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MARINE ECOTOXICOLOGICAL TESTING IN THE FEDERAL REPUBLIC OF GERMANY

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ABSTRACT

Activities in the field of marine ecotoxicology were initiated in Germany in 1966, when the Deutsche Forschungsgemeinschaft (DFG) sponsored a research programme involving an interdisciplinary group of scientists "which should deal with the effects on marine organisms of industrial and municipal waste water in coastal areas". This type of research has been carried out for a number of years, after which other agencies, such as the Bundesministerium für Forschung und Technologie (BMFT) and the Umweltbundesamt (UBA) became concerned with marine problems and supported specialized research programmes.

Research work has been performed in this context on different aspects of risk assessment of various compounds in the marine environment, without necessarily being especially concerned with test procedures, though the scientific basis for the conception of tests was respected.

Field experiments have been taken into consideration at an increasing rate and some aspects such as uptake and depuration of chemicals are to date tested in field versus laboratory experiments. New experimental tools (e.g. cages and caissons) for exposure of selected animals in estuarine and marine waters have been developed to test effects and fates of chemicals, such as organic compounds, heavy metals, and oil under more natural conditions in

coastal waters. More recently effect testing on field-exposed animals was included. Marine organisms preferably used in the experiments comprise bacteria, algae, hydroids, polychaetes, mussels, shrimps, and fish.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Legislation, Federal Republic of Germany, Review.

INTRODUCTION

In 1966 the Priority Programme "Littoral Zone Research - Pollution of Coastal Waters" was inaugurated by the DFG for the purpose of studying the impact of industrial waste components and municipal wastes on the coastal environment. The centre of this programme was a special working group of scientists which should investigate the influence of pollutants on the organisms of the coastal area in its broadest sense, in laboratory experiments as well as under natural conditions. Emphasis has been laid on the elaboration of the scientific basis necessary to explore the relationship between the pollutant introduced into the sea and its effects on the ecosystem, in order to establish methods for routine monitoring of marine pollution.

A number of potential pollutants, such as acids, pesticides, heavy metals, crude oil, and other organic wastes were included at that time in specified research projects, which may be summarized briefly as :

- pollution of the German Bight caused by titanium dioxide production ;
- effects of dumping of sewage sludge ;
- the behaviour of tensides ;
- the entry and accumulation of heavy metals in the sea ;
- accumulation, transfer and transformation of chlorinated pesticides in marine food chains ;
- marine pollution by mineral oils ;
- culturing of test organisms for bioassays on pollutant toxicity

The results of the Priority Programme "Pollution in Coastal Waters" have been reviewed from about 250 publications, in an interim report covering the activities from 1966 through 1974 (Caspers, 1975b). The research programme during this period was a comparatively broad one which also included more general aspects of marine biology and marine analytical chemistry. The three following series of contributions are more closely related to aspects of ecotoxicology :

1. Effects of pollutants, cultivation experiments :
   Caspers (1968) ; Halsband (1968abcd, 1969, 1970, 1971, 1974) ; Rheinheimer (1968, 1970, 1974) ; Bock and Mann (1969) ; Janovic and Mann (1969) ; Kayser (1969, 1970ab, 1971, 1973) ; Kühnhold (1969, 1972) ; Stripp and Gerlach (1969) ; Rachor (1970) ; Rosenthal and Stelzer (1970) ; Bock (1971, 1972) ; Mann (1971, 1972, 1973ab) ; Stelzer et al. (1971) ; Horstmann (1972) ; Karbe (1972) ; Schneider (1972) ; Schramm (1972) ; Schulz-Baldes (1972, 1973, 1974) ; Braum (1973) ; Rosenthal and Sperling (1974) ; von Westernhagen et al. (1974) ; Thormann (1975) ; Rachor and Dethlefsen (1976).

2. Fate of pollutants including analytical chemistry :
   Reimann (1968) ; Ernst (1969, 1970abc, 1973) ; Hickel (1969) ; Ehrhardt and Blumer (1971) ; Ehrhardt (1972, 1975, 1976) ; Weichart (1972) ; Gadow and Schäfer (1973) ; Kremling (1973) ; Caspers (1975a) ; Derenbach and Ehrhardt (1975) ; Ehrhardt and Heinemann (1975) ; Karbe et al. (1975).

