dry matter. Finally, in the same investigation, water concentrations of copper of 2.4 µg/L were measured in Studstrup and of 13 µg/L in the pleasure craft harbour of Marselisborg but these analyses are stated to be somewhat doubtful.

The copper content in harbour sediments from other localities in the area has also been analysed. The highest concentrations were found at the slipways in Bønnerup harbour (7,000-8,000 mg/kg dry weight), which is a combination of a pleasure craft and fishing harbour, and in Århus fishing port (1,600-2,400 mg/kg dry weight). The copper concentrations in the basins were 15-70 mg/kg dry weight in Bønnerup harbour and 100-400 mg/kg dry weight in Århus fishing port. In the sediment from Ebeltoft, Grenå and Hov Bedding, the concentrations were 280, 490 and 1,200 mg/kg dry weight, respectively (Jensen and Heslop 1997b).

The county of Funen has measured copper contents in sediment from 5 to 110 mg/kg dry weight in harbours (The County of Funen 1999). From the Little Belt, dated sediment cores have been analysed so that the temporal development of the copper content might be assessed. The measurements in the sediment cores showed a significantly increasing content of copper in the vicinity of Als, an upward trend at four stations and constant/varying concentrations at four other stations. The copper content in the cores varied from 19 to 46 mg/kg dry weight.

Sweden
In 1990 and 1993, the copper concentrations in water, sediment and aquatic plants were measured in the skerries of Stockholm (Greger and Kautsky 1990 and 1993, cf. Bard 1997). The measurements showed a significantly higher content of copper in the sediments in the vicinity of pleasure craft harbours and areas with heavy pleasure craft traffic. Copper concentrations of up to 1,3000 mg/kg dry weight were measured in sediments. Compared to less contaminated areas, increased copper concentrations were also found in aquatic plants. Similar measurements were performed in the vicinity of the Bullandö Marina, which is also situated in the skerries of Stockholm (Öhrn 1995, cf. Bard 1997). In April, 1993, before the start of the sailing season, the copper content in the water was 0.8-1.0 µg/L while, in June, it was 3.0-3.8 µg/L. The copper content in the sediment at the Bullandö Marina was only slightly increased when compared to the reference stations, at which the copper content was 30 mg/kg dry weight.

France
Measurements performed by the French authorities in the Arcachon Bay at the Atlantic coast from 1979-1991 showed an increase in the copper content in oysters (Claisse and Alzieu 1993). The increase was significant at two of four stations from 1982 to 1991. The increase in the copper content in oysters coincides with an increased consumption of copper-based antifouling products when the use of organotin TBT was regulated in 1982. The increase in the copper content was highest and significant in the oysters from the two stations in the inner bay. The increase was less and not significant at the stations in the outer bay, which may be explained by the fact that the rate of water renewal at these stations is higher than in the inner bay. The French measurements are unique because of the long time series and the extensive measuring programme carried out. Several conditions may influence the accumulation of copper in organisms and the direct relation between an increased copper content in water and an increased content of copper in oysters is not stated in the French studies.

Background concentrations
Compared to the above stated concentrations, the background concentration of copper is given as 25-35 mg/kg dry weight in Danish sediments and as 0.5-1.5 µg/L in seawater (Madsen *et al.* 1998). Swedish studies give a copper content of 0.3-0.8 µg/L in water from the Baltic Sea and of 0.2 µg/L in water from the Kattegat. The Swedish background values for sediment are given as 10-40 mg/kg dry weight in the Baltic Sea (Debourg *et al.* 1993).

In harbours and neighbouring waters, concentrations above normal of copper have thus been found in both sediments and water samples, i.e. in pleasure craft harbours up to a factor of 30 times the background concentration in sediments and up to a factor of 10-15 times the background concentration in water.

2.2 Transformation and bioavailability of copper in water and sediment

Bioavailability
Contrary to organic compounds used in antifouling products, metals are not degradable. In water, copper will occur dissolved in the water as well as sequestrated to particles. Copper may, however, occur in different forms (species) depending on e.g. the salinity, pH, content of organic matter, etc. of the water. The speciation of the copper decides whether live organisms can take it up (whether it is bioavailable) and thereby whether copper is toxic to the organisms.

It is often accepted that primarily the free copper ions (Cu^{2+}) may pass cell membranes and thus constitute the bioavailable and toxic part of copper (Campbell 1995). It has, however, been demonstrated that other

copper ions and lipid-bound copper may also pass cell membranes and may thus also be bioavailable (Allen 1993).

Sequestration
In seawater and fresh water, it is a well-known fact that sequestration of copper to organic substances is predominant (Bruland *et al.* 1991), which typically reduces the bioavailability of copper (Lewis 1995). It is, however, not that simple as there are differences in the sequestering properties of organic substances in relation to copper. E.g., Garvey *et al.* (1991) have demonstrated that humic acid reduces the toxicity of copper while fulvic acid does not have a similar effect. In all probability, the sequestration of copper to organic substances is very specific (Wells *et al.* 1998). It has been demonstrated that planktonic algae can excrete organic substances binding copper (Brand *et al.* 1986, cf. Wells *et al.* 1998). Planktonic algae that are exposed to increased copper concentrations may excrete such copper-binding substances (ligands) thereby reducing the bioavailability and potential toxic effect of copper (Wangersky 1986, cf. Paulson *et al.* 1994). The formation of colloids and subsequent aggregation, which eliminates copper from the water phase and transports it to the sediment, is considered another effect of the organic ligands (Wells *et al.* 1998).

Sedimentation, speciation and bioavailability
The transportation of copper to sediments will typically proceed via sedimentation of copper built into or adsorbed to particles (micro algae, clay particles, etc.). In open waters, the sedimentation of copper will primarily be controlled by the sedimentation of planktonic algae (Wangersky 1986, cf. Paulson *et al.* 1994), to which copper is sorbed and/or built in. In the sediment, a large number of chemical and biological transformations of importance to the speciation of copper will take place, including oxidation/reduction, dissolution/leaching and sequestration. The transformations will be controlled by sediment type (i.a. grain size and content of organic matter), digging and filtering activity of sediment-living invertebrates (bioturbation) and the oxygen conditions in the water and in the sediment.

Speciation of copper in sediments is controlled by dynamic and reversible processes (Calmano *et al.* 1990). E.g., copper sequestrated to reduced compounds (organic matter and sulfides) may be released from the sediment to the above water due to oxidation as a result of resuspension or bioturbation (Petersen *et al.* 1997; Ciceri *et al.* 1992; Westerlund *et al.* 1986), or a redistribution may take place sequestrating copper in oxidized compounds instead (e.g., ferric or manganese oxides and hydroxides). These compounds are considered unstable while sulfides and organic substances are characterized as more stable (Förstner *et al.* 1990; Calmano *et al.* 1990).

In anoxic sediments, e.g., in fine-grained sediments with high content of organic matter, copper will typically sorb to sulfides and organic matter while, at good oxygen conditions, copper will typically be sequestrated to compounds like ferric oxides, manganese oxides and hydroxides. Metal sulfides are recalcitrant but relatively easily and rapidly oxidized at good oxygen conditions (Förstner 1985).

The bioavailability of copper in sediments is an extremely complex phenomenon that does not depend only on the speciation and the sediment but also on the physiology and food choice of the exposed organisms (Slotton and Reuter 1995). It has been demonstrated that the bioavailability may be specific for individual species and that variations occur within the same species related to age, sex and size of the organism (Lewis 1995). Furthermore, it has been shown that the organisms take up more easily metals sorbed to easily digested food than metals sorbed to food hard to digest (Wang and Fisher 1996). Digestive enzymes in the intestine ensure a high utilization of the food (Forbes *et al.* 1998), which may also result in an increased uptake of copper from sediment.

Assessment of bioavailability

Increased concentrations of metals in aquatic sediments are widespread and the authorities must often consider whether the increased concentrations imply a risk of adverse effects on the ecosystem. Unfortunately, this problem is versatile as the bioavailability of metals varies a great deal in different sediments (Luoma 1989).

In attempts to predict the bioavailability of metals in sediments on the basis of chemical analyses, various extraction and fractionation guidelines have been developed for analyses of copper sequestrated to carbonates, manganese oxides, ferric oxides and organic substances (e.g., Förstner 1985). The problem in these extraction and fractionation guidelines is, however, to interpret which species are bioavailable. On the basis of investigations showing a correlation between the cadmium concentration in pore water in sediment and the acute toxicity of cadmium-added sediment to an amphipod (crustacean living in holes in the sediment), the assumption that the content in the pore water represented the bioavailable part of cadmium was proposed (Ankley *et al.* 1994).

In similar investigations of the effects of cadmium on other amphipods, Di Toro *et al.* (1990 cf. Ankley 1996) have demonstrated that the acute toxicity of cadmium may be predicted on the basis of the content of acid volatile sulfide (AVS). AVS is the fraction of sulfide in the sediment that is extractable with cold hydrochloric acid and is a measurement for the capacity of the sediment to sequestrate metals. If the sequestering capacity is exceeded, the concentration of cadmium in the sediment is increased and the amphipods die. Attempts have been made to use AVS for determining the bioavailability to amphipods of copper in sediments (Ankley *et al.* 1993, cf. Ankley 1996). AVS significantly overestimated the bioavailability of copper, which was explained by the presence of another sequestration phase than AVS.

The concept is based on the assumption that only the content in the pore water is available combined with a steady state consideration. This assumption cannot be expected to apply to sediment reworkers that swallow whole sediment particles and have digestive enzymes in the intestine for degradation of organic substances. Furthermore, the AVS method is limited in as much as it was developed to determine only the actual bioavailable fraction of metals and thus does not give a measurement for

the potentially bioavailable fraction that may eventually become bioavailable, e.g., in relation to a change in oxygen conditions.

No simple method based on chemical analysis has thus yet been found with which you can assess how large a part of the copper - especially in sediments - that is bioavailable and it is questionable under which conditions (sediment type, oxygen conditions) and for which organisms, the AVS method is valid.

2.3 Release and sequestration of copper in sediments

Dredging and dumping
In connection with resuspension of sediments, it was demonstrated that a considerable part of the sorbed copper may be released from the sediment. In laboratory experiments under natural conditions, it was found that up to 2% of the particle-sequestrated copper may be released to the water at resuspension (Petersen *et al.* 1997). An investigation of sediments at dumping sites at Cleveland Bay before and after a dredging and dumping concludes that copper in the sediment is sequestrated in labile fractions, which are potentially bioavailable and which are easily spread at resuspension (Reichelt and Jones 1994).

Measured release
It has been demonstrated that metals (especially copper) may be released from sediments to the water above sediments with oxidized surface (Luoma 1989). Release of copper from sediments was observed at the North American coast (Boyle *et al.* 1981 cf. Luoma 1989), in the North Sea (Kremling 1983, cf. Luoma 1989) and in several places in coastal areas (Windom *et al.* 1983, cf. Luoma 1989). *Fencing* experiments have shown that the copper release is larger from copper-contaminated sediments than from uncontaminated sediments (Hunt and Smith 1983).

Bioturbation
Bioturbation in sediments may be of great importance to remobilization of metals in the sediment. Sediment-living animals are characterized in relation to their search for food. Sediment reworkers swallow sand, mud and water without previous separation. The organic content in sediment is low compared to other types of food. In order to compensate for this, the sediment reworkers have to consume large amounts of sediment, some ingest 8-10 times their body weight a day. Sediment reworkers typically rummage about a lot in the sediment, which may mobilize buried metals. Suspension feeders feed on particles, which they filter from a current that they create between the water above the sediment and the sediment itself thereby increasing the exchange of substances above the sediment-water surface. All in all, the animals increase the contact between the sediment and the above water. Considering that the net deposition in marine sediments is only a few millimetres a year, the animals may contribute to bringing up old sediment to the surface and new sediment down to underlying layers. Peterson *et al.* (1996) found that bioturbation could significantly increase the bioavailability of metals in sediments through oxidation of sulfide compounds. They found that

metal/sulfide complexes were relatively unstable towards the oxidation taking place in connection with bioturbation.

The replacement of sulfide-containing water by oxygen-containing water will also remobilize sulfide-sequestrated metals (Emerson *et al.* 1984, cf. Förstner *et al.* 1990) as the oxygen content of the water above the sediment is of great importance to the sequestration and release of metals from sediments. Measurements showed that, during summer periods with poor oxygen conditions in the harbour at Corpou Christi Bay, cadmium was sequestrated to sulfides while measurements showed a release during winter months with good oxygen conditions (Holms *et al.* 1974, cf. Förstner *et al.* 1990). There is thus no immediate reason to suppose that copper sequestrated in sulfides may not become bioavailable on a long view.

2.4 Bioaccumulation and aquatic toxicity

2.4.1 Bioaccumulation

Copper is a micro-nutrient that live organisms need in small doses. Higher animals like fish can regulate the content of copper in their organism and, to some extent, they can accumulate copper in the lever but not in the muscles. If copper exists in the surroundings or in the food in very low concentrations, an accumulation may be the result of the organism utilizing copper as a nutrient. The interpretation of bioconcentration factors (BCF values) for an essential micro-nutrient like copper is thus difficult and no information is available in the investigations quoted on concentrations of copper and the requirements for copper of the organisms used. In short-term studies with algae (½-2 days), BCF values were measured at 1-40. In long-term studies with insects and mussels, the BCF values were considerably higher: In a 28-day study with mosquito larvae - in all probability in sediment - a BCF value of 5,830 was found; furthermore, BCF values of 5,000-10,000 were found in mussels during a period of 2-3 years (AQUIRE 1999). BCF values between 400 and 90,000 have been found in plankton and some lower organisms (Debourg *et al*. 1993).

2.4.2 Toxicity to aquatic organisms

Aquatic organisms

Table 2.1 gives an overview of the toxicity of copper to various groups of aquatic organisms measured in single-species laboratory tests. Table 2.1 illustrates that copper is very toxic with effect concentrations from only a few micrograms of copper per litre.

Table 2.1
Ecotoxicological data on effects of copper on aquatic organisms [A].

Taxonomic group	End point	Exposure time	Results [mg/L]
Algae	LC50/EC50 growth	1h-5d	0.01-0.55
Algae	NOEC*	2-3d	0.009-0.049
Algae	NOEC	19-20d	0.01
Crustaceans	LC50	2-4d	0.0075-0.32
Crustaceans	LC50 (dissolved Cu)	2d	0.019-0.084
Crustaceans	EC50 (reproduction)	7d	0.01-0.02
Crustaceans	NOEC (reproduction)	7-10d	0.04-0.22
Fish	LC50	4d	0.024-21
Fish	LC50 (dissolved Cu)	4d	0.098-0.60
Fish	EC50 (anormalities + hatching)	12d	0.075-0.19
Fish	NOEC (survival + hatching)	12-42d	0.01-0.12
Insects	LC50	1-10d	23.6-0.20
Molluscs (snails, mussels)	LC50	1-4d	0.03-9.3
Molluscs (mussels)	EC50 (closing)	1-6d	0.04-<0.02
Echinoderm	NOEC (reproduction + development)	½-1h	0.0031-0.066
Rotifers	LC50	1d	0.063
Rotifers	NOEC (movement)	3h	0.006
Worms	LC50	28d	0.044

[A]: AQUIRE 1999. Data of high quality have been selected among several hundred results from the AQUIRE database. The results are given as nominal, total concentrations of copper, and in general, the speciation is not given.

* The highest concentration at which no effects were observed (NOEC, No Observed Effect Concentration).

In Denmark, quality criteria have been specified for copper in fresh water and seawater of 12 µg/L and 2.9 µg/L, respectively (The Danish Ministry of Environment and Energy, 1996). It is, however, stated that the criteria are based on data that have not finally been quality assessed. On the basis of 65 single-species laboratory tests with marine organisms, a PNEC value for copper has been calculated at 5.6 µg/L (Hall and Anderson 1998). The calculation method used is based on the distribution of the sensitivity of the organisms tested, and the calculated PNEC value theo-

retically protects 95% of the species with 95% confidence. This is, however, twice the lowest NOEC value in Table 2.1 (0.0031 mg/L = 3.1 µg/L).

Ecosystem studies
Effects on natural planktonic algae have been measured at only a few micrograms of copper per litre. Chronic effects of copper on planktonic algae in marine ecosystem modelling were demonstrated from 1 µg/L (Gustavson *et al.* 1999). Comprehensive and well-documented experiments with micro algae and copper (Brand *et al.* 1986) show that, even at very low concentrations, copper may inhibit the reproduction of algae. In these studies, the effect of copper on 38 different clones of marine planktonic algae was examined in water, in which the metal-chelating properties were known. In this scientific article, the toxic effect of copper on the reproduction is related to the activity of free copper ions and it is concluded that copper may inhibit the reproduction of sensitive algal species even in uncontaminated waters where the copper concentration is low (0.1-0.2 µg/L). These studies distinguish themselves i.a. by relating the effect of copper to the activity of the free copper ions in the water and not only to the total copper concentration as so many other studies do.

Swedish investigations have shown copper concentrations of up to 3 µg/L in the vicinity of pleasure craft harbours in areas in which the background concentration of copper was 0.8-0.5 µg/L. At the actual copper concentrations, no effects on planktonic algae were found (Wängberg *et al.* 1995).

Bottom-living organisms
The results from the tests with organisms living in the sediment and at the bottom are presented in Table 2.2.

Table 2.2
Ecotoxicological data on effects of copper on bottom-living organisms.

Taxonomic group	End point	Exposure time	Results
Insects[A]	LC50	10d	0.20 mg/L
Insects[2]	LC50	10d	1,026 mg/kg DM[3]
Crustaceans[A]	LC50	10d	0.028 mg/L
Crustaceans[2]	LC50	14d	247 mg/kg DM[3]
Worms[A]	LC50	28d	0.044 mg/L
Crustaceans[1]	LC25	28d	998 mg/kg DM[3]
Crustaceans[1]	EC25 (growth)	28d	330 mg/kg DM[3]
Crustaceans[2]	LC50	10d	185 mg Cu_2O/kg DM ≈ 164 mg Cu/kg DM[3]

[A] AQUIRE 1999; [1] Borgmann and Norwood 1997; [2] Bard 1997; [3] DM = dry matter.

The three studies, in which the concentration is given in mg/L, may have been conducted in water without sediment. The other studies indicate that copper in sediment may cause effects on sediment-living animals at

concentrations exceeding 100 mg/kg (Table 2.2). This is well over twice as much as the highest of the background concentrations stated but much lower than the concentrations measured in harbour sediments.

2.5 Assessment of copper

Copper is an element and is thus not degradable. Copper can be "removed" from the aquatic environment by sorbing to and being buried in sediments outside the reach of organisms. Seen in a geological time perspective, large amounts of heavy metals have been discharged into the sea without causing serious ecotoxic effects as the sequestration of metals to the sediment has prevented this.

In the aquatic environment, copper will sorb to inorganic and organic substances and particles. These sequestering conditions contribute to the occurrence of various species of copper. It is uncertain which species are bioavailable, and no reliable measuring methods for assessment of the size of the bioavailable fraction are available. Furthermore, the bioavailability of copper is not constant and must be view in different time perspectives. A differentiation must thus be made between the actual and the potential bioavailability. The actual bioavailability will typically be considerably less than the potential bioavailability. Furthermore, bioavailability is species specific and may also depend on physiology, nutrition, age, size and sex of the organisms in question.

A permanent immobilization of copper can only occur at sequestration to particles and subsequent sedimentation on sediments with poor oxygen conditions with a permanent presence of sulfides. In reality, such conditions only exist in areas without resuspension, i.e., without bioturbation (macro fauna) and fishery with bottom trawl. The extension of these sediment types in Denmark is limited to a few holes in i.a. the archipelago south of Funen. Copper sorbed to particles that settle on sediments rich in oxygen with bioturbation will probably stay in the biological systems for many year. In deep waters, nutrients and trace metals, including copper, stay in the water phase as the particles attain to transformation in the water column before they reach the surface of the sediment.

Harbour sediments are typically anoxic and have a high content of sulfides which will bind copper. Therefore, copper is expected to be relatively strongly sequestrated in harbour sediments. A release from the sediment at resuspension induced by e.g. the propellers of ships can, however, not be excluded. At regular intervals, the sediments in the harbours are dredged and the material is dumped at selected localities. Copper may be released at dumping and, typically for dumping sites in Denmark, the sediment will subsequently be spread by current and wave action. Stable dumping sites are difficult to find in Denmark and copper in the harbour sediments must be expected to be spread over large areas in connection with dumping.

The toxicity of copper is dependent on the speciation and the bioavailability of copper in the water. The fact that copper is a micro-nutrient combined with the fact that the content of metal chelating substances

may greatly vary in time and space and that the sensitivity of different species varies much, make it very difficult to compare different investigations. The concentrations, in which effects are measured in laboratory tests, are generally higher than the background concentrations stated for copper in the environment but concentrations measured in and in the vicinity of harbours are at the same level as or higher than concentrations in which effects have been measured. The organisms that are most sensitive to copper are algae and crustaceans and, in ecosystem tests of the sensitivity of algae, effects were measured at copper concentrations on the same level as background concentrations.

3 Sea-Nine

This chapter contains an ecotoxicological assessment of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-on (DCOI), which is the active substance in Sea-Nine 211.

3.1 **Physico-chemical properties**

Table 3.1 summarizes the physico-chemical properties of DCOI.

Table 3.1
Physico-chemical properties of DCOI.

CAS No.	64359-81-5
Synonyms	4,5-dichloro-2-n-octyl-4-isothiazolin-3-on 4,5-dichloro-2-n-octyl-3(2H)-isothiazolone RH-5287
Classification	-
Molecular formula	$C_{11}H_{17}C_{l2}NOS$
Molar weight	282.23
Water solubility (20°C)	6.5 mg/L[1]
Vapour pressure (25°C)	$7.4 \cdot 10^{-6}$ mm Hg[1]
Octanol-water partition coefficient (log K_{ow})	2.8 (measured)[2]
Organic carbon-water partition coefficient (log K_{oc})	3.2 (measured)[3]

[1] Shade *et al.* 1993; [2] Jacobson 1993; [3] Howard 1991.

3.2 **Biodegradation of DCOI in the aquatic environment**

3.2.1 **Primary degradation in seawater**

Several studies have been made of the degradation of DCOI in the aquatic environment. It is stated that abiotic processes progress with half-lives of 9-12.5 days for hydrolysis and 13.4 days for photolysis. Biological processes are, however, of greater importance to the transformation of DCOI. Studies described by Shade *et al.* (1993) have shown that DCOI (10 µg/L) is transformed with a half-life of 11 hours in seawater with $7 \cdot 10^4$ bacteria/mL (total number of bacteria determined by counting in a microscope). Parallel tests with seawater samples with a lower number of bacteria (<1,000 bacteria/mL) resulted in longer half-lives for DCOI (Shade *et al.* 1993). These tests are not considered relevant as the biological activity of the seawater was unrealistically low. In a recent study, the transformation rate of DCOI (10 µg/L) was determined in seawater from the pleasure craft harbour of Jyllinge. The study demonstrated that 7.1% of the DCOI added remained after 72 hours at a

temperature of 12°C (Jacobson and Kramer 1999). On the basis of the measured concentrations of DCOI (Jacobson and Kramer 1999), the biological half-life may be estimated at 14 hours at 12°C (Appendix 1, Section 2.4.1).

3.2.2 **Mineralization and metabolites in aerobic sediment**

Transformation of DCOI

The aerobic half-life of DCOI is very short in marine systems with sediment and seawater. Analyses of samples from laboratory tests with sediment and seawater showed that DCOI was rapidly transformed into other chemical compounds. In bottles with a dosage of 0.05 mg/kg, less than 6% of the radioactivity added was intact DCOI at sampling on the first day of the test (day 0). At a dosage of 1 mg/kg, approx. 3.5% of the ^{14}C added was intact DCOI at sampling on day 1. In reality, the insignificant part of the parent compound recovered on day 0 represented a sampling after one hour as the preparation of the samples for analysis took approx. one hour. The very rapid transformation of DCOI makes it impossible to calculate an exact half-life, which is, however, for certain less than one hour (Lawrence *et al.* 1991a).

Mineralization and metabolites

During the 30-day test period, [^{14}C]DCOI was partially mineralized as 22% (0.05 mg/kg) and 8.7% (1 mg/kg) of the radioactivity added was transformed into $^{14}CO_2$ at 25°C. DCOI is primarily transformed into polar metabolites and into compounds that are not extracted from the sediment (Table 3.2). A comparison with HPLC chromatograms of 15 potential metabolites did not result in an unambiguous identification of the metabolites observed in the sediment tests. The most polar metabolite had the same analytical retention time as n-octyl malonamic acid ($C_8H_{17}NHC(=O)\ CH_2CO_2H$) and at least two other metabolites were linear structures in which the isothiazolone ring was broken (Lawrence *et al.* 1991a). Assessed on the basis of analyses of 15 known standards, more cyclic structures were formed at the initial primary degradation of DCOI. It is considered likely that the metabolites present in the tests after 30 days were linear compounds. The two metabolites found at the analysis of the sediment samples after 30 days were both more polar than the isothiazolone standards used. The rapid primary degradation of DCOI (more than 94% transformation after 1 hour) indicates a rapid reaction involving a chemically unstable bond, e.g. the N-S bond in the isothiazolone ring (Lawrence *et al.* 1991a).

