The Use of Mesocosms in Risk Assessment of Active Substances in Ballast Water Treatment

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Abstract

As a consequence of the adoption of the IMO Ballast Water Convention, several companies have developed ballast water management systems (BWMS). When a BWMS makes use of active ingredients, toxicity should be assessed according to MEPC guideline G9 in order to establish the ecological risk of the substance and the treated ballast water that is discharged. Acute and chronic laboratory tests (bioassays) are being used to assess the ecological risks of substances and treated ballast water. Bioassays are single species tests that give information on the direct effects on the individuals of the organism tested. Ecosystems, however, consist of several interacting species and, as a community, may react differently to a toxic substance and show recovery after exposure declines. Moreover, in most cases the exposure conditions in a field situation strongly deviate from a laboratory test beaker. Dissipation/degradation is, for instance, hardly addressed in laboratory toxicity tests. This is recognized in the legislation process of pesticides, where data collected under more field relevant conditions is used for what is called the 'higher tier risk assessment'. For this type of testing, mesocosms, or experimental ecosystems, are applied. Organisms from different taxonomic and functional groups are exposed simultaneously in outdoor ponds under realistic environmental conditions and exposure regimes. This allows for the assessment of direct and indirect toxic effects on a suit of organisms (the ecosystem) present in the test systems. Over the last decade, we have tested several pesticides in outdoor freshwater mesocosms for legislation purposes.In 2008 and 2009, we have conducted marine mesocosm experiments, in order to investigate the applicability of these systems for higher tier risk assessment of for instance active substances used in BWMS and the residue risk of treated ballast water at the moment of discharge. The results of these studies will be presented and

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compared to results of freshwater pond studies. Possibilities for improving the accuracy of the risk assessment of BWMS will be discussed.

Key words: Ballast Water, Marine Mesocosms, Community, Risk Assessment

1 Introduction

In 2004, the International Maritime Organization (IMO) adopted the "International Convention for the Control and Management of Ship's Ballast Water and Sediments" (IMO 2005), which would enter into force 12 months after ratification by at least 30 states representing 35% of world's merchant shipping tonnage. To guide the development of Ballast Water Management Systems (BWMS), the IMO has published a number of guidelines stating the requirements for performance and use of BWMS. For BWMS using active ingredients the Guideline G9 (MEPC 169/57) is especially important. It describes the procedures for ecological risk assessment for the receiving waters by evaluating the ecotoxicity of active ingredients and treated ballast water.

2 Risk Assessment Procedures

The procedure for the risk assessment that is applied for BWMS that make use of active ingredients, is basically similar to procedures adopted elsewhere (see for instance EU-TGD Part II, ECB 2003). It is based upon the PEC/PNEC ratio: When the PEC (Predicted Environmental Concentration) is larger than the PNEC (Predicted No Adverse Effect Concentration) an ecological risk is indicated. The PEC is based on calculations using biodegradation data and a dilution model. The PNEC is based upon toxicity data from literature or laboratory tests. The risk of underestimating the actual environmental impact by following this approach is acknowledged and uncertainty (assessment) factors are derived on bases of assumptions made concerning extrapolation from single-species short-term toxicity data to complex ecosystem effects. It is assumed that the most sensitive species determines the ecosystem sensitivity and that protection of the ecosystems structure will protect the community function.

For marine risk assessment, more conservative assumptions are made compared to freshwater risk assessment in order to protect the higher phylogenetic diversity in the marine ecosystem (Table 1). Reducing uncertainty by collecting more information on the toxicity of a substance, will result in a lower assessment factor. The usual way to reduce assessment factors is to produce data about the toxicity of the active ingredient for more species, representing more phylogenetic groups, and/or by performing chronic toxicity tests, and preferably use these data to calculate the species sensitivity distribution and to predict the hazardous concentration that leads to a potentially affected fraction of 5% of the species (Aldenberg & Jaworska 2000). Based on the reliability of the dataset that is used for this approach, the assessment factor could be reduced to 5 or even 1.

