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Overview of test-systems and other information relevant for evaluating harmful effects in the marine environment from the use of anti-fouling paints on ships

Norwegian Institute for Water Research

REPORT

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Abstract

The International maritime Organization (IMO) has agreed to a plan to implement a ban on the use of organotin for antifouling on large ships. One element in this plan was to establish a mechanism for addressing and evaluating antifouling systems other than those based on organotin. It was agreed that the mechanism for addressing antifouling systems should include a list of restricted antifouling systems. An expert group established by IMO will review proposals aiming at an inclusion of an antifouling agent/system on this list.
The environmental data available to the experts will be of a composite nature covering physical/chemical properties, toxicity tests and community related observations in experimental ecosystems (mesocosms) and in the field. Understanding the full environmental relevance of different types of test results is difficult, even for experts. This report is meant to be a guide to how different types of test results and observations can be evaluated but is not intended as a ready to use manual on how to perform stringent evaluations It is anticipated that the report will be a contribution from Norwegian Maritime Directorate (NMD) to the efforts in IMO to establish methods for addressing and evaluating antifouling systems proposed to be included in the list of restricted substances.

4 keywords, Norwegian	4 keywords, English
1. Antibegroing	1. Antifouling
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4. Evaluering	4. Evaluation

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Preface

This report was prepared on request from the Norwegian Maritime Directorate (NMD). The scope for the project was outlined in letter of 04.05.99 from Norwegian Institute for Water Research (NIVA) to NMD. The contract for the project was signed 24.06.99 (NMD) and 09.07.99 (NIVA). The scope of the project was agreed adjusted according to recommendations in NIVA´s letter of 10/11-99 to NMD. The project was financed through funding from The Norwegian Ministry of Environment (MD) to NMD.

The scope for the project was:

- Based on available information, produce an overview of test methods and other data relevant for environmental evaluation of antifouling agents.
- Suggest how field evidence of effects, results from experiments in mesocosms, results from toxicity testing and physical/chemical characteristics of the active agent(s) in an antifouling system can assessed with the aim to include them in a list of restricted substances.

It is planned that the report from the project will be submitted to the October 2000 MEPC meeting.

It is anticipated that the report can be a tool for expert groups in their evaluation of suggested hazardous antifouling systems.

Karstein Thingvold was contact person at NMD during the course of the project.

John Arthur Berge was project leader at NIVA.

Oslo, 14/4-2000

John Arthur Berge

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Summary

At the IMO MEPC meeting in November 1998, a plan was agreed, to ban on the use of organotin for antifouling on large ships. One of the elements in this plan was to establish a mechanism for addressing and evaluating antifouling systems other than those based on organotin. It was agreed that the mechanism for addressing antifouling systems would include a list of restricted antifouling systems rather than a list of approved systems. Any party may propose amendments to this list. An expert group established by IMO will review such proposals and report whether available information supports the contention of risk in the proposal.

The environmental data available to the expert group will be of a composite nature covering physical/chemical properties, toxicity tests at different levels of organisation (subcelullar to individual) and community related observations in experimental ecosystems (mesocosms) and in the field.

Understanding the full environmental relevance of different types of test results is difficult, even for experts. This report is intended as a guidance on how test results and observations can be evaluated but is not meant to be a detailed manual on how to perform stringent evaluations. It is anticipated that the report will be a contribution from the Norwegian Maritime Directorate (NMD) to the efforts in IMO to establish methods for addressing and evaluating antifouling systems proposed to be included in the list of restricted substances.

The aim of requiring information concerning the physical/chemical characteristics and toxicity data on active compounds, is to be able to address, predict and avoid possible harmful consequences in the field.

Three basically different methods may lead to the inclusion of an antifouling agent/system on a list of restricted substances. Each method involves different types of data/information. The three methods are:

- Screening - assessment based on inherent properties of the relevant antifouling compound
- Risk assessment based on theoretical predictions of field concentrations and effects
- Field evidence of effects from antifouling compounds in use

In addition possible control measures has to be considered in order to evaluate whether such measures reduce harmful effects to an environmentally acceptable level.

The relevance of the different methods depend on availability of information. One should seek to perform the evaluation at a level that maximise the use of data.

<u>Screening</u>
Screning and predictions of the effects of an antifouling system/agent are usually based on:

1. Inherent properties, physical/chemical characteristics and degradation rate
2. Toxicity tests including properties related to the assumed mechanism for the effect of the biocide (endocrine disruption, genotoxicity, mutagenicity).

Some inherent properties are so undesirable that the system/agent is recommended to be included in the list of restricted antifouling systems independent of other data. Inherent characteristics/properties of a compound that suggest its exclusion as part of an antifouling system for ships from an environmental point of view are:

- Systems that include antibiotics[1]
- Compounds with a predominant genotoxic or genotoxic effect
- Potent endocrine disrupters
- Substances with a log Pow>4-5 and molecular weight <700
- <10 % degradation in 28 days in a standardised test (not relevant for metals)
- Leaching rate for active substance >0.1%/day

[1]Substance that mainly has an anti bacterial effect (human and veterinary medicine) that might result in development of resistant bacteria in the field.

If the inherent properties are acceptable the expert group should move on to evaluate the results of standardised toxicity tests. Documentation on at least three species, representing different taxonomic groups should be required. Suitable alternatives are a mollusc reproduction test or a fish early life stage test. An example of a suitable test battery with endpoints and suggested criteria is shown below.

Species	Endpoint	Suggested criteria[1]	Suggested criteria[2]
Pelagic copepod (*Acartia*)	LC_{50}	<0.01 mg/l	0.01-0.1 mg/l
Planktonic algae (*Skeletonema*)	EC_{50}	<0.01 mg/l	0.01-0.1 mg/l
Mussel or fish	NOEC	<0.001 mg/l	0.001-0.01 mg/l

[1]Irrespectable of half life

[2]-Compounds with low degrability, half life >2 months)

Lack of data should not be an advantage for accepting an antifouling product. It is therefore important that lack of crucial data should result in including a substance in the list of restricted substances. This will provide an incitement for performing tests to acquire the relevant data.

Risk assessment

In the risk assessment results from toxicity tests are combined with calculations of concentrations likely to be found in the field. An environmental risk assessment consists of two main objectives:

- Estimate/predict/measure the realistic environmental concentration (PEC)
- Estimate the highest concentration that is likely not to give effects on organism (PNEC).

For the purpose of the risk assessment a standardised exposure scenario has to be defined for calculation of environmental concentration (PEC). The maximum acceptable volume of water effected by an antifouling agent is a matter of environmental politics more than science. Estimation of PEC should primarily adress conditions outside obviously impacted areas such as harbours.

The two estimates are combined in a fraction (PEC/PNEC). A PEC/PNEC>1 indicates that effects are probable.

Only a limited number of species can be tested for determination of PNEC. There is therefore a considerable probability that species not included in the tests are more sensitive. Assessment factors are therefore proposed in order to compensate for this uncertainty. Adjusted PNEC can be estimated from the toxicity data using assessment factors as proposed below.

Species	**End point**	**Assessment factor**
Alga *(Skeletonema costatum)* growth inhibition test	EC_{50}	500
Crustacea *(Acartia tonsa)*	LC_{50}	500
Mollusc reproduction test or Fish early life stage test	NOEC*	50

Field evidence

Predictions based on screening and risk assessment have failed if unexpected harmful field effects are found after a system/agent has been in use for some time. Field effects or other alarming supplementary environmental information (mesocosm experiments) on an antifouling system may trigger a call for a re-evaluation of the use of the biocide.

Priority based on environmental realism of tests/effects has therefore changed from inherent properites and standardised toxicity tests to:

1. Field evidence of effects
2. Mesocosm evidence of effects at environmentally realistic concentrations

It is difficult to establish a cause-effect relationship from field observations and unequivocally link the observed effect to a specific antifouling system/agent. It is generally simpler to restrict the use of a biocid based on its inherent properties, general toxicity or functional mechanism of the biocidal effect. The expert group should therefore redo the evaluation (screening and risk assessment) based on updated information on:

- Degradation and other inherent physical/chemical characteristics of the compound .
- Functional mechanism of the biocidal effect.
- Results from new toxicity tests.

In order to see if the characteristics:

- are so undesirable that the system/agent should be included in the list of restricted antifouling systems, independent of possible field effect,
- in combination with possible field/mesocosm effects are so undesirable that the system/agent is recommended to be included in the list of restricted antifouling systems

The expert group should secondly scrutinise the field and mesocosm observations of claimed effects in order to evaluate if the evidence is sufficient to tie the observations to the antifouling system. If such a cause/effect relationship is established the expert group must evaluate if the observed effects are sufficiently serious to recommend that the agent should be included in the list of restricted antifouling systems.

The suggested methods for evaluating if an antifouling agent system should be included in a list of restricted substances is designed purely from an environmental viewpoint related to the marine environment. It is appreciated that the expert group also has to consider other aspects. The challenge for the expert group is to reveal the inherent relevance of the different types of test results and data presented, and formulate a balanced united evaluation based also on non-environmental considerations and give a final recommendation.

1. Introduction

Materials submerged in seawater experience a series of physical, chemical and biological events resulting in the formation of a layer of attached organisms known as fouling. Fouling on ships promotes corrosion, creates roughness and thus reduces speed per unit of fuel, increase overall fuel costs and the emissions from the engine to the air, and reduce service intervals.

Organotin has been used as an effective biocide to prevent fouling since the seventies and is now the most commonly used antifouling agent in paint for the underwater hull of large ships.

Individual states and international bodies (EU) have been concerned with the unintended environmental effect of TBT seen world-wide. At the IMO MEPC meeting in November 1998, a plan for implementing a ban on the use of organotin for antifouling on large ships was agreed. Implementation of the plan requires an agreed Convention (Convention on regulating the use of shipboard antifouling systems that have adverse effects on the marine environment). The basic elements in the plan were:

- No new application of paints containing organotin after 1st January 2003.
- No use of organotin in antifouling systems for ships after 1st January 2008.
- Establishment of a mechanism for addressing (and evaluating) antifouling systems other than organotin-based systems.

At the IMO MEPC meeting in November 1999 it was agreed that the mechanism for addressing antifouling systems would include a list of restricted antifouling systems rather than a list of "allowed" systems. At present organotin compounds which acts as biocids are the only substances planned to be included in this list. Any party may propose amendments to this list (including new antifouling systems). Such proposals shall include adequate documentation of:

- Identity of the antifouling system addressed in the proposal
- Information suggesting that the antifouling system can cause unintended adverse concentrations/effects
- Preliminary recommendations on the type of restrictions that could be effective

An expert group established by IMO will review such proposals (initial or comprehensive). The expert group is planned to consist of government-designated experts with expertise in environmental fate, marine biology, economic analysis, risk management, or other fields of expertise necessary to review the proposal. The expert group will report to the appropriate body in IMO whether the information presented and any other relevant data that comes to light supports the contention of risk in the proposal.

Any decision to amend the list of restricted antifouling systems (for example including new substances) shall take into account the recommendations of the experts and only be adopted by the Parties of the Convention.

The environmental data available to the expert group will be of a composite nature covering physical/chemical properties, toxicity tests at different levels of organisation (subcelullar to individual) and community-related observations in experimental ecosystems (mesocosms) and in the field. The challenge for the expert group is to make a correct assessment of the different types of test results and data presented, and formulate a balanced evaluation and give a final recommendation.

Understanding the full environmental relevance of different types of test results is difficult, even for experts. In the present report we give an overview of test-systems and information relevant to evaluating possible harmful effects of putative antifouling agents in the marine environment.

The report is meant to be a guide to how compound related physical/chemical properties and different types of test results and observations can be evaluated. The report will prioritise the different types of data/test-results according to environmental relevance but is not meant to be a manual on how to perform the full evaluation. However, a proposed scheme for evaluation of antifouling agents will be presented to serve as an example on how different types of information may be interpreted and emphasised in the evaluation procedure.

The report is planned to be a contribution from the Norwegian Maritime Directorates to the process in IMO to establish the "Convention on regulating the use of shipboard antifouling systems that have adverse effects on the marine environment". If found appropriate the document will be submitted as a national contribution from Norway to the IMO-MEPC meeting in October 2000.

