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# LECTURE NOTES PREPARED FOR THE TRAINING WORKSHOP ON THE DESIGN OF MONITORING PROGRAMMES AND MANAGEMENT OF DATA CONCERNING CHEMICAL CONTAMINANTS IN MARINE ORGANISMS

(Athens, Greece, 22-26 June 1993)

Organised in the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean (MED POL - Phase II)

In cooperation with:

UNEP IAEA

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Athens, May 1993

# INTRODUCTION

The FAO/UNEP/IAEA Training Workshop on the design of monitoring programmes and management of data concerning chemical contaminants in marine organisms (Athens, 22-26 June 1993) is organised in the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL - Phase II) which constitutes the scientific and technical component of the Mediterranean Action Plan.

As stipulated in article 10 of the Barcelona Convention and in article 8 of the Land-based Sources Protocol, the Contracting Parties (Mediterranean countries) should establish pollution monitoring programmes. Such programmes are in fact in existence in most countries and copies of the data generated are transmitted to the Coordinating Unit for the Mediterranean Action Plan. In studying these data, through the help of experts, it has been come apparent that their utility is limited due to various gaps and inconsistencies.

The present workshop mainly aims to assist participants in their efforts to design or redesign their national monitoring programmes. The course will provide guidance on how programmes are designed by providing the criteria for the selection of species, contaminants, areas, etc. and by giving examples.

The course will also deal with the management and assessment of data.

The present document contains the material which will be presented by the lecturers. Some of the lecturers participate in activities of organizations responsible for the northern seas of Europe and especially of ICES and their experience will be valuable to the workshop. Some material will be distributed during he workshop. After the workshop, the lecturers will finalize their presentations which will be published in the MAP Technical Reports Series.

## TABLE OF CONTENTS


| | Page No. |
|---|---|
| Introductory lecture on background and scope by G.P. GABRIELIDES | 1 |
| Monitoring: its strategies, tactics and operational plans by S.R. CARLBERG | 9 |
| Water, sediment and fish; in which media should I look for the contaminants by S.R. CARLBERG | 37 |
| Which contaminants do I need to monitor? by S.R. CARLBERG | 43 |
| Correct sampling - What is that? by S.R. CARLBERG | 99 |
| Uptake, storage and elimination of chemical contaminants (metals, organochlorine compounds, radionuclides) in marine biota by C. CHASSARD-BOUCHAUD | 151 |
| Criteria for the selection of organisms for monitoring purposes by C. CHASSARD-BOUCHAUD | 169 |
| The role of consistency in the data collection process by R. FRYER | 181 |
| How many samples are necessary to detect important trends? by R. FRYER | 193 |
| Computerization of marine pollution data by A. AKSEL | 199 |
| Contaminant monitoring programmes using marine organisms: Quality Assurance and Good Laboratory Practice (Reference Methods for Marine Pollution Studies No. 57) | 201 |
| Alternative means of assessing data by N.W. GREEN | 235 |
| And so.... what action do we take by N.W. GREEN | 247 |

# INTRODUCTORY LECTURE ON BACKGROUND AND SCOPE

by

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## 1. INTRODUCTION

Pollution monitoring, in one form or another, started in the late sixties and early seventies. It was really an attempt to detect various contaminants in the marine environment and establish their levels. However, the data collected showed, in many cases, big variations which attracted the interest of research scientists. The variations studied,as far as biota are concerned, included that of contaminant concentration with the animal's age, length, weight, sex, sampling season and location. Data quality assurance (QA) programmes were initiated to minimise variations that could be attributed to sampling techniques, sample handling and chemical analysis. Early inter-calibration exercises for halogenated hydrocarbons showed variations of up to 1000%. Another interesting fact was that the concentrations of total mercury reported for seawater decreased through the years. This did not indicate a reduction in pollution but an improvement in the analytical procedures which paid more attention to sample contamination. Eventhough QA programmes are now implemented in all monitoring programmes the minute contaminant concentrations present in the marine environment still pose a problem.

By the end of the 1970's a large number of papers were published indicating the existing levels and possible variations of contaminants in the marine environment. The generation of all these data helped to convince Governments that action was necessary but could not be used to justify specific action, especially when difficult managerial decisions had to be taken and thus Governments thought they were not getting their money's worth. One of the first questions that scientists had to respond to, was whether the situation, as regards marine pollution, was improving or deteriorating. A positive answer would indicate that the measures taken by Governments were effective. However, an attempt to analyse the data statistically in order to provide a scientifically-based response to such a question brought to surface a number of problems which were not anticipated in advance. The major problem was that the real differences which scientists tried to observe were masked by natural and anthropogenic variations and that little effort was made to minimise the former ones. Consequently monitoring programmes had to be re-designed on a different basis.

## 2. OBJECTIVES

By far the most important step in designing monitoring programmes is the strict definition of the objectives of the programmes concerned. The purpose of marine pollution monitoring has been defined by a US interagency marine pollution committee as ".....to obtain time-series data for detecting significant changes.....to provide timely warning and other advice to management so that appropriate actions may be taken". However, most historical marine pollution programmes have proven useless in a management context (Segar and Stamman, 1986).

According to the Advisory Committee on Marine Pollution (ACMP) of the International Council for the Exploration of the Sea (ICES), "the ultimate purpose of monitoring is the control of exposure of the organism of interest, most likely to be first affected to the activity or contaminant in question, whether this target be Man or some specified element of the marine resource. Basically, monitoring looks at changes in the marine environment, and in practice, falls into one of the following three categories:

- monitoring for compliance purposes
- monitoring patterns and trends, or
- monitoring for research purposes

It should be noted that research purpose monitoring is generally the first and major step in establishing appropriate and efficient techniques for monitoring patterns and trends, and that in many cases the latter provides information that will be useful in the interpretation of compliance monitoring results" (ICES, 1988).

According to GESAMP (1991) the design of any monitoring programme should be based on clearly defined objectives and the formulation of testable hypotheses. Since monitoring is costly, data should only be collected that (a) are required to satisfy the objectives (b) are amenable to meaningful interpretation, and (c) have known precision and accuracy. Otherwise, technical and financial resources will be wasted and, in the case of compliance monitoring, the production of data of doubtful quality may limit their legal acceptance.

Compliance monitoring pre-supposes the existence of legal limits. These limits have different objectives and could be the maximum permissible level of a contaminant in seafood (protection of public health) or its concentration in an effluent (reduce inputs) or in the marine environment (any compartment or area). As stated above, monitoring for research purposes is essential in order to obtain the information necessary for designing programmes for management purposes. Programmes for such purposes should provide the manager with information that can enable him to (a) ensure that human health is not threatened, (b) ensure that unacceptable harm is not done to the marine ecosystem or resources, and (c) make informed decisions concerning continued, reduced, or expanded use of the sea for waste disposal and other activities.

Usually the objectives set are one or more of the following:

a) **the assessment of risks to human health** or the protection of human health. This is known as health-related monitoring and is normally carried out by national or local health services in a country. This could include monitoring of beaches and bathing waters for microbial pollution and monitoring of seafood for microbial and chemical pollution. In this case, fish samples are obtained from the market rather than sampled directly from the sea. The contaminant levels are normally compared to some standards legally adopted or not. Research oceanographic institutions do not normally show an interest in such activities; however, any biota samples analysed for other objectives and indicating contaminant concentrations higher than the accepted health limits are reported to the relevant authorities.

b) **the assessment of the effectiveness or efficiency of measures taken to reduce pollution.** Measures taken to reduce the level of marine pollution are primarily directed at the control and reduction of inputs of contaminants. This objective is therefore directly linked with objective (d) which monitors inputs. It is also necessary to control the quality of the marine environment with time and therefore trend monitoring has to be implemented.

c) **the assessment of damage to marine life** or the protection of marine life. Biological effects monitoring in a systematic manner is a relatively new activity. It can provide a measurement of the direct effect of adverse water and sediment quality on marine organisms. The basis of such monitoring is measuring the extent to which a specific biological response deviates from a normal value. There are reactions at the cellular level which are specific to certain groups of chemicals. Responses such as the induction of mixed-function oxygenases (MFOs) by organic compounds and of metallothionein heavy metals have the potential to be very sensitive and therefore act as "early warning" indicators. It is essential that biological monitoring should be integrated with chemical monitoring, so that the extent to which the measured effects can be ascribed to specific chemicals, can be established (GESAMP, 1991).

d) **the assessment of the inputs of contaminants** into the marine environment from various sources. This is known as load monitoring and is directly linked with objective (b). The main inputs are from land-based discharges. riverine sources, the atmosphere, and direct dumping.

e) **the assessment of the existing level of marine pollution** as a timely warning system. For this objective, water, sediments as well as organisms have to be monitored. As it can be seen, all objectives are inter-dependent and a careful programme design can satisfy them all.