Table 1. Overview of assessment factors to derive a PNEC for aquatic and marine ecosystems (ECB 2003)

Data Set	Aquatic	Marine
Lowest short-term LC50 from algae. crustacean, fish	1,000	10,000
Lowest short-term LC50 from algae. crustacean, fish + 2 additional marine groups	.	1,000
1 long-term NOEC from crustacean or fish	100	1,000
2 long-term NOECs from algae and/or crustacean and/or fish	50	500
Lowest long-term NOEC from algae, crustacean, fish	10	100
2 long-term NOECs from algae and/or crustacean and/or fish + 1 NOEC additional marine group	.	50
Lowest long-term NOEC from 3 fresh water or marine species + 2 NOECs additional marine groups	.	10
Species sensitivity distribution (SSD) method	5-1	5-1
Field data or mesocosms	case by case	case by case

The draw-back of this method is that the basic data is still based upon single-species laboratory experiments, which do not incorporate species interactions and recovery potential. Typically, the active ingredients used in BWMS are chemicals with a very short residence time. Chronic testing of these substances at a constant exposure concentration of the active ingredient is, therefore, not appropriate to study the environmental impact of the residue toxicity of discharged treated ballast water.

The same is the case for modern, rapidly degradable pesticides. In the legislation of pesticides this gap between laboratory and field is recognized and results from the 'first tier risk assessment' can be overruled when "it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on the viability of exposed species occurs -directly or indirectly- after use of the plant protection product according to the proposed conditions of use" (ECB 2003). For this 'higher tier assessment' under more realistic conditions, mesocosms, or experimental ecosystems, are applied.

3 Mesocosms as Tool for Risk Assessment

Each mesocosm study is designed to answer specific questions, nonetheless various guidance documents have been drafted that describe the basic principles of this kind of studies when performed for risk assessment. The most recent guidelines are the recommendations from the 'HARAP' (Campbell *et al.* 1999) and the 'CLASSIC' (Giddings *et al.* 2002) workshops. In De Jong *et al.,* (2008) guidance is given about the evaluation of mesocosm studies for risk assessment. In principle, organisms from different taxonomic and functional groups are exposed simultaneously in outdoor ponds under realistic environmental conditions and exposure regimes. This allows for the assessment of direct and indirect toxic effects on a suit of organisms (the ecosystem) present in the test systems, including recovery of the community once the toxic stress has disappeared (Figure 1).

