ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. 1984. G. Persoone, E. Jaspers, and C. Claus (Eds). State Univ. Ghent and Inst. Mar. Scient. Res., Bredene, Belgium. Vol. 1. 798 p.

MARINE ECOTOXICOLOGICAL TESTS WITH FISH

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ABSTRACT

Guidelines for laboratory tests with marine fish and their use in preventing sea pollution are discussed. Historically, they were used for identifying discharges and tidal waters which were acutely toxic to marine organisms so that remedial action could be taken quickly. As gross pollution abated, the need arose for predictive tests for assessing the environmental impact of new chemicals, new discharges and for setting environmental quality standards. Because of the problems of measuring sublethal and long-term effects on fish, invertebrate species are preferred as test organisms and fish tests remain restricted to measurements of acute lethality. A pressing need is procedures for correlating results of laboratory tests on marine organisms with events in the sea so that realistic environmental quality standards may be set.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Fish, Review.

INTRODUCTION

Since the industrial revolution at the beginning of the nineteenth century pollution of natural waters has been an ever-growing problem but only in the last thirty years has public awareness led to legislation for its prevention. The publicity given to fish kills and the disappearance of fish from streams, rivers, lakes and estuaries highlighted the problem so that high priority was given by controlling authorities to the restoration of fisheries which are invaluable as a source of food and recreation. It is not surprising therefore that fish were the preferred species used in laboratory experiments for identifying toxic discharges and substances acutely toxic to aquatic organisms. Having identified the sources of pollution the quality of natural waters was greatly improved by treatment of discharges to remove toxic materials or simply banning the toxic discharges.

In many countries priority has been accorded to legislation aimed at the protection of bodies of freshwater which are the sources of high quality potable water, so essential to man. In the United Kingdom, many highly industrialised estuaries were seriously polluted by sewage and industrial wastes whilst coastal waters have remained comparatively free of pollution and the high productivity of near shore fisheries has not been seriously impaired. Thus, the protection of tidal waters has always lagged behind the measures taken to protect freshwater and migratory fish. Initially, simple and inexpensive static procedures with freshwater fish were used for detecting toxic discharges, whilst toxic constituents were often identified by performing parallel tests on major constituents identified by chemical analysis. In this manner, a substantial body of knowledge has been acquired over the years about the acute lethality of chemicals to many species of fish, details of test procedures and those factors which influence experimental results.

These static tests for acute lethal toxicity provide information about the concentration of an effluent or chemical that leads to the death of a proportion of the test species under the conditions of test, and at best this information can only be used to give a first indication of the level which might be acutely toxic in the natural environment. It is not possible to predict how other species are likely to be affected nor is the nature and degree of adverse effects, arising from long-term (chronic) exposure, predictable from the acute tests. Recognising these shortcomings, the results of static tests are only used for setting consents for discharges after applying a substantial safety factor. The arbitrary value commonly applied in the early days of control was that the concentration in the receiving water of a potentially lethal effluent or constituent should not exceed one-tenth of the $LC50$ measured over 96 h in the laboratory. Provision was made that in the event of further evidence of deleterious effects being produced from field studies or laboratory investigations more stringent standards could be applied. Today in the light of existing knowledge very much larger safety factors are commonly applied, based largely on extensive laboratory derived data about toxicity to invertebrate species, as well as field data resulting from ecological and chemical monitoring of natural waters.

A major drawback of the static test is maintaining constant the concentrations of active constituents in the test solutions since normally they are only changed once a day. Constituents may be lost by aeration, biological breakdown, adsorption etc. This led to the early development of constant-flow procedures which usually eliminate the problem but require very large volumes of test solutions. The practical difficulties of transporting natural waters and effluents to the laboratory without change in composition rules out the use of flow-through procedures for assessing the toxicity of these waters. However, flow-through procedures have played a valuable part in laboratory investigations of the toxicity to fish of single substances and mixtures, particularly on volatile and readily degradable materials.

Concern for the marine environment only came into prominence in the seventies following the Stockholm Conference. In fact, a report published in 1969 by the International Council for the Exploration of the Sea states that only one country, the United Kingdom, had toxicity testing apparatus in continuous use for carrying out marine tests on a routine basis. These were acute static tests and the report also discusses the need for longer-term tests. Subsequently, the development of new methods associated with the registration of pesticides, the consenting of discharges to freshwater and notification procedures for new chemicals has led to the development of improved procedures for ecotoxicological testing with freshwater fish and many species of invertebrates. Many of these methods are applicable to marine species and it is often recommended in test guidelines that where there is concern for the marine environment the methods should be adapted for marine fish in seawater. In the absence of data for marine species data for freshwater species are often used for hazard assessment.