2. Biodegradation, physical and chemical properties of the biocide

The biodegradability and selected chemical and physical properties are the first properties documented for a compound. In our opinion it is possible already at this stage to screen a compound with respect to unwanted properties and in this way save expenses on further evaluation and product development for compounds which are obviously nvironmentally unacceptable.

2.1 General

The following outlines how different inherent properties of an antifouling agent may be used in an evaluation process where the ultimate goal is to decide whether the compound should be included in a list of restricted substances or not.

Some relevant threshold values for including the substance in a list of restricted substances are suggested.

2.2 Biodegradation

Biodegradation is the degradation of a molecule by means of enzymatic actions. Therefore the biodegradation is dependent on the organisms present, generally bacteria the most important and the active level of these. Microbial activity is generally limited by available nutrients and by temperature.

2.2.1 Relevant methods for measuring biodegradation

The biodegradative properties of organic compounds can be investigated by performing standardised tests. In ISO/TC 147/SC5 "Water quality - Guidance for the determination of biodegradability in the marine environment" 5 relevant methods for the marine environment are mentioned. These are:

- ISO 7827 "DOC die away test"
- ISO 10707 "The closed bottle test"
- ISO 10708 "The two phased closed bottle test",
- ISO 9439 "The CO_2 evolution test"
- ISO 14593 "The CO_2 headspace test".

OECD has also a marine test:

- OECD 306 "Biodegradation in seawater"

There are two options in the OECD test, which resemble the ISO 10707 and 10708 test.

In practice it is often observed that biocides cannot be tested in a sensible manner with these tests. The main reason for this is the minimum requirement of test material specified for the tests, around 2 mg/l, a concentration at which biocides often have an inhibitory effect on the microbial community and therefore results in no biodegradation as a result in the test.

In these cases ^{14}C-labelled material is recommended, this may however be regarded as too great a deviation from the standard method to be acceptable for classification purposes. As information on degradation is crucial in a marine risk assessment it is recommended that both a readily biodegradability test and a marine test is performed. Both tests should be performed at concentrations not inhibiting the bacterial activity. As the active biocide will occur in the environment in low concentrations, biodegradation at low concentrations becomes highly relevant.

None of the mentioned tests for the marine environment are acceptable for classification purposes for readily biodegradability. Compounds therefore need to be tested with respect to OECD 301A, B, C, D, E or F in addition to achieve this classification. Those tests are only relevant in the case that readily biodegradability becomes a requirement as they have little predictive value in a marine environmental risk assessment.

2.2.2 Temperature dependent degradation.

It is well documented that the biodegradation rate is temperature dependent, as is also the lag phase (period before any degradation is observable). In a risk assessment procedure, biodegradation rates at 20 ^{o}C is extrapolated to ambient temperatures by means of a Q_{10} factor. This is a factor that compensates for changes in microbial activity at a temperature interval of 10 ^{o}C. Q_{10} factors are often found in the range of 2.8 to 3.1. However values of 4-7 have also been found (Walker et al., 1997). This means that in order to get realistic rates, biodegradation test in marine water should preferably be performed at 2 temperatures in order to calculate the Q_{10} factor.

If the documentation for a biocide shows that the compound does not degrade in tests where concentrations are above the concentration that is inhibitory to microorganisms two alternatives are possible;

1) One may conclude that the compound is inadequately documented and ask for more documentation.
2) One may use the precautionary principle and assume that the compound is not degradable in the risk assessment. (Which inevitably will result in including the compound in the list of restricted substances).

The drawback with alternative 1) is that the IMO expert group may have difficulties in getting more documentation than follows from requirements made by national authorities. Another dilemma is that alternative 1) only causes a delay in the evaluation procedure with no consequence related to the use of the compound. Use of alternative 2) whereby the weight of acquiring additional evidence is placed on the producer/user of the product/compound is an incitement for conducting more tests.

2.2.3 Criteria for unwanted properties with respect to biodegradation

As biodegradation probably is one of the most important properties of a biocide in an environmental risk assessment perspective, a criterion should be set, indicating a threshold level below which compounds may be classified as unacceptable for release to the marine environment. This principle is based on the fact that the marine environment is the ultimate recipient of chemicals released by humane activities. Loss of persistent compounds from the marine environment may be extremely slow, and can for all practical reasons be ignored in risk assessment procedures. This implies that compounds released to the marine environment stay there unless they are biodegraded.

With respect to biodegradation the following criteria can be used as a threshold for when an anti-fouling agent should be included in list of restricted substances:

2.2.4 Biodegradation, Criteria for inclusion on a restriction list:

An active anti-fouling compound of primarily organic type should be included in the restriction list if its estimated biodegradation is less than 20 % per year under field conditions.
For standardised test this mean compounds that has no significant (<10 %) biodegradation in the course of a 28 days readily biodegradation test. Assuming a first order exponential degradation this is equivalent to a half-life of 80 days.

Metals and inorganic compounds are relevant to use in antifouling systems. Most metals are found naturally in the marine environment and as elements they do not biodegrade. They may therefore build up in the environment eventually leading to concentrations that gives harmful effects in non-target areas. For such compounds lack of degradation must be judged against the concentrations found naturally in the different environmental compartments and the toxicity and toxic mechanisms involved. The contribution from the use of the element to the concentrations found in the environment is also important.

2.3 Bioaccumulation

The property of a compound to accumulate in organisms is of concern, even in those cases where the compound does not have any obvious negative effects. Accumulation of a foreign compound in an organism may change the intracellular environment, causing subtle changes that effects the functioning of the organisms in ways that we at the moment cannot measure. It is this argument that is the basis for classifying compounds which are both bioaccumulative and persistent with the desription "may cause unwanted long term effects" (R53 according to Directive 67/548/EEC).

In an assessment the threshold for classifying a compound as "having bioacumulative properties" is triggered by the compound having a log P_{ow}>3 (see below under Chemical and Physical properties).

Bioconcentration tests are most commonly performed with fish according to OECD 305 guideline. "Bioconcentration, flow through" (there are several modifications in use). There are two important endpoints in these studies. 1) Maximum accumulation and 2) depuration rate. Maximum accumulation, denoted as bioconcentration factor (BCF) is a measure of how many times higher the concentration is within the fish compared to the exposure concentration in water. The depuration rate is measured as a reduction in the concentration in the fish (normally for 14 days) after transfer of exposed fish to clean water. A BCF value >100 is now used as evidence of bioaccumulative properties and for classification of the compound as bioaccumulative. In environmental risk assessment a compounds depuration rate is equally important. A rapid depuration rate indicates that the compound will not bioaccumulate in the food chain. When the depuration rate is low, i.e. a half-life of >30 days, one may assume that there is a potential for biomagnification in the food chain. This is typical for persistent non-polar halogenated pollutants (i.e. DDT).

In classification and labelling meetings a discussion has begun with respect to increase the classification threshold for BCF from 100 to 500.

Based on bioaccumulative properties, the proposed threshold criteria for inclusion of a compound on a list of restricted substances is that BCF>500 and half-life for depuration >30 days.

2.4 Chemical and physical properties

The Biocide directive (directive 98/8/EEC) requires that properties concerning chemical and physical properties must be documented. The most important from an ecological risk assessment viewpoint are: water solubility, vapour pressure, melting point octanol/water partitioning coefficient, molecular weight among others. In addition some specific requirements regarding the compounds use as a biocide (time to dry the anti fouling paint, release of active compounds, etc) are asked for.

Several of these properties are not independent, low solubility is related to a high partition coefficient and low release from paint.

Criteria for unwanted properties for chemical and physical properties.

Water solubility is an important property. One should therefore have solubility data also for saltwater. For polar compounds solubility is higher in salt water than freshwater while the opposite is generally the case of non polar compounds. High water solubility leads to higher leaching from the paint matrix and ensures that the compounds mainly stays in the water phase. However there is no risk directly connected to either high or low solubility.

Water solubility, Criteria for inclusion: none

The octanol/water partition coefficient is an important factor in environmental risk assessment because it to a large degree determines in which environmental compartment the compound is most likely to be found. A high partition coefficient represents a potential for bioaccumulation of the compound in organisms and also adsorption to organic matrices (i.e. sediment). The partition coefficient has been used for classification purposes and log P_{ow}>3 has been used as a threshold for bioaccumulation potential. There are arguments for increasing this threshold to 4-5, as a log P_{ow} of 3-4 only rarely results in a BCF >100 (see above). The main concern here is whether a high log P_{ow} is manifested in a high BCF and a low depuration rate. However in order to avoid that lack of data should be an advantage for accepting an antifouling product, criterion for non-acceptance based on P_{ow} should be applied. This will create an incitement for performing a bioaccumulation study or acquiring data on bioaccumulation by other means.

Bioaccumulation is also related to the size of the molecule in question. Generally organic substances with a molecular weight >700 do not bioaccumulate (TGD 1996).

Octanol/water partition coefficient (P_{ow}), criteria for inclusion in list of restricted substances:
Log Pow>4-5 and molecular weight <700 and the compound is not readily biodegradable and BCF data is unavailable.

The Vapour pressure indicates the tendency of compound to escape to the atmosphere. However it is unlikely that a compound that is going to be used as an active biocide in an anti-fouling paint is volatile to such a degree that it mainly will be found in the air compartment. There is no unambiguous evidence indicating a relation between vapour pressure and harmful effects of antifouling agents in the marine environment. Therefore this property should not be used as a criterion.

Vapour pressure, Criteria for inclusion in list of restricted substances: none

Leaching rate
Information on leaching rate of all active compounds of an anti-fouling system is a requirement in the biocide directive. A high leaching rate will indicate that high local concentrations may be expected in the vicinity of the vessel, especially during slow speed or in areas of high vessel density. However, this would also deplete the active compound fast and the anti-fouling effect would cease without frequent treatment.

As the main cause for concern is the environment, a high leaching rate leading to high local concentrations is clearly undesirable and requires a threshold limit.

Leaching rate, Criteria for inclusion: leaching > 0.1 %/d

Chemical and physical properties not mentioned above have generally little or no influence on a risk assessment, although some of the properties may have significance for human health. However, this is outside the scope of this assessment. Thus those properties are not relevant in the evaluation.

Other chemical and physical properties, Criteria for inclusion: none

3. Calculation of field exposure

3.1 General

An environmental risk assessment involves two operations:

1) Estimate/predict/measure the environmental concentration, in risk assessment often referred to as the PEC value (predicted environmental concentration).

2) Estimate the highest concentration that is likely not to give effects on organisms. This is often referred to, as the PNEC value (predicted no effect concentration).

In the risk assessment the ratio PEC/PNEC is used to quantify the risk.

A PEC/PNEC>1 indicates that effects are likely to occur. The confidence of the risk assessment will depend on the accuracy of the estimates of both PEC and PNEC.

3.2 Estimation of predicted environmental concentration

There is now a rapidly expanding need for models that calculate the concentration of a chemical in different environments. Within the EU a guideline has been developed that estimates environmental concentrations of chemicals used in industry (TGD 1996). This guideline does not incorporate marine systems, nor compounds that leach out of paints into water. The Biocide directive has suggested some models (Luttik et al., 1993 and Linders & Jager, 1997). An EU project has been established with the aim to design and validate a model to be used for anti-fouling products. The results from the project were scheduled to be ready in 1999.

3.3 Validation of predicted environmental concentrations

The establishment of theoretical PEC values is inherently so uncertain that large uncertainty factors have to be applied when using these in a risk assessment. Models are often validated for one or a few chemicals and often become much less predictive when used for compounds not similar to the ones they were validated for. Because of the great uncertainy of the estimates of PECs, it is neccessary to perform validation procedures in connection with the risk assessment for anti-fouling compounds. For new compounds not already in use there is no possibility of validating the estimated PEC in the environment. For such new compounds large scale mesocosm studies should be performed using downscaled models of vessels.
For compounds that are already in use there should be a requirement to perform a monitoring program with the aim to measure the concentration of the active compound in areas of high vessel density and at sites of special biological importance.