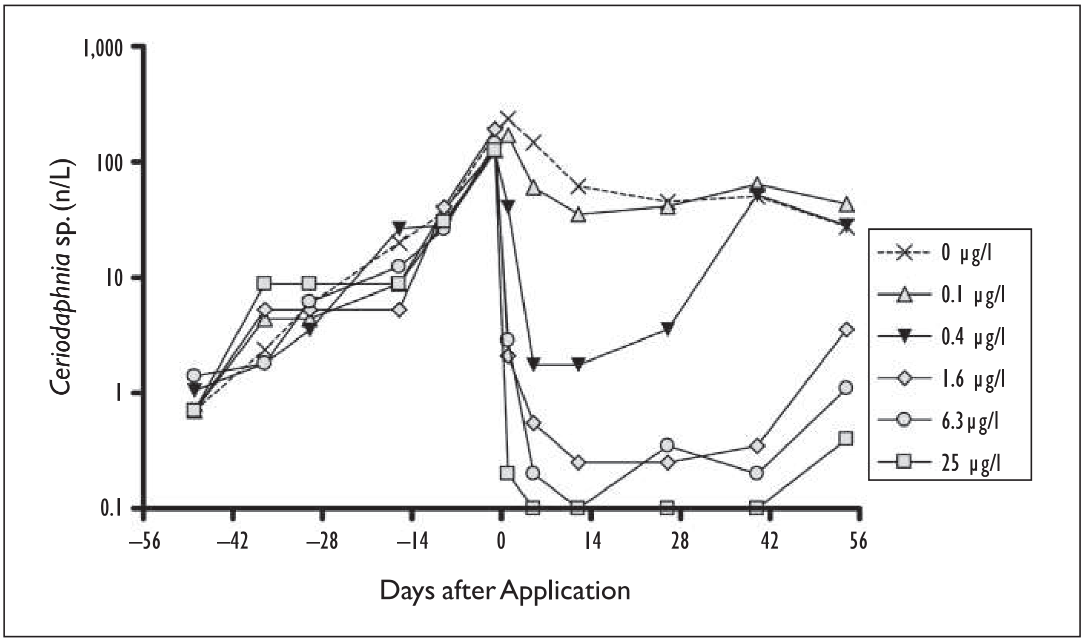

Figure 1. Impact of a single application (on day 0) of a rapid degradable insecticide in freshwater mesocosms on a zooplankton group. Presented are the average values of the duplicated treatments. The grey field indicates the range of the observations in the triplicated control (0 µg/l) mesocosms. Treatment level 0.1 µg/l has no impact, treatments 0.4 µg/l shows severe impact with recovery after 40 days. Higher treatment levels show indication of recovery at the end of the study. Example from a mesocosm study conducted in 2000 by IMARES.

The expert group that discussed the application of mesocosm data for risk assessment of pesticides during the HARAP workshop concluded that "If a field study (outdoor micro- or mesocosms) has been properly designed, executed, analysed and interpreted, the results may be used in risk assessment without applying an uncertainty factor" (Campbell *et al.* 1999). This conclusion was adopted by the European Commission in 2002 (Sanco 2002).

In mesocosm studies, agricultural pesticides are usually applied one or more times in a scheme representative of agricultural usage practise. Several test concentrations are created in duplo or triplo, as well as untreated controls. The experiment is then continued until at least 8 weeks after the last application in order to be able to specify the effect classes that are related to recovery of the most sensitive endpoints (De Jong *et al.* 2008). A similar approach would be applicable for testing of the active ingredients of BWMS, simulating one or more discharges resulting in specified concentrations of the active ingredient in an ecosystem.

For testing the impact of the discharge of treated ballast water on a receiving ecosystem, a specific experimental design will be necessary, as it would require replacement of a variable amount of water in the mesocosms. In some way this should also be applied to the controls, in order to be able to separate the impact of dilution from possible toxic effects.

4 Practical Experiences

During the past 20 years we gained broad experience with various types of mesocosms. Besides the standard stagnant ponds, work was done with flow through systems to study the chronic impact of complex effluents, tidal marine systems, enclosures of planktonic communities and mesocosms consisting of two connected compartments representing a pelagic surface system and a (dark) deep water benthic system (Bowmer *et al.* 1994; Foekema 2004; Foekema *et al.* 1998; Jak *et al.* 1998; Kaag *et al.* 1994, 1997, 1998; Kuiper 1977, 1984; Scholten *et al.* 1987). The fresh water mesocosms were constructed both as stagnant ponds and as flow-through systems, depending on the research questions. Until recently, for marine mesocosms stagnant systems were only used when a benthic compartment was not part of the study. In the studies with a benthic compartment, the research question was focused on the environmental impact of contaminated sediments, and it was believed that a stagnant system would be strongly affected by the organic enrichment that often accompanies contaminated sediments. However, for studying the fate of active ingredients in an ecosystem stagnant systems are most appropriate. For this reason, we have started experiments with stagnant marine mesocosms including a benthic compartment in 2008.