3. General aspects of biology and microbiology :
   Caspers (1967, 1975b) ; Weyland (1967) ; Giere (1968) ; Lorenzen (1971) ; Wachs (1971ab, 1972) ; Gerlach and Schrage (1972) ; Iturriaga and Rheinheimer (1972) ; Winter (1973).

In view of the importance of the pollution problem the Priority Programme was prolonged for some years. Special research projects were initiated since 1970 by the Federal Ministry for Research and Technology in the framework of the German governmental programme of environmental research. From 1976 - 1979 a prolonged Priority Programme was run, subdivided into four specific research topies : 1. analytical chemistry ; 2. bioindicators; 3. toxicity ; 4. fate of pollutants. During this period the attention was more sharply focussed on specified aspects of pollution. These

Table I. Species recently used in laboratory experiments or in test procedures under development. (A) : heavy metals ; (B) : organic compounds

Organism	Effect	Type of test compound	Reference
Bacteria			
Brackish and estuarine species	growth rate		Thormann and Weyland, 1979
Acinetobacter CS 13, (continuous culture)	growth rate	(A)	Than, 1980, 1983 (Fig. 1)
Bacillariophyceae			
Schroederella schroederi	growth rate	(A)	Kayser, 1977
Thalassiosira rotula	growth rate		
Dinophyceae			
Prorocentrum micans	growth rate, morphological alteration of cells	(A)	Kayser and Sperling, 1980
Ceratium furca	growth rate	(A)	Kayser, 1976,1977,1982
Scrippsiella faeroense			
Gymnodinium splendens			
Phaeophyceae			
Laminaria saccharina	photosynthesis growth rate	(A)	Markham et al., 1980
Hydrozoa			
Eirene viridula	toxicity	(A)	Karbe, 1972
Laomedea loveni	toxicity	(A)	Theede et al., 1979
Clave multicornis	toxicity	(A)	Fischer, 1978

Tunicata			
Ciona intestinalis	growth rate	(A)	Kayser, 1982
Ascidiella aspersa	toxicity		
Molgula manhattensis			
Bivalvia			
Mytilus edulis	bioaccumulation,	(B)	Ernst, 1975,1977,1979a,1979c
	elimination	(B)	
Polychaeta			
Nereis virens	bioaccumulation	(B)	Goerke and Ernst, 1977, 1980
Nereis diversicolor	elimination	(B)	Goerke, 1979, 1982,1984
Nereis diversicolor	growth rate, respiration rate	(A)	Müller, 1979
Crustacea			
Crangon crangon	growth rate, respiration rate	(A)	Müller, 1979
Tisbe holothuriae	dynamics of exploited populations	(A)	Hoppenheit, 1977 Hoppenheit and Sperling, 1977
Palaemon longirostris	elimination	(B)	Goerke, unpubl. work
Gammarus duebeni duebeni	bioaccumulation	(A)	Hoppenheit *et al.*, 1980
Echinodermata			
Psammechinus miliaris	early life stage	(A)	Klöckner and Rosenthal, 1983
Paracentrotus lividus	development		
Pisces			
Solea solea	bioaccumulation	(B)	Ernst and Goerke, 1974
	elimination	(B)	Ernst and Goerke, unpubl. work
Pomatoschistus microps	growth rate, respiration rate	(A)	Müller, 1979

studies have contributed significantly to a better understanding of the factors influencing the effects and fates of pollutants in the marine environment.

