

# The Road from Approval to Compliance

A.C. Sneekes & N.H.B.M. Kaag

**IMARES Wageningen UR, Dept. Environment. P.O.Box 57, 1780 AB  
Den Helder, The Netherlands. Email: [andrea.sneekes@wur.nl](mailto:andrea.sneekes@wur.nl)**

## Abstract

Ballast water treatment systems are faced with two contrasting challenges; the treatment needs to be very efficient in removing or killing organisms on the one hand, but should not pose an environmental risk at discharge on the other. At this moment a suite of techniques is used to assess the efficacy and the environmental risk of ballast water treatment systems and improvements are still going on. No single technique is sufficient on its own, but using a combination of techniques facilitates a “weight-of-evidence” approach.

The next challenge in controlling ballast water is compliance monitoring that should allow port authorities to determine the efficacy of a treatment and judge the environmental risk at discharge. Using the full battery of testing techniques for each system and at each discharge will be practically impossible. Therefore we propose to select a deliberate set of testing techniques on the basis of the information compiled during land-based and toxicity testing. Examples of such an approach will be presented based on our ballast water experiences in the last five years, but also many years of field monitoring.

**Key words:** Ballast Water, Efficacy, Environmental risk, Compliance Monitoring, Bioassays, Biological Early Warning Systems, Rapid Screening Assays.

## 1 Introduction

In 2004, the International Convention for the Control and Management of Ship's Ballast Water and Sediment was adopted. The goal of the convention was to reduce the number and rate of invasions of species outside their native range. This boosted the research and development of Ballast Water Management Systems (BWMS). The convention will not enter into force until ratification by 30 states representing 35% of merchant shipping tonnage. At 30 June 2010, already 26 States representing over 24% of world tonnage have signed the convention which makes the ratification getting closer and closer. Signed parties are, however, already obligated to prevent, minimize and ultimately eliminate the transfer of harmful aquatic organisms and pathogens through the control and management of ships' ballast water and sediments.

For the registration procedure, IMO has set out standards for development of BWMS, and new systems must be approved by the Administration in accordance with IMO Guidelines. Only looking at the environmental part, BWMS must be rigorously tested proving the efficacy of the system without causing greater harm than they prevent to their environment. Although both types of analyses have the same biological background, the endpoints are markedly different: efficacy aims 100% effect, environmental risk prevention aims no effects.

The development of Ballast Water Management Systems plus monitoring of the efficacy of the treatment without causing negative effects to the environment is a great challenge for technicians, chemists, biologists, but also policy makers. As the date of

ratification is getting closer, the pressure on port state authorities is rising. They pose the challenge in monitoring all BWMS that passed the IMO guidelines and enter their harbour for ballasting and/or de-ballasting on bases of efficacy, but also determining the lack of residual environmental risk.

This paper points out some challenges on bases of our ballast water experiences for the approval procedures in the past five years with regards to efficacy testing and (residual) environmental risks. And an ecotoxicological view on compliance monitoring built up from many years of field monitoring, specifically for the determination of the residual environmental risk.

## 2 Approval procedure

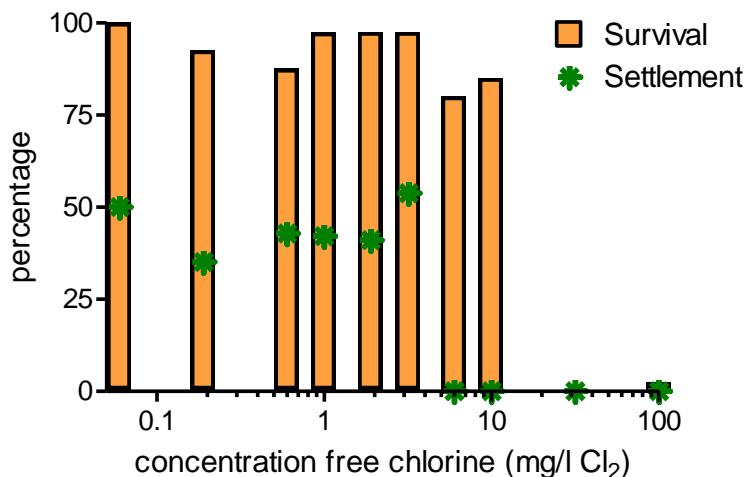
The approval procedure focuses on the main goal: preventing, minimizing and ultimately eliminating the transfer of harmful aquatic organisms and pathogens. For the Administration, guidelines for approval of a BWMS are described in G8. Testing the efficacy of the system by means of ship-board and land-based testing is a main part of these guidelines. Systems that make use of an active substance must also research the sustainable use of the substances concerning ship safety, human health and the aquatic environment as described in procedure G9.