There are a number of technical reasons why the development of marine testing with fish has lagged well behind the developments with freshwater fish. Firstly there is the difficulty and therefore the high cost of obtaining suitable marine species for testing at all seasons of the year. A further complication is obtaining a substantial and continuous supply of seawater, free of contamination by chemicals, suspended matter and plankton. Furthermore the growth of marine fish is relatively slow, giving life cycles extending over many months or years, so that any studies of growth, reproduction, etc. are prolonged and therefore expensive. Invertebrate species with short life cycles have many advantages over fish since several generations may be tested within days or weeks, so reducing handling problems and costs. There is also evidence that many invertebrate species are highly sensitive to pollutants, such as heavy metals, since the levels shown to have little or no adverse effects often approach the natural levels found in coastal waters which are free of pollution.

Whilst on theoretical grounds it is highly desirable to have available an extensive battery of ecotoxicological test methods for measuring both lethal and sublethal effects, the facts are that practically all routine tests with fish currently used for control purposes are restricted to short-term tests for lethality. Apparently the practical needs of controlling authorities have not justified the time, effort and expense of developing other types of tests with marine fish.

When occasionally a need arises for tests other than lethality to marine fish, investigations are normally of an "ad hoc" nature. Thus, the method will depend largely on the physical, chemical and biological characteristics of the pollutant and with species of fish, indigenous to the receiving waters. For example, it may be necessary to assess the likelihood of fish becoming tainted by chemicals released into the sea, or avoiding contaminated waters. From the literature, it may be possible to identify techniques which can be adapted for the "ad hoc" investigation or alternately the investigation may become a research project where new methods are developed and verified.

Another problem stems from the ability of aquatic organisms to bioaccumulate substances from the environment. If this has deleterious effects on the organism or its predators then the substance becomes a pollutant. Therefore, demonstrating that a particular substance is accumulated by fish is only the first step in assessing the likelihood of pollution. Obviously other species, particularly those at the end of the food chains, are particularly vulnerable because of the possibility of biomagnification.

AIMS AND OBJECTIVES

The primary aim of ecotoxicological tests with marine fish is to provide scientific information which can be used for deciding what are the acceptable environmental quality standards for chemicals or mixtures of chemicals in seawater in order to achieve the environmental quality objective of protecting marine fish and fisheries against pollution.

The IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Group of Experts on the Scientific Aspects of Marine Pollution state that pollution of the marine environment means "the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) which results in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activity including fishing, impairment of the quality for use of seawater, and reduction of amenities". Mance and O'Donnell (1983) has defined the environmental quality standards, EQS, as the concentration of a substance which must not be exceeded if a specified use of the aquatic environment is to be maintained.

Since it is impossible to reproduce in the laboratory or field trials the highly variable conditions likely to be found in estuaries or the sea, predicting acceptable environmental quality standards must be a value judgment which may be highly subjective as full environmental data are usually lacking. In fact, it is impossible to prove conclusively on scientific grounds that any substance is completely free of deleterious effects, so when setting standards it is common practice to apply substantial safety factors.

There are two basic elements in predicting the likelihood of pollution. Firstly, the maximum concentration of the biologically active form of each potential pollutant or mixture in the tidal waters, and secondly, the minimum concentration considered to be harmful to marine fish, the latter being derived from the results of ecotoxicological tests. If the maximum concentration of the potential pollutant in seawater is well below the harmful level measured in the laboratory, pollution is unlikely. However, as the margin of safety decreases, the greater the care required when defining and measuring the two elements.

Many pertinent questions then arise. Is it necessary to set standards which will protect all species of fish in the sea or should the standards be restricted to species found in the area of contaminated seawater ? As many important species spawn offshore whilst most discharges are made to inshore waters, should deleterious effects on fish eggs and larvae be considered, since they are frequently much more sensitive to pollutants than juvenile or adult fish ? These problems are usually resolved pragmatically by consultation between all interested parties, controlling authorities and dischargers, who jointly decide which species should be tested and the methods. Frequently there is an ongoing dialogue where after initial investigations further experimental work is agreed to fill gaps in knowledge and to confirm initial results.

In the past the use of short-term inexpensive tests allowed joint decisions to be taken quickly to abate or prevent gross pollution. The alternative, the counsel of perfection, usually entails long delays in the decision-making process and additional expenditure. Ideally, for each trophic level, the most sensitive form of the most sensitive species must be identified and long-term tests developed which will permit the identification of a "no detectable effect" level to be measured for each contaminant or mixture. However, there will always be concern whether or not this ideal is achieved and there is always the possibility of synergic or antagonistic effects arising from the multiplicity of chemicals in seawater. Thus it seems very unlikely that an EQS will ever be set without incorporating a substantial arbitrary safety factor. Once an EQS is applied it creates a precedent which may be extremely difficult to change even though evidence is subsequently produced that the standard is far too stringent. Once a stringent standard is universally applied the opportunity may be lost to produce information showing that the standard is unnecessarily severe.

Effective control of marine pollution may be achieved by applying EQSs to the receiving waters. A problem arises, however, deciding where in the sea the actual environmental concentrations should be measured for control purposes. The sea occupies some three-quarters of the earth's surface with an average depth of two and a half miles, hence in global terms dilution in the sea is virtually infinite. Furthermore, the concentration of most substances entering the sea is reduced by natural chemical, physical and biological processes. Thus for control purposes the environmental concentration of a substance must be related to a particular body of water.