LABORATORY TESTS

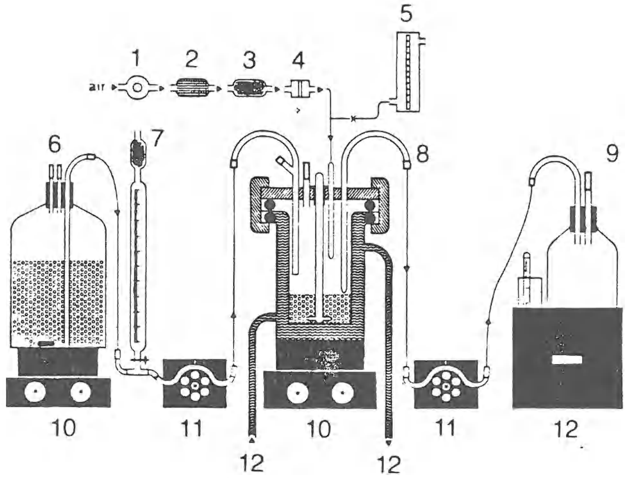

Fig. 1. Schematic view of a toxicity-test system with bacteria (Tan, 1983). 1 = membrane valve, 2 = activated charcoal, 3 = cotton plug, 4 = membrane filter, 5 = air-flowmeter, 6 = medium reservoir (10 l), 7 = medium flow-rate tube, 8 = culture vessel (1 l), 9 = effluent vessel (5 l), 10 = magnetic strirrer, 11 = peristaltic pump, 12 = temperature-control system.

The laboratory tests developed more recently to measure toxic effects and bioaccumulation of various pollutants on different marine species are given in Table I. An example of an experimental setup with bacteria is shown in Fig. 1. These tests were carried out with heavy metals, such as Zn, Cd, Hg. It should be noted that the majority of bioassays were conducted on single species. In order to draw ecologically valid conclusions other aspects should be taken into account as well, such as the effects of the toxicant at the population level (Hoppenheit, 1977 ; Hoppenheit and Sperling, 1977). Effects of environmental factors such as temperature and salinity should also be considered ; e.g. it was found that hydroids are more tolerant to Cd at low temperatures and high salinities and vice versa. These findings indicate that marine organisms living in brackish waters may be affected at lower pollution levels than those living under "optimal" marine conditions (Theede, 1980). As a consequence, critical pollutant concentrations established for normal marine conditions, are not necessarily applicable to estuarine conditions without taking into account specific safety factors or application factors.

Tests for the determination of bioaccumulation data were mainly conducted with organic compounds, such as pesticides and industrial chemicals. Bioaccumulation testing was systematically developed, using mussels (Mytilus edulis), polychaetes (Nereis virens and Nereis diversicolor), flatfish (Solea solea), and crustaceans (Palaemon longirostis) which appeared to be the most suitable test species. These organisms are widely distributed, can easily be collected and acclimated to laboratory conditions, and maintained in the laboratory for long periods at low food rations.

In view of the broad range of physicochemical properties of the substances to be tested, different methods of exposure and evaluation have been followed :

1. The determination of bioconcentration factors (BCF) in static exposures, in which the decrease of the pollutant concentration in the water was used to indicate the proximity of steady state conditions in the system (Ernst, 1977). This method is applicable to substances of low or moderate volatility and a water solubility allowing precise analytical measurements in the water without disturbing the system (Fig. 2).
2. If physicochemical properties of test compounds do not favour exposure according to 1), the animals are exposed to the toxicant in the water or the food without the implication or reaching the steady state level ; elimination rates can be measured when the pollutant level is high enough to allow a reliable analytical control of depuration. The elimination rate, determined as the time necessary for a 50 depuration from the initial level has been found to be an indirect measure for bioconcentration. For mussels (Mytilus edulis e.g.) the following relationship between the depuration half life $T_{1/2}$ (in h) and the bioconcentration factor was found (Ernst, 1984):