A positive identification of three metabolites was achieved in a later study in which a microbial enrichment culture proved suitable for achieving higher concentrations of metabolites (Mazza 1993). The culture was enriched after dosing aquatic sediment with DCOI (5 mg/kg). A comparison between HPLC chromatograms of metabolites formed in the enrichment culture and in sediment showed that the products were almost identical. By use of the enrichment culture and more analytical methods (i.a. HPLC and GC/MS), two essential metabolites were identified as N-(n-octyl) malonamic acid and N-(n-octyl) acetamide. Furthermore, a third quantitatively less important product N-(n-octyl) β hydroxy-

propionamide, which is probably formed at anaerobic degradation, was identified.

Table 3.2
Aerobic biodegradation of [^{14}C]DCOI (0.05 mg/kg), polarity and distribution of metabolites in sediment and seawater. Data from Lawrence et al. 1991a.

Time (days)	% of ^{14}C added				
	DCOI	Polar substances*	Non-polar substances**	CO_2	Non-extractable substances
0	5.1	41.1	1.2	0.0	62.0
1	-	44.7	0.65	0.55	62.2
2	-	27.6	1.3	3.3	55.3
5	-	27.0	0.3	8.1	66.8
9	-	23.9	0.7	8.2	59.0
15	-	22.3	-	8.4	56.5
20	-	24.8	-	9.1	78.0
26	-	20.3	-	14.2	67.0
30	-	13.1	-	21.9	63.5

-, not detected; * more polar than DCOI; ** less polar than DCOI.

The sediment from the aerobic biodegradation tests (Lawrence *et al.* 1991a) was further characterized as regards bound metabolites. Sediment samples sampled at the start of the tests and after 30 days were characterized by extraction with methylene chloride/methanol followed by extractions with HCl and NaOH (Kesterson and Atkins 1992a). Relatively water-soluble metabolites that are extracted with HCl, constituted <0.1% of the radioactivity added. Metabolites in the NaOH extract were further divided into fulvic acid and humic acid fractions containing 1.2% and 5.1%, respectively, of the ^{14}C added after 30 days. The metabolites that were not extracted by these procedures were probably bound to humin or clay and constituted 45% of the ^{14}C added after 30 days (Kesterson and Atkins 1992a). The results showed that the stable metabolites from DCOI were mainly bound to humic acid, humin and clay minerals in the sediment.

Studies with Danish sediments

The aerobic biodegradability of DCOI was examined by use of a clayey sediment (0.83 µg DCOI/g) and a sandy sediment (0.033 µg DCOI/g), which had both been incubated with their respective seawater (Appendix 2). Both sediments and their respective seawater had been collected at two localities in the Sound. The mineralization of [2,3-^{14}C]DCOI into $^{14}CO_2$ constituted 13% of the ^{14}C added in the clayey sediment and 24% of the ^{14}C added in the sandy sediment after 42 days' incubation at 15°C (Figures 3.1 and 3.2). The examination of the distribution of ^{14}C in the clayey sediment at the termination of the test after 42 days showed that 48% of the ^{14}C added was bound to humic acids, humin and clay miner-

als. These substances are expected to be little bioavailable. In the water phase of the test system or in the form of hydrolyzable compounds and fulvic acids, the substances more easily soluble in water altogether constituted 17% of the ^{14}C added after 42 days. Tests were made with glucose in order to examine the effect of the low concentration and the other experimental conditions on the mineralization of a readily biodegradable substance. The mineralization of glucose constituted 52% of the ^{14}C added in the clayey sediment and 60% of the ^{14}C added in the sandy sediment after 42 days. Methods and results are described in detail in Appendix 2.

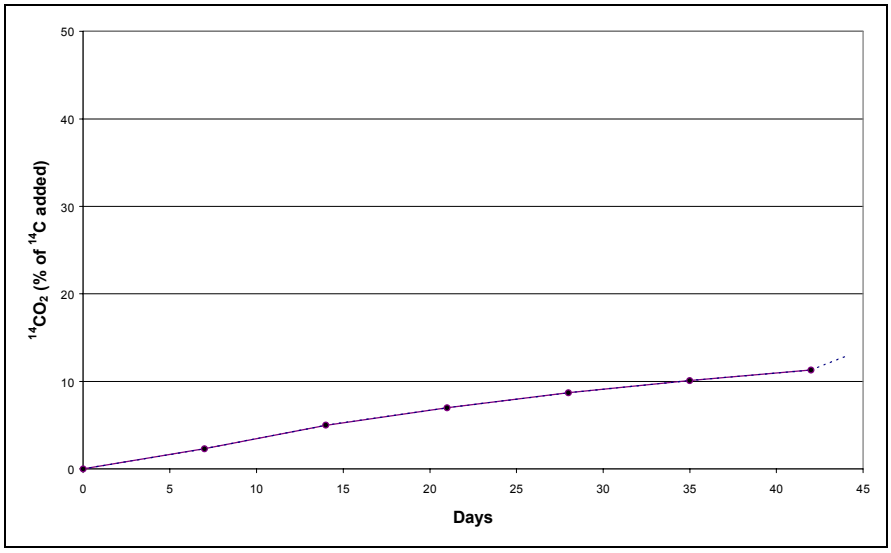

Figure 3.1
Mineralization of [^{14}C] DCOI (0.83 µg/g) in clayey sediment and seawater from the Sound (sediment LS). Aerobic conditions. Dotted curve represents $^{14}CO_2$ released by acidification.

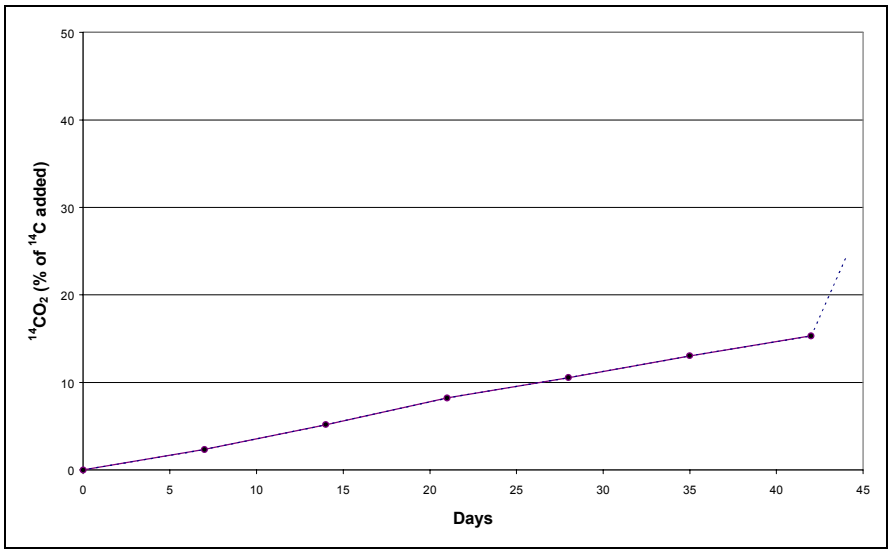

Figure 3.2

Mineralization of [^{14}C] DCOI (0.033 µg/g) in sandy sediment and seawater from the Sound (sediment SS). Aerobic conditions. Dotted curve represents $^{14}CO_2$ released by acidification.

In the study with the clayey sediment, water and sediment samples were sampled at the start of the incubation and after 28 and 42 days. Chemical analyses of DCOI and metabolites in these samples were made by Rohm and Haas (Spring House, Pennsylvania). The water samples turned out to have a low content of ^{14}C (2.5-6% of the ^{14}C added), which did not allow a more detailed characterization of metabolites. The analyses of the sediment samples from the same test showed that DCOI was transformed into compounds more polar than the parent compound and that a considerable part of the radioactivity added resisted extraction from the sediment (Table 3.3).

Table 3.3
Aerobic biodegradation of [^{14}C]DCOI into metabolites and carbon dioxide in seawater and clayey sediment from the Sound. HPLC analyses were only performed with sediment samples (sediment LS).

Time(days)	DCOI[1]	Polar substances[2]	Non-polar substances[3]	CO_2	Non-extractable substances
% of ^{14}C added					
0	0.37	46.4 ± 6.5	-	0	43.7 ± 4.6
28	-	20.4 ± 0.60	-	8.7 ± 0.35	51.0 ± 13.6
42	0.80 ± 0.92	18.5 ± 1.7	-	13 ± 0.52	49.2 ± 21.9

[1] determined by HPLC co-chromatography with DCOI standard; [2] more polar than DCOI; [3] less polar than DCOI; SD, standard deviations of three replicates; -, not detected.

3.2.3 **Mineralization and metabolites in anoxic sediment**

Transformation of DCOI

As was the case under aerobic conditions, DCOI was rapidly transformed into other chemical compounds in anoxic sediment. In the tests, only 2.0% (0.05 mg/kg) and 2.2% (1 mg/kg) of the radioactivity added were intact DCOI at sampling on the first day of the test (day 0). As the first sampling in reality represents a 1-hour sample, it can be demonstrated with certainty that the half-life of DCOI was less than 1 hour (Lawrence *et al.* 1991b).

Mineralization and metabolites

[^{14}C]DCOI (0.05 mg/kg) was only less mineralized in the anoxic sediment as the formation of $^{14}CO_2$ constituted between 6.7 and 9.5% of the radioactivity added throughout the entire test period of 365 days (Table 3.4). This level was attained after 61 days' incubation at 25°C. The comparative share, which was mineralized in the parallel test with a dosage of 1 mg/kg, constituted between 5.3% and 8.2% of the ^{14}C added in the period from day 61 to day 365 (Lawrence *et al.* 1991b). The products

formed by the degradation of DCOI were related to standards of 15 potential metabolites. The results show that, after 29 days, at least three metabolites more polar than DCOI had been formed. Although it cannot be excluded that one of these products resembles the parent compound, it is considered most likely that linear structures are in question. Furthermore, two metabolites less polar than DCOI were demonstrated. The identity of these non-polar substances cannot be established with certainty as they could not be related to any of the standards used. Table 3.4 shows that the quantitatively most important metabolites from DCOI are polar compounds. The polar metabolites are presumably composed of more linear compounds.

Table 3.4
Anaerobic biodegradation of [^{14}C]DCOI (0.05 mg/kg), polarity and distribution of metabolites in sediment and seawater. Data from Lawrence et al. 1991b.

Time (days)	% of ^{14}C added				
	DCOI	Polar substances*	Non-polar substances**	CO_2	Non-extractable substances
0	2.0	13.3	0.9	0.0	47.1
14	-[A]	25.3	2.1	1.1	41.4
29	-	23.0	1.5	4.0	41.6
61	-[A]	18.7	3.8	8.4	40.1
90	-	18.6	2.5	7.6	58.5
120	-	12.6	1.3	9.5	47.6
180	-	12.6	2.2	8.5	48.9
270	-	8.7	1.0	8.5	66.7
365	-	***	***	6.7	44.0

-, not detected (however: [A], low conc. detected, probably artifact); * more polar than DCOI; ** less polar than DCOI; *** sample lost.

Water-soluble metabolites from the transformation of DCOI constituted between 3.6% and 9.3% of the radioactivity added throughout the entire test period. Metabolites that were bound to the sediment and could not be extracted with methylene chloride/methanol constituted a constantly high part of between 40% and 67% of the ^{14}C added (Table 3.4). Further extraction with HCl and NaOH showed that relatively water-soluble metabolites constituted <0.1% while fulvic and humic acids constituted 0.6% and 3.6%, respectively, of the ^{14}C added after 365 days. Metabolites that were still bound to the sediment, probably to humin or clay, constituted 30% of the ^{14}C added (Kesterson and Atkins 1992b). The formation of metabolites binding to humic acids, humin and clay minerals in the sediment is in agreement with the results in the aerobic biodegradation tests (Kesterson and Atkins 1992a).

Studies with Danish sediments
The anaerobic biodegradability of DCOI (0.83 µg/g) was examined by use of a clayey sediment and its seawater (Appendix 2), which was also

used in the aerobic tests (cf. Section 3.2.2). Sediment and seawater was incubated under anaerobic sulfate-reducing conditions, which are normally prevalent in coastal marine sediments. The mineralization of [2,3-^{14}C] DCOI into $^{14}CO_2$ constituted 14% of the ^{14}C added after 56 days' incubation at 15°C (Figure 3.3). The examination of the distribution of ^{14}C in the clayey sediment at the termination of the test after 56 days showed that 45% of the ^{14}C added was bound to humic acids, humin and clay minerals. Altogether, water-soluble substances in the water phase of the test system and hydrolizable compounds and water-soluble fulvic acids constituted 7% of the ^{14}C added after 56 days. The mineralization of glucose, which was included as a readily biodegradable reference substance, constituted 59% of the ^{14}C added after 56 days. Methods and results are described in detail in Appendix 2.

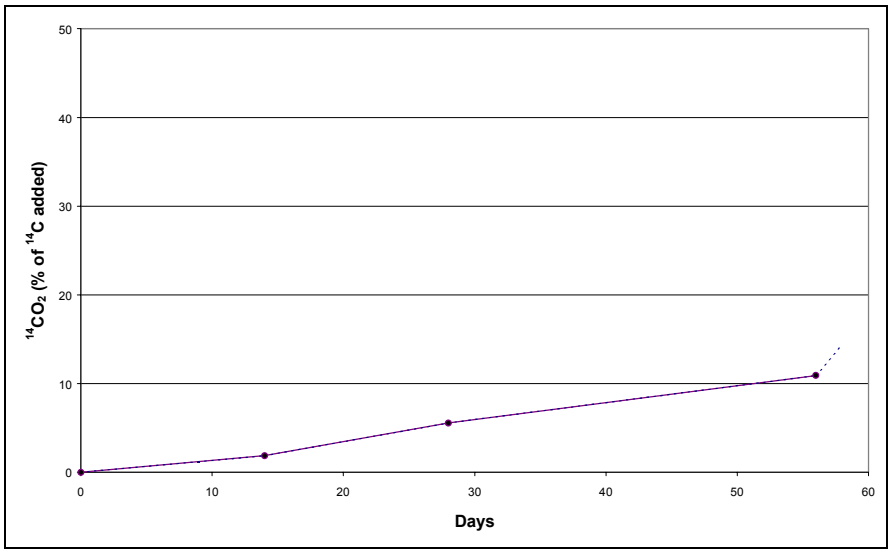

Figure 3.3
Mineralization of [^{14}C] DCOI (0.83 µg/g) in clayey sediment and seawater from the Sound (sediment LS). Anaerobic conditions. Dotted curve represents $^{14}CO_2$ released by acidification.

Water and sediment samples from the tests were sampled at the start of the test and after 28 and 56 days. Chemical analyses of DCOI and metabolites in the water samples were made by Rohm and Haas (Spring House, Pennsylvania).

The analyses of water samples sampled at the termination of the test after 56 days showed that 4.0 ± 2.4% of the ^{14}C added was present in the form of compounds with the same HPLC retention time as DCOI. In the same water samples, polar compounds constituted 13.7 ± 3.0% of the ^{14}C added. The sediment samples were not analyzed as they contained 3-4 times less radioactivity than the sediment samples from the aerobic tests (Table 3.3).

3.2.4 **Transformation and fate of DCOI in a harbour**

An investigation of the spread and removal of DCOI was carried out in the vicinity of a freshly painted ship and of another ship that had been painted a couple of months earlier. Both ships were lying in Korsør Harbour where the investigations were made on 26 and 27 October 1998. Those days, the temperature of the water was approx. 10°C and varied very little according the depth of the water (Steen *et al.* 1999). The wind was southwesterly (between approx. 240 and 255° on 26 October and approx. 200° on 27 October). The wind velocity was approx. 8-10 m/sec with wind blasts of up to 15 m/sec on 26 October and a little more on 27 October (Danish Meteorological Institute 1999). The entrance of Korsør Harbour points in a north-easterly direction why the water must be expected to have been pressed out of the harbour.

The concentration of DCOI in the water phase was measured along two transects: one perpendicular to the direction of the ships and the other in north-easterly direction, i.e. in the wind direction. Most of the samples were taken on 26 October. The samples were taken over a relatively short period of time (approx. 5 hours) and the measured concentrations can thus only be considered valid for the day in question. The highest concentrations measured of DCOI were <300 ng/L close to the ship's side (≤1 m) and decreased to <50 ng/L at a distance of approx. 30 metres from the ship. The concentration of DCOI in a distance of 2 metres from the ships (along the transect and perpendicular to the ships) varied very little according to the water depth why the vertical mixing was considered to be total.

Steen *et al.* (1999) have made model calculations in which Korsør Harbour was modelled as a one-dimensional box, in which the flow in and out of the harbour was neglected and in which the dispersion coefficient was varied between approx. 0.004-0.03 m^2/s. This interval is stated to be the end points of the expected variation interval of the dispersion coefficient of the harbour. Apart from the spread, a first order disappearance kinetics is assumed for DCOI. The simulations were made with three different rate constants for this first order process: 0 day^{-1}, 1 day^{-1}, and 1 $hour^{-1}$. As a result of the winds on 26 and 27 October, the dispersion must presumably have been high in the basin. By way of comparison it may be mentioned that the horizontal dispersion coefficient in Danish coastal waters typically varies between 0.04 and 5 m^2/s (Harremoës and Malmgren-Hansen 1989). As regards the two transects, the best correlation between the measured and the calculated DCOI concentrations was achieved by use of a rate constant of disappearance of between 1 $hour^{-1}$ and 1 day^{-1}.

With the rate constant, 1 $hour^{-1}$, a good correlation was achieved between measured and calculated values close to the ships for one transect while the concentrations of the other transect was underestimated at distances of more than approx. 8 m. For both transects, the calculated concentrations are lower than the measured concentrations at larger distances from the ships (approx. 30 m). When assuming a rate constant of disappearance of 1 day^{-1}, the calculated concentrations are higher than the measured concentrations close to the ships for both transects but lower than the measured concentrations farther away (approx. 60 m). There are thus

indications that the rate constant of the disappearance of DCOI close to the ships is higher than the corresponding constant farther away from the ships. On this basis, the rate constant of disappearance of the whole basin is considered to be between 1 $hour^{-1}$ and 1 day^{-1}, which corresponds to a half-life of between approx. 0.69 and 16.6 hours. This half-life includes biological and abiotic transformation as well as processes like sorption to suspended matter, sedimentation, potential vertical mixing and potential imperfection in the calculation of the dilution in the harbour.

3.3 Bioaccumulation and aquatic toxicity

3.3.1 Bioaccumulation

Studies on the bioaccumulation of DCOI in fish are available but not in other types of organisms (e.g. mussels). The ability of DCOI to bioaccumulate in fish has been examined in laboratory tests over 28 days by use of [^{14}C]DCOI. Two studies including chemical analyses of water and tissue samples have been made (Forbis *et al.* 1985; Derbyshire *et al.* 1991). In all tests, [^{14}C]DCOI was continuously added to a flow-through system. Chemical analyses showed that, at the final part of the tests, the concentration of DCOI was considerably lower than the nominal concentration (e.g. 4.5% and 0.55% of the ^{14}C added after 21 and 28 days, respectively (Leak 1986)) while DCOI was hardly measurable in the second test (Derbyshire *et al.* 1991). Presumably, the principal part of the remaining ^{14}C activity in the water represented one or more polar metabolites.

The BCF values found (measured as radioactivity) were more or less identical in the two studies. The BCF values were 130-200 for muscle tissue, 700-1,100 for internal organs and 600 for the whole fish (Forbis *et al.* 1985; Derbyshire *et al.* 1991). The chemical analyses demonstrated that only 1% of the radioactivity in the fish was intact DCOI (Leak 1986). In connection with the study by Derbyshire *et al.* (1991), HPLC as well as TLC was used for identifying ^{14}C labelled substances accumulated in the tissue of the fish. These studies indicate that it was most likely a question of substances without an isothiazolone ring structure and that the substances were built into the protein of the fish. The results indicate that DCOI was transformed in the water, after which it was mainly polar and probably linear compounds that were taken up in the fish. This assumption is confirmed by the biodegradation of DCOI (cf. Section 3.2.2; Lawrence *et al.* 1991a). It is thus considered likely that the measured BCF values should rather be related to metabolites of DCOI but as only a few of these metabolites are identified, the importance of the recorded bioaccumulation of labelled ^{14}C cannot be assessed.

3.3.2 Toxicity towards aquatic organisms

Aquatic organisms

The toxicity of DCOI has been examined in standard laboratory tests with a number of aquatic organisms living in fresh water and in seawater:

Fresh water:

- *Selenastrum capricornutum,* green algae (Forbis 1990)

- *Daphnia magna*, crustacean (Burgess 1990; Ward and Boeri 1990)
- *Oncorhynchus mykiss*, rainbow trout (Shade *et al.* 1993)
- *Lepomis macrochirus*, bluegill sunfish (Shade *et al.* 1993)

Seawater:
- *Skeletonema costatum,* green algae (Debourg *et al.* 1993)
- *Mysidopsis bahia*, mysid, crustacean (Boeri and Ward 1990)
- *Penaeus aztecus*, brown shrimp, crustacean (Heitmuller 1977)
- *Cyprinodon variegatus,* sheepshead minnow, fish (Shade *et al.* 1993)
- *Paralichthys olivaceus*, Japanese flatfish, fish (Kawashima 1997a)
- *Pagrus major*, red sea bream, fish (Kawashima 1997b)
- *Crassostrea virginica*, oysters (Roberts *et al.* 1990)

Furthermore, tests with one mussel and protozoans are quoted by Shade *et al.* (1993) and Debourg *et al.* (1993), respectively. In some of the tests, problems with maintaining a constant exposure concentration have been reported and not all results have been calculated on the basis of measured concentrations (see below). The result of these irregularities is an overestimation of the effect concentrations - resulting in an underestimation of the toxicity of the substance.

The results, which are compiled in Appendix 4, show that there was no big difference in the sensitivity of freshwater and marine organisms. Table 3.5 summarizes the effects on the different groups of organisms.

Table 3.5
Ecotoxicological data on effects of DCOI on aquatic organisms (see Appendix 4 for detailed data).

Taxonomic group	End point	Exposure time [days]	Results [mg/L]
Algae	EC50	4-5	0.0139-0.036
Crustaceans	EC/LC50	2-4	0.0047-1.312
Crustaceans	NOEC* (reproduction)	21	0.00063
Fish	LC50	4	0.0027-0.030
Fish	NOEC (early life stage, ELS)	35	0.006
Molluscs (snails, mussels)	EC/LC50	2-4	0.0019-0.850
Protozoans	100% effect	?	5

* The highest concentration at which no effects were observed (NOEC, No Observed Effect Concentration).

The results from the algal tests performed (Forbis 1990) are calculated on the basis of the nominal concentration. The report on one of the tests shows that the concentration of DCOI decreased during the whole test period. Only 48% of the nominal concentration was left after 48 hours and, at the end of the test after 72 hours, it was only possible to measure

the substance in the test vessels containing the highest concentration (Forbis 1990). The EC50 values stated are thus too high.

A 21-day reproduction test with daphnids (Ward and Boeri 1990) was conducted in such a way that is difficult to draw certain conclusions. This is due to the use of various concentrations of a solvent in relation to the addition of DCOI and to large variation in the data. The NOEC value stated represents the lowest concentration tested but the way in which the test has been conducted does not exclude that effects of DCOI may have occurred at this concentration as the effect may be dimmed as an unintentional result of the solvent. As the result of this test is the lowest NOEC value found in the tests, this value forms the basis of the calculation of PNEC for DCOI.

N-(n-octyl) malonamic acid

The acute aquatic toxicity of N-(n-octyl) malomanic acid, which is an important metabolite from the transformation of DCOI (cf. Section 3.2.2), has been investigated in tests with fish and daphnia. The toxicity of N-(n-octyl) malomanic acid was tested in static tests and the calculations are based on measured mean concentrations of the substance. The effect concentrations for N-(n-octyl) malomanic acid are given for daphnia (48 h): EC50 = 260 mg/L, NOEC = 16 mg/L (Sword and Muckerman 1994b) and for rainbow trout (96 h): LC50 = 250 mg/L, NOEC = 160 mg/L (Sword and Muckerman 1994a). In the daphnia test, there is large variation in data and the basis of the calculation of the result is not clearly defined. Daphnids lying on the bottom of the test vessels do not seem to have been included as “immobile”, which they should according to the method description used. The actual EC50 is estimated to be in the interval of 90-160 mg/L rather than 260 mg/L as stated in the report (Sword and Muckerman 1994b).

Even with the above reservations, it must, however, be concluded that N-(n-octyl) malomanic acid is several orders of magnitude less toxic than DCOI.

In connection with the investigations of N-(n-octyl) malomanic acid, QSAR calculations have been made of the toxicity of this metabolite and some substances with similar structure, which are important metabolites from the microbial transformation of DCOI. The results are given in Table 3.6.

Table 3.6
QSAR calculations of the toxicity and the potential bioaccumulation of four probable metabolites from the transformation of DCOI.

Substance	Calculated EC50 (48 h), daphnids [mg/L] *	Calculated EC50 (48 h), trout [mg/L] **	Calculated octanol-water coefficient [log K_{ow}] **
N-(n-octyl) malonamic acid	172	199	1.9
N-(n-octyl) acetamide	102	115	2.0
N-(n-octyl) oxamic acid	140	160	1.9
N-(n-octyl)-β-hydroxypropionamide	261	Not determined	Not determined

* From Sword and Muckerman 1994b.
** Personal comm., Andrew Jacobson, Rohm and Haas Company.