### 2.1 Efficacy Testing

The Ballast Water Performance Standard (Regulation D-2) stipulates that discharged ballast water shall contain less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension and less than 10 viable organisms per millilitre less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and discharge of the indicator microbes shall not exceed the specified concentrations.

The IMO criteria do not distinguish between survival and mortality, but between viable and non-viable. It seems that makes life easier, as organisms do not have to be killed. The only objective is that reproduction is made impossible. Determination of viability is, however, a complicated assessment. Mortality -how difficult it may sometimes be to assess unequivocally- is rather instantaneous. Viability needs long-term analyses, if ever it can be assessed beyond doubt.

An example of this was seen during the land-based tests of the ErmaFirst BWMS at the NIOZ test facilities. It appeared that barnacle cyprid larvae were not killed by the chlorine that was used as active substance. A laboratory test with different doses of hypochlorite showed that cyprid larvae of *Balanus amphitrite* were not killed below 10 mg/l free chlorine and even at 100 mg/l still surviving larvae were observed (Fig. 1). However, settlement of the cyprid larvae did not occur at 6 mg/l and higher. This means they are not viable, as the cyprid larva itself is a non-feeding stage which will eventually die of starvation when not settled. A 3-day test like this is of course no definitive proof, but also in the land-based test, no settlement was observed in the treated tanks (Kaag & Sneekes, 2010).



**Fig. 1** Graphical view of the results from the laboratory barnacle settlement test

Similarly, organisms can be temporarily disabled by a treatment. This may give the impression that they are dead, but after recovery they may appear to be very viable. For instance, when phytoplankton is treated with low doses chlorine, staining with neutral red reveals no viable cells during the first 24h. This is supported by measurements of chlorophyll-a and photosynthetic activity. However, within a few days the phytoplankton starts growing again vigorously in re-growth experiments. Extrapolation of the growth curves back to the treatment day indicate that the number of viable cells must have been much higher than is acceptable (1000-2000 cells/ml).

## 2.2 Environmental Risk

IMO obligates Parties not to cause greater harm than they prevent to their environment. In an ideal situation, all species available in the environment are screened for their sensitivity to the discharge water from a BWMS. As this is not possible, risk assessment relies on the representativeness of a group of test-species. BWMS that make use of an active ingredient must prove with means of biological toxicity tests, the so-called bioassays that there is no significant negative effect of ballast water discharge to the receiving environment. We have a suit of standardized bioassays available as shown in Table 1 that covers, but is not limited to, the three main groups used in risk assessment: Algae, Crustacean and Fish.

**Table 1** Typical standardized bioassays used in Ballast Water Research by IMARES Wageningen UR, the Netherlands.

Group	Species name	Application
Bacteria	<i>Vibrio fischeri</i>	fresh to saltwater
Algae	<i>Phaeodactylum tricornutum</i>	brackish-saltwater
	<i>Pseudokirchneriella subcapitata</i>	freshwater
Crustacea	<i>Artemia franciscana</i>	brackish-saltwater
	<i>Acartia tonsa</i>	saltwater
	<i>Daphnia magna</i>	freshwater
Mollusc	<i>Crassostrea gigas</i>	saltwater
Rotifer	<i>Brachionus plicatilis</i>	brackish-saltwater
	<i>Brachionus calyciflorus</i>	freshwater
Fish	<i>Solea solea</i>	saltwater

A wide range of bio-assays and species should be evaluated for the assessment of environmental risk. Our own experience not only with ballast water tests, but also with marine and freshwater effluents, single substances, concentrated extracts and sediments, as well as with numerous multi-species mesocosm experiments, shows that no single species or species group is the 'most sensitive' for all substances. For that reason an 'intelligent' choice of bioassays is necessary.