$$\log BCF = 1.327 \log T^{1/2} + 1.186$$

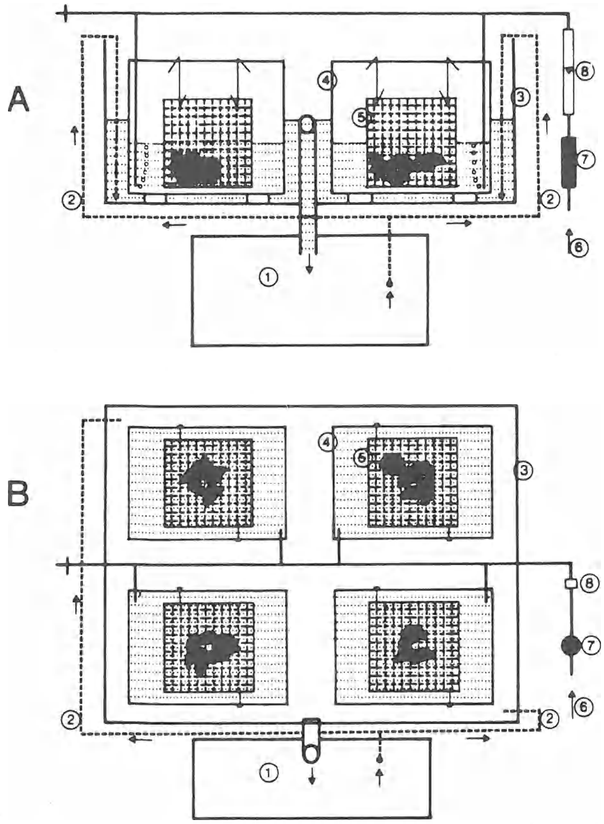

Fig. 2. Scheme of setup for bioaccumulation tests with mussels (from Ernst, 1979c). A = side view, B = top view, 1 = temperature-control system, 2 = water-flow, 3 = outer tank with water of constant temperature, 4 = glass tank with seawater, 5 = stainless steel baskets with test animals, 6 = air inlet, 7 = activated charcoal filter, 8 = air-flow meter.

Volatile substances and/or substances with low solubility may also be applied orally to the test animals at appropriate levels with a subsequent measurement of the elimination kinetics. A precise amount of chemicals can be administrated to animals trained for immediate uptake of food, via food particles dosed with the test compounds. This method was applied to flatfish

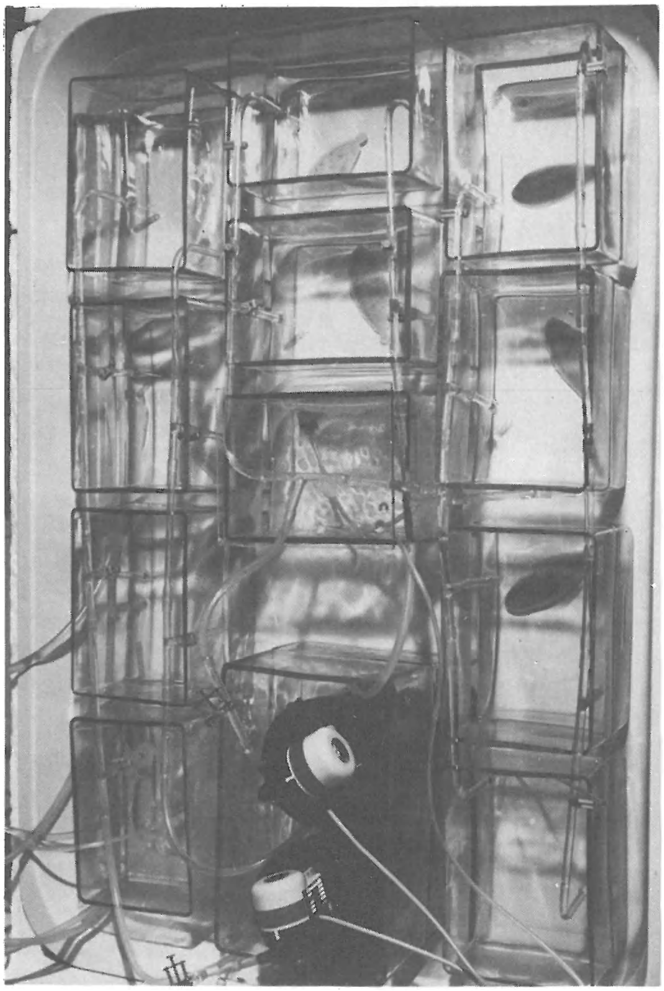

Fig. 3. Exposure tanks for biokinetic studies with flatfish, *Solea solea*. Water is circulated through activated charcoal filters during elimination (from Ernst and Goerke, 1974).