The results in Table 3.6 indicate that the probable metabolites from the transformation of DCOI are neither particularly toxic nor bioaccumulative in aquatic organisms.

Sediment-living organisms
Results of a 10-day test with the marine sediment-living crustacean, the amphipod *Ampelisca abdita* are available: LC50 = 320 mg/kg and NOEC = 6.9 mg/kg dry weight (Putt 1994). The test was made with ^{14}C-labelled DCOI and the concentrations were measured as radioactivity. At the end of the test, approx. 90% of the ^{14}C activity was attached to the sediment while the remaining part was distributed in the ratio of approx. 8:2 of pore water to the water above the sediment. No chemical analyses were made and the authors draw attention to the fact that the measured radioactivity is probably owing to metabolites and not to DCOI.

Algal communities
Acute and chronic effects of DCOI have been examined on communities of natural phytoplankton (planktonic algae) and epipsammon (micro algae living on grains of sand). Acute and chronic effects of DCOI on communities of phytoplankton have been found at a concentration of DCOI of 0.0003 mg/L (the lowest concentration in which effects were observed, Lowest Observed Effect Concentration, LOEC) (Arrhenius 1997). The acute effect of DCOI was a stimulation of the activity of the algae while the chronic effect was an adaptation to DCOI in a few days. Inhibition of the photosynthesis occurred at higher concentrations (EC50: 0.05-0.1 mg/L (95% confidence interval)). The study concludes that the effect of DCOI was still significant at the end of the test after 7 days. Communities of epipsammon were extremely tolerant to DCOI and the effect concentrations were several orders of magnitude higher than those for phytoplankton.

Effects of degradation of DCOI on aquatic toxicity
Laboratory tests have been made in which the effects of degradation on the toxicity towards aquatic organisms were tested for a number of anti-

foulants including DCOI, Irgarol 1051 and Diuron (Callow and Finlay 1995; Callow and Willingham 1996). In these tests, the substances were incubated in seawater, seawater enriched with of marine bacteria and in sterilized seawater. Changes in the toxicity as the result of degradation of the active substances were tested towards marine bacteria (counting of colonial bacteria), diatoms (*Amphora coffeaeformis*) and crustaceans (*Artemia salina*). The degradation tests were started at concentrations of the substances causing 80% effect on the algae (EC80 = 0.5 mg/L for DCOI) so that a potential decrease of the toxicity of the solutions could be traced. The results showed that the toxicity practically did not decrease in sterilized seawater, and that the transformation of the active substance into metabolites with low toxicity progressed most rapidly in the bacteria-enriched seawater. The diatom test showed that e.g. the toxicity of DCOI had been considerably reduced (from approx. 80% to approx. 20% inhibition) after two weeks in natural and in bacteria-enriched seawater and that tests incubated for 4, 6 and 8 weeks in these two types of seawater caused 10% or no significant inhibition (Callow and Finlay 1995). The half-life of the toxicity of DCOI was calculated at 8.5 days in natural seawater and 3 days in bacteria-enriched seawater (Callow and Finlay 1995).

The relation between degradation and sorption of DCOI and the acute toxicity towards the marine crustacean *Acartia tonsa* has been examined in the present study. The tests were performed in systems with the sandy sediment and its seawater from the Sound (Appendix 2), which was also used in the biodegradation tests. DCOI was added in a concentration of 100 µg/kg to the sediment-seawater systems. Water phase and sediment were separated 20 min. after dosing and use of the water phase in tests with *A. tonsa* caused a mortality corresponding to 35% of the test organisms. Stationary incubation in the dark at 20-25°C resulted in the fact that there were no mortal effects on *A. tonsa* after day 1 (Figure 3.4). Similar results were achieved when the sediment-water systems were incubated in the light at an intensity corresponding to 340 µmol/m^2 · s. Measurements made by VKI in the Sound show that, in the period from May to October 1998, the average light intensity was 420 µmol/m^2 · s in a depth of approx. 1 metre. The light intensity used was thus approx. 80% of the mean value calculated on the basis of the measurements in 1998. The test results with *A. tonsa* show that DCOI sorbs to sediment or is transformed into metabolites with a considerably lower toxicity than the parent compound. The methods used are described in detail in Appendix 3. A parallel test was made with zinc pyrithione (cf. Section 4.4).

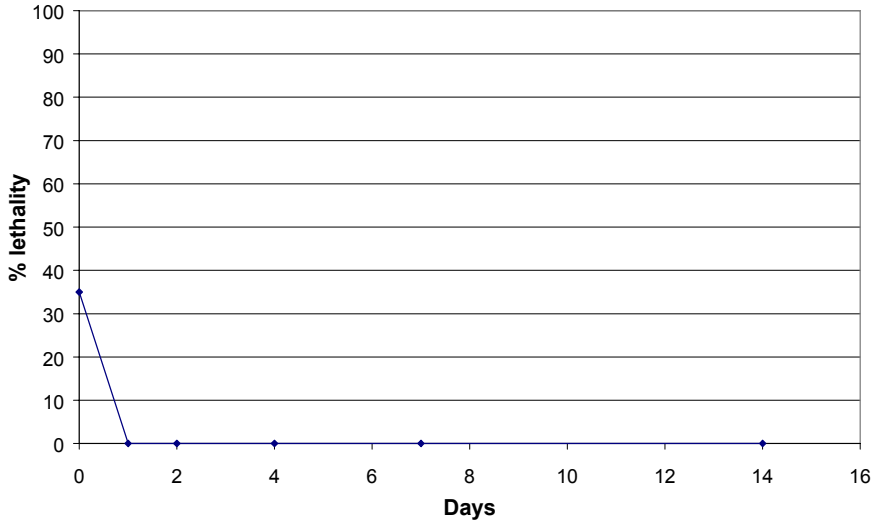

Figure 3.4
Effects of degradation of DCOI (100 µg/kg) dosed to sediment and seawater on the acute toxicity to Acartia tonsa (test performed in the dark).

3.4 **Risk assessment of DCOI**

Calculation of exposure concentrations (PEC)
In order to calculate the exposure concentrations (PEC, Predicted Environmental Concentration), a model was established, based on principles normally used for exposure assessments (EC 1996). The exposure assessments were made for two scenarios:

- A pleasure craft harbour (on the basis of the conditions in the pleasure craft harbour of Jyllinge)
- A busy navigation route (on the basis of the conditions at the Kronprins Frederiks Bro, Frederikssund)

The model and the two scenarios are described in detail in Appendix 1. For the parent compound and the most essential metabolites, the following exposure concentrations were calculated for each of the two scenarios:

- PEC (water column)
- PEC (sediment)
- PEC (sediment-pore water)

The three exposure concentrations were defined as the steady-state concentration of the sub-environment in question. I.e., the concentration which the calculated concentrations eventually approach when a continuous leaching of the parent compound to the water environment is simulated. The calculations of PEC have been made by use of realistic worst-case scenarios, which means that the parameters used in the model are based on realistically conservative assumptions, which results in the fact that, in practice, the calculated PEC values are seldom exceeded. The model used is not validated towards measured concentrations in har-

bour environments or navigation routes. More of the assumptions that form part of the simulation are of vital importance to the result of the calculations:

- The background concentrations for both the parent compound and the metabolites were assumed to be zero.

- 70% of the pleasure craft was assumed to have been painted with paint containing DCOI.

- The leaching rate of DCOI from bottom paints was calculated at 13 mg/m^2/day in harbours and 25 mg/m^2/day when sailing (Appendix 1).

- The primary biological transformation of DCOI into the expected metabolite N-(n-octyl) malonamic acid was assumed to proceed with a half-life of 14 hours in surface water at a temperature of 12°C.

The biological half-life of DCOI of 14 hours, which was assumed in the simulation, is established on the basis of an experimentally determined half-life in seawater at 12°C (Jakobson and Kramer 1999). The half-life of DCOI in seawater and not in seawater and sediment was chosen as the result of the simulation is exposure concentrations at a continuous leaching of DCOI to seawater after steady state was achieved. When the pleasure craft are taken out of the water at the end of the sailing season, DCOI will probably be rapidly eliminated as DCOI is either transformed in the water phase or sorbs to the sediment, in which it is transformed with a very short half-life (cf. Sections 3.2.2 and 3.2.3).

The exposure concentrations calculated for DCOI and its metabolites are approx. 50 times higher in the pleasure craft harbour than in the busy navigation route outside the harbour (Table 3.7).

Table 3.7
Calculated exposure concentrations (PEC) for DCOI and metabolites at steady state.

Scenario	Substance	PEC (water)	PEC (sediment, pore water)	PEC (sediment, sorbed)
		µg/L	µg/L	µg/kg
Pleasure craft harbour	DCOI	0.52	0.0015	0.12
	N-(n-octyl) malanomic acid	1.98	0.83	2.32
	N-(n-octyl) beta hydroxypropionamide	0.020	0.14	0.43
	N-(n-octyl) acetamide	0.050	0.084	2.42
	Other compounds	0.10		
Navigation route	DCOI	0.0061	0.00002	0.0014
	N-(n-octyl) malanomic acid	0.040	0.011	0.031
	N-(n-octyl) beta hydroxypropionamide	0.00071	0.0019	0.0058
	N-(n-octyl) acetamide	0.0018	0.0013	0.039
	Other compounds	0.0040		

Calculation of Predicted No-Effect Concentration (PNEC)
The Predicted No-Effect Concentrations (PNEC) are estimated for DCOI and N-(n-octyl) malonamic acid. The other stable metabolites from the transformation of DCOI are considered to have aquatic toxicity corresponding to that of N-(n-octyl) malonamic acid.

The available studies on the aquatic toxicity of DCOI are considered representative and the data include long-term studies with fish and crustaceans. The algal test may be interpreted as a short-term as well as a long-term test (EC 1996).

For DCOI, three NOEC values from long-term tests (fish, crustaceans and algae) are available, including the groups of organisms most sensitive in short-term tests (fish). On this basis, PNEC is calculated by dividing the lowest NOEC value, which is 0.00063 mg/L for crustaceans (Ward and Boeri 1990), by an assessment factor of 10 (EC 1996). This results in a PNEC of 0.00006 mg/L = 0.06 µg/L for DCOI. As already mentioned in Section 3.3.2, no unambiguous NOEC value can be derived from this long-term test with crustaceans (Ward and Boeri 1990). If this study is ignored, the results from one single long-term study with fish are available, in which NOEC was 0.006 mg/L. In this case, an assessment factor of 100 is applied, which results in a PNEC value calculated at 0.00006 mg/l = 0.06 µg/L, which is identical with the above calculated value.

Calculation of PNEC for N-(n-octyl) malonamic acid is based on the lowest effect concentration. As data primarily originates from short-term tests, an assessment factor of 1,000 is used for the lowest effect concentration. For N-(n-octyl) malonamic acid, the lowest reported LC50 = 250 mg/L for rainbow trout while the value for daphnia (EC50 = 260 mg/L) as already mentioned above is a moot point. For the calculation of a

PNEC for N-(n-octyl) malonamic acid, an LC50 value of 90 mg/L (towards daphnids) is used as this value is considered the actual effect concentration in the tests performed (cf. Section 3.3.2). This results in a PNEC value of 0.09 mg/L = 90 µg/L. The calculated PNEC for N-(n-octyl) malonamic acid is assumed to be representative of the other metabolites from the transformation of DCOI. The two calculations of PNEC are shown in Table 3.8.

Table 3.8
Calculation of PNEC for DCOI and N-(n-octyl) malonamic acid.

Substance	Lowest effect concentration	Value [µg/L]	Assessment factor	PNEC [µg/L]
DCOI	Long-term test NOEC crustaceans	0.63	10	0.06
N-(n-octyl) malonamic acid	Short-term test EC50 crustaceans	90,000	1,000	90

Risk quotient
On the basis of the above calculated PEC (water) values for DCOI and its metabolites given in Table 3.7 and PNEC values for DCOI and N-(n-octyl) malonamic acid, the risk quotients Rq = (PEC/PNEC) can be calculated as shown in Table 3.9.

Table 3.9
Calculation of risk quotients (Rq) for DCOI and its metabolites.

Substance	PNEC [µg/L]	Pleasure craft harbour		Navigation route	
		PEC [µg/L]	Rq	PEC [µg/L]	Rq
DCOI	0.06	0.52	8.7	0.0061	0.10
Metabolites	90*	2.2	0.02	0.047	0.0005

* N-(n-octyl) malonamic acid

The stated risk quotients are calculated on the basis of realistic worst-case scenarios (Appendix 1), which are i.a. based on the assumption that 70% of the pleasure craft are painted with a bottom paint containing DCOI. On the basis of the assumptions made in the simulation and of the calculated PEC values, it is considered likely that a risk of chronic ecotoxic effects within the pleasure craft harbour may exist as, presumably, DCOI will constantly be applied by leaching from bottom paints. The risk quotient for DCOI out of the harbour is less than 1 and here, the risk of ecotoxic effects is considered to be low.

Within as well as out of the pleasure craft harbour, a very small risk of ecotoxic effects of metabolites from the transformation of DCOI is considered to exist.

4 Zinc pyrithione

4.1 **Physico-chemical properties**

Table 4.1 gives an overview of the physico-chemical properties of zinc pyrithione.

Table 4.1
Physico-chemical properties of zinc pyrithione (Olin 1997).

CAS No.	13463-41-7
Synonyms	Bis(1-hydroxy-2[1H]-pyridinethionato-O-S)-(T4)zinc, Zinc Omadine
Classification (two products)	T, R22, R23, R41, R38 + X_n, R20/22, R36/38
Molecular formula	$C_{10}H_8N_2O_2S_2Zn$
Molar weight	317.68
Water solubility	6.0 mg/L
Vapour pressure (25°C)	Not volatile, solid substance
Octanol/water partition coefficient (log K_{ow})	0.97
Organic matter/water partition coefficient (log K_{oc})	2.9-4.0

4.2 **Abiotic degradation**

Photolysis
Zinc pyrithione is very rapidly transformed by photolysis. Experiments conducted under sterile conditions with a light:dark cycle of 12:12 hours have shown that, under exposure to light, the concentration of [pyridine-2,6-^{14}C]zinc pyrithione in pH 9 buffer was reduced to 33% of the radioactivity added in 15 min. Data from this study also demonstrated that less than 5% of the ^{14}C added occurred as zinc pyrithione after 1 hour of exposure to light. Similar results have been achieved when photolysis of zinc pyrithione was investigated by use of artificial seawater. In this study, the parent compound constituted 45% of the radioactivity added after 15 min while, after 24 hours, 1.3% of the added dose occurred as zinc pyrithione. The estimated half-lives of the photolytic transformation of zinc pyrithione was 13 min in pH 9 buffer and 17.5 min in artificial seawater (Reynolds 1995a).

Hydrolysis
Hydrolysis of [pyridine-2,6-^{14}C]zinc pyrithione has been investigated in aqueous solution at pH 5, 7 and 9 and in artificial seawater at pH 8.2. In general, zinc pyrithione was hydrolysis-stable at all of the pH values investigated (Reynolds 1995b).

4.3 Biodegradation of zinc pyrithione in the aquatic environment

Transformation of zinc pyrithione

Experiments with freshwater and marine sediments have shown that the transformation of zinc pyrithione in aquatic systems proceeds with an initial rapid rate followed by a slower rate. This is the result of the distribution between the water phase and the sediment, in which the degradation proceeds with different rates in the two sub-environments. The resulting two-phased transformation is observed in fresh water as well as in seawater and under both aerobic and anaerobic conditions (Ritter 1996, 1999a-e). The half-lives of removal of zinc pyrithione from the water phase via degradation and sorption to the sediment were between 0.5 and 0.6 hours. For the aerobic as well as the anaerobic systems, this removal resulted in <5% of the added dose remaining in the water phase after 6 hours. In the following second phase, the removal of sorbed pyrithione proceeded with a half-life of 4 days under aerobic conditions and 19 hours under anaerobic conditions, respectively (Ritter 1999a-e).

Zinc pyrithione reacts by transchelation in the presence of metals transforming zinc pyrithione into copper(II) pyrithione and other more stable metal-pyrithione complexes. The slower secondary transformation rate in studies performed at a low concentration of zinc pyrithione (0.05 µg/g) is probably due to the sorption of the metal-pyrithione complexes to the sediment (Ritter 1999a-e). In previous studies, in which a higher concentration of zinc pyrithione (3 µg/g) was used, the secondary transformation rate may be the result of the lower water solubility of copper(II)pyrithione being limiting to the transformation rate (Ritter 1996; Smalley and Reynolds 1996).

Zinc pyrithione is transformed to heterocyclic metabolites with one ring like omadine sulfonic acid and pyridine sulfonic acid. More other metabolites identified by Arch Chemicals are known to VKI but are given as NP1-NP5 in this project.

4.3.1 Mineralization and metabolites in aerobic sediment

The aerobic biodegradability of zinc pyrithione (3 µg/g) was investigated by use of water and sediments collected in freshwater and marine harbours in which maintenance of boats is carried out (Ritter 1996). Later investigations with seawater and sediment were made with both zinc pyrithione and copper pyrithione, which were added at a lower concentration of 0.05 µg/g (Ritter 1999a, b, d). In these studies, the degradation proceeded at the same rate and resulted in the same metabolites whether the pyrithione was added as the zinc or the copper complex. The greatest importance is attached to the results of the most recent experiments as the lower concentration of the parent compound results in more realistic mechanisms of sorption and degradation.

Mineralization and metabolites

After 84 days of incubation at 25°C, the mineralization of [pyridine-2,6-^{14}C]zinc pyrithione (0.05 µg/g) to $^{14}CO_2$ in seawater and sediment constituted 0.44% of the ^{14}C added (Ritter 1999a, b, d). A correspondingly low mineralization was observed in the previous studies, in which zinc pyrithione was added at a concentration of 3 µg/g (Ritter 1996). In the fresh water and sediment, the mineralization of zinc pyrithione was higher as 12% of the ^{14}C added was transformed to $^{14}CO_2$ after 30 days at 25°C (Ritter 1996).

The first stage of the aerobic degradation of zinc pyrithione is the formation of its disulfide, which is identified as omadine disulfide. In studies performed with zinc pyrithione at the concentration of 3 µg/g (Ritter 1996), omadine disulfide was formed as one of the most important metabolites. Omadine disulfide has almost the same chemical structure as zinc pyrithione and has been shown to be very toxic to aquatic organisms (Table 4.7). The presence of omadine disulfide was only transient as the further transformation of this metabolite caused omadine disulfide to constitute 2.8% of the radioactivity added after 30 days in the experiment with seawater and sediment (in the experiment with fresh water and sediment, the concentration of omadine disulfide was below the detection limit of 0.3 ng/g after 30 days). The demonstration of omadine disulfide in the studies, in which zinc pyrithione was added in 3 µg/g, is probably due to the kinetics of desorption and degradation at the concentration used, which is considered environmentally unrealistic. In the more recent experiments, in which the level of concentration was 0.05 µg/g (Ritter 1999a, b, d), omadine disulfide was not detected and omadine disulfide must thus be considered a transient metabolite in the biological transformation of zinc pyrithione into heterocyclic compounds with one ring. On the basis of the experiments made at a concentration of 0.05 µg/g, the most important metabolites from the aerobic degradation of zinc pyrithione are considered to be omadine sulfonic acid and pyridine sulfonic acid and two other metabolites called NP1 and NP2 (Table 4.2). NP2 was only demonstrated by extraction of the sediment with alkali. It is, however, not clear yet whether this metabolite was formed in the sediment before extraction or by a chemical reaction in the alkaline extract. Data from investigations of the transformation of copper pyrithione in anaerobic aquatic systems suggest that, most likely, NP2 was present in the sediment before the extraction (Ritter 1999a-e).

Table 4.2
Aerobic biodegradation of [^{14}C]zinc pyrithione into metabolites and carbon dioxide in seawater and sediment (data from Ritter, 1999a, b, d).

Time (days)	% of added dose						
	Zinc pyrithione	NP1	Omadine sulfonic acid	Pyridine sulfonic acid	NP2	Non-extractable	CO_2
0	49.1	16.2	-	-	20.5	6.2	-
1	9.8	25.8	-	-	40.7	14.2	0.01
3	11.7	36.0	-	-	22.0	22.2	0.02
7	1.5	41.5	4.2	4.5	17.3	24.8	0.05
14	1.6	26.4	17.2	6.8	13.5	28.5	0.08
21	3.0	5.6	33.7	10.2	10.5	31.8	0.16
30	1.1	7.2	35.3	11.8	8.2	27.8	0.23
42	1.8	8.0	28.6	11.9	8.0	34.3	0.33
63	1.2	-	28.9	17.4	7.9	29.7	0.39
84	2.0	1.7	31.7	24.7	4.4	30.4	0.44

-, not detected.

A considerable part of the metabolites sorbed to the sediment and resisted extraction with acetonitrile followed by two extractions with 0,1 N KOH. The percentage of these non-extractable ^{14}C labelled metabolites increased during the first fortnight and, in the period from day 14 to the end of the experiment after 84 days, it constituted approx. 30% of the ^{14}C added. The total recovery of the radioactivity added varied between 93 and 99% (Ritter 1999a, b, d).

Studies with Danish sediments

The aerobic biodegradability of zinc pyrithione (0.0037 µg/g) was examined in sediment and seawater from the same two locations in the Sound as in the study of DCOI, including a clayey and a sandy sediment (cf. Section 3.2.2 and Appendix 2). After 42 days' incubation at 15°C, the mineralization of [pyridine-2,6-^{14}C]zinc pyrithione into $^{14}CO_2$ constituted 2.8% of the ^{14}C added in the clayey sediment and 5.0% in the sandy sediment (Figures 4.1-4.2). The examination of the distribution of ^{14}C at the termination of the tests after 42 days showed that metabolites from the transformation of zinc pyrithione were primarily water-soluble compounds. In the test system with the sandy sediment, 65% of the ^{14}C added was recovered in the water phase of the test system while 22% was bound to hydrolizable compounds and fulvic acids in the sediment. In the test with the clayey sediment, 32% of the ^{14}C added was recovered in the water phase while the sediment contained 38% in the form of hydrolizable compounds and fulvic acids. Compared with this, metabolites bound to humic acids, humin and clay minerals constituted a minor part of between 3.6% (sandy sediment) and 16% (clayey sediment) of the radioactivity added. The results from the chemical analyses (Table 4.2) show that the ^{14}C remaining at the termination of the test was metabolites and not intact

zinc pyrithione. The metabolites are considered to have a high bio-availability as the radioactivity occurred especially in the form of water-soluble compounds. Methods and results are described in detail in Appendix 2.

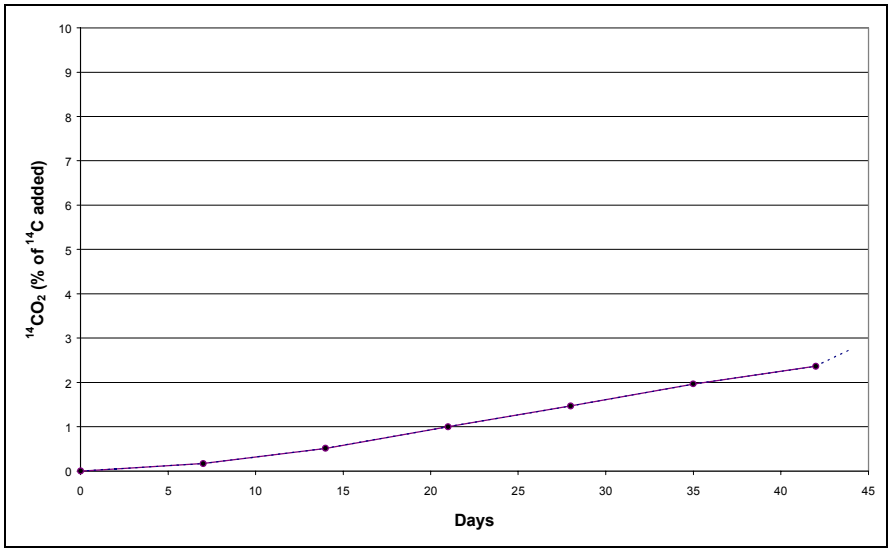

Figure 4.1
Mineralization of [^{14}C] zinc pyrithione (0.037 µg/g) in clayey sediment and seawater from the Sound (sediment LS) Aerobic conditions. Dotted curve represents $^{14}CO_2$ released by acidification.