4. Tests for effects of antifouling substances

Toxicity data may be used to predict possible effects in the environment and thereby avoid possible harmful consequences in the field by restricting its use. It is obviously impossible to test and monitor all aquatic organisms for possible effects of xenobiotic substances[1] or contaminants introduced into the environment. Neither is it possible to identify one single taxonomic group as the most sensitive. For this reason a limited number of species have to be selected, belonging to different taxonomic groups, e.g. an alga, a fish, a crustacean, with the hope that important mechanisms of toxicity, that could cause harmful effects in the field, will be expressed in one or more of these organisms.

As toxicity tests, where mortality is the endpoint, determine the acute or short-term toxicity of substances on chosen species, additional methods are needed to clarify the mechanisms involved and to determine and predict possible sublethal and/or long-term effects.

Tests can be performed at different levels of organisation. Although the anti-fouling agents all have the purpose to prevent the settling of organisms on solid surfaces, the mechanism to achieve this may vary. This means that the mode of action of the chemicals may differ, which again means that it will not be possible to identify one single endpoint as the most sensitive. A battery of tests is therefore required to achieve sufficient confidence in an assessment of environmental risk.

Tests at the following level of organisation are treated in the present chapter:

- Subcellular (molecular interactions)
- Cellular
- Organ
- Individual

Long-term effects and interactions between species can be tested directly in more ecologically relevant systems, e.g. mesocosms (see section 5) and from field experience (see section 6) or be inferred indirectly from methods that identify mechanisms of action. Such mechanisms can be identified at the level of molecular interactions and at the individual level.

The environmental compartment that will be the primary recipient of anti-fouling agents is the marine pelagic environment. There should therefore be a special focus on pelagic organisms. For compounds with a high log P value the sediment compartment becomes more relevant. Highly persistent substances may biomagnify in food webs and could ultimately affect marine mammals or birds. If a relevant substance is highly persistent and hydrophobic, consideration should be given to possible effects on marine birds and mammals. It is by now established that organotins accumulate in marine mammals and they have been linked to immune dysfunction. If the substance fulfils such criteria, data from studies on mammals should also be used in the assessment. In addition, exposure studies with birds should be considered. Assessments of tests specifically related to mammals and birds are outside the scope of this report.

[1] in this text, the terms “xenobiotic” (from greek, “foreign substance”) and “contaminant” will be used to describe substances that are introduced to the environment and may cause toxic effects in marine organisms.

4.1 Sublethal tests

4.1.1 Background

The current knowledge of mechanisms of action is largely derived from the medical sciences. Although most directly relevant to mammals, most of the mechanisms have been shown to be applicable to other vertebrates, e.g. fish, and also to invertebrates. It is however important to be aware that many of the ”expected” effects of contaminants have been studied in systems different to the ones that will have to be used in the marine environment.

To most aquatic species, exposure to contaminants will cause a general stress response. Threat from a predator or high turbidity may however also be stressors to marine organisms, eliciting a response similar to that caused by contaminants. It is therefore important to single out responses that are contaminant-specific and that are only to a limited extent affected by natural factors.

In the following, relevant methods are presented according to whether they relate directly to molecular interactions (section 4.1.2) or whether they relate to whole-organism responses (other than death, section 4.1.3).

4.1.2 The molecular approach

All effects of xenobiotics or contaminants are initially mediated through interactions with biological molecules. A foreign substance may interact with e.g. membranes, signalling substances, receptors, energy carriers, proteins and DNA. The availability of methods is obviously governed by the current knowledge. Most of the methods have been applied to marine organisms, but some are at present only applicable to fish, not invertebrates (Table 1). In all cases, it is important to use the appropriate tissue. For some methods, the metabolically most active tissue, i.e. liver in fish, is appropriate, whereas other methods require circulating cells (e.g. blood cells) to be used.

Reduced membrane stability is an effect that may be caused by a wide range of environmental contaminants (Table 1). The method has been used for both invertebrates and fish.

Many of the most well-known contaminants, e.g. cadmium, have strong inhibiting effects on general protein synthesis, which can be assayed using total mRNA in the tissue. There are high concentrations of total mRNA in cells under normal metabolism, so the method will be more sensitive if specific mRNAs are determined (otherwise the decrease may be so small as to be non-detectable compared to the total).

Table 1. **Overview of functions that may be affected by antifouling substances, methods to determine effects and marine species for which the method has been applied.**

Function affected	Method	Method available for marine organisms	Reference(s)
membrane stability	lysosomal stability	invertebrate, fish	Regoli 1992
protein synthesis	total mRNA	invertebrate, fish	Veldhuizen-Tsoerkan et al. 1990, Viarengo et al, 1980
energy transfer	ATP:AMP ratio	invertebrate, fish	den Besten et al. 1991, Ivanovici, 1980
Genotoxicity	various	fish	Ericson et al. 1996, McElroy et al. 1991
cell death/apoptosis	various	invertebrate, fish	LyonsAlcantara et al. 1998
intracellular signals	-	-	
receptor binding, endocrine disruption	estrogen androgen thyroid	fish	Pottinger and Moore, 1997, Thomas and Smith, 1993

There have been numerous uses of the status of cellular energy-carriers to indicate whether cells or tissues are affected by xenobiotics. The rationale for this approach is interesting, as most xenobiotics would be expected to cause an increased use of energy in the cell. The method would therefore provide a reasonable estimate for a general effect at the cellular level. The methods currently available would have to be improved.

There is currently a wide range of methods available to determine damage to or binding of xenobiotics to DNA. Binding or damage to DNA indicates possible carcinogenic and/or mutagenic effects of xenobiotics. In all organisms there is a continuous turnover of cells. Death and removal of cells can be effected through various processes, but the quantitatively most important appear to programmed self-destruction, or apoptosis. Various xenobiotics have been shown to accelerate this process, e.g. organotin compounds.

Disruption of intracellular signalling is a mechanism of action that has not been extensively studied, but is expected to be relevant. The methods are lacking at present to determine such effects in marine organisms. One kind of signalling is mediated through receptors. Such receptors recognise and associate with many endocrine and paracrine agents, e.g. hormones. Xenobiotics, e.g. xenoestrogens, have been found to bind to receptors and elicit the response normally reserved for the natural agent. Methods exist to isolate receptors and quantify the binding of xenobiotics to them. This approach has been used for marine fish species. Many substances have been found to have endocrine disrupting effects in *in vitro* studies, while very few have been shown to have an effect on e.g reproduction in *in vivo* studies in the field.

4.1.3 The whole-organism approach

The methods discussed in the previous section all relate to responses in intact organisms, but where the actual measurement is performed at the molecular level. The molecular approach is by its nature very sensitive, but may also be overprotective when used for regulatory purposes because regulating mechanisms within the organism may counteract the effect of antifouling substances.

By including methods that measure more or less essential functions of an organism, such regulating or homeostatic mechanisms will be taken into account. A set of the most relevant functions is listed in **Table 2**. Unfortunately, for many of the known functions the available methods are not really well established, at least not for marine organisms. Some references are given in the tables to work in the field. Energy allocation was also discussed in the preceeding section, but there are also available methods to assess whole organism responses, indicated in **Table 2**. Scope for growth in mussels has been widely used, but newer methods can be used with smaller organisms.

Some of the most subtle effects of biocids, e.g. organotins, on mammals involve immune dysfunction. The current state of knowledge on immune disruption in aquatic organisms is not sufficiently far advanced to be applied for regulatory purposes at present, but such methods will hopefully be available in the future.

There is currently a focus on endocrine disrupting compounds (EDCs) and there is a continuous development of methods. The effect of organotins on gastropods, causing intersex or imposex, is one prime example of endocrine disruption. Good general methods for invertebrates are lacking at present, but methods do exist for fish. There are currently protocols under development within OECD. There are similarly protocols under development for reproductive and developmental toxicity, unfortunately there is a main focus on freshwater species (zebrafish, fathead minnow, medaka) in this development.

The development of tumours or preneoplastic lesions needs a long-term exposure and has not as yet been used as a test. Methods to detect early stages of tumorigenesis were included in the previous section (DNA damage). There is not presently much knowledge of neurotoxic effects of xenobiotics on aquatic organisms, except for impairment of olfactory senses. In some areas, an inhibition of acetyl cholinesterase (AChE) has been observed in wild fish and invertebrates, indicating effects of organophosphate or carbamate pesticides. Some studies indicate that other factors may also affect this family of enzymes.

Table 2. Overview of mechanisms that may be affected by antifouling substances, methods to determine effects and marine species for which the method may be applied.

mechanism	method	method available for marine organisms	reference(s)
energy allocation	scope for growth cellular energy allocation (CEA)	mollusk zooplankton	Koehn and Bayne 1989
immune disruption	health, immune system	fish	Secombes et al. 1992
endocrine disruption	vitellogenin, egg shell protein synthesis	fish	Arukwe et al. 1997
reproductive toxicity	species-dependant	any	
developmental toxicity	species-dependant	fish	
carcinogeniicity	various	fish invertebrates	Payne et al. 1988
neurotoxicity	changed behaviour, acetyl cholinesterase inhibition	fish	

4.1.4 The use of sublethal effect methods (biomarkers)

There has been an increasing use of sublethal effect methods (biomarkers) over the past 10 years to assess effects of contaminants in the marine environment. The ecological impact of TBT was identified through its sublethal effect on bivalves (shell thickening) and gastropods (imposex). At present, there exist protocols for a range of methods, e.g. endocrine disruption, genotoxicity and mutagenicity, but there is also a continuous development of techniques. In addition to being used in separate tests, analyses of biomarkers can be applied in toxicity tests and in mesocosm experiments. Biomarkers are most relevant for detecting chronic effects after relatively short exposure. Genotoxic, mutagenic and estrogenic/androgenic effects are among the most immediately relevant. Such effects would not normally be detected in the toxicity tests currently used for regulatory purposes. In the current context, methods that detect genotoxic, mutagenic or endocrine disrupting effects are the most relevant to include in a test battery. It is expected that methods that address neurotoxicity and effects on the immune system will be developed within this decade, but such methods are not currently available.

4.2 Toxicity tests

4.2.1 Definitions

In this context toxicity tests are confined to test methods where the toxic effect of chemicals are studied on the organism or population level under defined conditions. Normally such tests imply exposure of test organisms in a concentration series of the chemical for a defined period. Various test endpoints, e.g. survival, growth, fertility and development may be recorded.

Toxicity test methods are categorised as ”acute” or ”chronic”, depending on the test duration in relation to the life span of the organism. OECD (1998) have proposed the following definitions:

Acute	Short exposure in relation to the life span of the organisms
Subchronic	The exposure period covers a significant part of the life cycle or covers life stages (e.g. early life stages) or life processes (e.g. reproduction) considered to be especially sensitive
Chronic	Effects observed during exposure through the entire life cycle of the organism

Testing at a range of concentrations allows calculation of EC_x- values, which represent the concentration that causes x % effect on the endpoint studied. When lethality is recorded, results are expressed as LC_x (lethal concentration). Chronic toxicity tests are often designed to allow estimation of NOEC, which is defined as the highest test concentration showing no significant effect on the test endpoint.

4.2.2 Applications of toxicity tests

Toxicity tests are widely used to provide data for various evaluation schemes designed for:

- hazard identification, ranking and classification
- effect assessment
- generic risk assessment

of chemicals.

It is well known that large variations in sensitivity may occur between organisms (Slooff et al. 1983). This is particularly the case for chemicals with a specific mode of action such as pesticides (LeBlanc 1984). No species or taxonomic group can be identified as the most sensitive one in a general sense (Blanck 1984, Cairns 1986). Usually, differences in sensitivity are smallest between closely related species, but interspecies variations in EC_{50} values may still be as high at 10^4 within a group of organisms such as planktonic algae (Blanck et al. 1984).

Because of the large variations in sensitivity, most evaluation schemes rely on batteries of toxicity tests. The test batteries are composed of species representing different taxonomic groups and important ecological functions (e.g. primary producers, herbivores and predators). An example is the

European Directive for Risk Assessment for New Substances (93/67/EEC) which requires toxicity tests with algae, *Daphnia* and fish as a minimum.

Risk assessment of chemicals involves comparing likely exposures with likely effects (Calow 1998). In risk assessment schemes the toxicity data is used to estimate the Predicted No Effect Concentration (PNEC) which is compared to the Predicted Exposure Concentration (PEC). Uncertainty factors are applied to derive the PNEC from the EC_x or NOEC-values obtained in toxicity tests. In general the uncertainty factor is reduced as the amount and quality of the toxicity data is increased. To avoid excessive testing, many risk assessment schemes uses a tiered approach, where successively more toxicity data is required to increase the precision in calculation of PNEC when necessary.