For example, the Oslo and Paris Commissions have decided on a Joint Monitoring Programme with the following objectives:

a) the assessment of possible hazards to human health

b) the assessment of harm to living resources and marine life (ecosystems)

c) the earliest possible assessment of the existing level of marine pollution

d) the assessment of the effectiveness of measures taken for the reduction of marine pollution in the framework of the Conventions.

The Cooperative Monitoring Programme of ICES includes only analysis of fish and shellfish and has the following three objectives:

a) the provision of a continuing assurance of the quality of marine foodstuffs with respect to human health

b) the provision over a wide geographical area of an indication of the health of the marine environment in the entire ICES North Atlantic area

c) to provide an analysis of trends over time in pollutant concentrations in selected areas, especially in relation to the assessment of the efficacy of control measures

The second stage, after defining the objectives, is to decide on the methodology to achieve them and thus design a suitable programme.

## 3. PROGRAMME DESIGN

There are a number of factors to be considered in the planning of a monitoring programme to meet specific objectives. Some of these are:

a) which contaminants should be measured;

b) in which matrices should they be measured;

c) where should the samples be collected;

d) when should the sampling be done and how frequently;

If biota are going to be analysed, which species and tissue; also what size and how many individuals should be collected. If sediment is the matrix, then sampling technique, grain size and extraction method have to be decided.

The selection of contaminants depends on the objectives of the monitoring programme, the likely sources and the analytical capabilities of the participating laboratories. Matrices should be selected accordingly having also in mind where a contaminant would be predominantly associated. For example, one should not look for chlorobiphenyls in water since the octanol: water partition coefficient indicates otherwise.

At any rate, I do not intend to expand on these issues since the lecturers will go into these in detail during the week.

## 4. THE MED POL PROGRAMME

The MED POL programme was initiated in 1975. Phase I, which ended in 1981, consisted of baseline studies and pilot projects. The Food and Agriculture Organization of the United Nations, through its General Fisheries Council for the Mediterranean, was responsible for the technical co-ordination of the following:

a) Baseline studies and monitoring of metals, particularly mercury and cadmium, in marine organisms

b) Baseline studies and monitoring of DDT, PCB's and other chlorinated hydrocarbons in marine organisms

c) Research on the effects of pollutants on marine organisms and their populations

d) Research on the effects of pollutants on marine communities and ecosystems.

When approving MED POL - Phase II the Contracting Parties decided that its specific objectives are designed to provide them with:

- information required for the implementation of the Convention and the protocols;
- indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the protocols;
- scientific information which may lead to eventual revisions and amendments of the relevant provisions of the Convention and the protocols and for the formulation of additional protocols;
- information which could be used in formulating environmentally sound national, bilateral and multilateral management decisions essential for the continuous socio-economic development of the Mediterranean region on a sustainable basis;
- periodic assessment of the state of pollution of the Mediterranean Sea.

MED POL Phase II is organised on four levels:

a) monitoring of sources of pollution providing information on the type and amount of pollutants released directly into the environment;

b) monitoring of nearshore areas, including estuaries, under the direct influence of pollutants from identifiable primary (outfalls, discharge and coastal dumping points or secondary (rivers) sources;

c) monitoring of offshore areas (reference areas) providing information on the general trends in the level of pollution in the Mediterranean;

d) monitoring of the transport of pollutants to the Mediterranean through the atmosphere, providing additional information on the pollution load reaching the Mediterranean Sea.

Monitoring type (a) in fact includes pollutants discharged directly into the coastal waters from land-based sources, pollutants dumped directly into the sea, pollutants dumped in emergency or released accidentally into the sea and substances reaching the sea through natural (weathering, hydrothermal etc.) processes from land-based (coastal) or maritime sources. Substances to be monitored are generally these listed in the Annexes of the protocols.

Monitoring types (a) and (d) are to assess pollution loads from land-based sources and reaching the sea through the atmosphere respectively, while types (b) and (c) refer to levels in the environment. In fact, the four types indicate that the programme is interested in load monitoring and establishment of pollution levels.

For general monitoring purposes of coastal waters the following priority parameters were initially indicated:

- total mercury in organisms and sediments;
- cadmium in organisms;
- high-molecular weight halogenated hydrocarbons in organisms and sediments;
- petroleum hydrocarbons in water, sediments and oil residues (tar balls) on sea-shores;
- faecal coliforms in recreational waters and edible bivalves;
- basic oceanographic and meteorological conditions.

It was envisaged that after 3 years the following additional parameters would be added to the list:

- cadmium in sediments;
- organic mercury in organisms and sediments;
- total arsenic in organisms;
- selenium in organisms;
- lead in organisms;
- polynuclear aromatic hydrocarbons in organisms;
- additional organics (such as carcinogenic compounds) in organisms;
- radionuclides in organisms;
- faecal coliforms in sediments;
- pathogens in waters, sediments and bivalves;
- ecological parameters, such as productivity and community structure.

On the basis of the experience gained during the first five years of implementation of the monitoring component of MED POL Phase II, it was proposed to continue with the above parameters but with minor changes as follows:

Category I parameters:

- Total mercury in organisms and sediments
- Organic mercury in organisms
- Cadmium in organisms and sediments
- High molecular weight halogenated hydrocarbons in organisms and sediments
- Faecal coliforms in recreational waters and bivalves

Category II parameters:

- Basic oceanographic and meteorological parameters (e.g. salinity, oxygen, temperature, chlorophyll, wind)
- Floating tar balls and tar balls on beaches
- Total arsenic in organisms
- Radionuclides in organisms
- Pathogenic microorganisms
- Polynuclear aromatic hydrocarbons in organisms

The species recommended for monitoring purposes represented different ecotypes:

a) Bivalves

Mytilus galloprovincialis (MG), or Mytilus edulis (ME), or Perna perna (PP) or Donax trunculus (DT).

M. edulis, P. perna or D. trunculus can only be monitored as alternative species if Mytilus galloprovincialis does not occur in the area.

b) Demersal fish

Mullus barbatus (MB), or Mullus surmuletus (MS), or Upeneus molluccensis (UM).

M. surmuletus or U. molluccensis can only be monitored as alternative species if Mullus barbatus does not occur in the area.

c) Pelagic carnivore fish

Thunnus thynnus (TT), or Thunnus alalunga (TA), or Xiphias gladius (XG).

d) Pelagic plankton feeding fish

Sardina pilchardus (SP). Other clupeids should only be monitored as alternative species if S. pilchardus does not occur in the area.

e) Shrimps

Parapenaeus longirostris (PL), or Nephrops norvegicus (NN), or Penaeus kerathurus (PK).

N. norvegicus or P. kerathurus can only be monitored as alternative species if P. longirostris does not occur in the area.

## 5. REFERENCES

ICES (1988), Report of the ICES Advisory Committee on Marine Pollution. ICES Cooperative Research Report no. 160, pp.18-23

GESAMP (1991), Global strategies for marine environmental protection. GESAMP Reports and Studies no. 45, 36 p.

Segar, D.A. and E. Stamman (1986), Fundamentals of marine pollution monitoring programme design, Mar.Pollut.Bull., 17:194-200

# MONITORING; ITS STRATEGIES, TACTICS AND OPERATIONAL PLANS

by

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## INTRODUCTION

The aim of this lecture is to make the course participant or reader familiar with some well established monitoring programmes, some of their results, their strong and weak sides as well as giving an illustration on where these programmes are moving in their further development.

The first few headlines are addressing basic questions in a provocative manner to set the scene for the further presentation and discussion of this theme. Therefore, the main text of the lecture is kept short since this presentation relies primarily on the material in the different annexes.

On the other hand the annexes contain some material that will not be presented in the lecture. This is included to the benefit of those who want to further study the subject. Consequently, the participant is invited to take notes during the lecture in order to connect the material to his or her own needs and experience of the subject.

## WHAT IS MONITORING?

Given different cultural backgrounds the word "monitor" may have different meanings: to supervise, to overview or to watch etc.

In a more environmental sense the word, in its more positive and active understanding, means "to act as a watchdog" that detects changes in the environment, regardless if these changes are positive or negative.