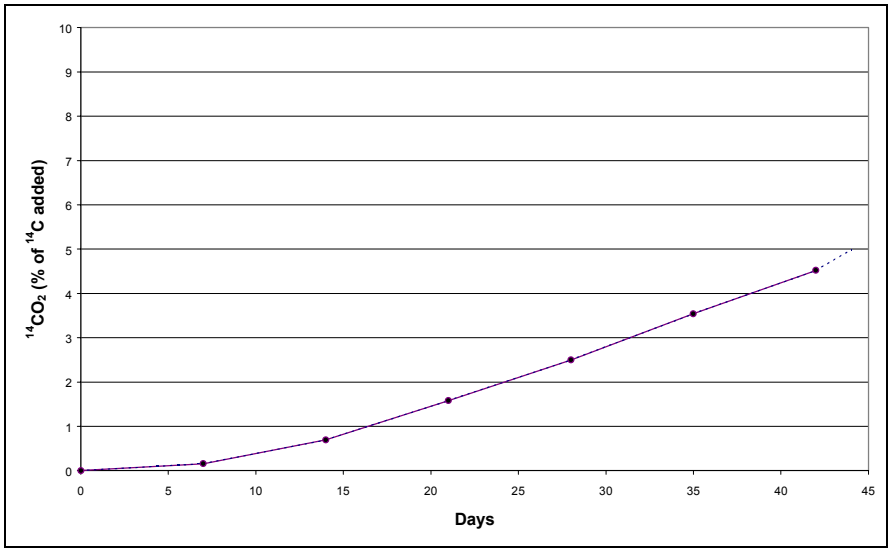

Figure 4.2
Mineralization of [^{14}C] zinc pyrithione (0.037 µg/g) in sandy sediment and seawater from the Sound (sediment SS) Aerobic conditions. Dotted curve represents $^{14}CO_2$ released by acidification.

Water and sediment samples from the tests were taken at the start of the tests and after 28 days. Chemical analyses of zinc pyrithione and metabolites were made by Arch Chemicals (Cheshire, Connecticut). These

analyses showed that zinc pyrithione was mainly transformed into omadine sulfonic acid, pyridine sulfonic acid and NP1 in both sediments (Table 4.3).

Table 4.3
Aerobic biodegradation of [^{14}C]zinc pyrithione into metabolites and carbon dioxide in seawater and sediment from the Sound.

Sample	Zinc pyrithione	NP1	Omadine sulfonic acid + pyridine sulfonic acid*	NP2	NP3	NP5	Non-extractable	CO_2
	% of ^{14}C added							
Day 0								
LS, water [1]	2.3	4.5	0.5	-	-	-	-	-
LS, sediment [1]	34.6	6.2	3.6	17.0	4.6	2.6	13.3	
LS, total [1]	36.9	10.7	4.1	17.0	4.6	2.6	13.3	
Day 28								
LS, water [2]	-	4.4	41.0	-	-	-	-	1.5
LS, sediment [2]	0.6	2.8	5.3	3.9	3.0	2.9	30.3	
LS, total [2]	0.6	7.2	46.3	3.9	3.0	2.9	30.3	
Day 0								
SS, water [1]	19.5	22.7	-	1.9	-	3.8	-	-
SS, sediment [1]	11.1	4.1	1.2	3.4	1.9	1.1	11.7	
SS, total [1]	30.6	26.8	1.2	5.3	1.9	4.9	11.7	
Day 28								
SS, water [2]	-	24.5	26.3	1.2	-	-	-	2.5
SS, sediment [2]	4.1	0.7	4.3	1.5	-	0.3	19.4	
SS, total [2]	4.1	25.2	30.6	2.7	-	0.3	19.4	

LS, clayey sediment; SS, sandy sediment;*, contained also NP4; [1], tests performed by Arch Chemicals; [2], tests performed by VKI; -, not detected.

4.3.2 **Mineralization and metabolites in anoxic sediment**

The anaerobic biodegradability of zinc pyrithione (3 µg/g) was investigated by use of water and sediments collected in the same freshwater and marine localities as in the aerobic experiments (Ritter 1996). Later investigations with seawater and sediment were made with both copper pyrithione and zinc pyrithione, which were added at a concentration of 0.05 µg/g (Ritter 1999a, c, e). In the assessment of the fate of zinc pyrithione under anaerobic conditions, the greatest importance is attached to the most recent results from experiments carried out at the concentration of 0.05 µg/g (Ritter 1999a, b, d).

Mineralization and metabolites

As was the case with the results from the aerobic biodegradation studies, the mineralization of [pyridine-2,6-^{14}C]zinc pyrithione to carbon dioxide was negligible in anoxic marine sediment. After 182 days of incubation

at 25°C, the formation of $^{14}CO_2$ constituted 0.9% of the ^{14}C added (Ritter 1999a, b, d).

In the previous studies, in which zinc pyrithione was added at a concentration of 3 µg/g, omadine disulfide was formed as a transient metabolite while an unsymmetrical disulfide of NP3 and 2-mercaptopyridine N-oxide was present throughout the entire test period of 91 days (Smalley and Reynolds 1996). The formation of these metabolites with two rings in considerable amounts (>10% of the radioactivity added) is probably the result of the kinetics of sorption and degradation at the concentration used. In the recent studies, in which the concentration of zinc pyrithione was 0.05 µg/g, neither omadine disulfide nor the unsymmetrical disulfide was detected (Ritter 1999a, b, d). The most important metabolite from the anaerobic transformation of zinc pyrithione added at a concentration of 0.05 µg/g was NP3 while lower concentrations of three other heterocyclic compounds with one ring (pyridine sulfonic acid, NP4 and NP5) were formed as a result of the further transformation of NP3 (Table 4.4). Small amounts of NP1 were formed immediately after the start of the test (<1% of the ^{14}C added; day 3) but this metabolite was transformed into other compounds and could not be detected after 14 days (Ritter 1999a, b, d).

Table 4.4
Anaerobic biodegradation of zinc pyrithione into metabolites and carbon dioxide in seawater and sediment (data from Ritter 1999a, b, d).

Time (days)	% of added dose						
	Zinc pyrithione	NP3	NP4	Pyridine sulfonic acid	NP5	Non-extractable	CO_2
0	30.1	21.2	2.1	1.0	5.2	7.4	-
1	4.7	62.0	3.5	-	6.8	5.8	-
3	0.3	74.5	3.0	0.1	3.1	7.9	-
7	0.1	78.1	1.5	0.8	2.7	9.1	-
14	-	54.5	5.9	3.3	8.2	14.8	-
22	-	38.0	8.8	1.4	5.4	18.2	0.1
30	-	32.9	7.4	2.0	4.7	19.2	0.3
63	-	14.3	8.5	2.2	5.5	29.4	0.6
90	1.8	13.7	3.4	2.2	4.0	34.3	0.3
182	-	2.3	7.9	4.3	1.2	52.7	0.9

-, not detected.

A considerable part of the metabolites sorbed to the sediment and resisted the extraction with acetonitrile and alkali. The concentration of non-extractable metabolites sorbed to sediment gradually increased throughout the test and constituted 53% of the ^{14}C added after 182 days. The total recovery of the radioactivity added varied between 90 and 102% (Ritter 1999a, b, d).

Studies with Danish sediments

The anaerobic biodegradation of zinc pyrithione (0.037 µg/g) was examined by use of the clayey sediment and its seawater (Appendix 2), which was also used in the aerobic tests (cf. Section 4.3.1). Sediment and seawater were incubated under anaerobic sulfate-reducing conditions, which are normally prevalent in coastal marine sediments. The mineralization of [pyridine-2,6-^{14}C]zinc pyrithione into $^{14}CO_2$ constituted 3.5% of the ^{14}C added after 56 days' incubation at 15°C (Figure 4.3). Compared with the tests performed under aerobic conditions, a larger part of the metabolites formed under anaerobic conditions was bound to humic acids, humin and clay minerals in the sediment. This part constituted 39% of the ^{14}C added after 56 days' incubation of the clayey sediment and its seawater. More water-soluble metabolites in the water phase of the system or bound to hydrolizable compounds and fulvic acids constituted, however, a considerable part of 35% in total of the radioactivity added. As in the aerobic tests, zinc pyrithione was transformed into metabolites (Table 4.5), of which several are considered to have a high bioavailability. Methods and results are described in detail in Appendix 2.

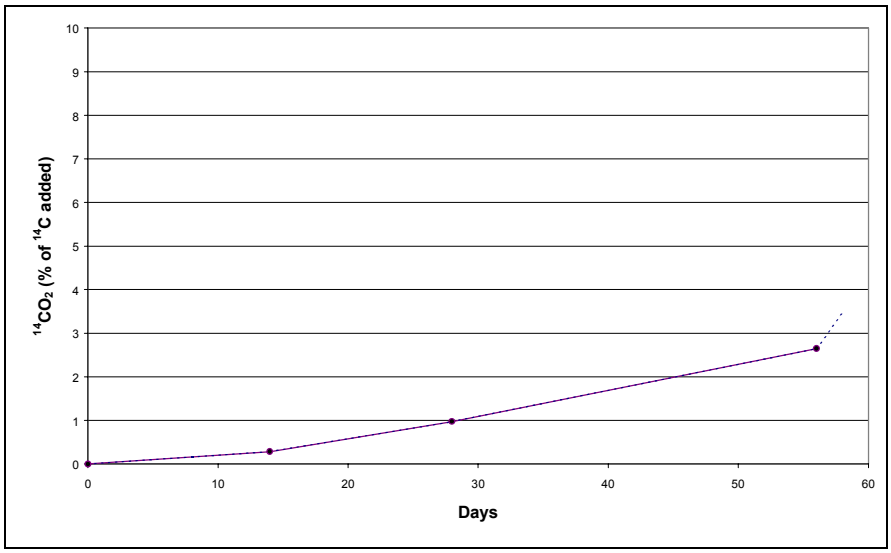

Figure 4.3
Mineralization of [^{14}C] zinc pyrithione (0.037 µg/g) in clayey sediment and seawater from the Sound (sediment SS) Anaerobic conditions. Dotted curve represents $^{14}CO_2$ released by acidification.

Water and sediment samples from the tests were taken at the start of the tests and after 28 days. Chemical analyses of zinc pyrithione and metabolites in these samples were made by Arch Chemicals (Cheshire, Connecticut). The results from the analyses performed showed that the quantitatively most essential metabolites under anaerobic sulfate-reducing conditions were the heterocyclic compounds with one ring, i.e. NP3 and NP5 (Table 4.5).

Table 4.5
Anaerobic biodegradation of [^{14}C]zinc pyrithione into metabolites and carbon dioxide in seawater and sediment from the Sound.

Time (days)	Sample	Zinc pyrithione	NP3	Omadine sulfonic acid + pyridine sulfonic acid*	NP1	NP2	NP5	Non-extractable	CO_2
		% of ^{14}C added							
0	LS, water [1]	15.1	13.7	0.8	0.5	-	-	-	-
0	LS, sediment [1]	19.0	18.9	6.3	3.3	1.1	2.7	11.0	
0	LS, total [1]	34.1	32.6	7.1	3.8	1.1	2.7	11.0	
28	LS, water [2]	-	0.7	10.2	-	-	21.2	-	2.6
28	LS, sediment [2]	-	21.2	3.2	-	-	1.3	25.7	
28	LS, total [2]	-	21.9	13.4	-	-	22.5	25.7	

LS, clayey sediment; *, contained also NP4; [1], tests performed by Arch Chemicals; [2], tests performed by VKI; -, not detected.

4.4 **Toxicity to aquatic organisms**

Zinc pyrithione
The toxicity of the active substance zinc pyrithione has been investigated in standard laboratory tests with a number of aquatic organisms living in fresh water (the green alga *Selenastrum capricornutum*, the crustacean *Daphnia magna*, the fish rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*)) and in seawater (the crustacean *Mysidopsis bahia*, the fish sheepshead minnow (*Cyprinodon variegatus*) and the oyster (*Crassostrea virginica*)) (Boeri *et al*. 1993; 1994a-e; Ward *et al*. 1994a). Furthermore, five species of freshwater fish were used at the same time in a single test (Olin 1997). *Pimephales promelas* was also used in this test and the results for this species agreed with the results in the standard test. Besides, *P. promelas* was the most sensitive of the five species. Because of the lack of stability of zinc pyrithione when exposed to light, the tests were carried out with subdued light and all tests - with the exception of the algal test - were made with constant renewal of the test solution (flow through). By so doing, the exposure concentration was successfully kept almost constant in all of the tests - even in the algal test (Ward *et al*. 1994a), in which the medium cannot be renewed, and all results are calculated on the basis of measured concentrations.

The results summarized in Appendix 5 show that the difference in sensitivity was not pronounced between the freshwater and the marine organisms. Algae are apparently the taxonomic group least sensitive to zinc pyrithione. Table 4.6 gives an overview of the toxicity of zinc pyrithione to various groups of organisms.

Long-term studies have been made with crustaceans (daphnids and small prawns) and fish (the most sensitive fish, *Pimephales promelas*, in a short-term test). In the studies with crustaceans, reproduction was ex-

amined and, in the study with fish, the development from egg to small fry was followed. The results in Table 4.6 indicate that fish are also the most sensitive group in long-term tests though the results with crustaceans and fish are of the same order of magnitude. The lowest NOECs are 0.0023 mg/L for crustaceans and 0.0012 mg/L for fish.

Table 4.6
Ecotoxicological data on effects of zinc pyrithione on aquatic organisms. All concentrations are measured concentrations, tests with animals were flow-through tests (see Appendix 5 for detailed data).

Taxonomic group	End point	Exposure time [days]	Result [mg/L]
Algae	EC50	5	0.028
Algae	NOEC*	5	0.0078
Crustaceans	EC50	2-4	0.0036-0.0063
Crustaceans	NOEC (reproduction)	21	0.0023-0.0027
Fish	LC50	4	0.0026-0.4
Fish	NOEC (early life-stage, ELS)	32	0.0012
Oyster	EC50 (shell deposition)	4	0.022

*: The highest concentration at which no effects were observed (NOEC, No Observed Effect Concentration).

Metabolites
The toxicity of the three metabolites has been investigated in the laboratory. The results of these tests are summarized in Table 4.7 together with the results from the tests with zinc pyrithione.

Table 4.7
Summary of results from aquatic toxicity tests with zinc pyrithione and three metabolites. All are short-term tests and the results are expressed as LC50 or EC50. Measured concentrations for zinc pyrithione and omadine sulfonic acid.

Taxonomic group	Zinc pyrithione L(E)C50 mg/L	Omadine disulfide* L(E)C50 mg/L	Omadine sulfonic acid L(E)C50 mg/L	Pyridine sulfonic acid* L(E)C50 mg/L
Algae	0.028	0.14	36	29
Crustaceans	0.0036-0.0063	0.0064-0.013	>127-71	72- >122
Fish	0.0026-0.4	0.03-1.1	59- >137	57- >127
Oyster	0.022	0.160	99	86

*: Data from Olin 1997.

It applies to all four substances (in Table 4.7) that they have been tested with one freshwater alga (*Selenastrum capricornutum*), one freshwater crustacean (*Daphnia magna*), one marine crustacean (*Mysidopsis bahia*), two freshwater fish (*Pimephales promelas* and *Oncorhynchus mykiss*) and one sea fish (*Cyprinodon variegatus*) and furthermore, a shell deposition test with the oyster species *Crassostrea virginica* (marine). Furthermore, pyridine sulfonic acid was used in a long-term test with the fish *Pimephales promelas* (Boeri *et al*. 1999).

In the algal test with omadine sulfonic acid, the concentration of the substance fell during the test. The concentrations used for calculating the effect concentration are measured at the start of the test and the real EC50 is probably somewhat lower than the value stated in Table 4.7 (EC50: 36 mg/L) (Boeri *et al.* 1994g). In the other tests, the results are calculated as the average of the concentrations at the start and at the end of the test (Ward *et al*. 1994b, c, d; Boeri *et al*. 1994f, h, i). If this method of calculation is applied to the results of the algal test, an EC50 = 23 mg/L is achieved.

The results show that while zinc pyrithione and omadine disulfide were very toxic to aquatic organisms (L(E)C50 in the order of 3-300 µg/L), omadine sulfonic acid and pyridine sulfonic acid were considerably less toxic (L(E)C50 in the order of >20 mg/L) (Olin 1977). In a long-term study with fish eggs and larvae, pyridine sulfonic acid gave no effects at a concentration of 0.01 mg/L (Boeri *et al.* 1999). Algae were the group of organisms most sensitive to the last two substances.

Effects of degradation of zinc pyrithione on aquatic toxicity

A parallel test, like the one described in relation to DCOI (cf. Section 3.3.2), was performed in order to examine the relation between degradation of zinc pyrithione and the acute toxicity towards *Acartia tonsa*. The studies were made in the same way as those of DCOI by use of sediment-seawater systems dosed with zinc pyrithione in a concentration of 25 µg/kg. Water phase and sediment were separated 20 min after dosing. The use of the water phase in tests with *A. tonsa* resulted in a lethality corresponding to 100% of the test organisms. The test results showed that stationary incubation in the dark or in the light (340 µmol/$m^2 \cdot s$) at 20-25°C resulted in the fact that no lethal effects on *A. tonsa* were observed after one day (Figure 4.4). The rapid detoxification demonstrates that zinc pyrithione was rapidly bound to the sediment or transformed to metabolites with considerably lower toxicity than the parent compound as was the case in relation to DCOI (cf. Section 3.3.2). The methods used are described in detail in Appendix 3.

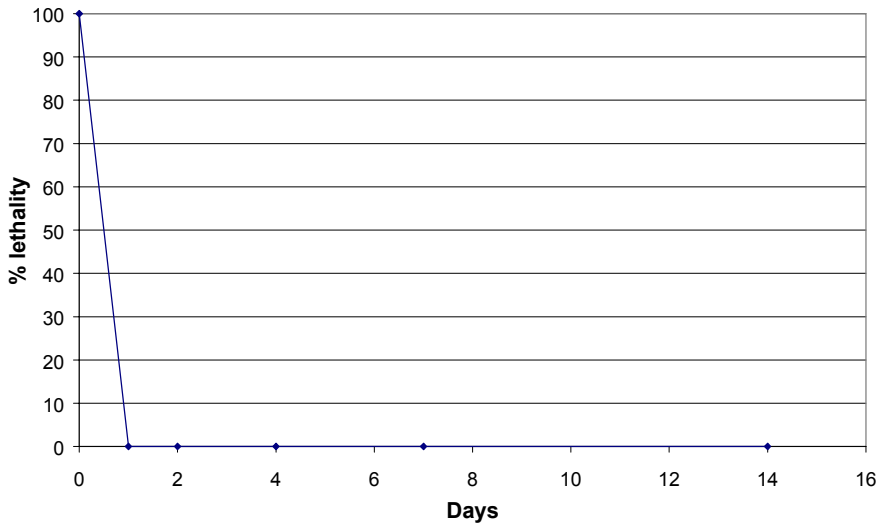

Figure 4.4
Effects of degradation of zinc pyrithione (25 µg/kg) dosed to sediment and seawater on the acute toxicity to Acartia tonsa (test performed in the dark).

4.5 Assessment of zinc pyrithione and metabolites

Zinc pyrithione is transformed very rapidly in aquatic systems. Tables 4.2 and 4.4 show that, after incubation for less than 24 hours, the intact zinc pyrithione constituted less than half of the radioactivity added (day 0). It is assumed that zinc pyrithione is transformed via the structurally comparable omadine disulfide, which is rapidly transformed to heterocyclic compounds with one ring under environmentally realistic test conditions. The tests performed with zinc pyrithione showed that the quantitatively most important metabolites were omadine sulfonic acid and pyridine sulfonic acid under aerobic conditions and NP3, NP4, NP5 and pyridine sulfonic acid under anaerobic conditions (Tables 4.2-4.5). The heterocyclic compounds with one ring are all considered to be recalcitrant and stable in aquatic systems. The biological degradation of zinc pyrithione results in a quantitatively considerable formation of metabolites that sorb to the sediment. This appears from the fact that, at the end of the aerobic biodegradation test after 84 days, approx. 30% of the radioactivity added was sorbed to the sediment while, in the anaerobic test, approx. 50% of the ^{14}C added could be recovered in the sediment after 182 days (Ritter 1999a, b, d).

The aquatic toxicity was investigated for omadine sulfonic acid and pyridine sulfonic acid, which were both considerably less toxic (L(E)C50 in the order of >20 mg/L) than zinc pyrithione and omadine disulfide (L(E)C50 in the order of 3-300 µg/L). Based on the chemical structure of the substances, the toxicity of the other metabolites with one ring is expected to be at the same level as the toxicity of omadine sulfonic acid and pyridine sulfonic acid. On this basis, the known stable metabolites from the transformation of zinc pyrithione under aerobic and anaerobic conditions are considered to have an aquatic toxicity that is between 1,000 and 10,000 times lower than the toxicity of zinc pyrithione (cf.

Table 4.7). The metabolites sorbed to sediment are not yet identified. As these metabolites could not be extracted from the sediment with acetonitrile and KOH, they are considered to have a low bioavailability and thus a low toxicity to aquatic organisms.

4.6 **Risk assessment of zinc pyrithione**

Calculation of exposure concentrations (PEC)
Exposure concentrations (PEC, Predicted Environmental Concentration) were calculated for a pleasure craft harbour (Jyllinge) and a busy navigation route by use of internationally recognized principles (EC 1996) as described in relation to DCOI (cf. Section 3.4). The model and the two scenarios are described in detail in Appendix 1. For parent compound and the most essential metabolites, the following exposure concentrations were calculated:

- PEC (water column)
- PEC (sediment)
- PEC (sediment-pore water)

The three exposure concentrations were defined as the steady-state concentration of the sub-environment in question. I.e., the concentration which the calculated concentrations eventually approach when a continuous leaching of the parent compound to the water environment is simulated. The model used is not validated towards measured concentrations in harbour environments or navigation routes. The exposure concentrations were calculated on the basis of the following assumptions:

- The background concentrations for both the parent compound and the metabolites were assumed to be zero.

- 70% of the pleasure craft was assumed to have been painted with paint containing zinc pyrithione.

- The leaching rate of zinc pyrithione from bottom paints was calculated at 21 $mg/m^2/day$ in harbours and 41 $mg/m^2/day$ when sailing.

- The average photolytical half-life of zinc pyrithione was calculated at 9.8 hours for the pleasure craft harbour of Jyllinge and 6.6 hours for Kronprins Frederiks Bro (cf. Appendix 1). It was not possible to quantify the influence of the presence of the pleasure craft and the shadow effects from the pier on the amount of light falling on the surface and calculations have thus been made with and without the inclusion of photolysis.

- The primary biological transformation of zinc pyrithione into heterocyclic compounds with one ring was assumed to proceed with a half-life of 12 hours in surface water at a temperature of 25°C.

The half-life for zinc pyrithione, which is assumed in the simulation, corresponds to a considerably slower transformation of zinc pyrithione than

the initial removal of the substance from the water phase in studies with seawater and sediment (cf. Section 4.3). Compared with the removal of zinc pyrithione from the water phase (Ritter 1999a-e), a longer half-life was used in the simulation as aquatic systems with sediment make sorption possible and normally have a larger potential for biodegradation compared with the degradation potential in the surface water. The reason for using a half-life for transformation of zinc pyrithione corresponding to the expected transformation in surface water is that the result of the simulation is exposure concentrations at a continuous leaching of zinc pyrithione after steady state was achieved. When the pleasure craft are taken out of the water at the end of the sailing season, zinc pyrithione will probably be rapidly eliminated as the substance is either transformed in the water phase or sorbs to the sediment, in which it is transformed with a very short half-life (cf. Sections 4.2 and 4.3).

The exposure concentrations calculated for zinc pyrithione and its metabolites are approx. 50 times higher in the pleasure craft harbour than in the busy navigation route outside the harbour (Table 4.8).

Table 4.8a
Calculation of PEC for zinc pyrithione and metabolites at steady-state. Photolysis included.

Scenario	Substance	PEC (water) µg/L	PEC (sediment, pore water) µg/L	PEC (sediment, sorbed) µg/kg
Pleasure craft harbour	Zinc pyrithione	0.56	0.00056	0.089
	NP3	1.22	0.25	0.68
	NP4	0.099	0.19	0.078
	Pyridine sulfonic acid	0.0080	0.068	0.040
	NP1	0.15	0.091	0.062
	Omadine sulfonic acid	0.012	0.48	0.47
	Other compounds	0.11	-	-
Navigation route	Zinc pyrithione	0.0053	0.00001	0.00090
	NP3	0.027	0.0028	0.0076
	NP4	0.0027	0.0022	0.00089
	Pyridine sulfonic acid	0.00040	0.00077	0.00045
	NP1	0.0032	0.0011	0.00077
	Omadine sulfonic acid	0.00046	0.0059	0.0058
	Other compounds	0.0032	-	-

Table 4.8b
Calculation of PEC for zinc pyrithione and metabolites at steady-state. Photolysis not included.

Scenario	Substance	PEC (water) µg/L	PEC (sediment, pore water) µg/L	PEC (sediment, sorbed) µg/kg
Pleasure craft harbour	Zinc pyrithione	1.7	0.0013	0.21
	NP3	0.00006	0.54	1.5
	NP4	0.20	0.43	0.18
	Pyridine sulfonic acid	0.016	0.15	0.090
	NP1	0.45	0.24	0.17
	Omadine sulfonic acid	0.036	1.3	1.3
	Other compounds	0.24	-	-
Navigation route	Zinc pyrithione	0.022	0.00002	0.0027
	NP3	0.00001	0.0072	0.019
	NP4	0.0059	0.0061	0.0025
	Pyridine sulfonic acid	0.00088	0.0022	0.0013
	NP1	0.013	0.0042	0.0028
	Omadine sulfonic acid	0.0019	0.022	0.021
	Other compounds	-	-	-

Calculation of Predicted No Effect Concentrations (PNEC)
Predicted No Effect Concentrations (PNECs) are estimated for zinc pyrithione and pyridine sulfonic acid. The other stable metabolites from the transformation of zinc pyrithione are considered to have the same aquatic toxicity as pyridine sulfonic acid.