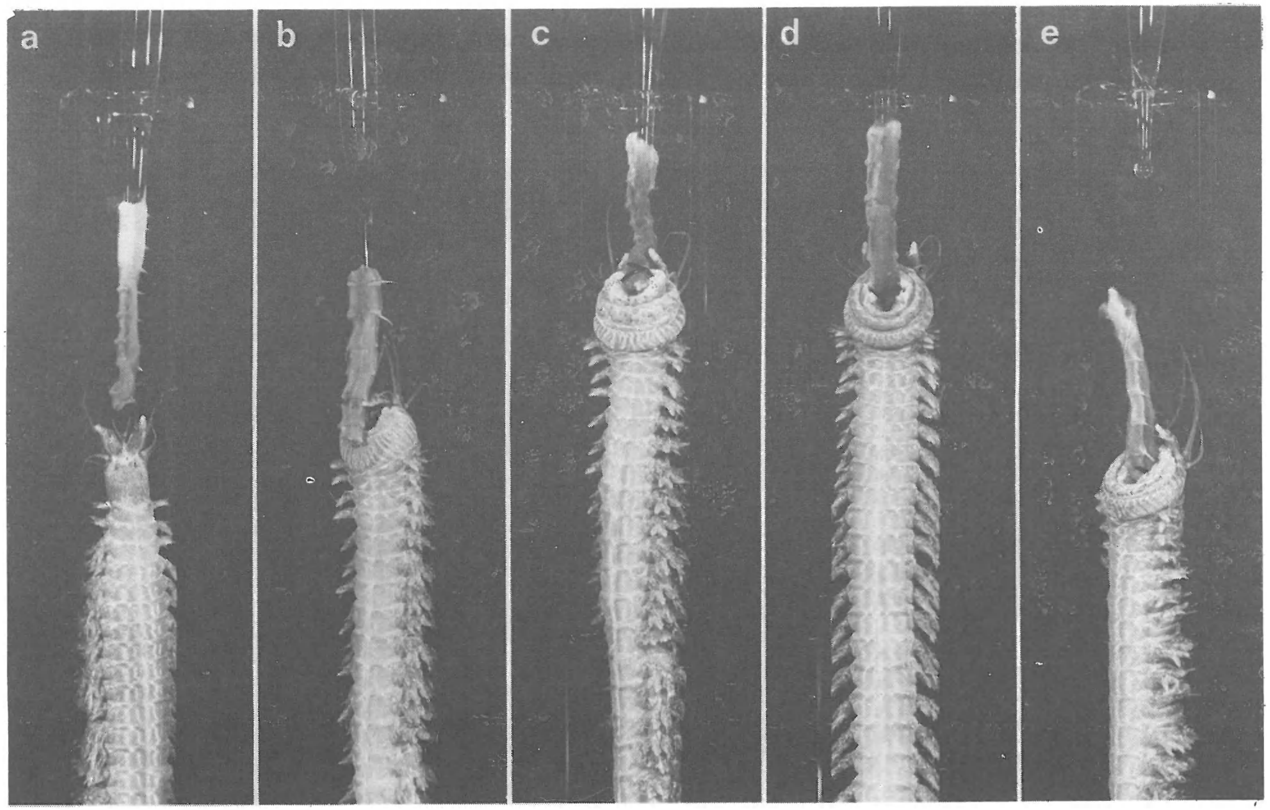

Fig. 4. Feeding technique of Neries virens with food particles dosed with test compounds (from Goerke, 1979).

(Ernst and Goerke, 1974) (Fig. 3), and was further refined to study the biokinetics of various pentachlorobipphenyls in the polychaete Nereis virens (Goerke and Ernst, 1977 ; Goerke, 1979) (Fig. 4.).