In estuaries and embayments it may be possible to measure steady state concentrations within a particular sector but in the open sea where there are few physical constraints on mixing processes, the concentration will decrease with distance from the point of entry.

At a specified time the precise dilution afforded will depend on the local meteorological and tidal conditions thus the concentration of a pollutant will vary both temporally and spatially. The area around the point of entry, the zone of initial dilution, is usually defined as the mixing zone, and subsequently as the diluted plume moves away, secondary dilution and dispersal take place due to natural turbulence and diffusion processes. There is a relationship between the initial and secondary dilution. If the initial dilution is small, secondary dilution will be very large, but should the initial dilution be large, the secondary dilution will correspondingly be reduced. The magnitude of dilution and dispersal processes in the coastal waters of the UK is such that from a point source of discharge, if the initial dilution of a conservative substance is of the order of one hundred in the mixing zone, secondary dilution within hours will usually be a further thousand times or more and eventually concentrations will fall to background levels. Consequently, controls are usually applied in such a manner that any deleterious effects are contained within the mixing zone, with the knowledge that away from the mixing zone detectable effects will be transitional and minimal. The problem is defining the size and position of the mixing zone, which changes with the tidal conditions, however, it is usually a very small part of the surrounding sea.

With the knowledge that dilution and dispersal rapidly reduce environmental concentration away from the outfall area, and that many substances are broken down or chemically changed in seawater, the possibility of long-term sublethal effects assume less practical importance than the problem of deleterious effects within the mixing zone. This is undoubtedly another reason for the lack of information in the literature about chronic effects of pollutants on marine fish. Furthermore, field investigations show that the effects of man-made changes in the sea are usually very small compared with the natural changes constantly taking place. Controlling authorities preoccupied with the problems of the mixing zone, may be aware of these other aspects, but rarely have the resources for their investigation.

Biological or chemical monitoring of the effects of pollutants in the sea is technically difficult and expensive. It is an area where much further research is required to verify that sublethal effects measured during ecotoxicological testing have real significance for the natural environment. Are they deleterious and therefore polluting ?

TYPES OF ECOTOXICOLOGICAL TESTS WITH FISH AND STANDARDISATION

Over the years there has been a proliferation in the number and types of tests available and this has led to a need for standardisation of tests to meet specific purposes. The aims of standardisation are to ensure that the methods are technically sound, that results from different laboratories are comparable, and to provide an equitable basis for setting environmental quality standards or consenting discharges.

Two main types of test are apparent, acute and chronic. The acute toxicity of a substance in the aquatic environment is normally expressed as the concentration of a substance in the water which has a harmful effect on 50 % of a batch of test organisms in a short exposure time, relative to the live span of the fish. The normal exposure time is 96 h so that tests can be completed within the working week. Chronic toxicity is that resulting from much longer periods of exposure usually at least several weeks which is a significant proportion of the life span of the fish.

A further categorisation of tests based on effect is common, distinguishing between lethal (in which death is the observed phenomenon) and sublethal (less than death) which includes effects on behaviour, growth, reproduction, biochemistry, and physiology. Thus it is possible to have acute lethal tests, chronic sublethal and so on.

Within each type of tests there are many different methods which might be used depending, for example, on the species involved or the precision of the results required. The most widely applied test remains the acute test for lethality, but a large part of the philosophy and fundamentals are common to all types of tests with fish. Consequently, during the past decade many working groups associated with both international and national organisations (ISO, OECD, EIFAC, EPA in the USA, Department of Environment in the UK, etc.) have been set the task of preparing standard procedures for fish tests but the outcome is usually a set of guidelines or recommended procedures. These provide a broad framework for tests, leaving the details to the operating laboratory.

GUIDELINES FOR FISH TESTS

Numerous publications (EIFAC, 1975 ; FAO, 1977, 1981) are available describing guideline procedures for laboratory tests with fish, and the fundamentals apply equally to both freshwater and marine species. The operator is given wide discretion in conducting the tests, hence well balanced judgment based on sound practical experience is required if the optimum information is to be obtained from a specific investigation. Various aspects of the guidelines are briefly considered in order to highlight some of the difficulties with tests on marine fish and those factors which determine the precision and accuracy of the results.

CONDITIONS OF TEST

Species

Availability of species in adequate numbers at all seasons of the year is a major problem, and of the many thousands of different species which frequent the seas only a few tens of species have been used for laboratory investigations. The sensitivity of the selected species depends on its age, size and previous history. The most widely used source of marine fish is collection from the wild so that the availability of eggs, larvae and juvenile forms is usually restricted to a few weeks in the year. Artificial spawning may be induced in commercial or laboratory reared stocks to overcome the problems of availability. Whatever the source, care must be taken to eliminate diseased or damaged stock and whilst it is desirable to have some measure of the condition of the test organisms this is rarely achieved with marine fish. Provided controls survive the experimental period with less than 10 % mortality, it is usual to assume that all the test organisms are not unduly stressed, except by the test substance.