One of several widely accepted definitions is that **monitoring is the repeated measurement of an activity or of a contaminant or of its effects, whether direct or indirect, in the marine environment.**

## WHY, AND WHAT, DO WE NEED TO MONITOR?

The environment is constantly changing. Most of these changes are certainly natural but Man and Man's activities cause several adverse changes as well as they may considerably change the speed and direction of many natural processes. Therefore, it is essential to review and describe the environmental status of an area and detect negative changes as early as possible in order to introduce corrective measures as early as possible. Another reason for monitoring is that when measures have been decided and introduced it is essential to follow the result and detect and report on the possible improvements. An example of the latter is the decision by ministerial

conferences within the North Sea and Baltic Sea communities to reduce by 50%, before the end of 1995, the discharges of nutrients and certain inorganic and organic contaminants.

Due to the high variability in the natural systems it may be very difficult to prove, or even to demonstrate, changes of that size. However, by setting realistic goals and objectives for our monitoring programmes we will be in a position to report results.

What do we need to monitor? Obviously we need to monitor those human activities that threaten the quality of the marine environment. This means nutrients and contaminants according to what is known about discharges of these substances.

However, measuring concentrations of contaminants does not tell us anything about their effects in a direct sense. In some cases we know at what contaminant concentration level we can expect a more or less dramatic effect in Nature but more often this dose-response relationship is not known. Therefore, it is essential that a good programme also includes monitoring of biological effects although it may be very difficult to link an observed effect with a certain concentration level of a certain contaminant.

In addition to this, there is a need to have "a monitoring of the unknown." WHY ? there is a very simple answer to that! We do not know the composition and content of all Man's discharges to the environment. Consequently, by definition, we do not know if we are monitoring all contaminants we should (most probably we are not) or if we are always looking for effects where they may or will occur.

Therefore, in addition to the biological effects monitoring mentioned above, in a good programme there should be a willingness and readiness to spend resources on surveys or studies having the potential of disclosing or revealing the unknown or the unexpected.

It is understandable if this cannot be done because resources are not available, <u>but it is a mistake to deliberately exclude this "watch-dog component" on the belief that we know exactly what should be monitored.</u>

A classical example of this is the discovery of PCB in the environment. In the (gas chromatographic) analysis of DDT in biota samples some strange and unexplained information (peaks in the chromatogram) appeared. The professional curiosity of one chemist led to retrospective analyses of archived biological samples from the Museum of Natural History (in Stockholm). Since all samples had an adequate historical record it was possible to trace back in time <u>when</u> these substances first appeared. Whith more detective work on industrial production, the PCBs came into focus and further analyses confirmed that the presence of PCBs was indeed the explanation for the peaks! Nobody would deny that PCBs have been, and still are, substances of great environmental concern.

**THIS CALLS FOR A STRATEGY - BUT WHAT EXACTLY IS A STRATEGY?**

Let us take it from the beginning! There is no unified way to define "strategy"! The word means different things to different people because they work in different organisations with different cultures!

The usual situation is the there is no clear distinction between "strategy" and "tactics."

In the figure in Annex 1 you can find one attempt (of several possible) to compare the thinking on this subject within the commercial business and the environment monitoring sector.The figure shows a pyramid with building blocks in three levels: strategy, tactics and operational plans.

In the commercial business sector one makes an important decision in setting up a company or starting a new activity, e.g. "We are going to be the biggest Volvo car supplier in Brazil." This statement is the business idea and it contains three crucial elements "Selling what" (Volvo cars), "How much" (the biggest car supplier) and "Where" (in Brazil).

This business idea is actually the strategy of the company! Their tactics will consist of such elements as whether they will set up offices and shops in all the places where they want to be present, or if they should work through local existing companies in a franchise system, whether they should offer leasing schemes or not, whether they should offer differentiated prices to different customer groups etc.

Their operational plans will describe in detail how to accomplish all this plus many other things as e.g. how to influence the market by advertising, how to deal with customer complaints etc.

In the field of monitoring a strategy could be "We must protect our national population from consuming shellfish that could cause shellfish poisoning." The tactics could include such elements as: is it sufficient to take samples from the catch that will be put on the market? should one sample some randomly selected areas or should one sample all areas where they fish plus all installations for shellfish aquaculture? Again, the operational plans would have to tackle practicalities such as frequency of sampling, methods for sampling, analysis and data reporting etc.

As international monitoring programmes are established by representatives from different administrations in different countries having different "cultural background" concerning language and management it is not at all surprising that the picture is less clear on how the words "strategy" and "tactics" are used. This is certainly not a criticism! The logical conclusion is that when these words are used it is advisable to find out what they stand for in the programme that is being discussed!

The International Council for the Exploration of the Sea (ICES), being a scientific advisory body to the Helsinki Commission and the Oslo and Paris Commissions, and a partner with the latter commissions within the North Sea Task Force, has worked out a monitoring strategy. The strategy is presented in detail in Annex 2. One may note in the figure in Annex 1 that the ICES strategy overlaps the areas of strategy and tactics and also that the operational plans of the Commissions (Guidelines) overlap the areas of tactics and operational plans. Again, this is not a criticism but rather a note that "the monitoring scheme" can be presented in different ways. There is no given answer to the question "which is the best or the correct way". The important point is that the monitoring must be part of a system with a holistic view as described in Annex 2!

## A COMPARISON OF SOME WELL ESTABLISHED MONITORING PROGRAMMES

1) The Joint Monitoring Programme of the Oslo and Paris Commissions

The Joint Monitoring Programme (JMP) of the Oslo and Paris Commissions (OSPARCOM) has been active since the principles of the programme were elaborated by the Joint Monitoring Group (JMG) in 1978.

The objectives and the structure (strategy and tactics!) of the programme is described in Annex 3 together with some other supplementary information. As can be seen in the annex the sampling is carried out at regular intervals. Also assessment of monitoring data for the various purposes is carried out in a regular way. Several assessment reports have been produced, either as internal documents for the OSPARCOM or as open publications.

2) The Monitoring Master Plan of the North Sea Task Force

The North Sea Task Force (NSTF) originates from a German political initiative to accelerate the work of the OSPARCOM, or more precisely, the work of the Commission as carried out for the North Sea area. A Ministerial Conference was arranged and one of the results was that OSPARCOM and ICES joined forces in 1988 and became equal partners in NSTF.

The Monitoring Master Plan (MMP) was designed with one overruling objective in mind; the 1993 Quality Status Report (QSR) of the North Sea as decided upon by the Ministerial Conference. The MMP focuses upon providing supplementary information to that collected under the JMP so that the QSR could give a complete description of the environmental situation in the North Sea. The MMP, therefore, was designed to: a) collect information on the distribution of well researched contaminants in areas normally not sampled, and, b) the occurrence and distribution of less well researched contaminants.

Examples from the structure of the MMP are given in Annex 4. The first assessment is the QSR that will be published in late 1993 as a preparation for the ministerial conference in 1994.

3) The Baltic Monitoring Programme of the Helsinki Commission

This programme was launched in 1979. There are two very interesting principles that makes this programme different from e.g. the JMP. The BMP Guidelines point out that for the understanding of the results of the environmental monitoring it is absolutely essential to understand the natural variations in the system. Therefore, such investigations are included in the monitoring (although partly on a voluntary basis)! Furthermore, it is stated that although all contracting parties are invited to contribute by monitoring in all subregions of the Baltic, there is a principle of strict regional responsibility. This puts the burden on identified countries to produce enough data from each subregion. The present programme is described in Annex 5.

The programme has produced one very extensive scientific assessment of the status of the Baltic marine environment published in 1981 and after that two periodic assessments (in 1986 and 1990). A third periodic assessment is being organised.

## STRENGTHS AND WEAKNESSES IN THE PROGRAMMES

The programmes bring Contracting Parties (member countries) and their institutions together in a cooperation within a coordinated plan. This is cost effective in the sense that the undeniably high costs for marine environmental monitoring is shared by all parties. Furthermore, it offers the possibility to collect more data from a large sea area than a single country normally could afford to do.

The fact that assessments of the quality of the marine environment are carried out jointly has both practical, scientific and political advantages. When an agreed assessment is ready it serves as a common managament instrument for further regulations e.g. discharge reductions etc.

As pointed out above, all three monitoring programmes have produced, and are producing, useful assessments.However, one should not deny that there are weak sides as well. The weaknesses are both in programme structure and in their execution. If they are not identified at an early stage they certainly become obvious in the assessment work. Therefore, it is important to create and maintain the feedback so that conclusions from the assessments are used also to improve the monitoring programmes and their plans.