The available studies of the aquatic toxicity of zinc pyrithione are considered representative and the data material includes long-term studies with crustaceans and the most sensitive group of organisms, i.e. fish. The algal test may be interpreted both as a short-term test and as a long-term test (EC 1996).

For zinc pyrithione, data are interpreted as including three NOEC values from long-term tests (crustaceans, algae and fish), which includes the group of organisms that was most sensitive in the short-term test (fish). On this basis, PNEC is calculated by dividing the lowest NOEC value, which is 0.0012 mg/L for fish, by an assessment factor of 10 (EC 1996). This results in a PNEC of 0.0001 mg/L = 0.1 µg/L for zinc pyrithione.

The result from the long-term test carried out with fish and pyridine sulfonic acid (Boeri *et al.* 1999) is not considered applicable for calculation of PNEC. This is due to the fact that the study used only one concentration (0.01 mg/L) at which no effects were measured. The result does thus not give any indications of the concentration area in which effects may be expected. Calculations of PNEC for pyridine sulfonic acid are thus based on the lowest effect concentrations shown in Table 4.7. The algal

test is the only test that may be considered a long-term test but this test alone is not adequate for making the calculations on the basis of NOEC (EC 1996). As all data were thus derived from short-term tests, an assessment factor of 1,000 is used with lowest effect concentration. For pyridine sulfonic acid, the EC50 value of 28.9 mg/L for algae (pyridine sulfonic acid) is used which results in a PNEC of 0.03 mg/L = 30 µg/L. The PNEC calculated for pyridine sulfonic acid is considered representative of the other stable metabolites from the transformation of zinc pyrithione.

Table 4.9 shows the two calculations of PNEC.

Table 4.9
Calculation of PNEC for zinc pyrithione and pyridine sulfonic acid.

Substance	Lowest effect concentration	Value [µg/L]	Assessment factor	PNEC [µg/L]
Zinc pyrithione	Long-term test NOEC fish	1.2	10	0.1
Pyridine sulfonic acid	Short-term test EC50 algae	28,900	1,000	30

Risk quotient
When transformation of zinc pyrithione by photolysis is included in the calculation of PEC, the risk quotient is calculated on the basis of PEC (water) for zinc pyrithione and the metabolites stated in Table 4.8. PEC (sediment, pore water) for the metabolites is higher than the corresponding PEC (water) when photolysis is ignored. In this case, PEC (water) for zinc pyrithione and PEC (pore water) for the metabolites are used for calculating the risk quotient. As PNEC values for zinc pyrithione and pyridine sulfonic acid are used, risk quotients (Rq = PEC/PNEC) are calculated as shown in Table 4.10.

Table 4.10
Calculation of risk quotients (Rq) for zinc pyrithione and its metabolites.

Substance	PNEC [µg/L]	Pleasure craft harbour		Navigation route	
		PEC^A [µg/L]	Rq^A	PEC^A [µg/L]	Rq^A
Zinc pyrithione	0.1	0.56 1.7	5.6 17	0.0053 0.022	0.05 0.22
Metabolites	30*	1.6 2.7	0.05 0.09	0.037 0.042	0.0012 0.0014

A, upper value, photolysis included; lower value; photolysis not included.
*, Pyridine sulfonic acid

The stated risk quotients are calculated on the basis of realistic worst-case scenarios (Appendix 1), which are i.a. based on the assumption that

70% of the pleasure craft are painted with a bottom paint containing zinc pyrithione. On the basis of the assumptions made in the simulation and of the calculated PEC values, it is considered likely that a risk of chronic ecotoxic effects within the pleasure craft harbour may exist as, presumably, zinc pyrithione will constantly be applied by leaching from bottom paints. The risk quotient for zinc pyrithione within the pleasure craft harbour is between 0.05 and 0.22 and here the risk of ecotoxic effect of zinc pyrithione is considered to be low. The risk quotient out of the pleasure craft harbour is probably closest to 0.05, in which photolysis has been included in the calculation of PEC as major shadow effects are not expected on a normal navigation route.

Within the pleasure craft harbour, a low risk of ecotoxic effects of stable metabolites from the transformation of zinc pyrithione is considered possible and this risk is considered very low in areas out of the pleasure craft harbour.

5 Non-biocidal paints

5.1 **Investigations of non-biocidal paints**

Field tests with mechanical cleaning of two non-biocidal marine bottom paints were carried out by the Danish Sailing Association and Hempel during the sailing season in 1998 (Danish Sailing Association and Hempel 1999).

Non-biocidal antifouling paints are defined and interpreted in various ways in different official connexions. In this report, the following definition applies: A non-biocidal antifouling paint does not contain any active substances (biocides) added in order to prevent fouling through the toxic effect of these substances. Examples of biocides that have been or are still used in antifouling paints in Denmark are: TBT, copper, Diuron, Irgarol, Nopcocide, Sea-Nine (active substance is DCOI), zinc pyrithione, etc.

In stead, the antifouling effect is achieved by a very smooth surface on which the fouling has difficulty in sticking to the paint (corresponding to a "non-stick" effect, often based on silicone). Also very hard epoxy-based paints are considered as non-biocidal alternatives. Very heavy fouling is then expected but these epoxy-based paints allow repeated mechanical cleaning without destroying the surface of the paint.

The two types of paint were an experimental silicone-containing paint, 86330, and an epoxy-based paint, High Protect 35651, which is a commercial product designed to prevent osmosis. The environmental properties of the two non-biocidal paints were examined in ecotoxicological laboratory tests of water samples from a leaching test with painted panels. The ecotoxicological tests included the marine green alga *Skeletonema costatum* and the marine crustacean *Acartia tonsa*. As effects of substances leaching from the paints were only examined in tests with two water-living organisms, a test setup ensuring a worst-case situation was applied in the leaching test.

In the leaching test, the ratio of the painted area to the surrounding amount of water was established in accordance with calculations based on the conditions in the pleasure craft harbour of Jyllinge. On the basis of estimations from the Danish Sailing Association (personal communication with Steen Wintlev-Jensen, Danish Sailing Association), the boats in the harbour are considered to be composed of 360 sailing boats with a total of immersed bottom area of 6840 m^2 (360 · 19 m^2) and 60 motor boats with a total of immersed bottom area of 1320 m^2 (60 · 22 m^2). In the pleasure craft harbour of Jyllinge, the area is approx. 31,500 m^2 and the average water depth is 2.3 m, after which the amount of water in the harbour can be calculated at 70,450 m^3 (cf. Appendix, Table A.1.). Based on these assumptions, the ratio of the painted bottom area of the pleasure craft of the harbour to the amount of water in the harbour is calculated at 0.11 m^2:1 m^3. The ratio of the painted area to the total amount of water in the leaching tests was 1.5 m^2:1 m^3. The painted surface per volume unit

was thus 13-14 times higher in the leaching test than this ratio would be if the boats in the pleasure craft harbour of Jyllinge were painted with the same paint.

5.2 Leaching and ecotoxicological tests

Test paints
The laboratory tests included two non-biocidal paints (1 and 2 below) and Hempel's Antifouling Nautic 76800, which is an organotin-based bottom paint for large-scale navigation. The test paints in the study were as follows:

1. An experimental 86330 paint (silicone-containing)
2. High Protect 35651 (epoxy-based)
3. Hempel's Antifouling Nautic 76800 (organotin-based)

Leaching tests
The leaching tests were performed in cast solid glass aquaria with 38 litres of filtered seawater. Four panels painted on both sides with test paint were submerged into each aquarium so that only the painted part of the panels made contact with the seawater. The painted surface of each panel was 150 cm^2. The water in the aquaria was continuously aerated with a weak current of atmospheric air and vaporized liquid was replaced once a week. The aquaria were covered with black plastic and placed at 20°C. Water samples from the aquaria were sampled after 0, 6, 13, 20 and 34 days. On the first day of the leaching test (day 0), 0.5 litres were sampled from each aquarium after 3 hours while, at all other samplings, 9 litres were taken from each aquarium. At each sampling of 9-litre water samples, one panel was removed from the aquaria so that the ratio of the painted area to the liquid volume remained unchanged throughout the leaching test.

Ecotoxicological test
The toxicity of the water samples was determined in a growth inhibition test with the marine green alga *Skeletonema costatum*, which was conducted in accordance with the procedures in the OECD Guideline for Testing of Chemicals No. 201 "Algal Growth Inhibition Test" (OECD 1984). The algal test was performed with water samples taken after 0, 6, 13, 20 and 34 days' leaching. The results from this test were used for selecting water samples for the examination of chronic effects on crustaceans and for determination of potentially bioaccumulable substances.

On the basis of the results from the algal test, the toxicity of water samples taken after 13 and 34 days was determined in tests with the marine crustacean *Acartia tonsa*. The toxicity towards *A. tonsa* was examined in a screening test for acute toxicity (ISO 1998) and in a test for chronic toxicity, which has been described in detail by the National Environmental Research Institute (NERI 1986) and Johansen and Møhlenberg (1987). The presence of hydrophobic, potentially bioaccumulable substances in the leachate was determined in accordance with the OECD Guideline for Testing of Chemicals No. 117 "Partition Coefficient (n-

octanol/water), High Performance Liquid Chromatography (HPLC) Method" (OECD 1989).

The materials and methods used are described in detail in the report "Ecotoxicological tests of leachates of antifouling paints" (Bjørnestad *et al.* 1999), which also contains a detailed description of the study results. The most essential results are summarized below.

Growth inhibition test with algae

The toxicity test with *S. costatum* showed that the leachate from High Protect 35651 did not inhibit the growth of the algae. However, Hempel's Antifouling Nautic 76800 as well as the experimental 86330 paint leached substances to the surrounding water, which caused an inhibition of the growth of the algae (Table 5.1).

Table 5.1
Inhibition of growth of Skeletonema costatum in tests of leachates in a concentration of 900 mL/L.

Test paint	Inhibition of growth (%)				
	Day 0	Day 6	Day 13	Day 20	Day 34
Control	< 1	< 1	< 1	< 1	< 1
High Protect 35651	< 1	< 1	< 1	< 1	< 1
Experimental 86330	2	48	100	100	100
Hempel's Antifouling Nautic 76800	100	100	100	100	100

As the results with the experimental 86330 paint (Table 5.1) were astonishing, Hempel's laboratory has performed more leaching tests using the method described above (personal communication with Susanne Holm Faarbæk, Hempel). In this test, leachates were sampled after 20 days, after which the toxicity of the coded water samples was determined by VKI. Water samples from two separate leaching tests with the experimental 86330 caused an inhibition of *S. costatum* of 78% and 100%, respectively. The leachate from another paint, 97003-057, which composition is very similar to that of the experimental 86330, caused no inhibition of the algal growth. The test with 97003-057 could, however, not be reproduced as a new laboratory batch of the paint, 97003-128, caused an inhibition of 90% of *S. costatum* (personal communication with Susanne Holm Faarbæk, Hempel).

While the additional leaching tests with the experimental 86330 paint generally confirmed the results in Table 5.1, the diverging results for the 97003 paint indicate that variations in the production or painting process has great influence on the leaching of substances from the painted surface. It has not been possible to shed light on these conditions in connection with this study.

Toxicity test with Acartia tonsa

Water samples from leaching test with High Protect 35651 caused no acute toxicity towards *A. tonsa* in screening tests. The chronic toxicity test, however, showed that undiluted water samples from the leaching test with High Protect 35651 inhibited the development of *Acartia* nauplii while no inhibition was observed when the water samples were diluted 10 times (100 mL/L) (Figure 5.1). As no effects were observed either on the egg production at a concentration of 100 mL/L, NOEC (No Observed Effect Concentration) was 100 mL/L for the leachate from High Protect 35651.

Contrary to High Protect 35651, the leachate from the test with the experimental 86330 paint was acutely toxic to *A. tonsa* as the lethality of adult *Acartia* was 100% for undiluted water samples taken after 20 and 34 days, respectively. Leachate diluted 10 times (100 mL/L) caused a lethality of 40% (20 days) and 20% (34 days), respectively. In the chronic toxicity tests, no nauplii developed into copepodites and adults at an impact of leachate diluted to 100 mL/L (water sample taken after 13 days) and 10 mL/L (water sample taken after 34 days) (Figures 5.2-5.3.). On the basis of these results, it is concluded that NOEC was less than 10 mL/L for the leachate from the experimental 86330 paint.

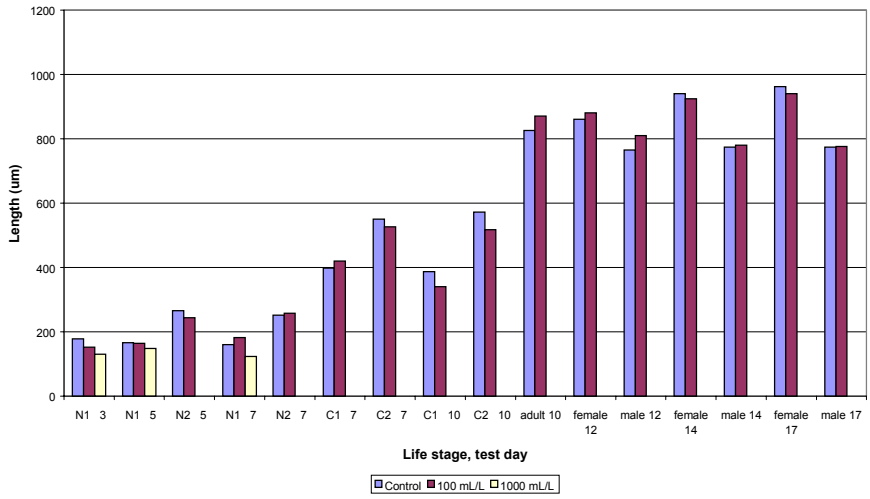

Figure 5.1
Development of nauplii, copepodites and adults in leachate from High Protect 35651, day 13.

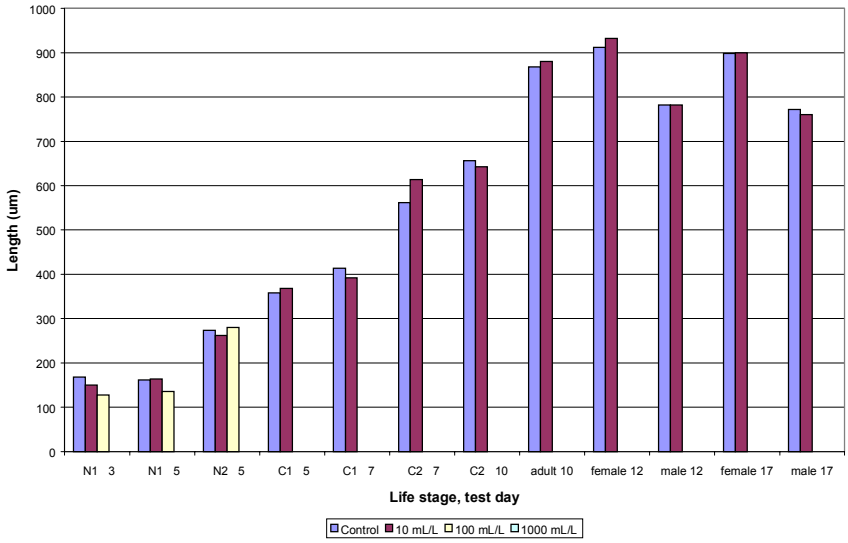

Figure 5.2
Development of nauplii, copepodites and adults in leachate from the experimental 86330 paint, day 13.

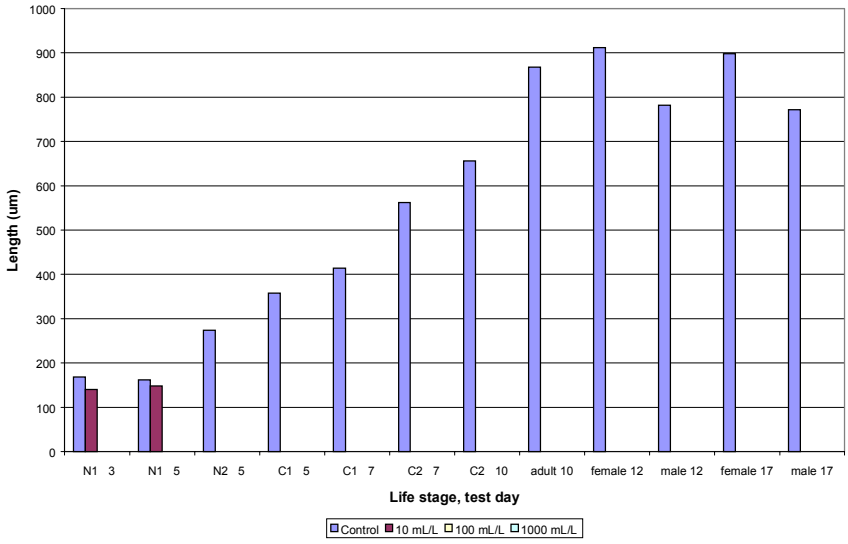

Figure 5.3
Development of nauplii, copepodites and adults in leachate from the experimental 86330 paint, day 34.

The water samples from the leaching test with Hempel's Antifouling Nautic 76800 had a high acute toxicity towards *A. tonsa* as the water sample taken after 20 days and diluted 100 times (10 mL/L) caused a lethality of 100%. No nauplii developed into copepodites and adults at an impact of leachate diluted to 0.1 mL/L (water sample taken after 13 days). On the basis of these results, it is concluded that NOEC was less than 0.1 mL/L for the leachate from Hempel's Antifouling Nautic 76800.

Table 5.2 gives the NOEC values for acute and chronic effects.

Table 5.2
NOEC values determined in tests with Acartia tonsa (NOEC, No Observed Effect Concentration).

	Day for sampling of leachate	NOEC acute (mL/L)	NOEC development (mL/L)	NOEC egg hatching (mL/L)
Control	34	1,000	1,000	1,000
High Protect 35651	34	1,000	100	100
Experimental 86330	34	<100	<10	10 [A]
Hempel's Antifouling Nautic 76800	34	<1	<0.1 [A]	-

[A], test performed with leachate taken after 13 days, -, not determined.

n-Octanol-water
partition coefficient

The n-octanol-water partition coefficient (log K_{ow}) is normally used as an expression of the inherent ability of chemical substances to bioaccumulate in water-living organisms. Substances with log K_{ow} >3 are considered potentially bioaccumulative. The test performed was a qualitative test, in which log K_{ow} was determined for substances in leachate but in which the concentration of the substances was not determined.

Six compounds with log K_{ow} >3 were demonstrated in a water sample from the leaching test with High Protect 35651 at neutral pH. There was, however, some analytical uncertainty as these compounds caused small areas in the HPLC chromatograms and four compounds were only demonstrated in one of the two analyses. At pH 2, twelve compounds with log K_{ow} >3 were found. Although the results should be interpreted with caution, the examination demonstrates the presence of potentially bioaccumulable substances in the leachate from High Protect 35651. Of these substances, the majority is considered to have a log K_{ow} between 3 and 4.

Four compounds with log K_{ow} >3 were demonstrated in a water sample from the leaching test with the experimental 86330 paint at neutral pH. At pH 2, 12-15 compounds were demonstrated with log K_{ow} >3. The results show that potentially bioaccumulable substances are leached to the surrounding water in leaching test with the experimental 86330 paint. As was the case with High Protect 35651, the majority of these substances is considered to have a log K_{ow} between 3 and 4.

The examinations of log K_{ow} have thus demonstrated that potentially bioaccumulable compounds may be leached from High Protect 35651 as well as from the experimental 86330 paint. The leached substances are, however, considered to have a low bioaccumulation potential as most of the substances have a log K_{ow} <3-4 and no compounds with log K_{ow} >5 have been demonstrated. Substances with log K_{ow} between 3 and 4 will typically have a bioconcentration factor of 100-575 (Veith and Kosian 1983).

5.3 **Assessment of non-biocidal paints**

The performed leaching tests were carried out with a ratio of the painted area to the surrounding liquid volume that was at least 13-14 times higher than the corresponding ratio in the pleasure craft harbour of Jyllinge (cf. Section 5.1). For both non-biocidal paints, High Protect 35651 and the experimental 86330 paint, water samples from leaching tests have markedly less effect than water samples from similar test with the commercial paint, Hempel's Antifouling Nautic 76800. Table 5.2 shows that leachates from High Protect 35651 and the experimental 86330 paint caused NOEC values for *A. tonsa* that were at least 1,000 and 100 times, respectively, higher than NOEC for leachate from Hempel's Antifouling Nautic 76800.

Water samples from the leaching test with High Protect 35651 caused no inhibition of *S. costatum* and chronic effects on *A. tonsa* were only observed with undiluted leachate (NOEC = 100 mL/L).

Water samples from the leaching test with the experimental 86330 paint were toxic to *S. costatum* and in acute and chronic tests with *A. tonsa* (NOEC, acute <100 mL/L; NOEC, chronic <10 mL/L). There are, however, problems in the leaching of substances from this type of paint that have not been fully examined (cf. the results with *S. costatum*). These problems should be further examined before a final assessment is made of the environmental properties of the paint.

6 Conclusion

The following conclusions may be drawn on the basis of the present study:

Bioavailable copper is very toxic to aquatic organisms. The potential toxic effect of copper on the aquatic environment is reduced by sorption to organic matter and sediments which causes the actual bioavailability of copper to be low. Disturbances of the sediment and the consequent change in oxygen conditions may, however, remobilize sequestrated copper and such changes may probably cause effects on sensitive organisms in the vicinity of harbours and dumping sites.

DCOI is rapidly transformed into metabolites in seawater in which half-lives of between 11 and 14 hours have been found. The transformation of DCOI is considerably quicker in aquatic sediment as half-lives of less than 1 hour have been demonstrated. DCOI is very toxic to aquatic organisms as the lowest effect concentrations (EC/LC50) are lower than 10 µg/L. The aquatic toxicity of the stable metabolite, N-(n-octyl) malomanic acid, is several orders of magnitude lower as the lowest effect concentrations (LC50) are estimated to be between 90 and 160 mg/L.

On the basis of realistic worst-case scenarios, risk quotients (PEC/PNEC) for DCOI have been calculated at 8.7 for the pleasure craft harbour and 0.1 for the navigation route. Based on the calculation prerequisites, it is estimated that, within the pleasure craft harbour, there is a risk of chronic ecotoxic effects as DCOI is assumed to be applied constantly by the leaching from bottom paints. The risk quotient for DCOI out of the pleasure craft harbour is less than 1, and here the risk of ecotoxic effects is considered to be low. The calculated exposure concentrations (PEC) are based on realistically conservative assumptions, which means that, in practice, the calculated PEC values are seldom exceeded. When the pleasure craft are taken out of the water at the end of the sailing season, DCOI will probably be rapidly eliminated as DCOI is either transformed in the water phase or sorbs to the sediment, in which it is transformed with a very short half-life.

Zinc pyrithione is very rapidly transformed by photolysis and biodegradation. Zinc pyrithione is very toxic to aquatic organisms as the lowest effect concentrations (EC/LC50) are lower than 10 µg/L. The toxicity of the stable metabolites, omadine sulfonic acid and pyridine sulfonic acid, is several orders of magnitude lower as the lowest effect concentrations (LC50) for these compounds are 36 and 29 mg/L, respectively.

By using the same realistic worst-case scenarios as for DCOI, the risk quotients (PEC/PNEC) for zinc pyrithione have been calculated to be 5.6-17 for the pleasure craft harbour and 0.05-0.22 for the navigation route. The lowest risk quotients are based on PEC values in which transformation of zinc pyrithione by photolysis is included in the calculations while the highest risk quotients are based on calculations in which photolysis is totally ignored. Based on the calculation prerequisites, it is

estimated that, within the pleasure craft harbour, there is a risk of chronic ecotoxic effects as zinc pyrithione is assumed to be applied constantly by the leaching from bottom paints. The risk quotient for zinc pyrithione out of the pleasure craft harbour is less than 1, and here the risk of ecotoxic effects is considered to be low. The risk quotient out of the pleasure craft harbour is probably closest to 0.05, in which photolysis has been included in the calculation of PEC as permanent shadow effects are not expected on a normal navigation route. As described for DCOI, the realistically conservative assumptions mean that, in practice, the calculated PEC values are seldom exceeded. When the pleasure craft are taken out of the water at the end of the sailing season, zinc pyrithione will probably be rapidly eliminated as a result of its short half-life in water and sediment.

Water samples from the leaching tests with High Protect 35651 caused no inhibition of the growth of *S. costatum* and chronic effects on *A. tonsa* were observed only in undiluted leachates (No Observed Effect Concentration, NOEC = 100 mL/L). Water samples from the leaching test with the experimental 86330 paint showed toxicity towards *S. costatum* and in acute and chronic tests with *A. tonsa* (NOEC, acute <100 mL/L; NOEC, chronic <10 mL/L). However, some factors seem to indicate that variations in the production or painting process may influence the leaching of substances from this type of paint. These problems should be further examined before a final assessment is made of the environmental properties of this paint. For both non-biocidal paints, water samples from leaching tests have significantly less effect than water samples from similar tests with the commercial paint, Hempel's Antifouling Nautic 76800. Leachates from High Protect 35651 and the experimental 86330 paint caused NOEC values for *A. tonsa* that were at least 1,000 and 100 times, respectively, higher than the corresponding NOEC values for leachate from the organotin-based paint.