Stress is likely to develop if fish are starved for long periods but starvation is unlikely to be of significance during acute tests. When feeding takes place during chronic tests there may be a substantial increase in the uptake of the test substance and consequently an increase in toxicity. This will depend on the nature of the test substance, the type of food and the frequency of feeding. It is unlikely that fish from the wild will be acclimated to the controlled conditions of the laboratory and time is required to allow for adaptation to the experimental regime of water temperature, dissolved oxygen and quality of water.

Test apparatus

Materials of construction are of greater importance than with freshwater tests because of the corrosive nature of seawater, hence plastics are often used which contain chemical stabilisers which may leach out and have deleterious effects, particularly on eggs and larvae. Test containers must not be such a shape and size to restrict the ability of the fish to move freely (a particular problem with flatfish) and should hold a sufficient volume of the test solution so that the concentration of test substance and dissolved oxygen are not unduly reduced during the test.

Dilution water

Availability of adequate supplies of seawater at inland sites presents many practical problems, particularly transport, whilst supplies taken from coastal waters may be polluted or contaminated. Following treatment to remove excess metals, synthetic seawater may be suitable for short-term tests but vital constituents may be lacking which may produce undue stress during chronic tests. Monitoring the levels of trace contaminants in seawater supplies is highly desirable but this is frequently very difficult and expensive.

Test solutions

Preliminary sorting tests with fish are necessary in order to select the optimum concentrations of test solutions and the concentrations should be maintained constant throughout the test. At least daily, the concentrations should be monitored by chemical analysis. During static tests substantial changes in test concentrations often occur, due to biological breakdown, aeration, etc. Changes are less likely in flow-through tests but even so monitoring concentrations by chemical analysis is desirable even though reliable dosing equipment is used for preparing serial solutions. Problems frequently arise because of the limited solubility of the test substance in seawater and recourse may be made to the use of low-toxicity solvents for preparing stock solutions of high concentration. The presence of these solvents in the test solutions may have an effect on the rate of entry of the test substance into the fish which may invalidate the experimental results.

The cost of developing and applying sensitive analytical techniques for monitoring test substances and contaminants in seawater is normally far greater than for freshwater. In practice chemical analysis may result in the total cost of an investigation being doubled or trebled. Many months of patient research using very expensive analytical equipment may be necessary to develop and validate a sensitive technique for measuring very low concentrations of complex organic compounds such as pesticides, weed killers, etc. in seawater.

PROCEDURE

Test organisms are normally selected at random from stock and distributed randomly within the test containers. Failure to randomise the animals may decrease the precision of the test since selection of active or inactive individuals can easily occur and lead to nonuniform distribution in test containers.

Precise definition of the effect under study is imperative. For example, in acute lethal toxicity tests care must be taken to define the criterion to measure death. Cessation of opercular movement for a period of ten seconds is a common criterion. Controls are essential for comparison of effects and an acute test would be invalidated if more than 10 % mortality were recorded in the controls. Failure to maintain constant the dissolved oxygen concentration, water temperature, salinity, and pH of the test solution may influence the results and should be monitored throughout the test.

RESULTS

There are numerous methods of recording and calculating the results of toxicity tests on fish and deriving statistical data. The method selected will depend on the use to be made of the data but there is much common ground amongst the methods acceptable to controlling authorities.

Toxicity is usually expressed in terms of the concentration which will produce a specified effect in a specified proportion of the population after a specified time. The measured statistic for a population response is usually the median, thus where a death is the response it becomes the median lethal concentration, the LC50. The concentrations are commonly calculated for 24, 48 and 96 h since the concentration-response relationships are of importance. If the concentration-response relationship plotted on a log-log paper is curvilinear and becomes asymptotic to the time axis, the asymptotic concentration can be interpolated. This value has probably greater practical and theoretical significance than a LC50 at some arbitrary time, since it gives a preliminary indication of the concentration which must not be exceeded if the death of the test species is to be avoided.

ACCURACY AND PRECISION

One of the basic requirements of all experimental results is a knowledge of the precision of the method and the accuracy of the results. The precision of the method is usually expressed as the confidence limits on the range of values within which, at a given level of statistical probability, the results from subsequent tests will fall, using exactly the same experimental conditions, and an equally representative batch of fish. Changes in the experimental conditions or in the susceptibility of the batch of fish, could give results outside the range expressed as confidence limits.

The accuracy of the results is best determined by replication of the experiments under exactly the same experimental conditions so that the variability of response of different batches of fish can be measured.

Because the resources for testing are limited, a balance usually has to be maintained between a few tests of high precision or more tests of limited precision, which give some idea of accuracy. In any event all efforts should be made to replicate all tests. Data for the accuracy and precision of marine tests is lacking but some indication is forthcoming from ring tests with freshwater fish. In 1979 the Commission of the European Community organised a ring test involving 51 laboratories. An ISO draft protocol was the basis for measuring the acute toxicity of five specially selected chemicals to the zebra fish Brachydanio rerio employing a static test procedure. Statistical analysis of the results showed that the standard deviation for reproducibility between the different laboratories is of the order of 20 % of the LC50 at 24 h for substances such as potassium dichromate which are readily soluble in water. As the solubility of test substance decreases the range of results becomes much wider. Thus, at best, the ratio of the highest LC50 to the lowest LC50 measured in the different laboratories might be expected to lie within a factor of two. This is a measure of the accuracy of the test method.