Generally there are insufficient data to allow an adequate assessment and essential information on inputs is often missing. Some of the major reasons are that the agreed monitoring plans are often not carried out completely and sometimes monitoring guidelines are not followed. This means e.g. that mandatory determinands are not measured and/or too few stations are sampled. Furthermore, information on the very necessary 'supporting parameters' (e.g., salinity, fat content) is often missing. As neither the JMP nor the MMP provide biological monitoring data, except for benthos, it would be recommendable to encourage the expansion of these monitoring programmes to cover also birds and mammals.

A strong point of the MMP is that, often for the first time, data were collected in areas far enough from local pollution sources to be used as a reference area.

The advantage of applying biological effect techniques is the fact that they integrate the effects of a wide range of stress factors, so they can provide information on the effects of substances that are not measured in the chemical monitoring programme. However, the application of certain techniques (benthic community studies, EROD, fish diseases and oyster larvae bioassays) is still under development and we will, therefore, have to wait some more years before they will be widely used on a routine basis.

## WHERE DO WE GO FROM HERE?

Under this heading some very recent information on the present work regarding developing and restructuring of the monitoring systems under the Conventions mentioned earlier will be described.

**ANNEX I**

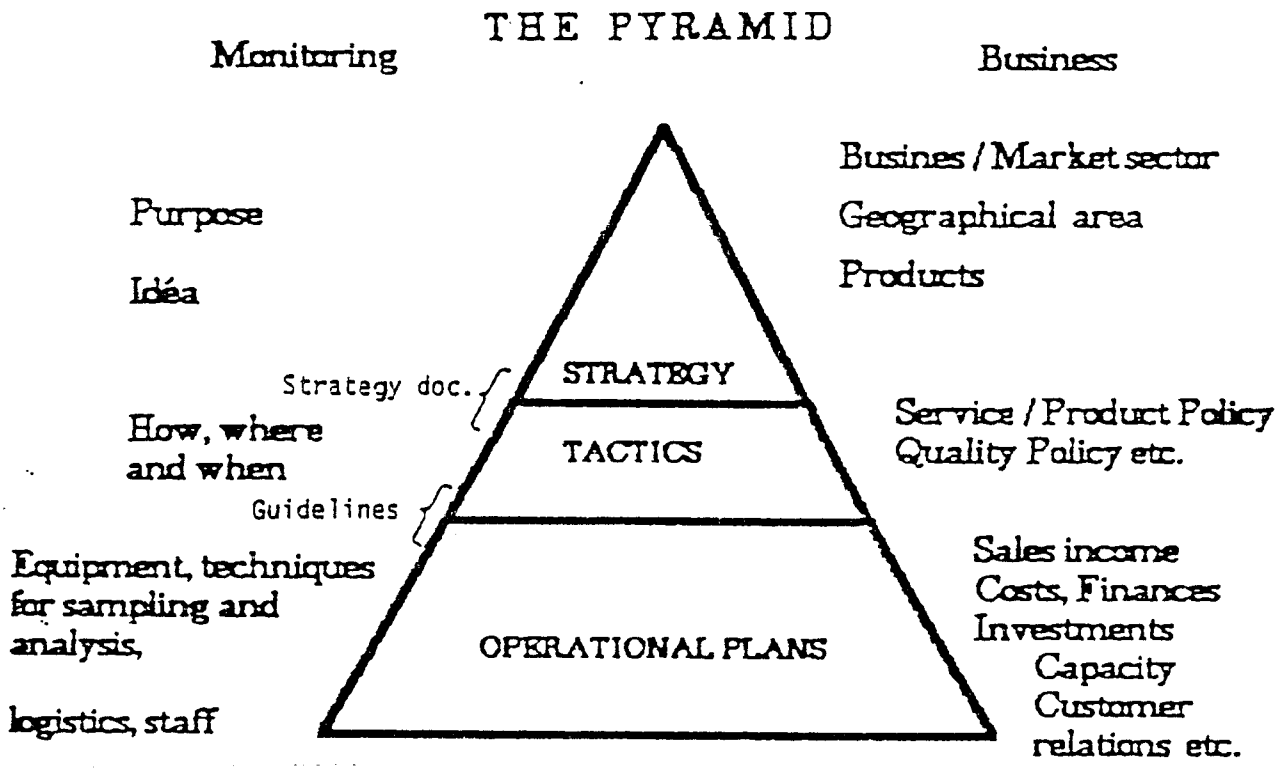


Figure indicating strategy, tactics and operational plans

# ANNEX II

## THE ICES monitoring strategy

**This text is an extract from the Report of the ICES Advisory Committee on Marine Pollution (ACMP) 1988. (Coop.Res.Rep. No 160).**

## 4. MONITORING STRATEGIES

Based on the report of the Working Group on Environmental Assessments and Monitoring Strategies (WGEAMS), the ACMP reviewed guidelines on the Philosophy, Principles, and Strategy of Monitoring, issued as an annex to the WGEAMS report. On the basis of this paper, the ACMP has prepared the following guideance on this topic.

### 4.1 Philosophy, Principles and Strategy of Monitoring

#### 4.1.1 Introduction

This paper is a revised and extended version of the previous advice on the question of monitoring in relation to the marine environment given by ACMP in its 1978 report (Cooperative Research Report No. 84, Annex 1).

#### 4.1.2 Definition

In the context of assessing and regulating environmental and human health impacts of anthropogenic activities, specifically the introduction of wastes, monitoring is the repeated measurement of an activity or of a contaminant or of its effects, whether direct or indirect, in the marine environment.

#### 4.1.3 Objectives

The ultimate purpose of monitoring is the control of exposure of the organism of interest, most likely to be first affected, to the activity or contaminant in question, whether this target be Man or some specified element of the marine resource. Basically, monitoring looks at changes in the marine environment, and in practise, falls into one of the following categories:

- monitoring for compliance purposes,
- monitoring patterns and trends, or
- monitoring for research purposes.

It should be noted that purpose monitoring is generally the first and major step in esablishing patterns and trends, and that in many cases the latter provides information that will be useful in the interpretation om compliance monitoring results.

#### 4.1.4 Strategies

All too often monitoring programmes continue unchanged long after they have ceased to produce useful data in the context of the original objective.

It is essential that monitoring should have a clearly defined objective, that the measurements made are designed so as to be usable in meeting that objective, and that the results be reviewed at regular intervals in relation to that objective. The monitoring scheme should then be continued, revised or even terminated, as appropriate.

Then, before any programme is drawn up and any measurements are made, the following questions should be addressed:

1) What exactly do we wish to measure?

2) Why do we wish to monitor a particular variable, contaminant or biological effect?

3) How can that measurement be achived and is monitoring the most appropriate approach?

4) In what compartment or at which locations can measurements most effectively be made?

5) For how long do we need to continue measurements in order to meet the originally defined aim?

Although much is now known about the marine environment, there is still a lack of basic knowledge and adequate description of the marine ecosystem as a whole. In order to be able to assess the quality and health of the environment, there is a need to be able to determinate natural variability and corresponding induced effects. This can only be achieved through monitoring programmes that include biological effects or produce data that can be compared to known and agreed effects levels, i.e., environmental quality standards.

Since the environment is subject to natural changes, e.g., climatic, it is important that an understanding is established of these natural changes and the way they might affect either contaminant levels or biological characteristics. This implies longterm data sets on parameters which establish the basic characteristics of the marine media, e.g., water temperature and salinity, transparency, chlorophyll levels, and nutrient concentrations.

There is also a fundamental need to recognise the requirements of decision-makers. It is especially necessary to recognise that they will require the results on finite time scales and that they will expect the results to be presented in a readily interpretable form. Thus, in common with the formulation of a regional assessment, part of which will be based on monitoring data, a basic requirement of monitoring is that it yields accurate data. These data, in turn, will provide the basis of sound, reliable advice to administrators on the need for environmental protection measures or the effectiveness of protection measures already introduced.

It is apparent that the responsibility to undertake monitoring programmes rests with organisations having interests in the nature and scale of particular areas and problems. From the single effluent outfall or river, to estuaries and shelf seas, through to the open ocean, there is a gradation of responsibility from local authorities to international organisations. However, with respect to the protection of the environment, global

consideration are of primary importance and, as far as possible. they should be taken into account in developing standards and scientifically based quality criteria.

With these strategic considerations as background, the following illustrates a practical approach to the planning of monitoring programmes.