In recent years it has been tried to predict bioconcentration potentials from physicochemical properties such as water solubility and the n-octanol/water partition coefficient, which in turn can be calculated from the molar fraction of those compounds which do not form hydrogen bonds (Yoshida et al., 1983) :

$$\log BCF = 0.542 \log P + 0.124$$ (Neely et al., 1974 ; fish)

$$\log BCF = 0.85 \log P - 0.7$$ (Veith et al., 1979 ; fish)

$$\log BCF = 0.74 \log P - 0.53$$ (Ernst, 1982 ; mussels)

$$\log BCF = -0.523 \log L + 4.53$$ (Ernst, 1982 ; mussels)

with P = n-octanol/water partition coefficient, and
L = water solubility in $\mu g.l^{-1}$.

It should, however, be taken into consideration that the calculation of the BCF from these equations, with acceptable margins of error, is limited by the test animals' ability to transform the compound metabolically, such as by conjugation of hydroxylated compounds (Kobayashi et al., 1970 ; Ernst, 1979ab), by hydroxylation, or by other reactions, making a bioaccumulation test indispensable.

IN SITU TESTS

The results of ecotoxicological testing in the laboratory have in many cases, to be extrapolated to the conditions of the natural environment. In order to check the validity of laboratory work various efforts have been made to perform "in situ" tests which approach more or less the "real nature" conditions. Work of this kind is, however, expensive. Until now some experience has been gained with two systems :

CAGE SYSTEM

The purpose of the cage system is to follow :

1. bioconcentration of pollutants by placing suitable test organisms into cages exposed to an environment in which the pollutant concentration is known or can be monitored by analyzing integrated water samples ;
2. elimination of pollutants from test animals "loaded" with specific pollutants in the laboratory and kept for well-determined periods of time in the natural environment.

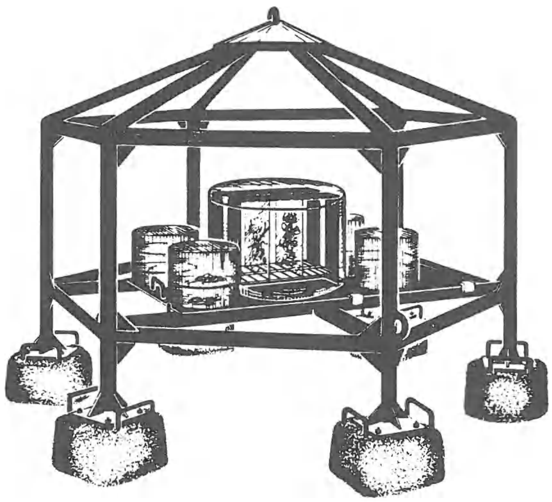

Fig. 5. Cage system for *in situ* tests (diameter approx. 2 m) containing small cages with mussels and tanks with polychaetes. Concrete feet allow mooring of the cage in shallow waters (from Ernst *et al.*, 1982).

An example of a cage as shown schematically in Fig. 5 illustrates the possibility of mounting various glass tanks in the system, for different test species, (*e.g.* polychaetes and mussels). In a number of experiments such cage systems were moored in the Weser estuary for periods of up to 8 weeks. In experiments on the biokinetics of polychlorinated biphenyls in the polychaete *Nereis virens* it was observed, that data from laboratory experiments were in good agreement with those obtained in a field study (Goerke and Ernst, 1984). Results of bioconcentration studies with the common mussel (Mytilus edulis), using the cage system combined with simultaneous water monitoring, for some organic chemicals, also matched quite well bioconcentration factors calculated from laboratory experiments (Ernst et al., 1982).