It appears from statistical analysis of all the results that the precision of the test method is satisfactory since the standard deviation for repeatability is only 10 % of the LC50 at 24 h. This indicates that, whilst a given laboratory may record a high degree of precision, it does not necessarily follow that there is a correspondingly high degree of accuracy. A laboratory may record little scatter amongst results of replicate tests carried out over a period of several weeks, but values may differ substantially from one laboratory to the next or at different seasons of the year in the same laboratory.

Obviously, the range of results would be far wider if different species of fish were used by each laboratory or different forms of the same species, namely eggs, larvae, juveniles or adults. Thus values of the LC50 for a named substance quoted in the literature may extend over several orders of magnitude.

APPLICATION OF ECOTOXICOLOGICAL TESTS WITH MARINE FISH

SETTING ENVIRONMENTAL QUALITY STANDARDS : EQSs

There is a growing need for environmental quality standards for existing substances and new chemicals which may contaminate or pollute seawater. Under EC legislation it is necessary to define EQSs for all grey list metals, and the first step is a survey of the scientific literature. Table I has been prepared from two recent surveys, Taylor (1982) and Mance and O'Donnell (1983), which summarise all available data about the levels of the metals copper, chromium, zinc, lead, nickel, and arsenic which are acutely or chronically toxic to marine fish or marine invertebrates. Surveys of other substances would be expected to reveal a similar paucity of relevant information. A number of important points arise from the table :

Table I. Acute and chronic toxicity of heavy metals in seawater (numbers between brackets indicate the literature references.

	Chromium	Lead	Zinc	Nickel	Copper	Arsenic
	LC50 for 96 h ($\mu g.l^{-1}$)					
Fish						
Menidia menidia	20 100(1)	No data	2 730 4 960(8)	7 960 (11)	113(larvae) (13)	16 033(19)
Cithorichtys stigmaeus	30 000(2)					
Fundulus heteroclitus	91 000(3)		60 000(9)	350 000(9)	3 200(14)	
Pseudopleuronectes americanus			83 000 4 920(8)		129(13)	
Trachinotus carolinus					360–510(15)	
Limanda limanda					170(16)	
Paralichthys dentatus					28(embryo) (13)	
Apeltes quadricus						14 953(19)
Aldrichetta fosteri	50 000(5)		10 500(5)			
Athennosoma microstoma	35 000(5)		38 000(5)			
Invertebrates						
Name of species	Mysidospis bahia	Capitella capitata	Mercenaria mercenaria	Heteromysis formosa	Acartia tonsa	Acartia clausi
Lowest recorded LC50	2 000(1)	> 1 000(6)	116(10)	152(11)	17(17)	508(19)
Number of species for which data are available	17	18	21	17	23	5

Chronic toxicity ($\mu g.l^{-1}$)

Fish	none	none	none	none	limited data	none
Invertebrates Name of species	Neanthes areanceo-dentata	Chaetoceros sp.	Mercenaria mercenaria	Lytechinus pictus	Campanularia flexuosa	Acartia clausi
Lowest concentration recorded for adverse effect	30(4)	60(7)	50(10)	58(12)	1.43(18)	508(19)
Nominal concentration of metal in unpolluted coastal water	0.5	1	3	0.5	0.5	2

(a) It shows that data for acute toxicity are restricted to a total of ten species of marine fish but for any one metal the number of test species is far less.
(b) Little relevant information is available about the chronic toxicity of these metals to marine fish. What information is available comes largely from extended tests for lethal effects. Far more information is available about chronic sublethal effects on invertebrate species, mainly effects on growth and reproduction.
(c) Values of the LC50 for 96 h show that invertebrate species are in general far more sensitive to the deleterious effects of the metals than the few named species of marine fish. The difference frequently exceeds a factor of 10.
(d) If the lowest recorded concentration for chronic adverse effects on the most sensitive species of invertebrate is compared with the limited data for acute lethality to marine fish, it appears that marine fish are relatively insensitive to the effects of the metals.

Difficulties arise when attempting to use the results of laboratory tests, particularly chronic sublethal effects on invertebrates, for setting EQSs. Whilst it may be clearly demonstrated in the laboratory that prolonged exposure to very low concentrations of metals may have an effect on the vital functions of test organisms, it does not necessarily follow that such effects occur in the sea with sufficient intensity to be regarded as deleterious. The difficulty is deciding the boundary between acceptable and unacceptable effects, a subjective decision. Should it be decided to set the standard at the level of "no detectable effect" during laboratory experiments it would be the sensitivity of the method which determines the acceptable level. How this relates to events in the natural environment is unlikely to be known.