It is first essential to identify the resource at risk and then the substances or activities most likely to threaten the resource it is desired to protect. This obviously requires, at an early stage, a fairly thorough assessment of what activities are already in progress and which substances are likely to enter the area in question and via which routes. Alternatively, information on inputs can be used to focus environmental monitoring effort on those substances or effects which are most likely to be encountered at levels considered to be significant. An understanding of input fluxes to the marine environment will frequently permit even sharper focussing of the monitoring effort.

The next step assumes the existence of maximum acceptable levels of inputs or effects in order to protect the resource in question. This requires an understanding of working relationship between rates of input and environmental concentrations, ideally via a model of exposure pathways, and the effects it is desired to avoid. It also assumes that a maximum acceptable level has been set or can be derived. Standards do not always exist and it is often argued that they cannot be defined. However, the use of simple data, even data from acute toxicity tests, can be used to derive preliminary quality objectives which will, if they incorporate appropriate safety factors, suffice pending the derivation of more accurate standards from more thorough biological testing.

4.1.5 Guidelines

The following general guidelines should provide some assistance in selecting the most appropriate monitoring techniques for the problem in question. Detailed guidelines on monitoring using marine organisms, sediments and sea water have been provided in past ACMP reports and are currently under review; details will be published separately as soon as the reviews are complete. If the following guidelines are followed, it is hoped that some of the effort currently devoted to routine monitoring can be deployed to research programmes designed to established a better understanding of the marine environment and what constitutes a pollution problem.

4.1.5.1 Contaminants

In the past, the selection of contaminants has been based largely on the black and grey lists of the various pollution prevention conventions. It is now apparent that some of those originally listed substances do not present serious pollution risks in a marine context, whereas other substances not identified in the lists probably do. This illustrates the need to review monitoring activities from time to time in order to assess the need for their continuation.

It is, therefor, recommended that the choice of which contaminants need to be monitored should depend primarily upon the perceived aim, i.e., why there may be concern, and secondly, on whether there is real reason for

concern in the area in question, i.e., is there an input of sufficient scale and is there a target likely to be affected. One certainly should not have to monitor regularly for all contaminants at all sites and it should not be necessary to use more than one substrate or effect to meet each aim. Thus, for example, if it is possible to analyse samples of fish liver for a range of metals and establish trends over time, it is not necessary also to measure any of these same contaminants in sea water for the same purpose. Matrix tables could be provided to cover the various options available and indicate the most appropriate choice.

The following table is given as an illustration.

Table 1

Marine matrix selection for contaminants monitoring in relation to the protection of human health.

| Matrix | Contaminant | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PCB | Lindane | Hg | Cd | Cu | Zn | As | Cr | Ni | Pb |
| Water | | | | | | | | | | |
| Sediments | | | | | | | | | | |
| Shellfish | + | + | + | + | | | [4] | | | + |
| Fish muscle | | | + | | | | [4] | | | |
| Fish liver | x[2] | x[2] | | [1] | [3] | [3] | | [3] | [3] | |

+ = primary matrix
x = secondary matrix

Notes and Qualifications:

[1] If fish liver is not a consumed fisheries product, ignore entry.

[2] If fish liver is not a consumed fisheries product and there remain human helth concerns, transfer attention to fish muscle.

[3] These contaminants are not normally of concern in respect to the comsumption of fisheries products.

[4] While arsenic exists in significant amounts, e.g., in plaice muscle and crustaceans, its chemical form makes it of little concern in respect to fish consumption and human health.

4.1.5.2 Biological Effects

At present, there is a wide variety of techniques available that are capable of demonstrating whether or not an effect occurs. Some are simple to conduct, others more complex, and not all are readily amenable to use in the

field. A difficulty in many cases is that although an effect is clearly detectable, its significance is unclear in terms of the well-being of the organism or species in question. Such techniques are not suitable for routine application to monitoring programmes and are probably best regarded at present as research techniques. From the standpoint of monitoring as defined in this document, the most useful biological effects are those that can be interpreted as being likely to affect adversely the ability of the species to survive, grow normally or reproduce. Ideally, the effect should be linked to a particular contaminant or source of contamination, but the fundamental requirement is that the effect is liable to have harmful consequences. Experience suggests that no one technique is likely to suit all situations.

4.1.5.3 Data Quality

Whilst it is obvious that good quality data are necessary at all times, attention should be paid to the level of accuracy and precision required. This can only be judged in relation to the aim. For example, if one is looking for trends at the ± 20% level, a high degree of precision will be called for (accuracy of data will also be important, in particular where several laboratories are to be used and data are referred to an agreed standard). However, if, on the other hand, one is demonstrating compliance with a standard which is several times higher than the concentrations actually being encountered, the level of precision (and accuracy) required is lower. There may be occasions when it is extremely difficult to measure accurately the parameter of interest, e.g., river inputs. In such situations, the limitations of the data must be clearly stated and, if comparisons are made between data from different sources, it is essential that the data compared be collected according to a common pattern, so as to eliminate differences related to procedures. Equally importnat is the statistical reliability of the original sampling design an the interpretation of the results.

In cooperative programmes involving several laboratories, it will of course be necessary to ensure that all participants are producing comparable data. For new contaminants this may not initially be possible and it may, therefore, be appropriate to allow a single laboratory with proven capability to conduct preliminary measurements in order to demonstrate the scale of a problem. If further measurements are considered necessary on a wider basis, it is almost certain that national authorities would wish to assure themselves of access to the data at the earliest possible opportunity. This would necessitate measures to assure the comparability of data produced by the different organizations in the different countries but the principle of having lead laboratories for particular contaminants, at least one per country, would facilitate achieving this end. The use of quality assurance procedures is strongly recommended.

4.1.5.4 Reporting Data

Once the monitoring programme is underway, it will be necessary from time to time to report the data to some coordinating centre so that they can reviewed and assessed relative to the originally stated aim and/or established standards or criteria. It is essential that the data be reported in adequate detail to meet this requirement. In this context, however, it should be noted that although it is now relatively easy to transmit data from centre to centre by tape, diskette or electronically, collecting and recording data involves effort and costs money. What is collected and transmitted should, therefore, be tailored to the need and be the minimum necessary to meet that need.

## 4.2 Future of ICES Monitoring Programmes

The ACMP discussed the future of monitoring programmes coordinated by ICES, on the basis of a review of this topic in the WGEAMS report and the monitoring strategy guidelines agreed in Section 4.1, above. The ACMP agreed to keep this topic under review in the light of impending developments, particularly those regarding the North Sea.

# ANNEX III

## "Principles and Methodology of the Joint Monitoring Programme"

Extract from the Oslo and Paris Commissions manual:

### A1: INTRODUCTION

The objective of this Introduction is to provide a brief description of the origin, development and present status of the Joint Monitoring Programme. It should not be regarded as substitute for the more precise and detailed information contained in the subsequent chapters of this Manual.

## PRINCIPLES AND METHODOLOGY OF THE JOINT MONITORING PROGRAMME

### INTRODUCTION

1. The obligation of the Oslo and Paris Commissions to conduct monitoring programmes is laid down in the texts of the two Conventions. Article 13 of the Oslo Convention reads as follows:

"The contracting Parties agree to institute, in cooperation with appropriate international organizations and agencies, complementary or joint programmes for monitoring the distribution and effects of pollutants in the area to which this Convention applies."

Article 11 of the Paris Convention is more elaborated and reads:

"The Contracting Parties agree to set up progressively and to operate within the area covered by the present Convention a permanent monitoring system allowing:

- the earliest possible assessment of the existing level of marine pollution;

- the assessment of the effectiveness of measures for the reduction of marine pollution from land-based sources under the terms of the present Convention.

For this purpose the Contracting Parties shall lay down the ways and means of pursuing individually or jointly systematic and ad hoc monitoring programmes. These programmes shall take into account the deployment of research vessels and other facilities in the monitoring area,

The programmes shall take into account similar programmes pursued in accordance with conventions already in force and by the appropriate international organisations and agencies."

2. Article 18 of the Paris Convention further states that the Commission shall, by unanimous vote, adopt programmes for monitoring as provided for in Article 11.