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Appendix 1: Model for calculation of exposure concentrations (PEC)

1. Introduction

This appendix describes the exposure model which was used for calculating the exposure concentrations PEC (Predicted Environmental Concentration) for DCOI and zinc pyrithione (ZPT) and their main metabolites.

The established model applies modelling principles that are generally used at the determination of PEC. Numerous models for calculating exposure are described in literature, i.a.:

- SimpleBox, which is a ”multi-compartment” model based on the fugacity principle (Fredenslund *et al.* 1995). This model is used in the Technical Guidance Document (TGD) for generic risk assessments of individual substances (EC 1996) and is thus incorporated in the edp model EUSES, which is an “electronic” edition of the TGD (European Chemicals Bureau 1997).

- Furthermore, EUSES includes a module for estimation of PEC for antifoulants.

- The CHARM model, which is used for risk assessments of offshore chemicals (Karman *et al.* 1996).

- EXAMS, which is an interactive computer program for simulation of the fate of environmentally hazardous substances in aquatic ecosystems (Burns *et al.* 1981).

2. Establishing a calculation model

In general, exposure assessments are composed of the following items:

- Setting up of scenarios describing the environmental parameters of importance to the emission and the fate of the substances

- Determination of the emission of chemicals

- Calculation of PEC in relevant sub-environments

- Sensitivity analysis in which the relative dependency of PEC of the parameters forming part of the standard scenarios and the relative dependency of PEC of the parameters of the substances are estimated.

These elements were also applied in the present exposure calculations.

2.1 Scenarios

Two standard scenarios have been set up for the calculation of exposure concentrations (PEC):

1. Pleasure craft harbour. The pleasure craft harbour of Jyllinge has been chosen as standard pleasure craft harbour. This pleasure craft harbour was selected as a realistic worst-case as the harbour has a large number of boats compared to the water volume of the harbour and a low water exchange. The whole harbour area is included in the scenario and total mixing is assumed for the entire harbour area.

2. Busy navigation route. The narrows of Kronprins Frederiks Bro (near Frederikssund) was chosen as standard navigation route. Partly because there is relatively heavy traffic of pleasure craft and partly because statistics have been made of the number of boats passing the bridge. The scenario comprises a water column with a length of 1 metre in the sailing direction and a width similar to that of the boats. Total mixing is assumed in the vertical direction of the water column.

Both standard scenarios are thus placed in Roskilde Fjord.

Each scenario was characterized as regards:

- Water exchange
  The net water exchange between Roskilde Fjord (inlet) and its mouth at Isefjord is assumed to correspond to the net water supply to the inlet, which is stated to be approx. $1.25 \cdot 10^{-4}$ $m^3 \cdot s^{-1}$ per metre of the length of the inlet (Harremoës and Malmgren-Hansen 1989). As Roskilde Fjord is approx. 38 km long (Harremoës and Malmgren-Hansen 1989), a total of approx. 410,400 m^3 water is supplied a day. With a surface area of approx. 125 km^2, this corresponds to a net water exchange of 0.003 m^3/m^2/day. The net water exchange is thus very low. DHI (1994) thus also indicates that the water level in Roskilde Fjord is primarily determined by wind conditions and tidal variations. The water level variations determine the currents and thus the water exchange of the inlet. The largest variations in water level are induced by the wind but the tide determines the regular minimum variations and thus which minimum water exchange occurs in a short view. At Hundested in the north, the normal range of the tide is approx. 20 cm, which corresponds to the daily variation in calm weather observed by the bridge guard at Kronprins Frederiks Bro near Frederikssund. At the bottom of the inlet near Roskilde, the tidal variations are as low as 6-7 cm (DHI 1994). Due to the wind, normally occurring variations over longer periods of time are, however, much larger.

  - For the pleasure craft harbour of Jyllinge, it is primarily northwesterly winds that may cause up to 1 metre high tides and southeast and southerly winds that may cause 0.5-1.0 m low tides. Data on the water level (for the years 1996, 1997 and 1998) for a station at Værebro Å (stream), which falls into Roskilde Fjord a few kilometres north of the pleasure craft harbour, state a mean daily total change in the water level of approx. 0.6 m/day

($0.6\ m^3/m^2/day$). The data on water levels were obtained from Ivar Thorstein Hansen, the County of Roskilde. This water level figure is considered to reflect the water exchange in the pleasure craft harbour of Jyllinge and is used in the model calculations.

- For the narrows at Frederikssund, the difference between daily minimum and maximum water depths (at times without high winds when the differences may be much more considerable) is stated by the bridge guard to be approx. 0.2 m. Furthermore, wind conditions will contribute to the water exchange. We have not succeeded in obtaining exact data on the water depth under the bridge, Kronprins Frederiks Bro, but the water exchange will probably as a minimum be at the same level as the water exchange in the pleasure craft harbour of Jyllinge and at Værebro. Therefore, a water exchange of approx. $0.6\ m^3/m^2/day$ was assumed for the narrows at Frederikssund.

For both scenarios, the concentration of substance in the water transported into the waters in question is considered insignificant.

- Water depth.

- Composition and characterization of suspended matter. The suspended matter was characterized with respect to the contents of organic matter. Furthermore, the suspended matter was considered negatively charged.

- Salinity.

- Temperature. In the present study, the temperature is put at 12.5°C, corresponding to the mean air temperature from April till September (the sailing season is typically from the end of April to the beginning of October).

- pH.

- The number of m^2 of bottom areas of ships that are in the waters per time unit.

- The percentage of the ships having bottom paint with the examined antifoulant (P).

- Water-holding capacity of the sediment. Apart from a minor content of organic carbon in the sediment, the composition of the upper sediment layer was assumed to be identical to the composition of the suspended matter. The sediment was assumed to be anaerobic.

- Number of boats in the waters in question.

  - Danish Sailing Association (personal correspondence with Steen Wintlev, Danish Sailing Association) has passed on information on the capacity (number of sailing and motor boats) of the pleasure craft harbour of Jyllinge. Furthermore, Danish Sailing Asso-

ciation has estimated the average wet surface of the boats to be approx. 18 m^2.

- Statistics on the passing-through of pleasure craft for 1995, 1996 and 1998 (Kronprins Frederiks Bro 1996, 1998, 1999) were used for determining the number of sailing and motor boats passing the bridge. Based on these statistics, the average daily number of passing-throughs in the peak season (May - October) was fixed at approx. 70 pleasure craft a day. The distribution of pleasure craft on sailing boats and motor boats and thus the average wet surface of the boats is assumed to be the same as that for the pleasure craft harbour of Jyllinge.

- Average time which the centre of gravity of the boat stay in the waters:
  - For the pleasure craft harbour, it was assumed that all the boats are in the harbour. Danish Sailing Association states that the berths are occupied from approx. mid-May till end-September. During summer holidays (1 July - 15 August), approx. a third of the boats are gone, and there are almost no visitors in the harbour as it is situated inconveniently to tourists on their way through Roskilde Fjord (personal correspondence with Steen Wintlev, Danish Sailing Association). Assuming that all berths are always occupied will thus overestimate the total leaching of antifoulants to the harbour.
  - Furthermore, Danish Sailing Association (personal correspondence with Steen Wintlev, Danish Sailing Association) states that the passing-through at Frederikssund takes place at relatively low speed because of the narrow fairway and the large number of boats. When passing through, the sea speed is estimated to be approx. 3-4 knots, corresponding to 5.5-7.4 km/h. The time that the centre of gravity of a boat stays in the water column in question is thus approx. $5.6 \cdot 10^{-6} – 7.5 \cdot 10^{-6}$ days.

Table B1.1 summarizes the parameters characterizing the two scenarios. These parameters are applied in the basic calculations.

Table B1.1
Standard scenario.

Parameter	Remarks	Unit	Standard scenario	
			Harbour	**Busy navigation route**
Selected location			Pleasure craft harbour of Jyllinge	Kronprins Frederiks Bro near Frederikssund
pH			7	7
Oxygen conditions (water column)			aerobic	aerobic
Oxygen conditions (sediment)			anoxic	anoxic
Salinity	From Harremoës and Malmgren-Hansen (1989)	‰	14.5	14.5

Parameter	Remarks	Unit	Standard scenario	
			Harbour	Busy navigation route
Temperature	From Fredenslund *et al*. (1995)	°C	12.5	12.5
Suspended matter	From Fredenslund *et al.* (1995)	mg/L	18	18
Organic carbon (OC) in suspended matter	From Fredenslund *et al*. (1995)	weight %	8	8
Organic carbon (OC) in sediment	From Fredenslund *et al*. (1995)	weight %	5	5
Water content in sediment	From Fredenslund *et al*. (1995)	weight %	80	80
Density of sediment	From Fredenslund *et al*. (1995)	kg/m^3	1,200	1,200
Water depth	Information from Danish Sailing Association and from Ulrik Petersen, bridgemaster at Kronprins Frederiks Bro (near Frederikssund).	m	2.3 The difference between high and low tides is stated to be approx. 1 m. Here, the daily tidal variation is put at 0.6 m. Min. depth at high wind: 1.4 m Max. depth at high wind: 2.9 m	4.2 Typical variation in 24 hours: 0.6 m Min. depth at high wind: 3.5 m Max. depth at high wind: 5.4 m
Area considered	Information from Danish Sailing Association.	m^2	Area of the pleasure craft harbour, approx. 31,500	2 · 1 m · width of the boats
Net sedimentation	From Bach *et al*. (1986)	m sediment/ year	0.01	0.01
Sedimentation rate	From Fredenslund *et al*. (1995)	m/d	1.0	1.0
Resuspension rate	Calculated by putting net sedimentation velocity at 0.01 m/year	m sediment/ year	0.017	0.017
Water exchange		$m^3/m^2/day$	0.6	0.6
% of vessels with examined bottom paint	Hempel (represented by M.W. Christoffersen) estimates that 30-70% of the boats in a harbour are painted with a bottom paint containing an organic active substance. In order to ensure a realistic worst-case estimate, this percentage is put at 70%	%	70	70
Number of boats per day	Based on information from Danish Sailing Association and statistics on the passing-through of pleasure craft at Kronprins Frederiks Bro (near Frederikssund)		400	70
Time that the centre of gravity of the boats stay in the waters		days	always there	$7.5 \cdot 10^{-6}$

2.2 Emission of antifoulants

The rate, at which the antifoulant leaches into the aquatic environment, is expressed as follows:

U = [leached substance per area per time unit].

The measuring of realistic leaching rates of antifoulants is causing great problems as the leaching rate depends on various factors such as:

- The time after painting. The leaching rate has often been demonstrated to decrease as a function of the time as a result of the falling concentration of the substances in the paint.
- Thickness of the coat of paint
- Liberation of other substances in the paint
- Outward circumstances, i.e. whether the boat is sailing or not, currents/water exchange, temperature, etc. The leaching rate is typically higher when the boat is sailing than when it is in port.

A draft standard (ISO 1999) is available from which the leaching rate can be calculated. The leaching rate is determined on the basis of an estimate of the life of the paint, in which it is simply assumed that all of the antifoulant will be released throughout the life of the paint. The first two weeks after the boat has been painted, a higher leaching rate is anticipated. After two weeks, the leaching rate is considered to be constant. The standard does not take into account that the leaching rate is probably higher while sailing than when the boat is in port, and the standard will thus be likely to overestimate the leaching rate when the boat is in port and underestimate the leaching rate while sailing. Furthermore, the draft standard proposes typical thicknesses of coating and lives of different paints (ISO 1999). On the basis of the proposals of the draft standard on coat thicknesses and lives, the thickness of the coat worn down in six months (corresponding to a sailing season) can be calculated to be 42 µm (soluble matrix), 38 µm (insoluble matrix), 45 µm (tin-based self-polishing paint) and 50 µm (tin-free self-polishing paint). These coat thicknesses are in good agreement with the estimates that Hempel has made, stating an average worn down coat of paint of 42 µm per sailing season for pleasure craft in Denmark (Hempel 1999c). Hempel has based their calculation on the amount of bottom paint sold in the Danish market a year and the number of sailing/motor boats of more than 6 m (corresponding to those painted) and their average bottom area.

In order to simulate the increased leaching rate while sailing, it is assumed that 60 µm of the coat of paint is worn off at constant sailing for 6 months. In order to simulate the lower leaching rate when the boat is in port, it is assumed that 30 µm of the coat of paint is worn off when the boat is constantly in port for 6 months. The above corresponds to the assumption that the boats are sailing for approx. 2 months of a sailing sea-

son and are in port for the remaining 4 months and that the leaching rate while sailing is twice the rate when not sailing.

On the basis of confidential information from Hempel on the content of antifoulants, dry matter and density (Hempel 1999b), the average leaching rates for the two types of antifoulants can be calculated. Table B1.2 gives the results of these calculations.

Table B1.2
Calculated average leaching rates.

Antifoulants	Leaching rate (U) (mg/m^2/day)	
	In port	While sailing
DCOI	13	25
ZPT	21	41

In the model, the total leaching of the active substance to the water per time unit is expressed as:

$$F[\text{mg/day}] = N \cdot A \cdot \frac{P}{100} \cdot U \cdot \tau$$

where

N is the number of boats being in the water area per day [boats/day]

A is the average wet bottom area [m^2/boat]

τ is the time that the centre of gravity of the boats stays in the waters in question [days]

P is the percentage of the boats that have been painted with the antifoulant in question [%]

2.3 PEC model

The model is divided into the following parts:

1. Mass balance in the water column
2. Mass balance in the sediment

Transformation in the water column

The following conditions in the water column were taken into consideration:

- The degradation rate of the parent compound and the subsequent formation of metabolites. Aerobic conditions in the water column were assumed. The effect of the temperature on the degradation rate was included by assuming that the degradation rate is halved when the temperature falls by 10°C (or vice versa). During the modelling period, temperatures are not so low that degradation stops.

- Abiotic transformation. Degradation of ZPT by photolysis is included. For the two investigated substances, hydrolysis is not consid-

ered a significant reaction (cf. Chapters 3 and 4). Like other reactions, degradation by photolysis is temperature dependent but the effect of the temperature is less than for other reactions. Schwarzenbach *et al*. (1993) thus states that a change in temperature of 10°C only changes the reaction rate by a factor of between 1.15 and 1.5. Therefore, the effect of the temperature on the degradation by photolysis is ignored in the present study.

- A first order degradation kinetics was assumed for all degradation reactions.

- No discrimination was made between dissolved substance and substance sorbed to dissolved organic matter (DOC).

- Sorption to the suspended matter was expressed as a linear adsorption.

- Linear sedimentation of the suspended matter was assumed.

- Resuspension of sedimentated matter. In the calculations, the resuspension rate was assumed to be constant.

The following conditions in the sediment were taken into consideration:

- Sedimentation of suspended matter from the upper water layer.

- Resuspension of sediment to the upper water layer.

- A first order anaerobic degradation. The sediment was assumed to be anaerobic for which reason only anaerobic degradation was included.

- Only the sediment formed during the simulation period was examined. This sediment layer was considered to be a completely homogeneous mixture.

For both the parent compound and its main metabolites, a mass balance was established for the water column and the sediment.

The following three PECs (Predicted Environmental Concentration) were calculated:

- PEC(water column)
- PEC(sediment)
- PEC(sediment, pore water)

These three concentrations were put equal to the steady-state concentration, i.e. the concentration that the calculated concentrations eventually approach when simulating a continuous leaching of the substance to the aquatic environment.

For all substances, the background level is assumed to be 0.

2.4 Data on active substances

2.4.1 DCOI

Aerobic degradation

Figure B1.1 shows the simplified degradation pattern which was assumed for DCOI. At first, DCOI was assumed to degrade into N-(n-octyl) malonamic acid which, while releasing CO_2, was transformed into N-(n-octyl) acetamide and N-(n-octyl) β hydroxy propionamide. These two metabolites were assumed to be transformed into a large number of different organic compounds, which were comprised under "Other metabolites". The half-life of this pseudo-reaction was assumed to be same as those of N-(n-octyl) acetamide and N-(n-octyl) β hydroxy propionamide. To a certain degree, these metabolites will be mineralized while forming CO_2 (half-life of this transformation is assumed to be the same for all "other metabolites").

The half-life of the transformation of DCOI into N-(n-octyl) malonamic acid was determined on the basis of a test in which the removal of DCOI was measured in seawater from the pleasure craft harbour of Jyllinge for a period of 72 hours at 12°C (Jacobson and Kramer 1999). By minimizing the total of the areas of the relative residues of DCOI (RRSQ) stated by Jacobson and Kramer (1999), using the following equation:

$$RRSQ = \sum_i \left(\frac{y_i(\text{observed}) - y_i(\text{estimated})}{y_i(\text{observed})} \right)^2$$

where

i	is a numeric reference to the observation
y_i(observed)	is the measured degradation (%)
y_i(estimated)	is the estimated degradation (%) assuming first order kinetics and assuming that y_i(estimated) at the time 0 = y_i(observed) at the time 0

half-lives can be calculated to be 12.8 hours (for replicate 1) and 15.3 hours (for replicate 2) with an average half-life of 14.1 hours (at 12°C). The other half-lives were estimated on the basis of the quantities which were considered to be present after 30 days' aerobic degradation (see Figure B1.1) in experiments carried out by Mazza (1993).

The estimated half-lives of the aerobic transformation of DCOI at 25°C are given in Table B1.3.

Table B1.3
Estimated half-lives (days) at 25°C of aerobic degradation of DCOI.

Parent compounds	Metabolites					
	DCOI	N-(n-octyl) malonamic acid	N-(n-octyl) β hydroxy propion-amide	N-(n-octyl) acetamide	Other metabolites	$^{14}CO_2$
DCOI	-	0.2	-	-	-	-
N-(n-octyl) malonamic acid	-	-	95	32.7	19.7	-
N-(n-octyl) β hydroxy pro-pionamide	-	-	-	-	19.7	-
N-(n-octyl) acetamide	-	-	-	-	19.7	-
Other metabolites	-	-	-	-	-	66.5
$^{14}CO_2$	-	-	-	-	-	-

Anaerobic degradation

The anaerobic degradation of DCOI was assumed to follow the same reaction pattern as the aerobic degradation. The degradation rates were, however, assumed to be slower for the anaerobic degradation.

The transformation of DCOI at aerobic and anaerobic test conditions is shown as a function of the time in Figure B1.2. Data from Table 3.2 of the main part of this report (aerobic conditions) and from Table 3.4 (an-aerobic conditions; concentrations set at 100%). It should be noted that the time axis depicting the anaerobic tests is 4.5 times longer than the time axis of the aerobic tests. Figure B1.2 shows that there is thus a fair correlation between the measured concentrations of the aerobic and an-aerobic tests, respectively. In the calculations, it was thus assumed that, under anaerobic conditions, the half-lives of the reactions are 4.5 times longer than under aerobic conditions.

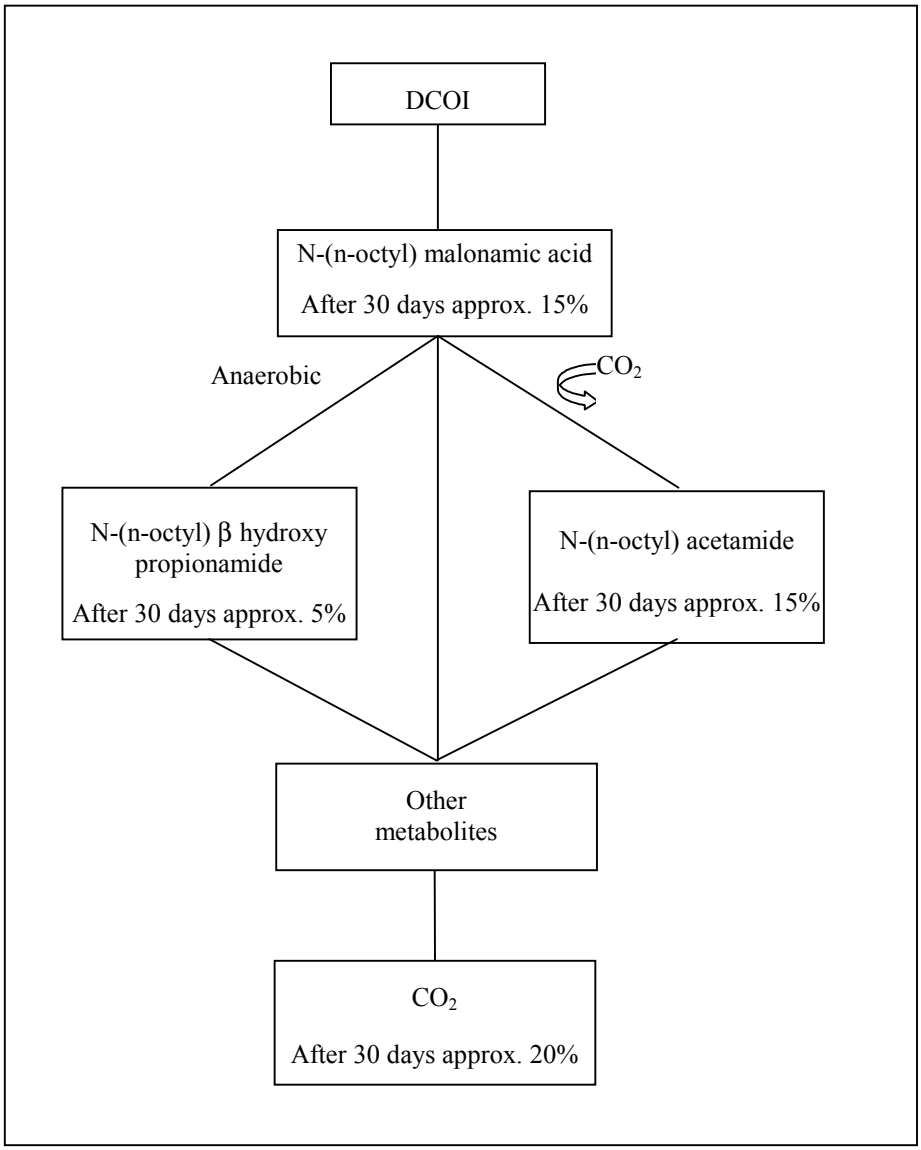

Figure B1.1
Degradation of DCOI.

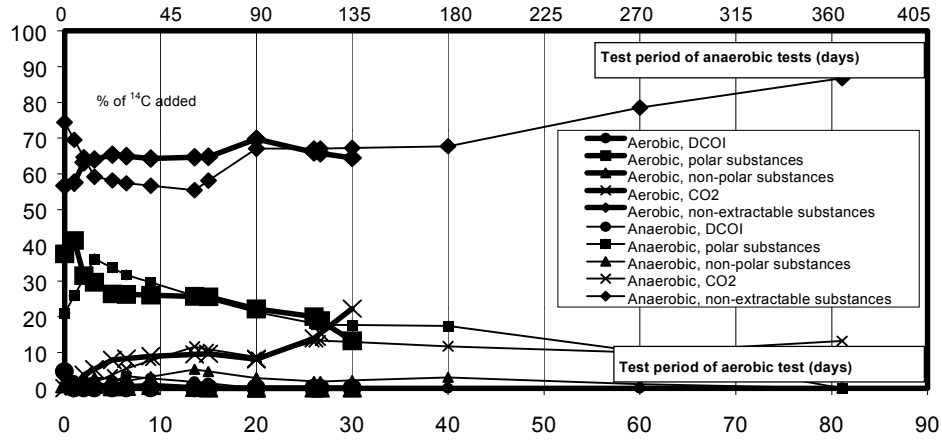

Figure B1.2
Degradation of DCOI under aerobic and anaerobic conditions.

Properties of the substance

Table B1.4 gives selected properties of DCOI and its metabolites. DCOI and the three metabolites were not assumed to be present in ionized form at pH = 7.

Table B1.4
Properties of DCOI and its metabolites.

Substance	Molar weight (g/mol)	Log K_{OW}	Log K_{OC}
DCOI	282	2.8**	3.2
N-(n-octyl)malonamic acid	215	2.00*	1.75*
N-(n-octyl) β hydroxy propionamide	201	1.77*	1.789*
N-(n-octyl) acetamide	171	2.74*	2.756*

* Calculated by means of K_{ow}Win (Syracuse Research Corporation 1996).
** Measured (data stated in the main report).

2.4.2 Zinc pyrithione (ZPT)

Biodegradation

Figure B1.3 shows the simplified biological degradation paths of ZPT which were simulated in the exposure calculations. It is a very simplified model compared to the very complicated degradation pathways of ZPT.

The following abbreviations are used:

- Zinc pyrithione ZPT
- Pyrithione PT
- Omadine disulfide OMDS
- Omadine sulfonic acid OMSo
- 2-Pyridine sulfonic acid PSoA

Other heterocyclic metabolites with one ring are given as NP1-NP5 (cf. Chapter 4 of the main report). The identity of NP1-NP5 is known to VKI.