Whilst data on toxicity to marine fish are usually limited to lethal effects, they provide the traditional starting point for setting realistic EQSs. Comparison of the LC50 for fish with the levels for sublethal effects on invertebrate species gives some guide to the magnitude of the safety factor which should be applied. From Table I it will be seen that acceptable safety factors may vary from one to several orders of magnitude. Obviously each standard must be judged on the merits of the data. Another starting point for deciding EQSs for metals is their natural level in seawater. The difference between the natural level and the EQS indicates the magnitude of the window for man-made inputs. In the absence of adequate ecotoxicological information there is a natural tendency to restrict the size of the window and so reduce the probability of pollution. However, it is unlikely that society will be prepared to pay the costs of implementing unnecessarily stringent standards and a balance has to be maintained between realistic and unrealistic environmental quality objectives.

DISCHARGES FROM LAND-BASED SOURCES AND OFFSHORE PLATFORMS

The control of discharges of liquid effluents into the sea from land-based sources and offshore platforms is normally achieved through a consent or licence given by a local or national authority in accordance with International Conventions. A consent normally stipulates the maximum rate of discharge and the maximum concentration of each constituent together with any other conditions which are necessary to prevent pollution. If the dilution in the mixing zone is calculated from hydrographical data and the quality of the receiving waters taken into account then consent conditions may be set to ensure that environmental quality standards are not exceeded outside the mixing zone.

Unfortunately, EQSs for the majority of substances have not been agreed, and there is always the possibility of synergic or antagonistic effects between different wastes or constituents of the receiving waters. Recognising this, controlling authorities normally require measurements to be made of the acute toxicity of representative samples of the discharge to indigenous species of fish in order to assess the minimum dilution in the sea to prevent acutely toxic effects. If the outfall is constructed so that this minimum dilution is rapidly exceeded within the mixing zone, controlling authorities are unlikely to withhold their consent for the discharge. If further ecotoxicological data are required about chronic effects on indigenous species of fish, then with the agreement of the controlling authority "ad hoc" investigations may be carried out.

Guidelines for test methods are usually produced by national authorities, including lists of recommended species of fish and invertebrates, but most guidelines also encourage the use of other indigenous species for testing with the approval of the controlling authorities. Each country appears to have its own particular list of recommended species for tests but this follows from the fact that availability is the prime concern and to a large extent availability depends on geographical factors including climate. Whilst fish are widely recommended for tests for acute toxicity and possibly bioaccumulation, invertebrate species are preferred for measuring sublethal effects.

OCEAN DUMPING

The system of control is similar to that for discharges from land-based sources. In order to comply with International Conventions, control of dumping is achieved through the issue of a permit or licence by the national authority. Ecotoxicological testing of the waste is required in support of an application for a permit and invaribly includes tests with marine fish for acute lethality as well as similar tests with invertebrate species. As far as is known there are no standard methods laid down by national authorities, only recommended procedures.

The Ocean Dumping Committee of the EPA, 1978, requires tests on a minimum of three species of marine organisms to be selected from a list which includes nine species of invertebrates and eleven species of marine fish. It is also stated that indigenous species should be tested whenever possible, and the tests should cover different taxonomic groups. Including in the recommended procedure is a life-cycle test with a marine fish, the sheepshead minnow Cyprinodon variegatus. The test requires from four to six months for completion and it is stated to have limitations. Sheepshead minnows are known to tolerate low dissolved oxygen and a wide range of temperature and salinity, therefore, they may be relatively insensitive to toxic substances.

In the United Kingdom, ecotoxicological tests in support of a licence application would normally be restricted to acute tests for lethality with one species of marine fish and one species of invertebrate, namely, the pogge Agonus cataphractus, and the shrimp Crangon crangon. By agreement with the controlling authority relevant data on other species are acceptable, particularly tests with species indigenous to the dumping area.

The results of tests with fish and invertebrates provide the basis for assessing the potential hazard by comparison with the predicted concentrations of the waste at the dumping site. A licence for a waste acceptable on toxicological grounds usually includes a specification of the rate of discharge which will give an acceptable margin of safety for species more sensitive than those used in the test.

EVALUATION OF THE HAZARDS OF HARMFUL SUBSTANCES CARRIED BY SHIPS

The marked increase in the transport of bulk chemicals by ships has increased the possibility of marine pollution resulting from spillages following collisions or strandings. After the International Convention for the Prevention of Marine Pollution from Ships, MARPOL 1973, the Joint Group of Experts on the Scientific Aspects of Marine Pollution, GESAMP, was commissioned to produce hazard profiles so that chemicals could be classified into groups. The aim is to reduce the risk of pollution by restricting each group of chemicals to a particular class of vessel which provides adequate containment of the chemical. Thus the most hazardous class may only be transported in purposely designed ships with robust storage tanks which are unlikely to be ruptured even after a collision or stranding.