3. The monitoring function is exercised for the two Commissions in many ways but notable within the framework of the Joint Monitoring Programme (JMP). The principles of the JMP were first elaborated by the Joint Monitoring Group (JMG) in 1978 and have been further developed since then, allowing the Commissions to fulfil the requirements of the Conventions. Each Commission also monitors the quality and quantity of pollutants introduced into the Convention area. The relevant information is compiled in reports on the quantities dumped (Oslo) or in reports on inputs to the sea via watercourses, direct discharges and via the atmosphere (Paris). The overall system allows the commissions to assess the consequences of decisions taken in implementation of the Conventions. The results of the Joint Monitoring Programme are reported to the Joint Meetings of the Oslo and Paris Commissions and published in the Commissions' Annual Reports.

Objectives of the JMP

4. It is the duty of both the Oslo and Paris commissions to examine the condition of the seas covered by the Conventions and the effectiveness of measures adopted. The JMP of the Oslo and Paris Commissions focuses on three compartments: marine organisms, seawater and sediments. Monitoring is carried out in more than sixty JMP areas for the following purposes:

(a) the assessment of possible hazards to human health;

(b) the assessment of harm to living resources and marine life (ecosystems);

(c) the assessment of the existing level of marine pollution (spatial distribution) and

(d) the assessment of the effectiveness of measures taken for the reduction of marine pollution in the framework of the Conventions (temporal trend assessment).

Purposes (a) and (b) are applicable only to marine organisms. For purpose (c), all three compartments are monitored; whereas for purpose (d) only organisms and sediments are monitored as a general rule.

5. In addition to these assessments, the JMG is also charged with the evaluation of the monitoring of dumping grounds for industrial wastes, sewage sludge and dredged spoils. Another task of the Group is the evaluation of inputs to the marine environment from land-based sources.

Principles of the JMP

6. When the JMP was planned in the late 1970s two criteria were agreed which should be fulfilled for data to be regarded as results of the Joint Monitoring Programme:

.1 the JMP should be based on existing national monitoring programmes rather than on a new joint programme as such;

.2 the JMP should be harmonised by use of uniform sampling procedures and by uniform treatment of samples, by intercalibration of the methods of analysis and by standard reporting procedures.

A third feature of the JMP which has proven to be important is the joint assessment of the collected data. The assessment procedures established enable the Commissions to obtain a clear picture of the current levels of monitored substances in the marine environment and to make the results available to the scientific community and to the interested public.

Geographical coverage

7. Although the JMP aims to achieve a coverage of the whole Convention area, it is recognized that each country has the option to select the areas and compartments which they monitor for each of the selected substances, in the light of inputs and levels found. Monitoring is therefore carried out at geographical locations (JMP areas) chosen by the Contracting Parties themselves and not imposed by the Commissions. In some areas organisms only are samples; in other areas only one (or more) of the declared purposes of the JMP are achieved.

8. Depending on the monitoring purpose, samples may be taken from different locations. For purpose (a) commercial fishing grounds near contaminant sources are the main targets. Purpose (b) and purpose (d) require sampling stations near known sources of contaminants in order to study their influences on the ecosystem or any effects of measures taken to reduce inputs from such sources. Sampling stations for purposes (a), (b) and (d) are normally situated in coastal areas. Purpose (c) monitoring requires a different sampling network extending to the more open sea areas in order to establish geographical gradients of contaminants.

9. The JMP now covers most of the coastal areas of Contracting Parties to the Conventions. It also includes some relatively open sea sites in the Irish Sea, North Sea and Skagerrak.

Frequency of sampling

10. The Commissions have agreed that sampling should be carried out according to the following frequencies:

Purpose (a):In even years. First time in 1984.
Purpose (b):On an experimental scale, no frequncy fixed.
Purpose (c):Every fifth year. First time in 1985.
Purpose (d):Annually. First time in 1983.

11. The monitoring of organisms for purpose (a), the assessment of possible hazards to human health, was last carried out in 1986. Reviewing the results of those data, the Commissions decided to accept that there is little evidence that present levels of contaminants in fish and shellfish pose a threat to human health anywhere in the Convention area. Consequently the sampling and analytical procedures for purpose (a) monitoring were replaced by a requirement that Contracting Parties provide information on national (food) standards or guidance values and how these have been compiled with. In addition, the data collected for purpose (c) and purpose (d) may be used to assess human health risks.

Determinands to be monitored

12. It was agreed that initially the JMP would cover only three substances: mercury cadmium and PCBs. In 1985, the Commissions agreed that mandatory determinands to be measured under the JMP should be:

in seawater: cadmium, copper, mercury and zinc;

in organisms: cadmium, copper, lead, mercury, zinc and PCBs;

in sediments: cadmium, copper, lead, mercury and zinc.

To the extent possible, Contracting Parties are requested to measure on a voluntary basis gamma-HCH in organisms and sediments and PCBs in sediments.

13. Substances other than those listed above are also studied on a voluntary basis within national monitoring programmes and the results are submitted to the JMP data bank.

Data Quality Assurance

14. In order to obtain comparable data, the JMG has developed a number of guidelines which have to be followed in sampling and analysis for the JMP:

- guidelines for the sampling and analysis of trace metals in seawater under the JMP;
- guidelines to be followed for sample collection, preparation and analysis of organisms in the context of the JMP;
- guidelines for temporal trend analysis of data on contaminants in fish samples for purpose (d) of the JMP;
- guidelines for the sampling and analysis of sediments under the JMP.

15. Besides these guidelines, the JMG has adopted specific advice from ICES to be followed in the JMP, with regard to biological effects monitoring and the monitoring of sediments.

16. For analytical quality assurance at inter-laboratory level a number of intercalibration exercises have been carried out. Such intercalibration exercises, which are normally organized by ICES, have covered trace metals in seawater and in estuarine waters, PCBs in seawater, trace metals and PCBs in biological tissues and trace metals in sediments. A current intercalibration exercise on analyses of CBs in marine media takes account of recent developments in the analytical methods available for such measurements.

17. In order to restrict costly intercalibration exercises to a minimum, the use of certified reference material for the internal laboratory assurance of the quality and comparability of the data is recommended.

Data handling

18. The monitoring data is submitted to ICES, which holds the Commissions' monitoring data bank, using standardized reporting formats. The data are accompanied by commentaries giving a national interpretation of

the results. All data are submitted to a screening and validation procedure before final inclusion in the Commissions' data bank.

Data assessment

19. Pre-assessment of the monitoring data are carried out by invited experts in the framework of an Ad Hoc Working Group on Monitoring, and make use of ICES' statistical treatment facilities. The final assessment reports are examined and endorsed by the JMG itself, before being submitted to the Commissions for approval and publication.

North Sea Monitoring Master Plan

20. In 1988, the Oslo and paris Commissions and ICES jointly established the North Sea Task Force (NSTF) in response to a request of the Second International Conference on the Protection of the North Sea to enhance scientific knowledge and understanding with regard to the North Sea. Where possible, the NSTF will make use of the results of existing JMP monitoring in carrying out its main responsibility: the preparation in 1993 of a regional assessment (Quality Status Report) of the North Sea.

21. As one of the tools for fulfilling this task the NSTF has developed a Monitoring Master Plan (MMP) for the North Sea. Mandatory determinands under the MMP are cadmium, copper, lead, mercury and zinc, and also CBs, alph-HCH, gamma-HCH, HCB and a range of nutrient determinands.

Future plans

22. With regard to the JMP, the Commissions agreed in 1988 that the principal aim at present should be to consolidate the JMP. It was agreed that this could best be achieved by monitoring existing JMP areas more regularly for the specified parameters and in accordance with the agreed frequencies and guidelines for sampling, analysis and assessment.

23. The 1990 purpose (c) monitoring will focus on sediments and on a study of organisms aiming at supplementing the 1985 baseline study on organisms. The 1990 monitoring under the MMP and the monitoring within the framework on the JMP in that year will be closely coordinated. It is expected to get results also from locations within the Convention area which, as yet, have not been covered by monitoring activities within the JMP. Both the MMP and the JMP will incorporate in 1990 as many biological effects studies as possible. Also in 1990 the Paris Commission will embark upon a comprehensive study of riverine inputs and a comprehensive atmospheric monitoring programme. The data obtained in these studies will be used in conjunction with JMP data in order to allow an overall assessment of the status of the marine environment.

## ANNEX IV

## Examples from the structure of the NSTF/MMP

## Extract from the North Sea Task Force Monitoring Master Plan (NSTF/MMP). (North Sea Environment Report No 3).

1.1.3 The assessment of the existing level of marine pollution (JMP purpose (c) - Table 2)

Water

A distinction is to be made between nearshore and offshore waters. In the Former, marked salinity gradients occur, and contaminant distribution is more likely to be influenced by riverine or land-based inputs. In the latter, which is more from the above-mentioned inputs of contaminants, gradients are normally substantially less marked.