Two main degradation paths are assumed:

1) Primarily under aerobic conditions:
   ZPT → OMDS → NP1 → OMSo + other compounds

2) Primarily under anaerobic conditions:
   ZPT→ NP3 + OMDS → NP4 → PSoA + other compounds

It was assumed that OMDS, NP3, PSoA and OMSo were further transformed into other compounds, which, to a minor degree, are mineralized.

The half-life of the primary reaction (ZPT → PT→ OMDS + NP3) was set at 0.5 days. The other half-lives were estimated on the basis of the quantities found in the aerobic and anaerobic degradation tests in which the concentrations of substance were measured as a function of the time (these tests are discussed in the main report).
The estimated half-lives of the aerobic and anaerobic degradation are given in Tables B1.5 and B1.6. Measured and calculated concentrations are depicted in Figure B1.4.

Table B1.5
Model simulation of aerobic biodegradation of zinc pyrithione. Estimated half-lives (days) at 25°C.

Parent compound	Metabolites							
	ZPT	OMDS	NP4	PSoA	NP1	OMSo	Other compounds	CO_2
ZPT	-	0.5	-	-	-	-	-	-
NP3	-	-	-	-	-	-	50	-
OMDS	-	-	4.0	-	2.0	-	4.0	-
NP4	-	-	-	15.0	-	-	-	-
PSoA	-	-	-	-	-	-	250	-
NP1	-	-	-	-	-	15.0	-	-
OMSo	-	-	-	-	-	-	80	-
Other compounds	-	-	-	-	-	-	-	2,000
CO_2	-	-	-	-	-	-	-	-

Table B1.6
Model simulation of anaerobic biodegradation of zinc pyrithione. Estimated half-lives (days) at 25°C.

Parent compound	Metabolites								
	ZPT	NP3	OMDS	NP4	PSoA	NP1	OMSo	Other compounds	CO_2
ZPT	-	0.5	30	-	-	-	-	-	-
NP3	-	-	-	50	-	-	-	50	-
OMDS	-	-	-	4.0	-	2.0	-	4.0	-
NP4	-	-	-	-	15.0	-	-	-	-
PSoA	-	-	-	-	-	-	-	5.0	-
NP1	-	-	-	-	-	-	15.0	-	-
OMSo	-	-	-	-	-	-	-	80	-
Other compounds	-	-	-	-	-	-	-	-	6,000
CO_2	-	-	-	-	-	-	-	-	-

Photolysis

As mentioned above, the degradation of ZPT by photolysis is included in the model simulations. ZPT is assumed to be transformed into NP3 in the photolytical degradation.

In daylight about noon, the photolytical half-life of ZPT was estimated at:

- 1.78 min without cloud cover
- 3.74 min with cloud cover

The tests were made in September on the parallel approx. 42°N, where the degradation rate of ZPT in seawater was followed (Fenn 1999). The tests were made in curved tubes. A first order rate constant k_P can be es-

timated to be 0.18 min^{-1} (without cloud cover) and 0.08 min^{-1} (with cloud cover). A factor of 2.2 was used in order to correct for the curving of the tubes.

At a cloudless sky, it is assumed that the measured photolytical rate constant may be described by (cf. Schwarzenbach *et al.* 1993):

$$k_P = \Phi \cdot k_a^0$$

where

Φ is the so-called quantum yield, which is here assumed to be independent of the wavelength [-]. Φ states the amount of molecules exited by the light that are transformed into another compound.

k_a^0 is the specific rate of light absorption [time-1]. This rate was calculated by (Schwarzenbach et al. 1993):

$$k_a^0 = \int_\lambda \ln(10) \cdot W(\lambda) \cdot D(\lambda) \cdot \varepsilon(\lambda) d\lambda$$

λ is wavelength [nm].

$W(\lambda)$ is the light intensity at various wavelengths [milliEinstein/cm^2/time/nm]. The light intensity in the autumn on the parallel 40°N was borrowed from Zepp and Cline (1977).

$D(\lambda)$ is the ratio of the average length of the trajectory of the light to the depth of a water element, which can be assumed to be completely mixed [-]. $D(\lambda)$ is here assumed to be equal to 1 for all wavelengths.

$\varepsilon(\lambda)$ is the so-called molar extinction coefficient [(mol/l^{-1}/cm]. For ZPT, these values are borrowed from Fenn (1999).

The light deflection of the cuvette has been taken into consideration.

Φ was thus determined to be 0.07.

Then the American program GCSOLAR (U.S. EPA 1999) was used for calculating the photolytical half-life. This program uses the so-called attenuation coefficients, α[1], which are applied in order to calculate to which degree the water absorbs the light as a function of the depth.

The attenuation coefficient for the water at Kronprins Frederiks Bro is determined on the basis of measured Secchi disk transparencies at two stations in the vicinity of the bridge (Counties of Roskilde and Frederiksborg 1997). In the summer, the shortest Secchi disk transparency is approx. 2.5 m. By using data from Calkins (1977), the following correlation between the Secchi disk transparency and the attenuation coefficient α was found at Secchi disk transparencies of less than 4 m:

[1] If the light intensity at the surface is expressed as I_0 and the light intensity in the depth z as I, the correlation between I and I_0 is described as $\log_{10}(I/I_0) = -\alpha \cdot z$

$$\alpha\ [m^{-1}] = 3.05 - 0.57 \cdot \text{Secchi disk transparency [m]}.$$

The attenuation coefficient for the pleasure craft harbour of Jyllinge was found on the basis of literature data on coastal areas (Zafirioiu 1977). The values are here stated as a function of wavelength and with a minimum and a maximum value. The highest values are used in the present calculations.

GCSOLAR does not take the effect of the cloud cover on the photolysis rate into account. The American program EXAMS (Burns *et al.* 1981), which can also be used for simulating the photolysis of substance, does, however, take the effect of the cloud cover on the photolysis rate into account. By using EXAMS, it was estimated that, compared to the half-life at a blue sky, the half-life will be approx. 50% higher at a cloud cover of 60% which is the average cloud cover in Denmark from April to September (Danish Statistical Office 1996).

GCSOLAR can calculate the average photolytical half-life for each of the seasons: Spring, summer, autumn and winter and on various lattitudes (however, only lattitudes divisible by 10). The average photolytical half-life of ZPT for the seasons spring, summer and autumn and on the lattitudes 50° and 60° was found to be 9.8 hours for the pleasure craft harbour of Jyllinge (6.5 hours at blue sky) and 6.6 hours for the narrows at Kronprins Frederiks Bro (4.4 hours at blue sky).

The degradation by photolysis is slightly dependent on the temperature. A dependency of the photolytical degradation on temperature is, however, not included in the present calculations.

The photolytical half-life was determined for open waters where cloud cover and the falling light intensity down through the water column are taken into consideration but where the boats in the harbour and the shadow effects of the pier are not taken into account. Therefore, two types of calculations have been made, one in which the degradation by photolysis is taken into account and another in which the photolytical degradation is ignored. The actual conditions will probably be somewhere between these two reflections but it is not possible right away to quantify the importance of the shadow effect of the boats and the pier on the amount of light actually falling on the water surface. For the busy navigation route under the bridge (Kronprins Frederiks Bro), there will be limited admittance of sunlight right under the bridge while there will be no important shadow effects in the other part of the navigation route.

Properties of the substance

The properties of zinc pyrithione and its metabolites are summarized in Table B1.7.

The different heterocyclic metabolites with one ring have a low calculated log K_{OW}, which indicates a high water solubility. The sulfonic acids are also expected to be very strong acids for which reason they are probably fully ionized at the prevailing pH in the two waters. For these two compounds, the sorption to sediment and suspended matter is thus expected to be low. The calculated K_{OC} values are, however, used for estimating the sorption to suspended matter and to the sediment.

Table B1.7
Properties of zinc pyrithione and its metabolites.

Substance	$LogK_{OW}$	$LogK_{OC}$
ZPT	0.97**	2.9-4.0
NP3	1.50*	1.728*
OMDS	-2.35*	3.355*
NP4	-2.36*	0.912*
PSoA	-2.35*	1.072*
NP1	-4.50*	1.131*
OMSo	-4.49*	1.291*
Other compounds	-3.0***	1.1***

* Calculated by means of K_{ow}Win (Syracuse Research Corporation 1996).
** Measured (data stated in the main report).
*** Fictive

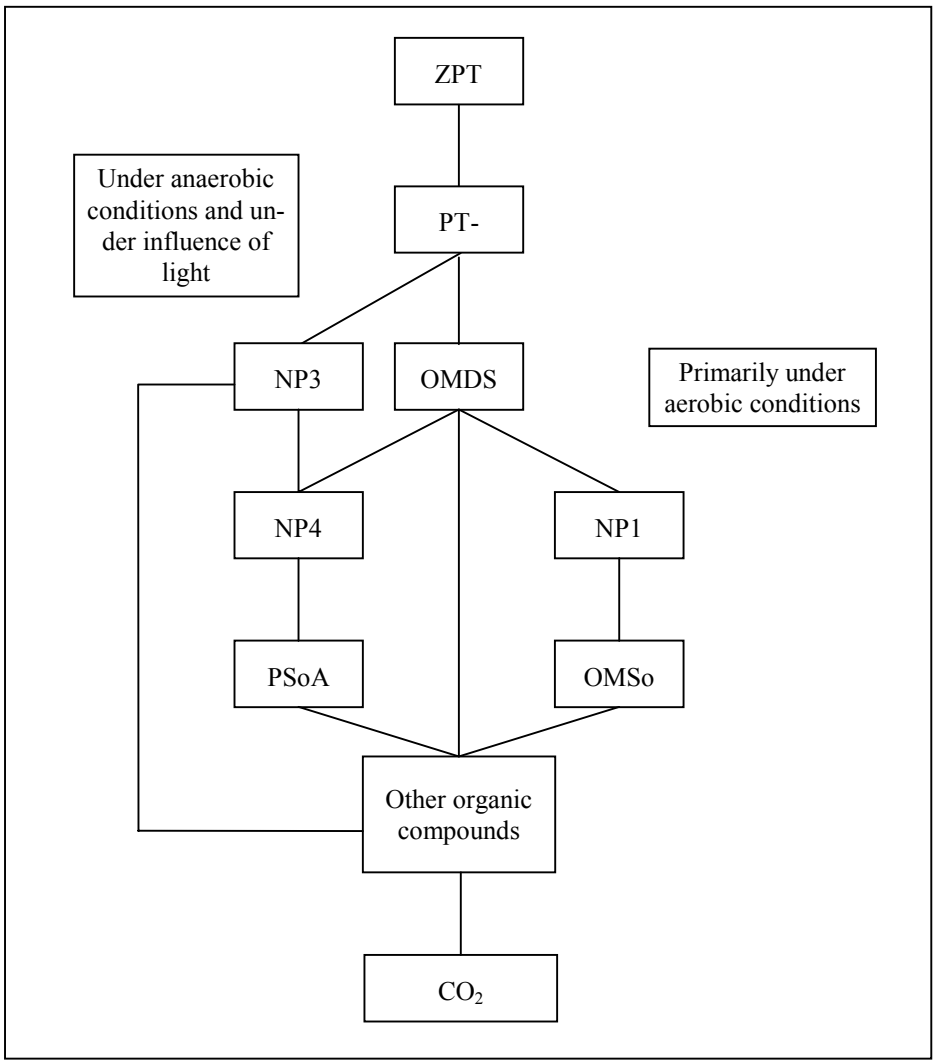

Figure B1.3
Aerobic and anaerobic degradation of zinc pyrithione.

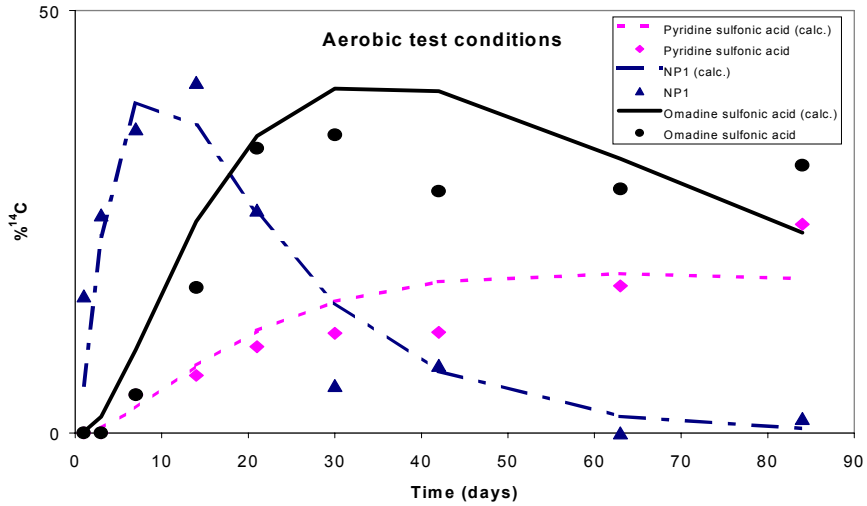

Figure B1.4a
Calculated and measured concentrations of zinc pyrithione and its metabolites. Aerobic experimental conditions.

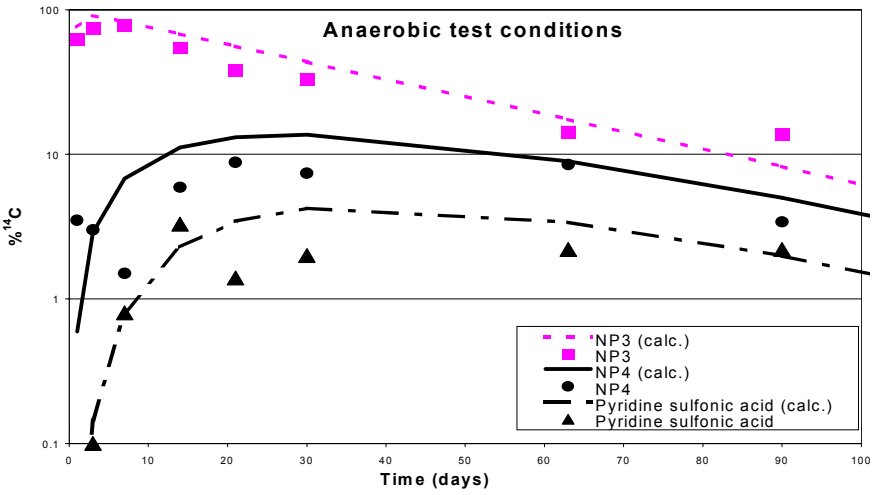

Figure B1.4b
Calculated and measured concentrations of zinc pyrithione and its metabolites. Anaerobic experimental conditions.

3. Calculation results

Table B1.8 gives the calculated PEC values for the two scenarios and the various substances. As mentioned above, the concentrations are steady-state concentrations.

It appears from Table B1.8 that the highest calculated concentrations were found for the pleasure craft harbour. Here, the calculated concentrations are approx. 100 times higher than the concentrations calculated for the busy navigation route.

For the parent compounds, the following steady-state concentrations for the water phase, PEC(water), were estimated:

- DCOI: 0.52 µg/L (pleasure craft harbour)
  0.006 µg/L (busy navigation route)
- Zinc pyrithione: 0.56 µg/L (pleasure craft harbour, photolysis included)
  1.7 µg/L (pleasure craft harbour, photolysis not included)
  0.005 µg/L (busy navigation route, photolysis included)
  0.022 µg/L (busy navigation route, photolysis not included)

Table B1.8a
Calculation results of DCOI.

Substance	Scenario	PEC(water) µg/L	PEC(sediment, pore water) µg/L	PEC(sediment, sorbed) µg/kg
DCOI	Pleasure craft harbour	0.52	0.0015	0.12
N-(n-octyl) malonamic acid		1.98	0.83	2.32
N-(n-octyl) β hydroxy propionamide		0.020	0.14	0.43
N-(n-octyl) acetamide		0.050	0.084	2.42
Other compounds		0.10		
DCOI	Navigation route	0.0061	0.00002	0.0014
N-(n-octyl) malonamic acid		0.040	0.011	0.031
N-(n-octyl) β hydroxy propionamide		0.00071	0.0019	0.0058
N-(n-octyl) acetamide		0.0018	0.0013	0.039
Other compounds		0.0040		

Table B1.8b
Calculation results of ZPT.

Compound	Scenario	With photolysis			Without photolysis		
		PEC(water) µg/L	PEC (sediment, pore water) µg/L	PEC (sediment, sorbed) µg/kg	PEC (water) µg/L	PEC (sediment, pore water) µg/L	PEC (sediment, sorbed) µg/kg
ZPT	Pleasure craft harbour	0.56	0.00056	0.089	1.7	0.0013	0.21
NP3		1.22	0.25	0.68	0.00006	0.54	1.5
NP4		0.099	0.19	0.078	0.20	0.43	0.18
PSoA		0.0080	0.068	0.040	0.016	0.15	0.090
NP1		0.15	0.091	0.062	0.45	0.24	0.17
OMSo		0.012	0.48	0.47	0.036	1.3	1.3
Other compounds		0.11	-	-	0.24	-	-

Compound	Scenario	With photolysis			Without photolysis		
		PEC(water) µg/L	PEC (sediment, pore water) µg/L	PEC (sediment, sorbed) µg/kg	PEC (water) µg/L	PEC (sediment, pore water) µg/L	PEC (sediment, sorbed) µg/kg
ZPT	Navigation route	0.0053	0.00001	0.00090	0.022	0.00002	0.0027
NP3		0.027	0.0028	0.0076	0.00000	0.0072	0.019
NP4		0.0027	0.0022	0.00089	0.0059	0.0061	0.0025
PSoA		0.00040	0.00077	0.00045	0.00088	0.0022	0.0013
NP1		0.0032	0.0011	0.00077	0.013	0.0042	0.0028
OMSo		0.00046	0.0059	0.0058	0.0019	0.022	0.021
Other compounds		0.0032	-	-	-	-	-

4. Sensitivity analysis

The calculation results are conditional on i.a. the values assigned to the different parameters.

A sensitivity analysis of the effect of the following parameters on the calculated concentrations of the parent compounds (DCOI and ZPT) was made:

- Harbour scenarios. Supplementary PEC calculations were made for five pleasure craft harbours (cf. Section 4.1 below).

- Temperature. The temperature was varied between 5°C and 15°C as this was considered a typical variation in the temperatures for the months from May till September (cf. Section 4.2 below).

- Water exchange. The water exchange was varied between 0 $m^3/m^2/d$ corresponding to no water exchange and 1 $m^3/m^2/d$ which would occur under specific conditions (cf. Section 4.2 below).

- Sedimentation rate. The sedimentation rate was varied between 0.7 m/d, corresponding to the net sedimentation being almost 0, and up to 1.5 m/d (cf. Section 4.2 below).

- Leaching rate. The total leaching rate was varied between 50% and 200% of the total leaching rate used in the basic calculations (cf. Section 4.2 below).

4.1 Harbour scenarios

Supplementary calculations were made for five other pleasure craft harbours. A characterization of these harbours is given in Table B1.9. These data were obtained by Hempel and passed on to VKI.

The water exchange in the harbours was set at 0.6 $m^3/m^2/day$ for all harbours with the exception of the harbours of Svendborg and Horsens. For the pleasure craft harbour of Horsens, the water exchange was set at 0.8 $m^3/m^2/day$. The pleasure craft harbour of Svendborg is a pile-built har-

bour in Svendborg Sund. Therefore, the flow conditions are assumed to correspond to those of Svendborg Sund. An average flow rate of 0.5 m/s was assumed for the pleasure craft harbour of Svendborg corresponding to the amplitude of the drastic periodic velocity variation caused by the tides in Svendborg Sund (Harremoës and Malmgren-Hansen 1989).

Table B1.9
Harbour scenario (prepared by Hempel).

Harbour	Water depth (m) Average	Flow rate just outside the harbour (m/s)*	Number of boats in the harbour	Boat size (m^2)	Area of harbour (m^2)	Difference of height (m)	Difference of height max. (m)	Number of daily changes	Mouth (m)
Jyllinge	2.3 (map)	low (0.2)	400 (DS)	18 (DS)	31,500 (map)	0.3-1.5 (DS)	1.5	3 (DS)	25 (map)
Grenå	3 (HO)	high (1.5)	250 (HO)	18 (HO)	98,000 (HO)	0.3-1 (HO)	2.3	2 (HO)	40 (map)
Svendborg	2.5 (HO)	high (2.0)	150 (HO)	18 (HO)	20,000 (HO)	0.3 - 0.5 (HP)	2	4 (HO)	200***
Horsens	2.8 (HO)	low (0.3)	650 (HO)	18 (HO)	61,000 (map)	0.6 (HO)	2.5	2 (HO)	100**
Rungsted	2.7 (map)	medium (0.5)	776 (HO)	18 (HO)	160,000 (map)	0.3-1.2 (HO)	2	2 (HO)	40 (map)
Egå Marina	2.5 (HO)	high (1.5)	750 (HO)	18 (HO)	112,500 (HO)	0.3-0.5 (HO)	2	2 (HO)	50 (HO)

Brackets indicate source of information.
HO: Harbour office
DS: Danish Sailing Association
HP: Harbour pilot
Figures in brackets are estimates based on our and others knowledge of the harbour environments.
Boat size in m^2: 28-30 feet boat as the average (all harbours).
**: $4 \cdot 35$ m from map 100 m used
***: the harbour is open 200 m used

Note: Difference of height is used as a parameter in stead of tides. As the tidal variation in Denmark is very small (10-40 cm), the primary water exchange is made by wind and currents. Therefore, the difference in height is an overall assessment of the different parameters.

Information was obtained from (conversations with):
Jyllinge: Steen Wintlev DS + map received
Grenå: Benny Andersen
Svendborg: Kurt Hansen
Rungsted: Finn Rosdahl
Horsens: Hilmer Christoffersen
Egå Marina: Dan Nilsson (harbour master Børge Heilbach)

The calculated steady-state concentrations for these harbour scenarios are given in Table 1.10.

Table B1.10
Calculated steady-state concentrations.

Harbour	Number of boats per harbour volume in relation to number of boats per harbour volume in the pleasure craft harbour of Jyllinge	PEC(water) (ZPT) (µg/L)		PEC(water) (DCOI) (µg/L)
		With photolysis	Without photolysis	
Jyllinge	1.0	0.56	1.70	0.52
Grenå	0.2	0.11	0.28	0.08
Horsens	0.7	0.49	1.14	0.35
Rungsted	0.3	0.02	0.08	0.02
Egå Marina	0.5	0.29	0.81	0.25
Svendborg	0.5	<0.01	<0.01	<0.01

Table B1.10 indicates that the pleasure craft harbour of Jyllinge results in the highest calculated concentrations. The main reason for this is that there are relatively more boats in the pleasure craft harbour of Jyllinge compared to the volume of water needed in order to dilute the leached chemical (Table B1.10). The scenario used is thus a conservative scenario as assumed at the selection of the pleasure craft harbour of Jyllinge.

4.2 Sensitivity analysis of other parameters

Tables B1.11a and B1.11b show the relation between the calculated concentration of the parent compound in the water phase after changing the parameter and the calculated concentration of the standard scenario.

Tables B1.11a and B1.11b indicate that, within the variation assigned to the individual parameters, it is the total leaching rate that causes the largest variations in the calculated concentrations. The sedimentation rate only slightly influences the calculated concentrations of the parent compounds.

Table B1.11a

Sensitivity analysis of PEC(water) of DCOI. The figures indicate the relation between the calculated concentration of the parent compound in the water phase, after changing the parameter, and the calculated concentration in the standard scenario.

Parameter	Pleasure craft harbour of Jyllinge	
	PEC(water)	PEC(water)
Standard scenario	1.0	1.0
Temperature 5°C	1.5	1.6
Temperature 15°C	0.9	0.9
No water exchange	1.2	1.1
167% increased water exchange	0.9	0.9
70% lower sedimentation rate	1.0	1.0
150% higher sedimentation rate	1.0	1.0
50% less leaching	0.5	0.5
200% more leaching	2.0	2.0

Table B1.11b

Sensitivity analysis of PEC(water) of ZPT. The figures indicate the relation between the calculated concentration of the parent compound in the water phase, after changing the parameter, and the calculated concentration in the standard scenario.

Scenario	Parameter	With photolysis	Without photolysis
		PEC(water)	PEC(water)
Pleasure craft harbour of Jyllinge	Standard scenario	1.0	1.0
	Temperature 5°C	1.1	1.4
	Temperature 15°C	1.0	0.6
	No water exchange	1.1	1.6
	167% increased water exchange	0.9	0.6
	70% lower sedimentation rate	1.0	1.2
	150% higher sedimentation rate	1.0	1.0
	50% less leaching	0.5	0.5
	200% more leaching	2.0	4.0
Busy navigation route	Standard scenario	1.0	1.0
	Temperature 5°C	1.2	1.5
	Temperature 15°C	0.9	0.9
	No water exchange	1.1	1.2
	167% increased water exchange	0.9	0.9
	70% lower sedimentation rate	1.0	1.0
	150% higher sedimentation rate	1.0	1.0
	50% less leaching	0.5	0.5
	200% more leaching	2.0	2.0

Appendix 2: Examination of the mineralization of DCOI and zinc pyrithione in marine sediments

1. Introduction

The mineralization of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-on (DCOI) and zinc pyrithione was examined in laboratory tests with marine coastal sediments. The tests were performed under aerobic and anaerobic conditions using test concentrations of ng/g, which is assumed to result in environmentally realistic transformation kinetics. In the anaerobic experiments, sulfate-reducing conditions were established by adding sulfate. Marine coastal sediments contain considerable amounts of sulfate (Sørensen *et al.* 1979). Glucose was included in the tests in order to examine the mineralization of a readily biodegradable substance at low concentrations and under the given test conditions.