The GESAMP hazard evaluation procedure, IMCO 1982, describes the evaluation of ecotoxicological data about direct toxic effects to living resources and bioaccumulation. Direct toxic effects are ranked according to LC50s for 96 h measured with juvenile or adult species of fish or crustaceans. Where data are not available for marine species but available for freshwater species they have been used after due consideration of the possible effects on toxicity of the different water medium.

GESAMP has rated chemicals as highly toxic if the LC50 for 96 h is less than 1 $mg.l^{-1}$, moderately toxic from 1 to 10 $mg.l^{-1}$, slightly toxic from 10 to 100 $mg.l^{-1}$, practically nontoxic from 100 to 1 000 $mg.l^{-1}$, and finally nonhazardous greater than 1 000 $mg.l^{-1}$. The ranking is not rigidly applied as each substance is judged on its own particular merits by the expert group taking into account sublethal or indirect effects.

The GESAMP rankings for bioaccumulation are also value judgments. A substance rated +, would be bioaccumulated to a significant extent and known to produce a hazard to aquatic life or human health. The next rank, Z, includes substances bioaccumulated with attendant risk to aquatic organisms or human health, however with a short retention time of one week or less.

T-ranking includes substances bioaccumulated and liable to produce tainting of seafood. Finally, the O-category, where there is no evidence to support the other ratings.

The majority of the information on bioaccumulation arises largely from "ad hoc" studies with specific compounds or research projects. Proposed standard practices for measuring the bioconcentration of chemicals with fishes are under consideration but, depending on the method, species, and environmental conditions employed, different results may be obtained from the same test material. It appears that standard methods for measuring the extent of bioconcentration are some way off. What is also lacking are methods of determining whether bioaccumulated materials are harmful to fish or predators, including man. Such investigations are normally of an "ad hoc" or research nature and not suitable for standardisation.

SUMMARY AND CONCLUSIONS

A limited number of marine species of fish are used for investigations in the laboratory of the toxicity of substances to marine organisms and for setting environmental quality standards in order to prevent pollution. With a knowledge of the concentration of substances or mixtures which are acutely toxic to marine fish and allowing a margin of safety, it is possible to set standards which will prevent the death of fish in the open sea. Traditionally, this is the first step in protecting marine fisheries, which are of great economic importance for man.

If the margin of safety is substantial, marine fish may also be protected to some extent against chronic lethal and chronic sublethal effects of pollutants. Because of the difficulties of obtaining suitable species of fish for testing throughout the year and their relatively long life cycle, fish do not lend themselves to long-term investigations which may be very expensive because of the problems of containment and providing adequate supplies of uncontaminated seawater. Invertebrate species from different trophic levels with relatively short life cycles are much more suitable for investigations in the laboratory of sublethal effects, for example, growth rates, reproduction, behavioural response, etc. These tests are far less expensive than comparable tests on fish, for obvious reasons.

Because of the proliferation of test methods for measuring the acute toxicity of substances to fish, some standardisation appears necessary, if only to ensure that the methods are technically sound and that the results from different laboratories are directly comparable. Only guidelines or recommended methods have so far been produced, which provides a degree of flexibility for the operators, since a few laboratories engaged in tests with marine fish could comply with rigid test protocols.

Various aspects of these guidelines have been highlighted which have a bearing on the precision and accuracy of experimental results. Whilst the precision of the method may appear highly satisfactory from replication of tests in one laboratory, it does not necessarily follow that the results are of comparable accuracy. Ring tests with freshwater fish using a standard protocol suggest that, at best, the accuracy could be a factor of two between the highest and lowest results from different laboratories. With marine fish collected from the wild, less accuracy would be expected as there is no accepted method of measuring the condition of fish used in tests. The use of species of fish indigenous to the receiving waters is frequently encouraged by controlling authorities, and also simulation of the conditions of the discharge so that the maximum amount of relevant information may be obtained. Further "ad hoc" tests for tainting or behavioural responses may be required since the response depends largely on the chemical and physical properties of the test substances, together with the appropriate method of testing which is adopted.

It is evident that invertebrate species of marine organisms are far more widely used in laboratory tests for chronic effects than fish, thus the trend in ecotoxicological testing may be said to be away from fish. To what extent this will continue will largely depend on current research on the effects of pollutants on marine fish. Whatever species or methods are used for testing, it is impossible to reproduce in the laboratory the constantly changing conditions found in the sea, and it follows that there will always be insufficient ecotoxicological data for predicting acceptable environmental quality standards. Standards will be value judgments which may be highly subjective. In the absence of adequate data there is a natural tendency to set stringent standards. If a standard is unnecessarily severe and it is applied universally the opportunity may be lost for acquiring field data confirming the stringency of the standard. Thus a pressing need for ecotoxicological resarch is the development of procedures for correlating the results of laboratory tests with events in the sea so that realistic environmental quality standards may be set.

LITERATURE CITED

Commission of the European Communities. 1979.
Interlaboratory ring test concerning the study of the ecotoxicity of a chemical substance with respect to fish. Environment and Consumer Protection Service, Brussels. 31 p.