The mesocosms used, are circular glass-fibre basins with a diameter of approx. 180 cm, partly buried to buffer the systems from fluctuations in air temperature, as well as for practical reasons. On the bottom a 20 cm layer of clean sediment (medium-fine sand) is created, after which the mesocosms are filled with a layer of 60 to 140 cm of natural water including phytoplankton and zooplankton. The water is 2 mm filtered to remove larger (predatory) species, but to maintain the natural plankton community. Specific macrofauna species are added to create a test community. In freshwater mesocosms, vascular plants may be introduced; in marine systems macro-algae. Subsequently, all mesocosms are interconnected through an overflow basin and the water is circulated amongst all mesocosms during one month to ensure a homogeneous water quality and plankton communities in all systems. Before applying a test substance, the circulation is ended and the mesocosms are isolated from each other. Internal circulation is created when necessary. Based on our expertise and experience we have described procedures that ensure a good replication of our mesocosms.

Water quality parameters (oxygen content, pH, nutrients, chemistry, etc.) and phytoplankton and zooplankton development are monitored on a regular basis.

Macrofauna is mostly sampled at the end of the test to assess survival, growth and, depending on the species, population development.The first stagnant marine mesocosm test ran from December 13th, 2007 to August 25th, 2008. Macrofauna was introduced on January 11th, 2008. Four shallow (water depth 60 cm) and four deep (water depth 140 cm) mesocosms were installed. Two of each were ended on April 21st, 2008 to assess development in winter and early spring. Water temperatures declined to near zero during December, but were 6°C in January when macrofauna was introduced. The temperature fluctuated between 4 and 8°C until the end of March, after which it continuously increased to more than 22°C early July.

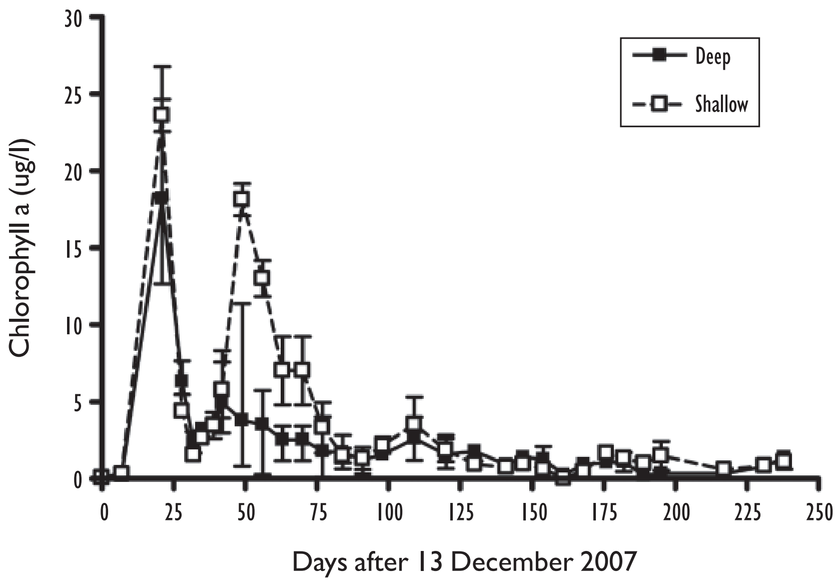

Figure 2. Development of phytoplankton in marine mesocosms based upon chlorophyll-a measurements. Presented is the mean and standard deviation of 4 (until Day 110) or 2 replicates.

Macrofauna species showed good survival (>90%) throughout the experimental period. Growth was mainly observed between April and August. Populations of the mudshrimp *Corophium volutator* developed from approx. 80/m^2 in January, to 500 (shallow) and 2,000 (deep) in April and 125,000 (deep) and 350,000 (shallowp)/m^2 in August. An exception was the lugworm *Arenicola marina*, which lost considerable weight between April and August. Most likely, the systems could not supply enough food to sustain 20 lugworms/m^2. This is a remarkable difference with flow-through mesocosms, in which we can introduce at least 80 lugworms/m^2 (Kaag *et al.* 1997). In the second experiment that ran from early April to early August 2009, only 8 lugworms/m^2 were introduced, allowing growth during summer.