Some indication regarding the most sensitive species may be extracted from literature and specific database (e.g. Aquire, EUCLID). It has to be considered, however, that most bioassays were developed for use in standard laboratory conditions in order to derive the dose-response for single substances. Treated ballast water, however, should be considered an effluent and needs another method of approach. The composition of the water to be tested is sometimes far from standard and specific water characteristics often are out of the specifications for the specific test. Although these are a result of the treatment, they may obscure effects of the active substance itself and manipulation of the water characteristics may be needed, as well as selection of other test species.

To conclude, no single assessment is sufficient to determine either the efficacy of a BWMS, or the safe concentration of ballast water at discharge. It is the combination of chemical analysis of active substances, different methods for viability analysis, a suit of bioassays and literature/database data that together give a picture of performance of a BWMS and potential environmental risks. In ecotoxicology this is called a 'weight-of-evidence' approach.

### **3 Compliance Monitoring**

Compliance monitoring aims at two questions: has the treatment been sufficient and -if active substances are used- does the discharged water pose a risk to the receiving environment.

Most BWMS are tested under controlled circumstances and experts have tested the water quality rigorously according to IMO criteria. Some discrepancies in the guidelines must be acknowledged and raise new challenges for monitoring. For example, the guideline asks to test a BWMS at two different salinities. When chosen for two high salinities (>20 psu), this might not reveal any problems, but when tested at low salinity (freshwater conditions) problems with the efficacy or residual toxicity of the system might occur. Some systems need a certain composition of the water in order to produce the active substance. A minimum salinity is for instance needed to produce sufficient chloride by electrolysis. This may be achieved in freshwater by adding brine, although this changes the basic characteristics of the water.

Another challenge can be found in temperature. Most often a system is tested in one continent under favourable conditions with respect to temperature and biology, the so-called biological season, in order to meet the specified criteria for inlet water. Physical/chemical reactions differ with different temperatures and can result in for instance insufficient degradation of the compound for a short trip, showing toxicity at discharge. But also, organisms found in winter time compared to summer time can react differently to the BWMS. Often, resting stages of organisms are more robust as they need to survive less favourable conditions.

#### **3.1 Efficacy Testing**

Testing the efficacy of the system faces the same problems as we have seen in the registration procedure: how to sample and recognize viable organisms. We will not go into detail on this subject, except for the following observation. Research on board of

ships, as has been presented on several occasions by Mr. Gollasch of GoConsult (e.g. Gollasch & David, 2010), has shown that the number of organisms may vary heavily between successive samples taken. Statistical analysis suggests that numerous samples have to be taken in order to obtain statistically sound results. On the other hand, Mr. Fuhr of NIOZ (Fuhr et al., 2010) suggested that the three samples should be taken immediately when discharge starts, as the analyses are very time consuming. Our own results of viable organism counts in three successive samples taken in-line during intake and discharge of treated ballast water give some practical nuance to this. At discharge, the treated water had been stored for 5 days in two 100 m<sup>3</sup> tanks in a ship. For organisms <50 micron, as well as organisms >50 micron, the numbers varied less than one order of magnitude between replicates. Also, in our data, either all three samples were in compliance, or none of them was. This might suggest that one sample taken when discharge starts could give indication to judge whether to further investigate by sampling or not.

### 3.2 Environmental Risk

The question is whether active substances in the discharged ballast water still pose a risk to the receiving environment. Chemical analysis may be used to assess the concentration of an active substance in the ballast water before discharge. Reliable chemical analyses, however, usually take some time and not every chemical laboratory is able to analyze all potential active substances. For some substances in-line monitoring of the concentration is used as part of the BWMS and can be used as first indication. Laboratory analyses may be used as a way to check on these systems. An alternative may be the use of rapid test-kits which are available for many substances. These kits do have their limitations with regard to precision, but are very quick. The main problem with many of these kits is that their detection limits are often much higher than stated in the specifications due to the composition of the water. It may, therefore, not always be possible to measure as low as the safe concentration determined for the BWMS.