EIFAC. 1975.
Report on fish toxicity testing procedures. EIFAC Technical Paper No. 24. 25 p.

FAO. 1977.
Manual of methods in aquatic environment research. Part 4 Basis for selecting biological tests to evaluate marine pollution. FAO 1977, Fisheries Technical Paper No. 164. FAO, Rome. 31 p.

FAO. 1981.
Manual of methods in aquatic environment research. Part 4 Basis for selecting biological tests to evaluate marine pollution. FAO 1981. Fisheries Technical Paper No. 208. FAO, Rome. 54 p.

IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP. 1982.
Joint Group of Experts on the Scientific Aspects of Marine Pollution : GESAMP Report and Studies No. 17.The evaluation of the hazards of harmful substances carried by ships. IMCO 1982. 32 p.

International Council for the Exploration of the Sea. 1969.
Report of ICES Working Group on Pollution of the North Sea. Corp. Res. Rep. Series A No. 13, ICES 1969. 61 p.

Mance G. and A.R. O'Donnell. 1983.
Application of the European Committee's directive on dangerous substances to list II substances in the UK. Wat. Sci. Techn. 16:159-165.

Ocean Dumping Committee of EPA. 1978.
Bioassay procedures for the Ocean Disposal Permit Programme by EPA. Ocean Disposal Bioassay Working Group, EPA Environmental Research Laboratory, Gulf Breeze, Florida. 121 p.

Taylor D. 1982.
Confidential report of Brixham Laboratory ICI to WRC.

LITERATURE CITED IN TABLE I

1. US Environmental Protection Agency. 1980.
Ambient water quality criteria for chromium. Ref. No. 81-117467 Oct.

2. Mearns A.J., P.S. Oshida, M.J. Sherwood, D.R. Young, and D.J. Reish. 1946.
Chromium effects on coastal organisms. J. Wat. Pollut. Control. Fed. 48:1929-1939.

3. Eisler R. and R.J. Hennekey. 1977.
Acute toxicities of Cd^{2+}, Cr^{6+}, Hg^{2+}, Ni^{2+}, and Zn^{2+} to estuarine macrofauna. Arch. Environ. Contam. Toxicol. 6:315-323.

4. Oshida P.S. 1977.
A safe level of hexavalent chromium for a marine polychaete. Southern California Water Research Project, El Segurido, California, Annual Report. 169-180.

5. Negilski D.S. 1976.
Acute toxicity of zinc, cadmium, and chromium to the marine fishes, yellow-eye mullet and small-mouthed hardyhead Aust. J. Mar. Freshwat. Res. 27:137-148.

6. Reish D.J., J.M. Martin, F.M. Piltz, and J.Q. Wand. 1976.
The effects of heavy metals on laboratory populations of two polychaetes with comparisons to the water quality conditions and standards in Southern Californian marine waters. Wat. Res. 10:299-302.

7. Hollibaugh J.T., D.L.R. Seibert, and W.H. Thomas. 1980.
A comparison of the toxicity of ten heavy metals to phytoplankton for Saanich Inlet B.C. Canada. Estuar. Coast. Mar. Sci. 10:93-150.

8. US Environmental Protection Agency. 1980.
Ambient water quality criteria for zinc. Ref.No. EPA 440/5-80-07 Oct. 169 p.

9. Eisler R. and G.R. Gardner. 1973.
Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. J. Fish Biol. 5:131-142.

10. Calabrese A., J.R. McInnes, D.A. Nelson, and J.E. Miller. 1977.
Survival and growth of bivalve larvae under heavy metal stress. Mar. Biol. 41:179-184.

11. US Environmental Protection Agency. 1980.
Ambient water criteria for nickel. Ref. EPA No. 440/5-80-060.

12. Timourian H. and G. Watchmaker. 1972.
Ni uptake by sea urchin embryos and their subsequent development. J. Exp. Zool. 182:379-384.

13. US Environmental Protection Agency. 1980.
Ambient water quality for copper. Ref No. EPA 440/5-80-036, Oct. 122 p.

14. Jackin E., J.M. Hamlin, and S. Sonis. 1970.
Effects of metal poisoning on five liver enzymes in the killifish (Fundulus heteroclitus). J. Fish. Res. Bd Can. 27:383-393.

15. Birdsong C.L. and J.W. Avault. 1971.
Toxicity of certain chemicals to juvenile pompano. Prog. Fish Cult. 33:76-80.

16. Taylor D. 1982.
Confidential report of Brixham Laboratory ICI to WRC.

17. Sosnowski S.L. and J.H. Gentile. 1978.
Toxicological comparison of natural and cultural populations of Acartia tonsa to cadmium, copper and mercury. J. Fish. Res. Bd Can. 35:1366-1369.

18. Moore M.N. and A.R.D. Stebbing. 1976.
The quantitative cyclochemical effects of three metal ions on the lysosomal hydrolase of a hydroid. J. mar. biol. Ass. UK. 56:995-1005.

19. US Environmental Protection Agency. 1980.
Ambient water quality criteria for arsenic Ref No. PB82-117327 Oct.