Typically, after installation of mesocosms, enhanced phytoplankton development is observed (algal bloom), the magnitude of which depends on the nutrient status of the systems. Once the zooplankton and other grazing animals are established, the phytoplankton community falls back to a lower level. This development is shown in Figure 2 for the first experiment using two types of stagnant marine mesocosms.

Experiment 1 was started in winter, when production is low and nutrient levels are relatively high. This rapidly resulted in a pronounced bloom of the phytoplankton, followed by a second bloom in shallow mesocosms. Later in the season, when the temperature increased, grazing by zooplankton (Figure 3) and macrofauna and competition of periphyton resulted in a lower standing stock of phytoplankton.

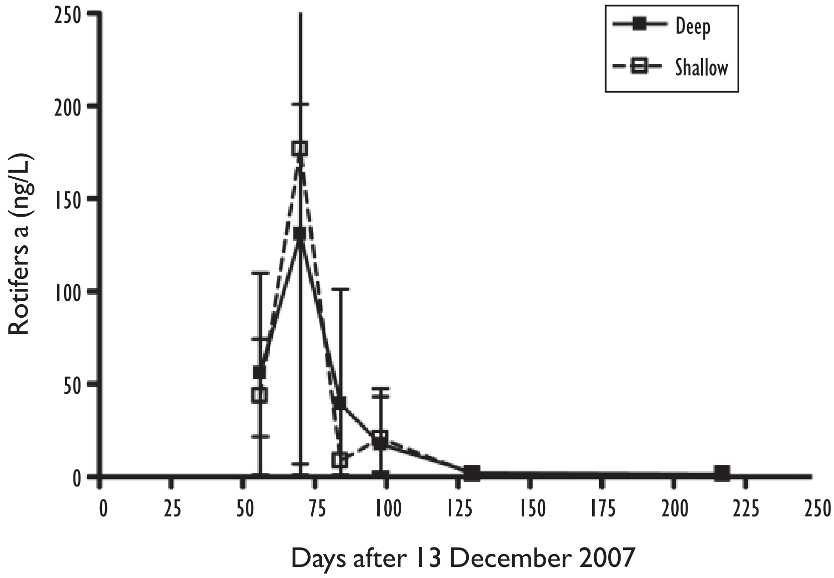

Figure 3. Number of adult copepods per liter in marine mesocosms. Presented is the mean and standard deviation of 4 (until Day 110) or 2 replicates.

Application of a toxicant may initiate a new and at higher concentrations persistent bloom of phytoplankton. At intermediate concentrations, the bloom may be transient due to recovery of the grazing by zooplankton.

5 Conclusions

Mesocosms can form a valuable tool for ecological risk assessment of discharged treated ballast water. Especially when the Ballast Water Treatment System is based on the application of rapidly degradable active substances. A carefully conducted mesocosm study will not only yield NOEC and LOEC values at the population level for a suit of organisms, but also at community level. Moreover, if the duration of the test is sufficient and recovery is observed, it is also possible to assess a NOEAEC (No Observed Ecological Adverse Effect Concentration). In accordance with pesticide regulations this NOEAEC could be applied as PNEC (Predicted No Adverse Effect Concentration) for risk assessment without using an assessment factor.

Agricultural pesticides are usually applied one or more times in a scheme representative of agricultural usage practise. Several test concentrations are created in duplo or triplo, as well as untreated controls. The experiment is then continued until at least 8 weeks after the last application in order to be able to specify the effect classes that are related to recovery of the most sensitive endpoints (De Jong *et al.* 2008). A similar approach would be applicable for testing of the active ingredients of BWMS, simulating one or more discharges resulting in specified concentrations of the active ingredient in an ecosystem. Test procedures for such studies are already available.

However, the most realistic scenario is the discharge of treated ballast water in an ecosystem, as will occur in/near international harbours. Even without causing an effect, in the mesocosms this will result in a dilution of the plankton community that is present. In order to separate the toxicological effects from this dilution effect, a comparable dilution should be achieved in the control mesocosms. Appropriate test procedures have to be developed to cope with these scenarios.

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