4.2.3 Selection criteria for toxicitytests of anti-fouling agents

In the case of anti-fouling agents, toxicity tests can be applied in a preliminary toxicity ranking step and/or as a basis for a risk assessment. Since these chemicals by purpose have a high potential to affect biota, a simple ranking based on toxic properties may not be adequate. The evaluation should also include a generic risk assessment, where effect is related to predicted exposure of non-target organisms.

For assessment of chemicals for a specific use pattern such as anti-fouling agents, test methods should be selected to ensure that the most likely environmental side effects are disclosed. This implies that tests should include particularly sensitive taxonomic groups or endpoints, which are relevant for the predicted exposure situation. In this context, it is probable that a selection of toxicity tests and biomarkers will provide such guidelines.

The environmental compartment that will be the primary recipient of anti-fouling agents is the marine pelagic environment. The organisms should therefore be representative for this environment. In addition, the following criteria may be used for selection of appropriate test methods:

- Reproducibility
- Ecological relevance of test organism and endpoint
- General sensitivity
- Technical performance
- Availability of test organism
- Cost

The test methods selected should preferably be standardised on the international or national level to avoid questions related to method validation. A review of methods for aquatic toxicity tests has recently been performed by OECD (1998). The purpose was to identify test methods that should be given priority in development of new test guidelines in the OECD Chemicals Testing Programme. The review covers nationally or internationally standardised methods in which the species tested or endpoints studied are not represented in the existing OECD Guidelines.

124 marine toxicy test methods were evaluated for practical feasibility, validity, and usefulness in prognoses and level of standardisation using a scoring system. As a result of the evaluation, candidates for standardisation were selected. For the marine pelagic environment, 28 test methods were selected as candidates for standardisation for use in hazard assessment schemes (see **Table 3**). The complete list of test methods evaluated, the criteria used for evaluation and the scores obtained for each test method are shown in Appendix I.

If possible, tests for evaluation of anti-fouling agents should be selected among those listed in **Table 3**. However, if the specific conditions related to the properties and use pattern of this group of products indicate that other targets have to be considered, alternative test methods should be sought. It should be realised that development of new test methods for this purpose would entail a significant workload. In the case that evaluation of chemicals will be based on existing information on toxicity, guidance for evaluation on the available test data could be sought in Appendix 1. An important issue here is the fact that the selected test organisms are robust species that do not necessarily reflect the most sensitive species.

Table 3. Marine toxicity tests recommended for use in risk assessment schemes in the pelagic environment. (Extracted from OECD 1998). Reference numbers refer to Appendix I. a.o.=and others.

Acute toxicity tests

Taxonomic group	**Species**	**Long/short time test**	**Endpoint**	**Reference no.**
Algae, macro	*Gracilaria tenuisipitata*	ST	growth	8
Crustaceans	*Mysidopsis bahia* a.o.**	ST	survival	21
	Peneaus aztecus a.o.**	ST	survival	23
	Acartia tonsa a.o.**	ST	survival	35 (ISO)
Rotatoria	*Brachionus plicatilis*	ST	survival	61
Fish	Cyprinodon *variegatus* a.o.**	ST	survival	OECD 203
	Cymatogaster sp. a.o.**	ST	survival	OECD 203

Table 3(continued).
Subchronic tests

Taxonomic group	**Species**	**Long/short time test**	**Endpoint**	**Reference no.**
Algae, macro	*Champia parvula*	ST	growth, reproduction	16 (EPA)
	Porphyra yezoensis	LT	growth	5
	Ceramium strictum	ST	reproduction	1
Crustacea	*Mysidopsis bahia*	LT	survival, reproduction	24
	Acartia tonsa	ST	survival, fertility	66
	Centrophages hamatus	ST	survival, fertility	32
	Eurytemora affinis	ST	fertility	39
Fish	*Menidia peninsulae*	ST	ELS, survival, growth, hatchability	47 (OECD draft)
	Menidia peninsulae a.o.**	LT	ELS, survival, hatchability, growth, malformation	55 (OECD)
Cnidaria (Coelenterata)	*Eirene vitridula* *Cordylophora caspia*	LT	asexual reproduction	76 76
Echinodermata	*Lytechinus pictus*	ST	reproduction, fertility	42

Table 3(continued).
Chronic tests

Taxonomic group	Species	Long/short time test	Endpoint	Reference no.
Algae, micro	*Skeletonema costatum*, a.o.**	ST	growth	60 (ISO)
Crustacean	*Mysidopsis bahia*	LT	survival, growth	14 (EPA)
	Acartia tonsa	LT	reproduction, survival	101
Fish	*Cyprinodon variegatus*	LT	reproduction, growth, survival, developmant	74 (EPA)
	Clupea harengus	LT	ELS*, hatchability, survival, growth	47 (OECD draft)
	Gasterosteus aculeatus	LT	ELS*, survival, hatchability	55 (OECD)
Echinodermata	*Stronglyocentrotus* sp. a.o.**	ST	fertility	42
Mollusca	*Crassostrea* sp. a.o.**	ST	reproduction	25 (ASTM)
Protozoan	*Uronema marinum*	ST	growth	28

*ELS = early life stage
**a.o.=and others

4.2.4 Proposed test requirement

Documentation on toxicity to at least three species, representing different taxonomic groups should be required as a basis for evaluation of anti-fouling agents. Since the primary environmental compartment that will be exposed to anti-fouling agents is the pelagic environment, a planktonic alga, representing the primary producers and a crustacean zooplankton, representing herbivore consumers, should be included. The third test should cover chronic or subchronic effects on an invertebrate or fish. Suitable alternatives are a mollusc reproduction test or a fish early life stage test. An example of a suitable test battery is shown in **Table 4**.

Table 4. Example of a suitable "test battery" for compounds expected to be introduced to the marine environment.

Test organism	Group	Category	Endpoint(s)	Reference (Appendix I)
Skeletonema costatum	alga	chronic	growth	60 (ISO 10253)
Acartia tonsa	crustacea	acute	survival	35 (ISO 14669)
Crassostrea sp. a.o.**	mollusc	chronic	reproduction	25 (ASTM)
Menidia peninsulae a.o.**	fish	sub-chronic	ELS, survival, growth, hatchability	47 (OECD draft)

In case the evaluation will be based on existing data, expert judgement may be used to evaluate whether the available data may be considered equivalent to the information preferred in the scheme for assesment of hazard and risk.

4.3 Proposed evaluation system for approval of anti-fouling agents

The evaluation of new systems/agents for anti-fouling should include an initial screening based on the generic properties of the chemicals with cut-off values for combinations of toxicity and biodegradation. Evaluation of systems/agents that have been in use for some time must also be based on possible field evidence of harmful effects (chapter 5 and 6).

Other inherent characteristics/properties of compound that from an environmental point of view suggest its exclusion as part of an antifouling system for ships are:

- Systems that include antibiotics[1]
- Substrances with predominantly genotoxic, mutagenic or carcinogenic properties
- Potent endocrine disrupters

[1]Substance that mainly has an anti bacterial effect (human and veterinary medicine) that might result in development of resistant bacteria in the field.

Initial screening
Based on the toxicity data and information on degradability, an initial screening should be performed to identify chemicals with non-acceptable properties.

Risk assessment
For chemicals that are not disapproved because of inherent properties, a generalised risk assessment should be performed. For this purpose a standardised exposure scenario has to be defined for calculation of environmental concentration (PEC) (see chapter 3).

The PNEC will be estimated from the toxicity data using assessment factors as proposed below. Note: Data from three test categories must be available, and the data giving the lowest PNEC used for risk assessment.

Table 5. Suggested assessment factor.

Species	**End point**	**Assessment factor**
Alga *(Skeletonema costatum)* growth inhibition test	EC_{50}	500
Crustacea *(Acartia tonsa)*	LC_{50}	500
Mollusc reproduction test or Fish early life stage test	NOEC*	50

5. Mesocosm experiments for evaluating effects of antifouling substances

5.1 Introduction

Natural marine systems are generally too large and unpredictable for conducting finely tuned experiments on the possible effects of biocids. The majority of biological and chemical/physical conditions are in continuous change, because of natural cycles or the impacts from human beings. Experimental ecosystems or mesocosms (in-door tanks/aquarium to large outdoor enclosures or enclosed bays), on the other hand, can be more easily controlled, thereby serving as simulation model ecosystems.

Mesocosms are regarded as an essential tool in studying marine pollution to bridge the gap between single-species toxicity tests (chapter 4) and the natural environment. Mesocosms can be dosed with known realistic concentrations of the chemical compound in question and a range of effects both at the individual and the community level can be measured. Also mesocosms may be naturally variable and have a complicated structure, but the possibility of repeated and replicated experimentation of both manipulated and control-units means that cause and effect are more readily established. There are few standards folowed when performing mesocosm tests. Tests are usually designed according to the specific scientific questions being addressed. Endpoints in such test may be on all levels of organisation (cell to community).

Regulatory limits on biocids have initially not been based on sophisticated understanding of how ecosystems are affected. Limits/regulations are generally based on how a species or a selected limited number of species reacts to a toxic compound in a small, controlled space (see chapter 4). Such tests do not take into account the more complex reactions a contaminant undergoes in the field and gives no account of secondary effects due to species interactions. Consequently, it is difficult to extrapolate with predictive confidence from lab findings to field conditions. And as a result, environmental regulations must often rely on research that is relevant for a limited number of species.

It is because of such experimental limitations that mesocosms have been used to simulate conditions in aquatic systems and in solving problems in pollution (Pilson, 1990). The long-term goal for using mesocosms is to develop models and acquire results that can be used to account for the complex interaction and feedback mechanisms of aquatic systems.

In order to investigate long-term effects, experiments can be performed, for instance to study the effects of biocids on benthic communities. Because of the time involved, these experiments are usually not applied in the process of authorising permits/regulations on a short time basis. Mesocosm experiments are useful in cases where the extrapolation of laboratory testing to field condition needs validation or when effects not covered by standard tests are suspected.

Mesocosms may simulate pelagic and benthic (hard bottom and soft bottom) systems in shallow and deep waters. The results from several kinds of mesocosm experiments are relevant for excluding the use of a potential unacceptable biocide. It should however be kept in mind that experience from field investigations generally is more environmentally relevant than similar mesocosm experiments. Field investigations should therefore in general be replaced with mesocosm experiments only for compounds not yet in use or if the cause and effect is not known.

Antifouling agents are generally deliberately designed to be toxic in order to prevent benthic organisms to settle on the hull. High toxicity is alone not sufficient to exclude the use of an antifouling agent. The crucial question is if the agent cause unacceptable effects in organisms in non-target habitats. The major challenge in designing a mesocosm experiment relevant for evaluating a biocide and possibly excluding an unacceptable antifouling agent is related to:

- Finding a relevant mesocosm system
- Selection of realistic concentrations for the test
- Selection of endpoints
- Criteria for what is regarded an unacceptable effect level (at realistic concentrations) in the selected endpoint

The most important consideration when selecting the mesocosm system to be used is which compartment the biocide is most likely to end in after it has been released from the hull. The physical and chemical properties of the agent and its mechanism for antifouling will give some guidance on what kind of mesocosm experiments are relevant. The concentrations to be tested should be based on calculations of exposure in non-target areas.

Selection of representative endpoint is difficult. In general endpoint should be chosen in order to

- address possible effects at the community level at environmentally realistic concentrations.
- address possible effects related directly to the functional mechanism of the agent.