2. Materials and methods

Sediment and seawater

Sediment samples and their seawater were collected at two localities in the Sound. The two sets of sediment and water samples can be described as follows:

Clayey sediment (LS)

- Location: The Sound, 55°50'642N - 12°40'854E; depth (sediment), 22 m; depth (water), 20 m.
- Texture: coarse sand (0.25-2 mm), 0.9%; fine sand (0.063-0.25 mm), 26.4%; silt and clay (<0.063 mm), 65.8%; organic matter, 6.9%.
- Number of bacteria (water): $3.1 \cdot 10^3$ per mL.
- Number of bacteria (sediment): $1.5 \cdot 10^5$ per g.

Sandy sediment (SS)

- Location: The Sound, 55°50'030N - 12°37'534E; depth (sediment), 4 m; depth (water), 2 m.
- Texture: coarse sand, 91.5%; fine sand, 7.3%; silt and clay, 1.0%; organic matter, 0.2%.
- Number of bacteria (water): $2.1 \cdot 10^3$ per mL.
- Number of bacteria (sediment): $4.8 \cdot 10^4$ per g.

The number of bacteria in seawater and sediment was determined as the bacterial count by embedding a known amount of the sample in Bacto Marine Agar 2216 (Difco). Sediment (approx. 1.6 g) is mixed with 9 mL fosfate buffer and is then treated as a water sample. The bacterial count is determined as the number of colonies occurring after 72 hours' incubation at 21°C. Sediment and water samples were stored separately stored in the dark at 4°C until use.

Chemicals

[2,3-^{14}C]DCOI (Lot Nos. 853.0208 and 853.0209; 15.9 mCi/mmol, 56.44 µCi/mg, radio-chemical purity 98.6%) was supplied by Rohm and Haas Research Laboratories (Spring House, Pennsylvania). [^{14}C]zinc pyrithione (Lot. No. 3228-143; 157.63 mCi/mmol, 0.50 mCi/mg) and [UL-^{14}C]D-glucose (Lot 48H9476 Sigma; 212.5 mCi/mmol, 1.18 mCi/mg, dissolved in ethanol:water, 9:1) was supplied by Arch Chemicals (Cheshire, Connecticut). All other chemicals are commercially available and of analytical purity.

Aerobic biodegradation tests

The aerobic biodegradation tests were made with sediment LS as well as sediment SS. Stock solutions of [^{14}C]DCOI (in methanol), [^{14}C]zinc pyrithione (in dimethyl sulfoxid) and [^{14}C]glucose (in deionized water) were added to 300-mL glass flasks with 10 g sediment (wet weight) and 50 mL seawater which had beforehand been aerated with atmospheric air for approx. 20 hours. For [^{14}C]DCOI, the stock solution was added to the test flasks with 0,5 g dried sediment and the methanol was allowed to evaporate before more sediment and water were added. The other substances were added by mixing the stock solution with the sediment, after which seawater was added. The resulting concentrations of the three model compounds are indicated in Table B2.1. Two glass pipes were placed in the test flasks, one pipe with 1N KOH (3 mL) for absorption of $^{14}CO_2$ and another with ethylene glycol for trapping other gaseous compounds. The flasks were closed with rubber stoppers and aluminium screw caps and placed in the dark at 15°C.

The mineralization of the substances was followed by determining the ^{14}C activity which was trapped in the glass pipes. Each week, samples were taken for liquid scintillation counting and the test flasks were placed in the dark without stoppers and caps for approx. 10 min in order to exchange the gas phase of the flasks with atmospheric air. Then the contents of the glass pipes were replaced with fresh KOH or ethylene glycol. At the end of the test after 42 days, CO_2 in the water phase was released after acidification of the sediment-water system to pH 1-2 by addition of concentrated sulfuric acid.

Anaerobic biodegradation tests

The anaerobic biodegradation tests were only made with sediment LS. In the same way as in the aerobic tests, the stock solutions of the three model compounds were added to 117-mL serum flasks with 30 g of sediment (wet weight) and 30 mL seawater. The resulting concentrations of the three model compounds are indicated in Table B2.1. Before use, the seawater was pre-treated with an addition of a fosfate buffer (27 mg KH_2PO_4 and 112 mg $Na_2HPO_4 \cdot 12H_2O$ per litre) in order to stabilize pH and a redox indicator (1.0 mg resazurin per litre) in order to control that anaerobic conditions were present throughout the test. Sulfide (0.5 g $Na_2S \cdot 9H_2O$/ kg) was added to each serum flask as reducing agent while sulfate (Na_2SO_4; 25 mM in the water phase) was added as electron acceptor in order to establish sulfate-reducing conditions, which are characteristic of marine sediments. A glass pipe with 1N KOH (3 mL) was placed in the serum flasks for absorption of $^{14}CO_2$. Throughout all of the above procedure, the serum flasks were carefully aerated with oxygen-free N_2 gas until they were closed with 1-cm butyl rubber stoppers and aluminium screw caps. The test flasks were placed in the dark at 15°C

The mineralization of the substances was followed by determining the ^{14}C activity (from $^{14}CO_2$) which was trapped in the glass pipe with KOH. Sampling of KOH for liquid scintillation counting was made after 14, 28 and 56 days after which the liquid in the glass pipe was replaced with fresh KOH. As part of the carbon at the mineralization of the model compounds may be transformed into methane, $^{14}CH_4$ was determined by injecting 2-mL gas samples into scintillation vials which were modified so that the caps could hold a septum (Zehnder *et al.* 1979). Before use, a hole was made in the screw cap of each scintillation vial and a butyl rubber septum inserted. 20 mL of a liquid scintillation cocktail (Insta gel II plus, Packard) was added to the scintillation vials. It turned out that the cocktail was capable of absorbing approx. 2/3 of the methane added in pilot tests. At the end of the test after 56 days, CO_2 in the water phase was released after acidification of the sediment-water system to pH 1-2 by addition of concentrated sulfuric acid.

Table B2.1
Aerobic and anaerobic mineralization. Initial concentrations of model compounds.

Model compound	Test type	Initial concentration (µg/g)
[^{14}C]DCOI	Aerobic conditions Sediment LS	0.83
[^{14}C]DCOI	Aerobic conditions Sediment SS	0.033
[^{14}C]DCOI	Anaerobic conditions Sediment LS	0.83
[^{14}C]Zinc pyrithione	Aerobic conditions Sediment LS	0.037
[^{14}C]Zinc pyrithione	Aerobic conditions Sediment SS	0.037
[^{14}C]Zinc pyrithione	Anaerobic conditions Sediment LS	0.037
[^{14}C]Glucose	Aerobic conditions Sediment LS	0.025
[^{14}C]Glucose	Aerobic conditions Sediment SS	0.025
[^{14}C]Glucose	Anaerobic conditions Sediment LS	0.025

Recovery of remaining ^{14}C

After the termination of the aerobic and anaerobic tests, the ^{14}C remaining in water sediment was determined by the following procedures. In the test, in which [^{14}C]DCOI had been added at a concentration of 0.033 µg/g, and in the tests with [^{14}C]glucose, the remaining radioactivity was determined after liquid scintillation counting of sub-sample of the water phase and incineration of 0.1-g sediment samples in excess of oxygen. In the other tests, the ^{14}C remaining in the sediment was determined by extraction with 6N HCl followed by 1N NaOH, after which the extracted sediment was incinerated in excess of oxygen (Madsen and Kristensen 1997). This method makes it possible to determine fractions of ^{14}C,

which are bound in the form of hydrolyzable compounds, humic and fulvic acids or to humin/ clay minerals.

Chemical analyses

DCOI and metabolites from the transformation of DCOI were analyzed and characterized by Rohm and Haas (Andrew Jacobson, Rohm and Haas Research Laboratories, Spring House, Pennsylvania). Water samples were treated with solid phase extraction (SPE) by use of acetate:methanol (1:1) before analysis in HPLC. Sediment samples were extracted twice with acetonitril: 0.01 N HCl (4:1) followed by extraction with dichloromethane for analysis in HPLC.

Zinc pyrithione and its metabolites were determined by Arch Chemicals (James C. Ritter, Department of Ecotoxicology, Cheshire, Connecticut). Sediment samples were extracted with acetonitril followed by two extractions with 1,0 N KOH. Then the water samples and the extracts from sediment were derived before analysis in HPLC (Arch Chemicals 1999b).

3. Results

The cumulated development of $^{14}CO_2$ from the mineralization of DCOI and zinc pyrithione is shown in the Figures 3.1-3.3 (Section 3.2 of the main report) and Figures 4.1-4.3 (Section 4.3 of the main report). The total mineralization and distribution of the radioactivity remaining at the end of the tests are shown in Tables B2.2-B2.4. The amount of ^{14}C in absorbers with ethylene glycol (aerobic tests) constituted <0.1% of the radioactivity added.

Table B2.2
Mineralization of DCOI in sediment and seawater under aerobic or anaerobic conditions. Distribution and recovery of ^{14}C after an incubation of 42 days (aerobic test) or 56 days (anaerobic test).

Retrieved ^{14}C activity (% of added $^{14}C \pm$ SD)							
Sediment/ test type	CO_2	Water phase	Sediment, hydrolyzable compounds[B]	Sediment, fulvic acid[C]	Sediment, humic acid[C]	Sediment, humic and clay minerals[D]	Total retrieval
SS, aerobic	24.3 ± 0.58	19.1 ± 1.9	-	-	-	-	70.0[E]
LS, aerobic	12.9 ± 0.52	2.0 ± 0.35	1.4 ± 0.35	14.0 ± 0.92	23.8 ± 0.92	24.2 ± 2.4	78.3
LS, anaerobic	14.5 ± 0.19 [A]	0.9 ± 0.12	1.0 ± 0.10	5.0 ± 0.16	14.7 ± 0.46	30.3 ± 1.4	66.4

[A], hereof 0.06% as $^{14}CH_4$; [B], extraction with HCl; [C], extraction with NaOH; [D], incineration of extracted sediment; [E], incineration of non-extracted sediment; SD, standard deviations between four replicates; not determined.

Table B2.3

Mineralization of zinc pyrithione in sediment and seawater under aerobic or anaerobic conditions. Distribution and recovery of ^{14}C after an incubation of 42 days (aerobic test) or 56 days (anaerobic test).

Retrieved ^{14}C activity (% of added $^{14}C \pm$ SD)							
Sediment/ test type	CO_2	Water phase	Sediment, hydrolyzable compounds[B]	Sediment, fulvic acid[C]	Sediment, humic acid[C]	Sediment, humic and clay minerals[D]	Total retrieval
SS, aerobic	5.0 ± 1.2	65.2 ± 1.4	8.3 ± 0.22	13.2 ± 0.52	2.6 ± 0.17	1.0 ± 0.13	95.3
LS, aerobic	2.8 ± 0.06	31.5 ± 0.66	5.3 ± 0.10	32.7 ± 1.0	9.4 ± 0.16	7.0 ± 0.39	88.7
LS, anaerobic	3.7 ± 0.59 [A]	18.8 ± 4.0	2.6 ± 0.15	13.7 ± 1.6	5.3 ± 0.88	33.4 ± 1.9	77.5

[A], hereof 0.11% as $^{14}CH_4$; [B], extraction with HCl; [C], extraction with NaOH; [D], incineration of extracted sediment; SD, standard deviations between four replicates.

Table B2.4

Mineralization of D glucose in sediment and seawater under aerobic or anaerobic conditions. Recovery of ^{14}C after incubation of 42 days (aerobic test) or 56 days (anaerobic test)

Sediment/ test type	CO_2	Water phase	Sediment[B]	Total retrieval
SS, aerobic	59.6 ± 2.0	6.0 ± 0.75	12.5 ± 0.89	78.1
LS, aerobic	52.3 ± 0.76	4.6 ± 0.48	25.5 ± 0.39	82.4
LS, anaerobic	58.5 ± 1.9[A]	3.1 ± 0.38	17.3 ± 0.10	78.9

[A], hereof <0.01% as $^{14}CH_4$; [B], incineration of non-extracted sediment; SD, standard deviations between four replicates.

Appendix 3: Examination of the effect of degradation and sorption on the aquatic toxicity of DCOI and zinc pyrithione

1. Introduction

The effect of degradation and sorption on the toxicity of 4,5-dichloro-2-n-octyl-4-isothiazolin-3-on (DCOI) and zinc pyrithione was examined in laboratory tests with the marine crustacean *Acartia tonsa*. As especially zinc pyrithione is degradable by photolysis, the tests were made in the dark as well as at a constant exposure to light.

2. Materials and methods

Sediment and seawater

This test was made with the sandy sediment (SS) and its seawater as described in Appendix 2.

Chemicals

DCOI (Lot No. 14-SS-18F; purity, 99.86%) was supplied by Rohm and Haas Research Laboratories (Spring House, Pennsylvania). Zinc pyrithione (Lot. No. J116659; purity, 98.9%) was supplied by Arch Chemicals (Cheshire, Connecticut).

Bioassays

DCOI and zinc pyrithione were added from stock solutions with methanol (50 µL) and dimethyl sulfoxid (50 µL), respectively, to 55-mL serum flasks with 0.5 g dried sediment. The methanol from the stock solution of DCOI was allowed to evaporate. Seven grammes of sediment (dry weight) was then added together with 35 mL of the respective seawater, which, before addition, had been adjusted to a salinity of 3.2%. The initial concentrations were 0.1 µg/g for DCOI and 0.025 µg/g for zinc pyrithione. As the gas phase of the test system, pure oxygen was added, after which the serum flasks were closed by Teflon-coated rubber septa and aluminium caps. One series of serum flasks were incubated in the dark while a parallel series of flasks were incubated at constant exposure to light. Both series of test flasks were incubated at a temperature of 20 ± 2°C. The light-exposed serum flasks were incubated bottom up and with a distance of 5 cm to fluorescent tubes (Pope FID 18W/33; a total of 14 tubes), which had been mounted with spaces of approx. 1.8 cm in between. The average light intensity at the distance of 5 cm from the light source was measured at 458 ± 32 µmol/m^2 · s in air, which was extrapolated to 342 µmol/m^2 · s in water. Measurements performed by VKI in the Sound have shown that the average light intensity in a depth of approx. 1 m (0.6-1.4 m) was 548 µmol/m^2 · s in 1997 and 420 µmol/m^2 · s in 1998 (the measurements in 1997 as well as in 1998 were made in the period from 9 May till 30 September by daylight).

Three replicate serum flasks from bioassays were harvested after 0; 1; 2; 4; 7 and 14 days. Before sampling, the flasks were shaken after which they were placed in the dark in order for the sediment to settle. The water phase from each replicate was carefully transferred to centrifugal vials

and, after centrifuging (1,500 rpm for 15 min), the supernatant was stored in a deep-freeze until use in the test with *A. tonsa*.

The acute toxicity to *A. tonsa* was tested by use of the ISO/FDIS 14669 procedure with the modification that the test was made in the dark in order to prevent transformation by photolysis of zinc pyrithione. Controls in this test included:

- Supernatant (t = 0 d) from test system without biocide
- Supernatant (t = 0 d) from test system without biocide with addition of 50 µL methanol as in the test
- Supernatant (t = 0 d) from test system without biocide with addition of 50 µL dimethyl sulfoxid as in the test
- Supernatant (t = 14 d) from test system without biocide incubated in the dark as in the test
- Supernatant (t = 14 d) from test system without biocide exposed to light as in the test
- Seawater from the Sound (sediment SS; cf. Appendix 2) adjusted to a salinity of 3.2%
- Seawater from the Kattegat adjusted to a salinity of 3.2%

Further information on the toxicity test with *A. tonsa* is given in the report "Ecotoxicological tests of leachates of antifouling paints" (Bjørnestad *et al.* 1999).

The effect of dosing with biocides on the number of bacteria in the test system was determined as the bacterial count (cf. Appendix 2). The number of bacteria in an untreated control sample was $9.1 \cdot 10^5$ per mL after 1 day's incubation while the corresponding numbers were $1.5 \cdot 10^5$ and $1.4 \cdot 10^5$ per mL in samples with dosages of DCOI and zinc pyrithione, respectively.

Appendix 4: Ecotoxicological data on DCOI

Table B4.1
Ecotoxicological data on DCO1.

Species	Parameter, end point	Exposure time	Result mg/l	Comments	Data source
Algae					
Selenastrum capricornutum	Growth EC50	120 h	0.032	Static nominal concentration, below detection limit at the end of exposure	Forbis 1990
Selenastrum capricornutum	Growth EC50	120 h	0.036	Nominal concentration	Debourg 1993
Skeletonema costatum	Growth EC50	96 h	0.018-0.026	Nominal concentration	Debourg 1993
Skeletonema costatum	Chlorophyll EC50	96 h	0.0201	Static, nominal concentration	Shade *et al.* 1993
Skeletonema costatum	Growth EC50	96 h	0.0139	Static nominal concentration	Shade *et al.* 1993
Crustaceans					
Daphnia magna	Immobil. EC50	48 h	0.0052	Flow-through, analytical confirmation	Burgess 1990
Daphnia magna	Immobil. EC50	48 h	0.0092	Static nominal concentration (+ xylene)	Shade *et al.* 1993
Daphnia magna	Immobil. EC50	48 h	0.0216	Static nominal concentration (solvent stripped)	Shade *et al.* 1993
Daphnia magna	Reproduction EC50	21 d	0.0012	Flow-through, analytical confirmation, problems with solvent	Ward & Boeri 1990
Daphnia magna	Reproduction LOEC	21 d	0.0011	Flow-through, analytical confirmation, problems with solvent	Ward & Boeri 1990
Daphnia magna	Reproduction NOEC	21 d	0.00063	Flow-through, analytical confirmation, problems with solvent	Ward & Boeri 1990
Daphnia magna	Reproduktion MATC	21 d	0.00083	Flow-through, analytical confirmation, problems with solvent	Ward & Boeri 1990
Mysidopsis bahia	LC50	96 h	0.0047	Flow-through, analytical confirmation	Boeri & Ward 1990
Panaeus aztecus	LC50	96 h	0.0124	Static nominal concentration	Shade *et al.* 1993
Panaeus aztecus	LC50	96 h	0.016	Static nominal concentration	Heitmuller 1977
Crab	LC50	96 h	1.312	Static nominal concentration	Shade *et al.* 1993
Fish					
"Fish"	LC50	?	0.010-0.030	Nominal concentration	Debourg 1993
Lepomis macrochirus	LC50	96 h	0014	Flow-through, analytical confirmation	Shade *et al*. 1993
Lepomis macrochirus	LC50	96 h	0.029	Static nominal concentration (+ xylene)	Shade *et al.* 1993

Species	Parameter, end point	Exposure time	Result mg/l	Comments	Data source
Lepomis macrochirus	LC50	96 h	0.017	Static nominal concentration (solvent stripped)	Shade *et al.* 1993
Oncorhynchus mykiss	LC50	96 h	0.0027	Flow-through, analytical confirmation	Shade *et al.* 1993
Oncorhynchus mykiss	LC50	96 h	0.0097	Static nominal concentration (+ xylene)	Shade *et al.* 1993
Oncorhynchus mykiss	LC50	96 h	0.0077	Static nominal concentration (solvent stripped)	Shade *et al.* 1993
Cyprinodon variegatus	LC50	96 h	0.0205	Flow-through, analytical confirmation	Shade *et al.* 1993
Cyprinodon variegatus	LC50	96 h	0.017	Static nominal concentration	Shade *et al.* 1993
Cyprinodon variegatus	Early life-stage LOEC	35 d	0.014	Flow-through, analytical confirmation	Shade *et al.* 1993
Cyprinodon variegatus	Early life-stage NOEC	35 d	0.006	Flow-through, analytical confirmation	Shade *et al.* 1993
Cyprinodon variegatus	Early life-stage MATC	35 d	0.0092	Flow-through, analytical confirmation	Shade *et al.* 1993
Paralichthys olivaeus	LC50	96 h	0.0061	Semi-static nominal concentration	Kawashima 1997a
Pagrus major	LC50	96 h	0.0052	Semi-static nominal concentration	Kawashima 1997b
Others					
Protozoa	100% effect	?	5	Nominal concentration	Debourg 1993
Mussel	LC50	96 h	0.850	Flow-through, no analysis	Shade *et al.* 1993
Mussel embryo	Growth + dev. EC50	48 h	0.0019	Static nominal concentration	Shade *et al.* 1993
Crassostrea virginica (oyster)	EC50	48 h	0.012-0.024	Nominal concentration, declining exposure concentration	Roberts *et al.* 1990
Crassostrea virginica (oyster)	NOEC	48 h	0.010-0.018	Nominal concentration, declining exposure concentration	Roberts *et al.* 1990
Oyster embryo	EC50	48 h	0.0069	Natural seawater, static nominal concentration	Shade *et al.* 1993
Oyster embryo	EC50	48 h	0.024	Natural seawater, static nominal concentration, low concentration at the end of exposure	Shade *et al.* 1993
Oyster embryo	EC50	48 h	0.0121	Synthetic seawater, static nominal concentration, low concentration at the end of exposure	Shade *et al.* 1993

Appendix 5: Ecotoxicological data on zinc pyrithione

Table B5.1
Ecotoxicological data on zinc pyrithione.

Species	Parameter, end point	Exposure time	Result mg/l	Comments[1]	Data source
Algae					
Selenastrum capricornutum	EC50	72+120 h	0.028	Static measured concentration	Ward *et al.* 1994a
Selenastrum capricornutum	LOEC	120 h	0.018	Static measured concentration	Ward *et al.* 1994a
Selenastrum capricornutum	NOEC	72+120 h	0.0078	Static measured concentration	Ward *et al.* 1994a
Crustaceans					
Daphnia magna	EC50	48 h	0.0036	Flow-through, measured concentration	Boeri *et al*. 1994d
Daphnia magna	LC50	48 h	0.0082	Flow-through, measured concentration	Boeri *et al*. 1994d
Daphnia magna	NOEC	48 h	0.0011	Flow-through, measured concentration	Boeri *et al*. 1994d
Daphnia magna	NOEC reproduction	21d	0.0027		Olin 1997
Mysidopsis bahia	LC50	96 h	0.0063	Flow-through, measured concentration	Boeri *et al*. 1993
Mysidopsis bahia	NOEC	96 h	0.0016	Flow-through, measured concentration	Boeri *et al*. 1993
Americamysis bahia	NOEC reproduction	50 d	0.0042	Flow-through, measured concentration	Arch Chemicals 1999a
Americamysis bahia	NOEC length/weight of offspring	50 d	0.0027	Flow-through, measured concentration	Arch Chemicals 1999a
Fish					
Cyprinodon variegatus	LC50	96 h	0.4	Semi-static, measured concentration	Boeri *et al*. 1994c
Cyprinodon variegatus	NOEC	96 h	0.2	Semi-static, measured concentration	Boeri *et al*. 1994c
Ictalurus punctatus	LC50	96 h	0.035	Flow-through, nominal concentration	Olin 1997
Lepomis macrochirus	LC50	96 h	0.021	Flow-through, nominal concentration	Olin 1997
Notemigonas crysoleucas	LC50	96 h	0.02	Flow-through, nominal concentration	Olin 1997
Oncorhynchus kisutch	LC50	24 h	10	DC = M	AQUIRE 1999
Oncorhynchus mykiss	LC50	96 h	0.0032	Flow-through, measured concentration	Boeri *et al*. 1994b
Oncorhynchus mykiss	NOEC	96 h	0.0016	Flow-through, measured concentration	Boeri *et al*. 1994b

Species	Parameter, end point	Exposure time	Result mg/l	Comments[1]	Data source
Oncorhynchus tshawytscha	LC50	24 h	10	DC = M	AQUIRE 1999
Pimephales promelas	LC50	96 h	0.0026	Flow-through, measured concentration	Boeri *et al*. 1994a
Pimephales promelas	NOEC	96 h	0.0011	Flow-through, measured concentration	Boeri *et al*. 1994a
Pimephales promelas	LC50	96 h	0.04	Flow-through, nominal concentration	Olin 1997
Pimephales promelas	NOEC early life-stage	32 d	0.00122	Flow through, measured concentration	Boeri *et al.* 1999
Ptychicheilus oregonensis	LC50	24 h	10	DC = M	AQUIRE 1999
Salmo gairdneri	LC50	96 h	0.0032	Flow-through, measured concentration	Olin 1997
Salmo gairdneri	NOEC	96 h	0.0016	Flow-through, measured concentration	Olin 1997
Salvelinus fontinalis	LC50	96 h	0.008	Flow-through, nominal concentration	Olin 1997
Others					
Crassostrea virginica (oyster)	LC50	96 h	0.022	Measured concentration	Boeri *et al*. 1994e
Crassostrea virginica (oyster)	NOEC (shell deposit)	96 h	0.010	Measured concentration	Boeri *et al*. 1994e

1: DQR = Data Quality Rating. In AQUIRE, the studies are evaluated according to the completeness of the descriptions, with a so-called Documentation Code (DC): I = incomplete, M = moderate, C = complete.

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