Bioassays are useful alternative for the assessment of residual toxicity in ballast water. Unlike the situation described for land-based testing, there is no need to apply a whole battery of tests. Based upon the information taken up in the applications for Final Approval for each active substance cq. BWMS, the most sensitive species can be selected. It is, therefore, recommended to make this information accessible to port authorities.

Algae quite often belong to the most sensitive species, which would make the algae growth inhibition test a suitable generic bioassay for compliance monitoring. This bioassay, however, lasts 3 days which is not very practical when a direct decision on the acceptability of the ballast water is needed. The same is true for most other bioassays. The typical test duration for ecotoxicological tests is 24h to 96h. The Microtox® Basic Test, using the bacteria *Vibrio fisheri* and comparable systems, may give results within one hour. Unfortunately, this test is often not sufficiently sensitive.

The way to move on here is to explore the possibilities of Biological Early Warning Systems (BEWS) and other rapid on-line screening assays. Several BEWS are routinely used for monitoring the quality of surface water that is used as source for drinking water. Others BEWS, marine as well as freshwater, are or have been used for the on-line monitoring of water quality near calamities (spills), or sewage/waste water discharge points (Sneekes et al., 2005; Butterworth et al. 2001). Most BEWS are based on changes in behaviour in response of organisms to a chemical cue. This change is registered and an alarm triggered if a pre-set threshold (No. of animals responding, duration of response) is exceeded. Some experience of the operator is needed, as an alarm may also be triggered by the change of water characteristics (from holding water to ballast water for instance),

sudden noises, etc. Usually, these alarms can readily be distinguished from chemical cues by the duration of the response and the pattern of recovery. Some additional research is needed to explore the applicability of BEWS, especially with regards to ease of use, sensitivity to different active substances used, detection limits and whether different BEWS can be interchanged.

Other potentially useful methods are rapid screening assays that are based upon standard bioassays. An example is the algae PAM-test. This test does not measure growth (inhibition) after several generations, but the direct inhibition of the photosynthetic system of the algal cells. In ecotoxicological testing, often an exposure period of 4h is used. In our tests with ballast water we have seen that a sensitive species may show a very pronounced response (to a high concentration) within 30 min.

In the end, it is not up to us to decide how to perform compliance monitoring. This is a policy issue. We hope that this contribution gives some information that may help to make such decisions and we are always willing to discuss and clarify specific issues addressed. If, however, we have succeeded in clarifying some obscure issues, we will now blow some new smoke:

The whole discussion on environmental risk and residual toxicity focuses on discharged ballast water. But what about toxicity of the water taken in? The water in most industrial harbour areas is not known for its ecological quality. This issue surfaced this autumn when we encountered severe toxic effects of the untreated ballast water control. This made an assessment of the success of the neutralisation impossible. In compliance monitoring there is no untreated control and the effect may be unduly attributed to the BWMS used. And even if it is clear that it originates from intake, would you allow discharge anyway?

## References

- Butterworth F.M., A. Gunatilaka & M.E. Gonsebatt (eds.) (2001): Biomonitoring and biomarkers as indicators of environmental change 2. A handbook. Kluwer Academic/Plenum Publishers, New York.
- Fuhr F., J. Finke, P.P. Stehouwer, S. Oosterhuis & M. Veldhuis (2010): Factors influencing Organisms Counts in Ballast Water Samples and Their Implications. In: Bellefontaine N., F. Haag, O. Linden, J. Matheickal (eds.) (2010): proceedings of the IMO-WMU Research and Development Forum. 26-29 January 2010. Malmö, Sweden.
- Gollash S., M. David (2010): Recommendations how to Take a Representative Ballast Water Sample. In: Bellefontaine N., F. Haag, O. Linden, J. Matheickal (eds.) (2010): proceedings of the IMO-WMU Research and Development Forum. 26-29 January 2010. Malmö, Sweden.
- Kaag N.B.H.M., A.C. Sneekes (2010): The sensitivity of barnacle cyprid larvae to chlorine. IMARES report C103/10.
- Sneekes A.C., W. Koops, N.H.B.M. Kaag, V.G. Blankendaal (2005): Response to harmful substances other than oil spilled at sea (HASREP). Task 3a: Investigation of the use of a flow-through musselmonitor® for monitoring incidental chemical releases at sea. TNO-report R 2005/342.