It is generally accepted that the biocid has the designed effects in the environment in the immediate surroundings of the ship. It is however more questionable to accept the use of a biocide that may results in harmfull effects (individual level or general reduction in diversity and/or production) at concentrations that are realistic on a long-term basis outside harbour areas. Whether a antifouling agent is acceptable or not is related to

- the severity of the effect observed
- size of the area (distance from ship water volume) effected

5.2 Pelagic communities

Obviously the first compartment the biocide will enter, is the water surrounding the ship. Several pelagic mesocosm systems have been constructed. The most commonly used approach is the use of floating plastic bags (Grice, 1984) or enclosing a marine water column on land like in the MERL (Pilson et al. 1977) facility. In both cases the enclosures contain a planktonic community which can be manipulated and the response followed through time. Pelagic plankton ecosystems can be maintained in such systems under near-natural conditions for a few days to a few weeks (Takahashi, 1990). Pelagic communities have a natural high variability. Planktonic mesocosms can also be used in combination with sediment systems (MERL) for studies on degradation of antifouling agents and transport to the sediment (Adelman et al. 1990).

Whether an antifouling agent is acceptable or not is related to:

- the effect observed
- area (distance from ship/water volume) effected

The maximum acceptable effect/distance (or volume of water) is a matter of environmental politics more than science.

Clear effects from an antifouling agent in planktonic mesocosm experiments simulating the concentration in the immediate vicinity of a ship are expected (at least for settling organisms) and not sufficient for prohibiting or restricting the use of the agent. Significant effects on diversity (distribution of number of individuals on species) or primary production at concentrations likely to occur on a long-term basis outside harbour areas and along shipping routes, are generally not regarded as acceptable.

Diversity effects may be mediated through a combination of direct toxic effects and through ecological interactions like changes in competition and predation. There exists no generally accepted methods at present to assess changes in the pelagic system.

5.3 Soft bottom communities

A depositional sedimentary softbottom environment is a geomorphic unit in which deposition takes place. In such areas marine sediments may be sinks for harmful substances. Transport to the sediment from the overlying water takes place through adsorption onto solid particles, precipitation and coprecipitation and through the sedimentation of biological detritus.

Results from soft bottom mesocosm experiments are especially relevant for antifouling agents that may end up in such sediments. Hydrofobic antifouling compounds may adsorbe to particles and through deposition end up in the sediments in the intertidal or the subtidal.

Effects of antifouling agents on natural benthic communities can be studies in mesocosms by manipulating the content of the biocide in the inflowing sea water or in the sediment in the mesocosm. Sediments for setting up such mesocosm experiments can be sampled with boxcorer or a grab and transported to the mesocosm facility were the experiments on more or less intact sections of the sedimentary environment can be performed.

Such experiments will allow an evaluation of effects of different concentrations of a compound on benthic communities and can also address questions related to the sediment chemistry. A comprehensive system for performing such experiments on natural sediments have been developed (Berge et al. 1986) and have since been refined and used for testing of chemicals used during drilling operations offshore which will end up on the sea floor (Schaanning et al. 1997).

Soft bottom mesocosms may also be of an intertidal type (Farke et al. 1984) and may also include experimental units placed directelly both in the intertidal (Christie and Berge, 1995) and subtidal (Berge 1990, Widdicombe and Austen, 1998, Matthiessen and Thain, 1989).

Arange of endpoints can be studied. Some of the most commonly used are related to:

- diversity (distribution of individuals among species)
- production (growth)
- bioaccumulation
- reproduction
- recolonization- and extinction rates
- behaviour (bioturbation)

Only a few endpoints are usually studied, and the possibility of missing an effect is therefore usually large. On the other hand if significant (in a statistical sense) effects at environmental realistic concentrations of the biocide, are documented this calls for concern.

The structuring forces in sediment communities are complicated and may interact with possible effects of the antifouling agents. If key species are effected, disproportionate effects on community structure/diversity may be seen.

5.4 Rocky shores

5.4.1 Littoral Mesocosms -Rocky Shore Ecosystems

The literature reveals few experimental ecosystems dealing with hard-bottom benthos probably because hard bottom in situ studies are relatively easy to perform (Bakke, 1990) at least as long as controlled dosage of chemicals are not involved.

Littoral mesocosms have however been used in investigations on effects of chemicals like oil (Bokn et al. 1993), nutrients (Bokn et al. in press) and chlorate (Rosmarin et al. 1994) on rocky shore ecosystems in boreal areas. In tropical and semitropical seas coral reefs may be important in shallow waters. Effects of UV radiation have been studied in coral reef mesocosm experiments (Santas et al. 1998).

Communities can bee established in rocky shore ecosystems by transplantation of rocks covered by algae and animals from the littoral zone. Rocks can be positioned on steps covering the different levels of the littoral and upper part of the sublittoral zones. Establishment of an intertidal community can continue by self-propagation and from larvae, zygotes and spores entering the mesocosm with supplied seawater.

Such systems allows controlled exposure during sensitive stages of the organisms lifespan.
Long term effects of antifouling agents can be tested in intertidal mesocosms.

The most commonly used endpoints are related to

- diversity (distribution of individuals among species)
- primary production
- secondary production
- reproduction
- recolonization- and extinction rates

Benthic algae are important primary producers in many rocky shore communities. Many shallow water invertebrate species rely directly or indirectly on the supply of organic carbon from the benthic algae. Bentic algae also increase the structural heterogeneity of rocky shores and shallow water environment and thus contribute further to the high diversity that can be found in such areas. If experiments indicate harmful effects on benthic algae at environmental realistic concentrations, their key position gives cause for special concern.

Table 6. Important endpoints in mesocosem experiments for evaluating harmful effects of antifouling substances.

Mesocosm community	**Endpoint**	**Criterion for unacceptable effect**
Pelagic	Primary production Diversity Sublethal responses	Reduced primary production and/or diversity at concentrations realistic to be found outside harbours and major shipping routes.
Soft bottom	Density of key species Diversity Sublethal responses	Reduced diversity at concentrations realistic to be found outside harbours and major shipping routes.
Rocky shore	Primary production Density of key species Diversity Sublethal responses Individual performance	Reduced primary production and/or diversity at concentrations realistic to be found in rocky shores outside harbours and major shipping routes.

6. Effects in the field

The use of an antifouling system/agent may result in observations of harmful effects in the field. Field observations of effects can only be made after the compound has reached the threshold concentration for eliciting the response. The biological entity studied must also have the time to develop the effect(s) in question. This means that the compounds/systems in question has been in use for some time (probably years) before investigators have any chance of observing changes in the field.

A cause/effect relationship is difficult to establish from field observations alone and generally requires some sort of experimentation (field experiments, mesocosm studies) in order to tie the observed effect to the antifouling system/compound. Significant (in the statistical sense) effects observed outside harbour areas that can be tied to the use of the antifouling system/agent are cause for particular environmental concern.

Before being approved nationally, the antifouling compound has to meet specified national requirements and regulations. This process implies that a certain amount of compound related information is available.

The main object of acquiring toxicity data is to predict and avoid possible harmful consequences in the field. If, after some time, such effects are observed, a new situation has occurred which call for a new evaluation. In such a situation the observed field effects must be evaluated against possible negative consequences of prohibiting/limiting the use of the agent.

Field observations of a cause and effect relationship for a compound should always be used in preference to other data.

Harmful effects of an antifouling system/agent are most likely to be observed near harbours and sheltered areas and along major shipping-routes. The species that potentially could be effected in a specific area is dependent on local occurrence and the distribution of the compound in the field.

It is impossible to give any general recommendations on what might be sensitive species or species groups. Field effects/observations related to the following should be considered particularly serious:

- diversity (distribution of individuals among species)
- primary production
- secondary production
- reproduction/endocrine disruption
- recolonization- and extinction-rates
- residues in seafood and marine mammals and birds

The community/population effects related to the above list are generally more radical than effects at the individual or sub-individual level. It is however important to bear in mind that responses at the community level may be triggered by unidentified effects at a lower level of organisation. Effects at the sub-individual level are less important if they do not result in effects at the community or population level.

Tributyltin has been used as an effective biocide in antifouling paints since the seventies and is for the time being a commonly used antifouling agent in paint for the underwater hull of large ships. High environmental concentrations of TBT and field effects have been reported for a variety of marine species. The nature of the majority of these effects was such that they were not detected by traditional toxicity testing.

The concern about high levels of residues in seafood is mainly related to possible human health problems but may also indicate harmful effects in marine organisms. This is examplified by the prominent levels of butyltin residues found in marine mammals in coastal waters of developed nations that may pose a considerable toxic threat to some coastal species of cetaceans (Tanabe, 1999).

The field evidence of harmful effects has enforced restrictions on the use of TBT as an antifouling agent on small boats. It is mainly the observations of harmful field effects (especially effects on reproduction in gastopods) and accumulation in marine organisms outside target areas that has triggered the process that probably will end up with a ban on the use of TBT on large ships by 2003/2008.

As long as biocides are used for antifouling some harmful effects are likely to occur outside the volume of water in the immediate vicinity of the ship. As mentioned previously, the maximum acceptable effect/distance (or volume of water) is a matter of environmental politics more than science. The ideal is that antifouling systems/agents only should effects organisms that approach or try to settle on the hull as would be the case for ships that can apply non-stick systems. For antifouling systems that rely on biocid the maximum acceptable effect-distance (or volume of water) in practice have to be longer/larger.

Some biological effects are probably acceptable in sheltered harbour areas with little water renewal but not in the intertidal or shallow subtidal along major shipping routes. It will be an important part of the expert panel work to define the influence areas where effects are acceptable.

7. Assessment procedures for antifouling compounds

The main objection for knowing the physical/chemical characteristics and acquiring toxicity data on compounds introduced to the environment is to address, predict and avoid possible harmful consequences in marine ecosystems.

The expert group may find that important data for the evaluation are not available. It is therefore important that the assessment procedure chosen allows a conclusion even in those cases were some information may be lacking. It is also important that lack of data does not promote a continued use of a harmful compound.

Below, 3 different assessment methods are suggested. In this way a flexible system of methods is introduced that allows the assessment of antifouling substances in the whole range from new substances not yet in use to compounds with very long track records. There are three different assessment methods that may lead to the inclusion of an antifouling agent/system on a list of restricted substances. The methods are arranged in order with respect to increasing access to detailed information, however each may be used independently of the other.

Each method involves different types of data/information. The three methods are:

- Screening assessment based on inherent properties (including mode of action) of the antifouling compound
- Risk assessment based on theoretical predictions of field contentrations and effects
- Field evidence of effects from antifouling compounds in use

In addition possible control measures have to be considered in order to evaluate if they are sufficient to remove/reduce harmful effects to an environmentally acceptable level.

Although it is stressed that the assessment methods are independent of each other they do to some degree utilise the same information as graphically depicted in **Figure 1**. The scheme outlined in **Figure 1** is not a genuine hierarchic system as for instance proven harmful effects in the field (block 5 in **Figure 1**) alone is sufficient to include the compound in the restricted list.

7.1 Screening assessment based on inherent properties of antifouling compound

This assessment method may primarily be used for screening of compounds under development for possible uses as antifouling compounds. It is based on the principle that there exist properties of compounds that are incompatible with release to marine environment. In this context it must be remembered that the sea is the final compartment of many chemicals and that it is a common resource for all the people on earth, which in principle means that all activities should ensure sustainable development in the sea. The assessment data needed are described in detail in chapter 2 and 4.

1. Inherent properties, physical/chemical characteristics and degradation rate.
2. Toxicity tests including properties related to the assumed predominant mode of action (endocrine disruption, genotoxicity, mutagenicity) of the biocide.

Block 1 in **Figure 1** summarises threshold limits for inherent properties that independent of other data, may be perceived as so undesirable that the system/compound is recommended to be included in the list of restricted antifouling systems.
Inherent properties of compound that from an environmental point of view suggest its exclusion as part of an antifouling system for ships may be:

- Systems that include antibiotics
- Compounds with a mainly genotoxic or mutagenic effect
- Potent endocrine disrupters
- Substances with a log Pow>4-5 and molecular weight <700
- <10 % degradation in 28 days in a standardised test
- Leaching rate for active substance >0.1%/day

Low degradation rate for a biocide should automatically exclud it as part of an antifouling system for ships, as build up in the environment will eventually lead to concentrations that can couse harmful effects in non-target areas. Another argument for excluding systems using chemicals with a low degradation rate is that restitution time of the environment will be long after the use of the system for some reason have ceased.

Metals do not biodegrade and will of course have to be exempted from this threshold limit. Most metals are also found naturally in seawater. In general on may conclude already at this point that method 1 is not a suitable assessment method for metals and that these should be evaluated on a PEC/PNEC approach.