CAISSON SYSTEM

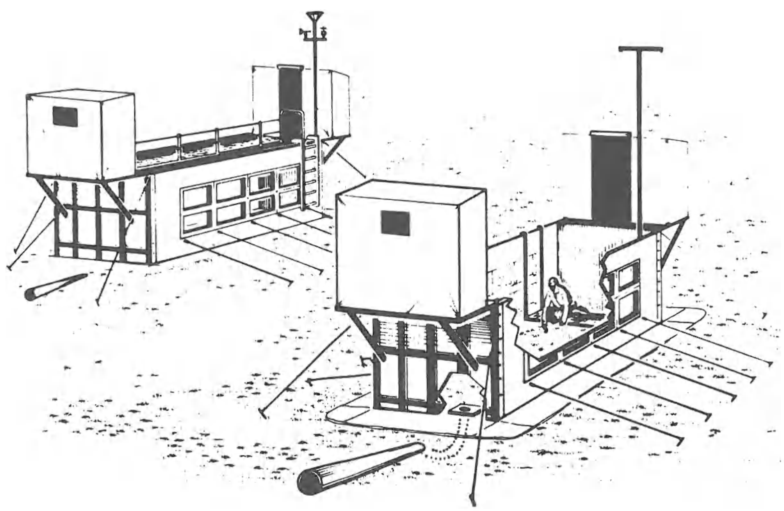

Fig. 6. Bremerhaven caissons as an example for in situ testing in tidal areas (from Farke et al., 1984).

The caisson system has been developed as a mobile instrument for field experiments in ecosystem and pollution studies in intertidal areas. The system is 5.6 m long and encloses an area of 13 m^2 of sediment (Fig. 6). By means of a tube system connected to the surrounding area the caisson can be flooded twice a day in the tidal rythm and pollutants can be dosed automatically. Due to the periodical exchange of water, only short-term tests are possible on plankton and seston ; long-term studies can be carried out with the benthic organisms enclosed in the system (Farke et al., 1984). In experiments conducted so far in the German Wadden sea, the accumulation of lead and chromium was investigated in molluscs, polychaetes, oligochaetes, and a crustacean (Schulz-Baldes et al., 1983).

Another promising approach in measuring in situ effects of pollutants is based on the idea of sampling animals from their natural environment to assess the possible effects resulting from pollutants to which they had been exposed. This type of study was conducted on flounder and herring by determining the reproductive success in relation to the levels of organohalogen compounds in the gonads and livers of the fish. For PCB and DDE threshold concentrations were found which affected the viable hatch of these animals (von Westernhagen et al., 1981 ; Hansen et al., 1983). In this connection it is interesting to note that early life-stages of sea urchins can be used for detecting changes in water quality. The usefulness of larval bioassay techniques with Psammechinus miliaris and Paracentrotus lividus for pollution monitoring was demonstrated by Klöckner and Rosenthal (1983).

CONCLUSIONS

Generally spoken the lowest toxicant concentrations necessary to produce deleterious effects are still substantially higher in most toxicity tests than those actually observed in the environment.

For the determination of acute toxicities a certain number of marine tests are available, some of which require further refinement for routine testing. To the contrary a much larger number of toxicity tests is available using freshwater species. Taking into account that for some compounds the application factors converting acute LC50 values to MATC values exhibit, for freshwater organisms, striking similarities with the factors obtained with marine species, it seems that there is no urgent need to develop new tests with marine species except if the new tests reduce the costs considerably.

There are considerable gaps, however, in our knowledge on the long-term effects of pollutants at subacute levels, and especially on the combined effects of organic compounds which occur in high numbers at different concentrations in estuarine and coastal waters (Weber and Ernst, 1983). Although there are safety margins of several orders of magnitude between actual pollutant concentrations in the open sea and their toxic concentrations as derived from laboratory data, the situation in estuaries is becoming more precarious, since the pollutant concentrations are usually much higher in these areas.

As mentioned above, for a number of organic chemicals bioconcentration factors may be predicted from their physicochemical properties. If one looks more carefully, however, at the components involved in these relationships it is striking that most of them belong to the group of more persistent chemicals. Organic compounds of different structure may be metabolized more or less easily in the test organisms, which will result in much lower BCFs than calculated from their solubilities or partition coefficients. Hence it followsthat atpresenta calculation of the BCF should not be used as a substitute for a bioaccumulation test. Although such tests are available for a number of species their general acceptance has still to be proven.

In situ systems for testing bioaccumulation, elimination, and toxic effects under natural conditions have been developed and are successfully used in the estuarine environment and tidal flats. However, their operation is expensive and their use will always be restricted to special problems.

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