The use of water analysis to reflect current levels of marine contamination is attractive in that it concerns the important aqueous phase, the environment in which both biota and sediment exist. However, considerable efforts are still required to improve the comparability of analytical performance among laboratories engaged in sea water analysis in member countries. The requirements for precision and accuracy of analysis at low concentrations limit the the number of determinands that can be considered in offshore waters to mercury, cadmium, copper, zinc, lead and lindane, all at secondary matrix level. Even in these cases, it would be essential for each laboratory to establish in-house quality control procedures, and for rigorous attempts to be made to establish comparability between laboratories, with particular attention being paid to lead.

In nearshore waters subject to anthropogenic influences, concentrations may be somewhat more variable, and chromium and nickel analyses might be added to the above list. The same quality assurance precautions would be needed. In nearshore waters it is necessary to take account of any correlation between contaminant concentrations and salinity, and of the influence of the concentration and composition of suspended matter on the dissolved contaminants.

Sea water is not a matrix of choice for CBs, as the octanol:water partition coefficients indicate that the compounds would be predominantly associated with sediment or biota.

The concentrations of arsenic naturally present in sea water make the discrimination of anthropogenic influences from natural processes difficult and, therefore, sea water is not indicated as an appropriate matrix.

There are some sheltered bays or lagoons in which the inputs of contaminants are sufficiently large to cause marked elevation of contaminant concentrations in sea water, or which changes in concentrations can be expected. As agreed be the Commissions, in such areas it might be appropriate for national authorities to give more prominence to water analysis in monitoring programmes, but this should not be regarded as general recommendation.

Sediments

There is very considerable emphasis laid on the use of surficial sediments as primary matrix for most of the contaminants. Participating laboratories should take full account of the most recent advice on the selection of sampling locations and methods (see, e.g., Section 15, 1986 ACMP Report (Coop.Res.Rep. No 142); Annex 2, 1983 ACMP Report (Coop. Res.Rep. No. 124); Annex 2, 1984 ACMP Report (Coop.Res.Rep. No. 132)). Areas of high sedimentation and low bioturbation rates are particularly favourable. It is also necessary to subject the samples or data to appropriate normalisation procedures to compensate for the natural distribution of contaminants in relation to the texture, provenance and grain-size of the sediment.

Biota

Both sediment and shellfish are indicated as primary monitoring matrices for tributyl-tin (TBT). Whilst the main area of concern over TBT is its effects on shellfish, particularly molluscs, for example, oysters and dogwhelk, these organisms are by nature of limited geographical distribution. TBT, and its derivatives DBT (dibutyl-tin) and MBT (monobutyl-tin), can be found in sediments, especially near shipyards and busy shipping lanes, in harbours and marinas and, at least until recently, in the vicinity of some mariculture operations. The monitoring of sediment for these compounds would allow the use of a single matrix in a wider range of environment (e.g., into low salinity areas of estuaries) than would be possible using a single molluscan species.

Matrix Table 2

In relation to the assessment of the existing level of marine pollution
(i.e., contamination [JMP Purpose (c)]
(This matrix table must not be considered independently of the preceding text)
(see also extensions to this table, from the 1990 Report of the ACMP, p. 36)

| Matrix | Contaminant | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PCB/CBs | Y-HCH | Hg | Cd | Cu | Zn | As | Cr | Ni | Pb | TBT |
| Nearshore water | | P | P[1] | P[1] | P[1] | P[1] | | P[1] | P[1] | P[1] | S |
| Offshore water | | S | S[1] | S[1] | S[1] | S[1] | | | | S[1] | |
| Surficial sediments[2] | P | | P | P | P | P | P[5] | P | P | P | P |
| Shellfish | S[3] | S[3] | S[1] | S[1] | | S[1] | | | | S[1] | P |
| Fish muscle | | | T[1,4] | | | | S[1,4] | | | | |
| Fish liver | S[4] | | T[1,4] | | | | | | | T[1,4] | |

P: primary matrix
S: secondary matrix
T: tertiary matrix

Notes and Qualifications:

1. Potential addition/alternative to sediment measurements in areas where sediment conditions are not wholly favourable.

2. Should be accompanied by organic carbon measurements and appropriate normalisation procedures, following the most recent ICES guidelines for monitoring contaminants in sediments.

3. Could be carried out on an opportunistic basis, as may provide additional information on distribution.

4. Sedentary species only (e.g., flatfish).

5. The signal-to-noise ratio for discriminating between anthropogenic and natural influences is extremely low.

CBs: Chlorobiphenyls on an individual basis, congener Nos. 28, 52, 101, 118, 138, 153 and 180.

**ANNEX V**

**The Baltic Monitoring Programme**

BALTIC SEA ENVIRONMENT PROCEEDINGS

NO. 27 A

# GUIDELINES FOR THE BALTIC MONITORING PROGRAMME FOR THE THIRD STAGE

PART A.
INTRODUCTORY CHAPTERS

BALTIC MARINE ENVIRONMENT PROTECTION COMMISSION
- HELSINKI COMMISION -
December 1988

## PREFACE

The Guidelines for the Third Stage of the Baltic Monitoring Programme (BMP) are based on the Guidelines for the Second Stage of the BMP, published by the Commission as Baltic Sea Environment Proceedings No. 12 (BSEP No.12). They have been revised by an expert group nominated by the Commission. The group was chaired by Dr. Gunni Aertebjerg and experts from all the baltic Sea States participated in the work, with assistance from the International Council for Exploration of the Sea (ICES) and experts of the Baltic Marine Biologists (BMB).

The ninth meeting of the Helsinki Commission (15-19 February 1988) accepted the Guidelines in general as HELCOM Recommendation 9/7. The Commission recommends that the Governments of the Contracting Parties to the Helsinki Convention should apply the Guidelines for the Third Stage of the BMP, i.e. from 1989 to 1993, and also, whenever possible, to follow the Guidelines in the monitoring of the internal waters as well. The data is to be submitted to the data bases of the Commission, as specifies in the Guidelines.

The Guidelines for the Third Stage of the BMP are published in the BSEP series as four separate volumes (27 A, 27 B, 27 C, 27 D) and also as one combined volume of loose sheets.

The contents of the Guidelines for the Third Stage of the BMP is as follows:

BSEP 27 A; Part A;Introductory Chapters
27 B; Part B;Physical and Chemical Determinands in Sea Water
27 C; part C;Harmful Substances in Biota and Sediments
27 D; part D;Biological Determinands

Volumes B, C and D are intended to be used together with Part A, which contains general information on e.g. station networks, sampling requirements and data submission.

Any correction or proposals for improvements concerning the content of these Guidelines are welcomed, and to be addressed to:

Baltic Marine Environment Protection Commission
- Helsinki Commission -
Mannerheimintie 12 A
SF - 00100 Helsinki
Finland

Tel.: 90 - 602 366
Tlx.: 125105 hlcom sf
Tfx.: 90 - 644 577

Possible comments concerning the formats prepared by the ICES should be addressed to the ICES, accordingly, as indicated in the formats.

# A. INTRODUCTORY CHAPTERS

## 1. Introduction

### a) The aim of the baltic Monitoring Programme

The aim of the baltic Monitoring Programme (BMP) is to follow the long-term (annual and long periods) change (trends) of selected determinands in the Baltic ecosystem.

Monitoring data form a part of the background information for an appropriate assessment of the state of the marine environment and for a forecast of possible man-induced changes. In order to register such man-induced changes, the natural changes of different elements of the ecosystem must be known. Therefore, monitoring will often include registration of more or less "natural" conditions. In its more restricted sense, the term is applied to the regular measurement of contaminant levels in relation to set standards or in order to judge the effectiveness of a system of regulation and control. Monitoring does not encompass experimental laboratory studies and scientific investigations, which, nevertheless, may be off importance in the planning of future monitoring activities.

### b) General outline of the programme

The monitoring programme is intended for implementation in several stages.

The first stage (1979-1983) was experimental in character and served as a pilot programme comprising a limited number of stations and measurements but, nevertheless, providing a basic coverage of the major aspects concerned. The second stage (1984-1988) provided for a more frequent coverage of representative stations in the main sub-areas of the Baltic, in addition to the requirements of the first stage.

The experience gained during the two previous stages has now been used to further improve the programme for the third stage, that will start in 1989. The duration of the third stage will be five years.