Biocides are by intention toxic as their purpose is to avoid growth of any kind of organism on structures submerged in the sea. Setting absolute threshold limits without considering other properties is therefore difficult. However one may also argue that using extremely toxic compounds increases the risk of massive effects locally in case of accidental spills or danger of misuse for other purposes. In **Figure 1**) threshold values have been suggested for some standardised tests relevant for the marine environment although they here are dependent on information regarding biodegradation. Although standardised test are indicated any toxicity test results of good quality should in principle be valid in this context. **Table 7** indicates test and species that may replace the most frequently used test organisms.

Again lack of data should not be an advantage for accepting an antifouling product. It is therefore important that lack of crucial data should result in its inclusion in the list of restricted substances. This will provide an incitement for performing tests for acquiring the relevant data.

7.2 Risk assessment based on theoretical predictions of field contentrations and effects

This method is particulary suited for compounds that are well documented with respect to chemical, physical properties and that are well tested with respect to effects as indicated in **Figure 1**. In the risk assessment results from toxicity tests are combined with the theoretical estimation of concentrations likely to be found in the field (se chapter 3.1).

For the purpose of the risk assessment a standardised realistic exposure scenario has to be defined for calculation of environmental concentration (PEC) (see chapter 3). Estimation of PEC should primarily simulate conditions outside accepted influence zones.

A PEC/PNEC>1 indicates that effects are probable. The validity of the estimated risk is of course dependent on the accuracy of both estimates of PEC and PNEC. PEC estimates are based on an idealised scenario with concern to the behaviour of the compound. It is also assumed that the exposure scenario incorporates realistic worst case parameters with respect to leaching, water exchange and boat frequency. In spite of such a conservative approach the derived PEC value may underestimate actual concentrations in the field. Another uncertainty is the derivation of PNEC, which only consider a limited number of robust species. There is therefore a considerable probability that species not included in the tests are more sensitive. Assessment factors are noted to compensate for this uncertainty.
Suggested assessment factors are proposed below in **Table 8**. It is important to stress that data from the three test categories must be available, and the data giving the lowest PNEC is used for risk assessment.

Table 8. Suggested assessment f actors.

Species	**End point**	**Assessment factor**
Alga *(Skeletonema costatum)* growth inhibition test	EC_{50}	500
Crustacea *(Acartia tonsa)*	LC_{50}	500
Mollusc reproduction test or Fish early life stage test	NOEC*	50

If a PEC/PNEC>1 is achieved for a compound one may perform the evaluation again including various control measures. Examples of control measures are: reduced leaching by changing the properties of the paint matrix, reducing the number of vessels that are allowed to use the antifouling system and reducing the content of the compound in the paint. If such control measures are shown to reduce the PEC/PNEC to below 1, control measures must be invoked in order that the compound is not entered in a restriction list.

7.3 Field evidence

Possible evidence (direct or indirect) of field effects is the third approach and is most relevant for compounds that have been in use for some time after which "unexpected" harmful field effects have had the time to develop. Field effects or other alarming supplementary environmental information (block 5 in **Figure 1**) on an antifouling system may release a call for a re-evaluation of the use of the biocide.

The expert group to perform such an evaluation may in addition to the claimed field effects be faced with updated information on physical/chemical characteristics and toxicity tests (other than the information available when the agent was approved at the national level).
It is however the field observations (possibly also supplementary mesocosm results) that has the most ecological realism and therefore should be given the heaviest weight in such a second evaluation.

Priority based on environmental realism of tests/effects has therefore changed from inherent properites and standardised toxicity tests to:

1. Field evidence of effects
2. Mesocosm evidence of effects at environmental realistic concentrations

It is difficult to establish a cause/effect relationship from field observations and tie the observed effect to the antifouling system/agent. It is generally more easy to restrict the use of a biocid based on its inherent properties, general toxicity or functional mechanism of the biocidal effect. The expert group should therefore, perform a new evaluation (screening and risk assessment) based on the updated information.

The expert group should secondly scrutinise the field and mesocosm observations of claimed effects in order to evaluate if the evidence is sufficient to tie the observations to the antifouling system. If such a cause/effect relationship is established the expert group must evaluate if the observed effects are sufficiently serious to recommend that the agent should be included in the list of restricted antifouling systems.

7.4 Control measures

If harmful field effects are probable and countermeasures are not sufficient to avoid such effects the substance should from an environmental point of view be included in the list of restricted substances.

Suggestions of controlmeasures aiming at a reduction of environmental concentrations are outside the scope of this report and are only briefly mentioned.

With the exception of leaching rate, it is difficult to anticipate countermeasures directly related to block 1 - 3 in **Figure 1** as the criteria for including the substance in the list of restricted substances are mainly related to inherent physical, chemical or toxic properties of the active substance.

The most obvious counter measures are efforts related to reducing the environmental concentration of the active compound and thereby reducing apparent harmful effects. Relevant countermeasures are largely dependent on how the antifouling system is meant to function, how the ships using the system are operated and the harmful effects observed. Suggestions for control measures aiming at reducing environmental concentrations are therefore highly technical and tightly tied to the antifouling system in question.

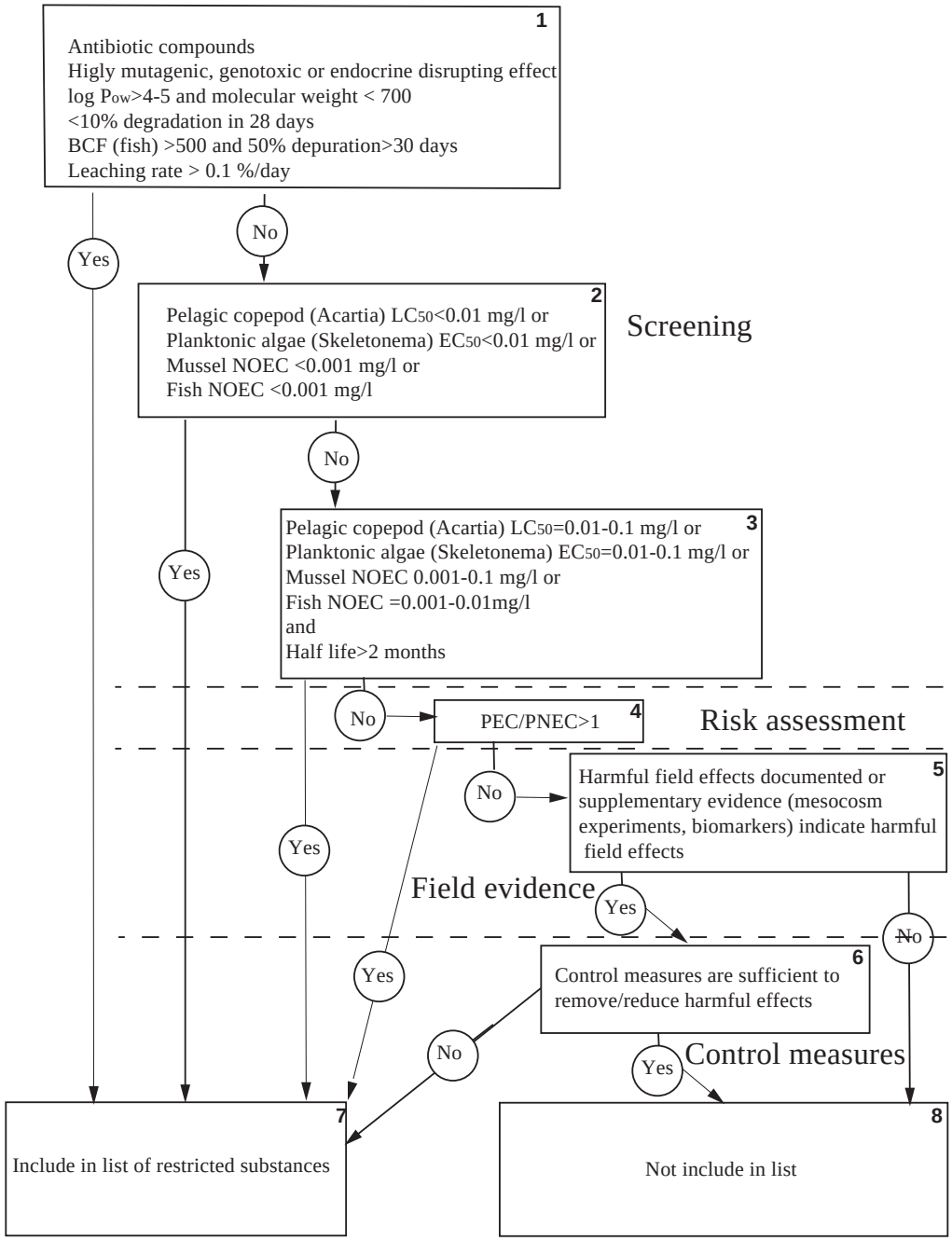

Figure 1. Suggested procedures for evaluating if an antifouling compound should be included in list of restricted substances.

7.5 Concluding remark

Understanding the full environmental relevance of different types of test results is difficult, even for experts. This report is meant to be a guide to how different types of test results and observations can be evaluated but is not meant to be a ready to use manual on how to perform a stringent evaluation.

The suggested methods for evaluating if an antifouling agent system should be included in a list of restricted substances (**Figure 1**) is designed purely from an environmental viewpoint related to the marine environment. It is appreciated that the expert group also has to consider other aspects like possible effects on shipyard workers, costs to international shipping and the availability of suitable alternatives. The challenge for the expert group is to reveal the inherent relevance of the different types of test results and data presented, and formulate a balanced united evaluation based also on non-environmental considerations and give a final recommendation.

In order to secure a comprehensive national discussion and feedback on the content of this report it should be distributed to relevant parties in Norway. Their comments should be considered before the document planned for presentation to the IMO-MEPC meeting in October 2000 is finalised.

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Appendix A.

APPENDIX I.

Evaluation of toxicity test methods. Extract from OECD Detailed Review Paper on Aquatic Testing Methods for Pesticides and Industrial Chemicals (OECD 1998)

Criteria for evaluation of toxicity testing methods

ITEM	RATING	
PRACTICAL FEASIBILITY OF THE TEST METHOD		
Technical performance	A	The performance of the method is comparable to internationally adopted routine tests with algae, crustaceans and fish (acute, subchronic and chronic, respectively)
	B	More difficult to perform than the existing routine tests, but within the ability of routine test laboratories
	CC	Extremely difficult to perform, requires special training of staff and/or equipment not expected to be available in laboratories performing routine testing.
Duration of long term tests	A	8-28 days
	B	29-60 days
	C	> 60 days
Availability and maintainence of test organisms	A	Sufficient documentation for relatively easy maintainance in the laboratory for several generations
	B	Cannot be held in culture under laboratory conditions, but can easily be maintained for at least twice the test duration (acclimatisation period and test duration) and can easily be purchased from supplier or sampled during most seasons (<6 months) in sufficient quantities
	CC	Insufficient documentation for minimum maintenance in the laboratory, cannot be easily purchased from supplier, or can be sampled only during a limited period of the year (<6 months)
Exposure system	A	Static, semi-static and flow-through systems are all described and sufficiently documented fro the method
	B	Only static and semi-static procedures have been described and documented
	C	Only static procedures have been described and documented
Costs, equipment	A	Normal laboratory equipment for routine testing (e.g. OECD Test Guidelines) is sufficient
	B	Low levell of investments may be needed (less than 10 000 EU/US$)

	C	High level of investments may be needed (more than 10 000 EU/US$)
Cost, labour	A	Corresponding to short-term routine tests (e.g. OECD TEst Guidelines)
	B	Corresponding to long-term routine tests (e.g. OECD Test Guidelines)
VALIDITY OF METHOD		
	C	More laborious than long-term routine tests
Reproducibility	A	The LC/EC50 values for reference chemicals tested at different laboratories lie within a factor of 5
	B	The LC/EC50 values for reference chemicals tested at different laboratories lie within a factor of 10
	C	The LC/EC50 values for reference chemicals tested at different laboratories lie above a factor of 10
Sources of potential error	A	Potential critical phases are few, suffficiently documented, and should not be critical for routine laboratories
	B	Potential critical phases are few, but not all of them are suffficiently documented. The critical steps may be of significance for the performance of the test
	CC	A relatively large number of critical steps are involved, which are not sufficiently documented
Range of tolerance to environmental conditions	A	The test organism can tolerate the test conditions used in terms of temperature, oxygen, pH, light regime, feeding, salinity a.o., as well as the range and variations of these during the test and maintenance
	B	Range of tolerance is documented, but tolerance to some of the environmental parameters may cause problems for routine laboratories
	CC	Some of the environmental conditions are likely to give problems for routine testing and maintenance
USEFULNESS IN PROGNOSES		
Geographical representativeness	A	Test organisms is represented in many geographical areas (cosmpoplite)
	B	Test organism is represented in one geographical area only
	CC	Endemic species, relicts and other organisms with a very narrow geographic distribution
Ecological representativeness	A	The test organism in its tested life stage is a representative of a typical life form of the taxonomic group and may be a dominating or ecologically important species and thus be of importance for the structure of the ecosystem
	B	The test organism in its tested life stage is a representative of a typical life form of the taxonomic group but does not dominate its natural environment
	CC	The organism represents a specialised type of life form within the taxonomic group and does not dominate its natural environment
Extrapolation of endpoints: usefulness and significance	A	Ecologically highly relevant endpoints: at community or population level, e.g. population growth, age

in risk assessments		structure, fecundity. For microorganisms, functional endpoints are used
	B	Ecologically relevant endpoints: survival/growth of individual or groups of organisms, behavioural responses etc.
	CC	Less ecologically relevant endpoints related exclusively to specific toxic mechanisms, physiological or biochemical endpoints at the organism level
General sensitivity	A	The species/system is documented to be highly sensitive to a range of chemicals
	B	In general, as sensitive as organisms presently applied (in OECD Test Guidelines)
	C	In general, less sensitive than the organisms presently applied (in OECD Test Guidelines)
Relevance of exposure route and test conditions	A	The abiotic and biotic conditions in the test and route of exposure during the test simulate well conditions in the natural habitat of the species. The organism is tested in a water-only system
	B	The route of exposure or the abiotic/biotic conditions in the test differs significantly from the natural habitat of the organism
	CC	Both the route of exposure and the abiotic/biotic conditions in the test differs significantly from the natural habitat of the organism
LEVEL OF STANDARDISATION		
	AA	International standard/guideline
	A	National standard/guideline or the method has been subject to national (or international) ring-testing (at least five laboratories), or internastional draft standard is in progress
	B	National standard method/guideline, but not yet ring-tested or national draft guideline in progress
	C	Method published in an international peer-reviewed journal or protocol with sufficient documentation for publication

Phytoplankton

	Skeletonema costatum	*Phaeodactylum tricornutum*	*Skeletonema costatum*	*Thallasiosira pseudonana*	*Isochrysis galbana*	*Skeletonema costatum*	*Tetraselmis suecica*	*Prorocentrum lima*	*Skeletonama costatum*	Natural phytoplankton	Various sp.
Reference no.	60	60	19	27	27	30	30	30	4	4	75
Trophic level	P	P	P	P	P	P	P	P	P	P	P
Duration	C/ST	C/ST	C/ST	C/ST	C/ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	C/ST
Technical performance	A	A	A	A	A	A	A	A	A	A	A
Duration of long term test											
Availability of test organism	A	A	A	A	A	A	A	A	A	A	A
Exposure system	C	C	C	C	C	C	C	C	C	C	C
Cost, equipment	A	A	A	A	A	B	B	B	C	C	A
Cost, labour	A	A	A	A	A	A	A	A	A	A	A
Reproducibility	A	A	-	-	-	-	-	-	-	-	-
Souces of potential error	A	A	A	A	A	B	B	B	B	B	A
Range of tolerance to environmental conditions	A	A	A	A	A	A	A	A	A	A	A
Geographical distribution	-	-	-	-	-	-	-	-	-	A	-
Representativeness of the test organism	A	B	A	-	-	-	-	-	-	A	A
Extrapolation of endpoints	A	A	A	A	A	CC	CC	CC	A	A	A
General sensitivity	B	B	B	B	B	B	B	B	B	B	B
Relevance of exposure route and test conditions	A	A	A	A	A	A	A	A	A	A	A
Standardization	AA	AA	A	A	A	C	C	C	C	C	B
Relative evaluation	**A**	**A**	**A**	**A**	**A**	**C**	**C**	**C**	**B**	**B**	**A**

Macro algae

	Gracilaria tenuistipitata	*Champia parvula*	*Ceramium strictum*	*Ascophyllum nodusum*	*Porphyra yezoensis*
Reference no.	8	16	1	7	5
Trophic level	P	P	P	P	P
Duration	SC/TC	SC/TC	SC/TC	SC/TC	SC/LT
Technical performance	A	B	A	B	A
Duration of long term test					A
Availability of test organism	A	B	A	A	A
Exposure system	B	C	C	C	B
Cost, equipment	A	A	A	C	A
Cost, labour	A	A	A	A	A
Reproducibility	-	-	-	-	-
Sources of potential error	B	B	B	B	B (-)
Range of tolerance to environmental conditions	-	-	-	-	-
Geographical distribution	B	-		B	A
Representativeness of the test organism	-(B)	-	A	A	B/A
Extrapolation of endpoints	B	B	B	B	B
General sensitivity	B	-	-	-	-
Relevance of exposure route and test conditions	A	B	B	B	B
Standardization	C	A	C	C	C
Relative evaluation	**B**	**A**	**B**	**C**	**B**

Crustacea warm water

	Mysidopsis bahia	*Mysidopsis bahia*	*Mysidopsis bahia*	*Mysidopsis bahia*	*Mysidopsis bahia*	*Mysidopsis bahia*	*Mysidopsis bigelowi*	*Mysidopsis almyra*	*Artemia salina*	*Artemia salina*	*Penaeus aztecus*	*Penaus duorarum*	*Penaeus setiferus*	*Holmesimysis costata*	*Holmesimysis costata*	*Acartia liljeborgi*	*Temora stylifera*
Reference no.	17	14	21	22	72	24	62	62	2	70	23	57	57	72	26	72	72
Trophic level	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	H	H
Duration	AC/ST	C/LT	AC/ST	C/LT	AC/ST	SC/LT	SC/LT	SC/LT	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST
Technical performance	A	B	A	B	A	B	B	B	A	A	A	A	A	A	A	A	A
Duration of long term test	-	A	-	A	-	A	A	A	-	-	-	-	-	-	-	-	-
Availability of test organism	A	A	A	A	-	A	A	-	A	A	B	B	B	-	-	-	-
Exposure system	C	B	A	A	C	A	A	A	C	C	A	A	A	A	A	C	C
Cost, equipment	A	A	A	A	B	A	A	A	B	B	A	A	A	A	A	B	B
Cost, labour	A	B	A	B	A	B	B	B	A	A	A	A	A	A	A	A	A
Reproducibility	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-
Sources of potential error	A	B	A	B	A	B	B	B	B	A	B	B	B	B	B	B	B
Range of tolerance to environmental conditions	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Geographical distribution	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B
Representativeness of the test organism	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Extrapolation of endpoints	B	A	B	A	B	A	A	A	CC	CC	B	B	B	B	B	B	B
General sensitivity	A	A	A	A	-	A	-	-	B	B	B	B	B	-	-	-	-
Relevance of exposure route and test conditions	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A
Standardization	A	A	A	A	C	A	A	A	C	C	A	A	A	C	C	C	C
Relative evaluation	**A**	**A**	**A**	**A**	**B**	**A**	**A**	**B**	**CC**	**CC**	**A**	**A**	**A**	**B**	**B**	**B**	**B**

Crustacea, cold water

	Nitocra spinipes	*Nitocra spinipes*	*Nitocra spinipes*	*Acartia tonsa*	*Tisbe battagliai*	*Nitocra spinipes*	*Acartia tonsa*	*Acartia hudsonica*	*Acartia tonsa*	*Acartia tonsa*	*Acartia tonsa*	*Crangon crangon*	*Balanus improvisus*	*Centropages hamatus*	*Centropages hamatus*	*Eurytemora affinis*	*Gammarus tigrinus*
Reference no.	41	?	36	35	35	35	33	34	40	65	66	10	68	32	37	39	38
Trophic level	O	O	O	H	O	O	H	H	H	H	H	O	O	H	H	H	D
Duration	AC/ST	SC/LT	AC/ ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	AC/ST	C/LT	SC/ST	AC/ST	AC/ST	SC/ST	AC/ST	SC/ST	AC/ST
Technical performance	A	B	A	A	A	A	A	A	A	B	B	AC/ST	B	B	B	B	A
Duration of long term test	-	A	-	-	-	-	-	-	A	A	-	-	-	-	-	-	-
Availability of test organism	A	A	A	A	A	A	A	A	A	A	A	B	CC	B	B	B	-
Exposure system	C	A	C	C	C	C	C	C	A	B	B	B	C	A	A	B	B
Cost, equipment	A	B	A	A	A	A	B	B	B	A	A	A	A	B	B	B	A
Cost, labour	A	B	A	A	A	A	B	B	B	B	B	A	A	B	B	B	A
Reproducibility	A	-	A	A	A	A	-	-	-	-	-	-	-	-	-	-	-
Sources of potential error	A	CC	A	A	A	A	CC	CC	CC	B	B	B	B	B	B	B	B
Range of tolerance to environmental conditions	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	B
Geographical distribution	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Representativeness of the test organism	B	B	B	A	B	B	A	A	A	A	A	B	B	B	B	B	B
Extrapolation of endpoints	B	A	B	B	B	B	B	B	B	A	A	B	B	A	A	A	B
General sensitivity	B	B	B	A	B	B	A	A	A	A	A	C	B	B	B	B	-
Relevance of exposure route and test conditions	A	A	A	A	A	A	A	A	A	A	A	B	B	A	A	A	A
Standardization	A	C	A	AA	AA	AA	C	C	CC	C/LT	C	C	C	C	C	C	C
Relative evaluation	**A**	**C**	**A**	**A**	**A**	**A**	**C**	**C**	**CC**	**B**	**B**	**C**	**C**	**B**	**B**	**B**	**B**

	Bacteria		**Protozoa**		**Rotatoria**		**Cinidaria**					
	Photobacterium sp		*Uronema marinum*		*Brachionus plicatilis*		*Diploria stringosa*	*Aurelia sp.*	*Cordylophra caspia*	*Eirene viridula*	*Aurelia aurita*	
Reference no.	29		28		61		59	31	76	76	78	
Trophic level	D		O		H		C	C	C	C	C	
Duration	AC/ST		C/ST		AC/ST		AC/LT	SC/LT	SC/LT	SC/LT	SC/LT	
Technical performance	A		A		A		CC	B	B	B	B	
Duration of long term test					-		C	A	A	A	B	
Availability of test organism	A		A		A		B	B	A	A	A	
Exposure system	C		C		C		C	C	B	B	B	
Cost, equipment	C		A		A		A	A	A	A	A	
Cost, labour	A		A		A		C	B	B	B	B	
Reproducibility	A		-		A		-	-	-	-	-	
Sources of potential error	A		A		A		CC	B	B	B	B	
Range of tolerance to environmental conditions	A		-		-		-	-	B	B	B	
Geographical distribution	A		-		A		A	A	B	B	A	
Representativeness of the test organism	B		-		-		A	A	B	B	B	
Extrapolation of endpoints	CC		A		B		B	B	A	A	A	
General sensitivity	B		-		B		-	-	-	-	-	
Relevance of exposure route and test conditions	CC		A		A		A	A	A	A	A	
Standardization	A		C		A		C	C	B	B	C	
Relative evaluation	**C**		**B**		**A**		**C**	**B**	**B**	**B**	**C**	

	Echinodermata								**Mollusca**								
	Arbacia punctulata	*Lytechinus pictus*	*Lytechinus variegatus*	*Arbacia punctulata*	*Strongylocentrolus droebacii*	*Strongylocentrolus sp.*	*Dendraster exentricus*		*Mytilus californianus*	*Mytilus edulis*	*Mytilus edulis*	*Crassostrea gigas*	*Crassostrea virginica*	*Mercenaria mercenaria*	*Mytilus edulis*	*Crassostrea virginica*	*Crassostrea gigas*
Reference no.	42	42	69	15	42	42	42		73	9	6	25	62	62	62	20	67
Trophic level	O	O	O	O	O	O	O		H	H	H	H	H	H	H	H	H
Duration	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST		SC/ST	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST	SC/ST
Technical performance	A	A	A	A	A	A	A		B	A	A	A	A	A	A	A	A
Duration of long term test	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-
Availability of test organism	B	B	B	B	B	B	B		B	B	B	B	B	B	B	B	B
Exposure system	C	C	C	C	C	C	C		-	C	A	C	C	C	C	A	C
Cost, equipment	A	A	A	A	A	A	A		B	A	B	A	A	A	A	A	A
Cost, labour	A	A	A	A	A	A	A		B	A	B	A	A	A	A	A	A
Reproducibility	-	-	-	-	-	-	-		-	-	-	-	-	-	-	A	A
Sources of potential error	A	A	A	A	A	A	A		B	A	A	A	A	A	A	A	A
Range of tolerance to environmental conditions	A	A	A	A	A	A	A		B	A	A	A	-	-	-	-	A
Geographical distribution	B	B	B	B	B	B	B		B	B	B	B	B	B	B	B	B
Representativeness of the test organism	B	B	B	B	B	B	B		A	A	A	B	B	B	A	B	B
Extrapolation of endpoints	A	A	A	A	A	A	A		B	B	B	B	B	B	B	B	B
General sensitivity	B	B	B	B	B	B	B		-	B	B	B	B	B	B	-	B
Relevance of exposure route and test conditions	A	A	A	A	A	A	A		A	A	A	A	A	A	A	A	A
Standardization	B	B	C	B	B	B	B		C	C	C	A	A	A	A	A	C
Relative evaluation	**A**	**A**	**A**	**A**	**A**	**A**	**A**		**B**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**

Fish warm water

	Atherinops affinis	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Cyprinodon variegatus*	*Fundulus heteroclitus*	*Fundulus heteroclitus*	*Fundulus similis*	*Lagodon rhomboides*	*Leiostomus xanthurus*	*Menidia beryllina*	*Menidia beryllina*	*Menidia beryllina*
Reference no.	46	11	12	51	53	52	43	44	55	53	58	53	53	53	13	18	50
Trophic level	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Duration	AC/ST	SC/ST	SC/LT	SC/LT	AC/ST	SC/LT	AC/ST	SC/ST	SC/LT	AC/ST	SC/-	AC/ST	AC/ST	AC/ST	SC/ST	AC/ST	SC/ST
Technical performance	A	A	A	A	A	A	A	A	A	A	CC	A	A	A	A	A	A
Duration of long term test	-	-	A	A	-	A	-	-	B	-	-	-	-	-	-	-	-
Availability of test organism	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B
Exposure system	C	B	B	A	A	A	A	A	A	A	C	A	A	A	B	A	A
Cost, equipment	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
Cost, labour	A	A	B	B	A	B	A	A	B	A	C	A	A	A	A	A	A
Reproducibility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sources of potential error	B	B	B	B	A	B	A	A	B	A	C	A	A	A	B	A	A
Range of tolerance to environmental conditions	B	B	B	B	A	B	A	A	B	A	B	A	A	A	B	A	A
Geographical distribution	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Representativeness of the test organism	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Extrapolation of endpoints	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
General sensitivity	B	B	B	B	B	B	B	B	B	B	-	B	B	B	B	B	B
Relevance of exposure route and test conditions	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A
Standardization	C	A	A	A	A	A	A	C	A	A	C	A	A	A	A	A	C
Relative evaluation	**B**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**C**	**A**	**A**	**A**	**A**	**A**	**A**

Fish warm water (cont.)

	Menidia beryllina	*Menidia menidia*	*Menidia menidia*	*Menidia menidia*	*Medinia peninsulae*	*Medinia peninsulae*	*Medinia peninsulae*	*Menidia sp.*	*Menidiae sp.*	*Oligocothus maculosus*	*Opsanus bita*	*Cyprinodon variegatus*
Reference no.	46	54	52	55	54	47	55	51	53	53	52	74
Trophic level	C	C	C	C	C	C	C	C	C	C	C	C
Duration	AC/ST	AC/ST	SC/LT	SC/LT	AC/ST	SC/ST	SC/LT	SC/LT	AC/ST	AC/ST	SC/LT	C/LT
Technical performance	A	A	A	A	A	A	A	A	A	A	A	B
Duration of long term test	-	-	A	A	-	-	A	A	-	-	B	C
Availability of test organism	B	B	B	B	B	B	B	B	B	B	B	A
Exposure system	C	A	A	A	A	A	A	A	A	A	A	A
Cost, equipment	A	A	A	A	A	A	A	A	A	A	A	B
Cost, labour	A	A	B	B	A	A	B	B	A	A	B	B
Reproducibility	-	-	-	-	-	-	-	-	-	-	-	-
Sources of potential error	B	A	B	B	A	A	B	B	A	A	B	A
Range of tolerance to environmental conditions	B	A	B	B	A	A	B	B	A	A	B	A
Geographical distribution	B	B	B	B	B	B	B	B	B	B	B	B
Representativeness of the test organism	B	B	B	B	B	B	B	B	B	B	B	B
Extrapolation of endpoints	B	B	B	B	B	B	B	B	B	B	B	A
General sensitivity	B	B	B	B	B	B	B	B	B	B	B	A
Relevance of exposure route and test conditions	A	A	A	A	A	A	A	A	A	A	A	A
Standardization	C	A	A	AA	A	A	AA	A	A	A	A	AA
Relative evaluation	**B**	**A**	**A**	**AA**	**A**	**A**	**AA**	**A**	**A**	**A**	**A**	**AA**

Fish cold water

	Citharichthys stigmaeus	*Clupea harengus*	*Clupea harengus*	*Coregonus albula*	*Cymatogaster sp.*	*Gadus morhua*	*Gadus morhua*	*Gasterosteus aculeatus*	*Gasterosteus aculeatus*	*Gasterosteus aculeatus*	*Morone saxatilis*	*Paralichtys denatus*	*Paralichtys sp.*	*Parophrys vetulus*	*Platichthys flesus*	*Platichthys stellatus*	*Pleuronectes platessa*
Reference no.	53	53	47	48	53	45	47	3	53	55	52	53	53	53	56	53	49
Trophic level	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Duration	AC/ST	AC/ST	SC/LT	SC/ST	AC/ST	SC/LT	SC/LT	AC/ST	AC/ST	SC/LT	SC/LT	AC/ST	AC/ST				
Technical performance	A	A	A	CC	A	CC	A	A	A	A	CC	A	A	A	CC	A	CC
Duration of long term test	-	-	A	-	-	B	A	-	-	A	B	-	-	-	C	-	B
Availability of test organism	B	B	B	-	B	B	B	B	B	B	CC	B	B	B	B	B	B
Exposure system	A	A	A	A	A	-	A	C	A	A	A	A	A	A	A	A	-
Cost, equipment	A	A	A	C	A	-	A	A	A	A	A	A	A	A	B	A	-
Cost, labour	A	A	A	C	A	C	A	A	A	B	B	A	A	A	C	A	C
Reproducibility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sources of potential error	A	A	A	B	A	-	A	A	A	B	CC	A	A	A	CC	A	-
Range of tolerance to environmental conditions	A	A	A	-	A	-	A	A	A	B	CC	A	A	A	B	A	-
Geographical distribution	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B
Representativeness of the test organism	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	A
Extrapolation of endpoints	B	B	B	B	B	B	B	B	B	B	B	B	B	B	C	B	B
General sensitivity	B	B	B	-	B	B	B	B	B	B	B	B	B	B	-	B	-
Relevance of exposure route and test conditions	A	A	A	-	A	-	A	A	A	A	B	A	A	A	B	A	-
Standardization	A	A	A	-	A	-	A	A	A	A	B	A	A	A	C	A	C
Relative evaluation	**A**	**A**	**A**	**C**	**A**	**C**	**A**	**B**	**A**	**AA**	**C**	**A**	**A**	**A**	**C**	**A**	**C**

Fish cold water (cont.)

	Scophthalmus maximus
Reference no.	79
Trophic level	C
Duration	
Technical performance	A
Duration of long term test	-
Availability of test organism	A
Exposure system	C
Cost, equipment	A
Cost, labour	A
Reproducibility	B
Sources of potential error	B
Range of tolerance to environmental conditions	A
Geographical distribution	B
Representativeness of the test organism	B
Extrapolation of endpoints	B
General sensitivity	B
Relevance of exposure route and test conditions	A
Standardization	B
Relative evaluation	**C**

Appendix B.

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Appendix C. Abbreviations/explanations

Appreviation etc.	Explanation
AChE	Acetyl cholinesterase
ADP	Adenosine diphosphate (associated in energy transfer for a range of different cellular activities)
AMP	Adenosine monophosphate (associated in energy transfer for a range of different cellular activities)
Androgenic	effect similar to male sex hormones (testosterone)
Antifouling agent	Compound preventing the settlement of organisms on substrates submerged in water
a.o.	And others
Apoptosis	Programmed cell-death
ASTM	American Standards for Testing and Materials
ATP	Adensosine triphosphate (provides a common source of energy for a range of different cellular activities)
BCF	Bioconcentration factor (concentration in organism/concentration in water)
Biocide	Compound used to kill weeds or other undesirable organisms
Biomarker	A cellular or physiological response to pollution
Bioturbation	The process of reworking the sediment performed by benthic organisms
Carcinogenic	Promote development of cancer
Copepod	Common group of animals (Crustaceans) in planktonic communities
DDT	Dichlorodiphenytrichloromethane (biocide previously used in agriculture)
DNA	Deoxyribonucleic acid (material of inheritance)
EC50	The concentration that gives 50% reduction in the endpoint (often photosynthesis) measured
EDC	Endocrine disrupting compound
ELS	Early life stage
Endocrine disruption	Disturbance of the regulation performed by an organisms hormonal system
Estrogenic	Effect similar to the female sexhormone estrogen
EU	European union
Fouling	Series of physical, chemical and biological events resulting in the formation of a layer of attached organisms on surfaces submerged in water
Genotoxic	Harmful effect on the genetic material
Half life	The time needed to degrade a substance 50 % (assumes an exponential degradation course)
IMO	The International maritime Organization
Imposex	Morphologic changes in the reproductive system of neogastropods (example dogwhelk) caused by TBT
in vitro	By derivation, means "in glass". In general applied to biological processes studied isolated from the whole organism.
in vivo	Within the living organism
Intersex	Morphologic changes in the reproductive system of periwinkles caused by TBT
ISO	International Organization for Standardization
LC50	The concentration that gives 50% mortality in a toxicity test
log Pow	Logarithm (base=10) to the water/octanol partitioning coefficient
LT	Long time test
Lysosom	Intracellular membrane bound particle involved in digestion and removal of cell

	material and autolysis
MD	Norwegian Ministry of Environment
MEPC	Marine Environment Protection Committee
Mesocosm	Experimental ecosystems (in-door tanks/aquarium to large outdoor enclosures or enclosed bays)
mRNA	Messenger Ribonucleic acid (substance involved in the translation of the structure of DNA into the structure of protein)
Mutagenic	Promote development of a mutation
NIVA	Norwegian Institute for Water Research
NMD	Norwegian Maritime Directorate
NOEC	Highest concentration that is likely not to give effects on an organism (safety factor considered)
OECD	Organization for Economic Co-operation and Development
Organotin	Organic compound containing the metal tin (exsample=TBT)
Paracrine	Effects of signaling substances on adjacent cells
PEC	Predict (estimate/measure) environmental concentration
PNEC	Predicted No Effect Concentration
Preneoplastic lesions	Early morphological change in a process that is thought to lead to cancer (tumor formation)
Q_{10}	Factor that describes how temperature dependant a process is
ST	Short time test
TBT	Tributyltin (substance used for antifouling)
Tumorigenesis	Formation and development of a tumor
Vitellogenin	Protein (yolk precursor protein) produced in the liver of female fish during sexual maturation. Production is regulated by estrogen.
Xenobiotic	Substances that are introduced to the environment (from Greek, "foreign substance")
^{14}C	Carbon (atomic weight=14)