The objective of the third and further stages is an optimization of the programme according to the experience gained and knowledge available in order to provide data for the preparation of more comprehensive assessments of the state of the Baltic marine environment.

### c) Monitoring in coastal waters

Bearing in mind that the Baltic Monitoring Programme in the open sea (outside territorial waters) is in general supplemented by national monitoring programmes and input studies in coastal waters in accordance with Article 4, Paragraph 3 of the Convention, it is recommended that the determinands and methods included in the Guidelines should be used whenever possible. In addition, other determinands may be used when deemed necessary for the understanding of regional problems.

In order to achieve an appropriate assessment of the state of the marine environment of the Baltic Sea Area, the Contracting Parties are requested to report compiled results of coastal monitoring in a generalized form. The status of coastal waters should be reported on a voluntary basis every fifth year, in order to be available for the preparation of the periodic assessments. The first reports should be available in 1993.

To facilitate the comparability of such reports, their contents should preferably be arranged as follows:

1. Introductory remarks
1.1 General description of the national monitoring programme for coastal waters
1.2 General review of national monitoring activities carried out during the preceding 5 years
1.3 Introductory review of other available information

2. Information on results of monitoring in coastal waters and related studies
2.1 Hydrographic and basic hydrochemical determinands
2.2 Harmful substances in sea water and sediments
2.3 Harmful substances in selected species
2.4 Biological determinands

3. Potential items for the periodic and/or specific assessments, and other related proposals

4. Conclusions

d) Monitoring programme for the third stage

The Baltic Monitoring Programme is described in the Guidelines in four main parts, as follows:

A. Introductory Chapters
B. Physical and Chemical Determinands in Sea Water
C. Harmful Substances in Biota and Sediments
D. Biological Determinands

In these revised Guidelines, a new code system for BMP stations is applied with references given to the former names of the stations.

The list of determinands to be monitored is divided into two groups: determinands which are essential for inclusion in the programme (obligatory determinands), and determinands which are desirable, bur for certain reasons cannot be made obligatory at this stage (tentative determinands). The tentative determinands include determinands for which suitable intercalibration among laboratories should be carried out successfully before their inclusion as obligatory in the general monitoring programme, and determinands which still require considerable effort with regard to both development of methods and intercalibration.

The analysis of harmful substances in sediments is not at present included in the BMP. However, consideration of this item is under way within ICES, and advice in relation to studies of sediments will be provided later. A reporting format for data on contaminants in sediments has been inserted in the present Guidelines (Section C.III.2.).

The species chosen as test organisms and the sampling procedures recommended for monitoring harmful substances in biota are to provide a picture of the levels of harmful substances in the organisms studied, and to determine trends in their levels over time.

Human health aspects associated with the consumption of contaminated fish are covered to a certain extent by the sampling of fish species of importance in human consumption, but they have not been given primary consideration in the selection of sampling procedures for the BMP. The sampling requirements directly concerned with human health are generally different from those for the assessment of trends in contaminant levels in the marine environment and, therefore, both cannot be fully covered in the same programme. Thus, it has been assumed that human health aspects will be dealt with more directly in national programmes concerned with fish and shellfish taken for human consumption.

In terms of joint or co-ordinated programmes, it can still be difficult to obtain results which are comparable from country to country. Such comparability should be a long-term goal associated with a co-ordinated monitoring programme and it should be facilitated by the use of good laboratory practice and quality assurance programmes, including the conduct of intercalibration exercises when appropriate. For the present, emphasis can best be placed on the development of trend analysis in each country, which has been shown to provide very valid results.

Part C of the Guidelines contains recommendations for only those species of organisms which are specifically selected for the BMP. However, sampling procedures have also been drawn up for selected coastal water species in order to enable comparability of the results obtained on the national level.

Part D concerns biological determinands. For the purpose of assessing the nature, extent and effects of pollution in the baltic Sea Area, regular monitoring of ecological determinands forms an important basis. Microbiological determinands are a new subject of the BMP, included in the present Guidelines.

It seems realistic to implement the BMP on a moderate basis with a few strategic stations in different parts of the baltic Sea, but with enough determinands to give a reasonable overall picture of the ecological changes in the sea.

## 2. Station grid and maps

The contracting Parties to the Helsinki Convention are invited to participate in the Baltic Monitoring Programme on a national, bilateral and multilateral basis in order to achieve an optimal spatial and temporal coverage of the Baltic Sea Area.

The Contracting Parties have agreed ti implement the Baltic Monitoring Programme generally according to a responsibility principle reflecting their wishes to concentrate the main part of their monitoring activities on certain areas.

The main responsibilities are as follows:

| | |
|---|---|
| The Baltic Proper | Finland, the German Democratic Republic, Poland, Sweden and the USSR |
| - The Gulf of Bothnia | Finland and Sweden |
| - The Gulf of Finland | Finland and the USSR |
| - The Sound and the Kattegat | Denmark and Sweden |
| - The Great Belt | Denmark |
| - The Bay of Kiel and the Bay of Mecklenburg | The German Democratic Republic and the Federal Republic of Germany |

# WATER, SEDIMENTS AND FISH; IN WHICH MEDIA SHOULD I LOOK FOR THE CONTAMINANTS ?

by

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(Preliminary version)

## 1. INTRODUCTION

The greater part of the inorganic contaminants discharged to the marine environment from various sources are also available in Nature as natural compounds. This is essential to underline since it has some important consequences from a monitoring perspective:

* there are certain natural concentration levels (background levels)
* there are established mechanisms for their distribution
* there are established mechanisms for their interaction with biological processes
* organisms have in many cases developed a tolerance to a certain degree (e.g. a certain concentration) to these substances

On the other hand, the major part of the organic contaminants disharged are synthetic which means that they are unknown in Nature. From a monitoring perspective this means that:

* there are no natural concentration levels (background levels)
* there are sometimes no established mechanisms for their distribution
* they often interfere with natural processes in organisms
* organisms have in some cases a natural or developed a tolerance to a certain degree (e.g. a certain concentration) to these substances
* in many cases they are extremely toxic to organisms.

## 2. SOME PROPERTIES OF INORGANIC CONTAMINANTS, MAINLY METALS

A short repetition of some well known facts of relevance:

* most of the pure metals are practically insolube in water
* many inorganic metal salts have a moderate to high solubility in water, whereas others are almost insoluble

* many organic metal salts have at least a moderate solubility in water

* once the metal is in solution it will not evaporate but it will undergo various chemical reactions.

## 3. DISTRIBUTION OF METALS, ACCUMULATION AND INTERACTION WITH BIOLOGICAL PROCESSES

A metal that is discharged to a water area in its non-dissolved state simply comes as a particle e.g. as mineral particles from mining. The particle will settle to the sea bottom usually within a rather short distance. If the particle is very small it may become adsorbed on organic material such as humic substances or ingested by organisms together with their food. From its position on the sea floor or in the organism it may then undergo chemical reactions that bring the metal into solution. If the particle has sedimented it usually will dissolve only very slowly. Therefore, metals concentrate in sediments and, if the sedimentation rate is sufficiently high and no or little resuspension occurs, sediments can provide an excellent record of contaminants input over shorter or longer periods.

A metal discharged as a dissolved substance will interact with the chemical environment in the water in different ways. The metal may react chemically and form an insoluble compound. When e.g. cadmium is discharged in an area where the bottom water is stagnant and contains hydrogen sulphide, insoluble cadmium sulphide will form when the two water masses are mixed. The cadmium sulphide will precipate and, while it may be carried a geographical distance by water currents, it will eventually sediment to the bottom.

A dissolved metal that does not precipitate will not stay as a "naked" positively charged ion but will form various complexes with the negative ions in the water. This will in many cases increase the solubility of the metal. Furthermore, different complexes of the same metal may have different (degree of) biological effects.

The dissolved metal, or the metal adsorbed to organic particles and ingested by organisms such as zooplankton, may interact with biochemical reactions e.g. in enzymatic processes. Metals may also become linked to proteins and accumulate in certain tissues as e.g. in filter feeding mussels or in fish liver.

The concentration of a metal (or rather its complexes!) may certainly increase in the water if there is a major discharge. However, since the water masses are in almost constant motion it is unlikely that metal concentrations will become high with the exception of near the discharge point and in very local areas with limited water exchange.

It is important to underline that the examples given are parts of a system that is dynamic. Although the processes have different speeds the metal may pass from one compartment of the environment to the other, and often in a reversible way. Refer to Figure 1 for a principal presentation and to Figure 2 for a more detailed illustration.

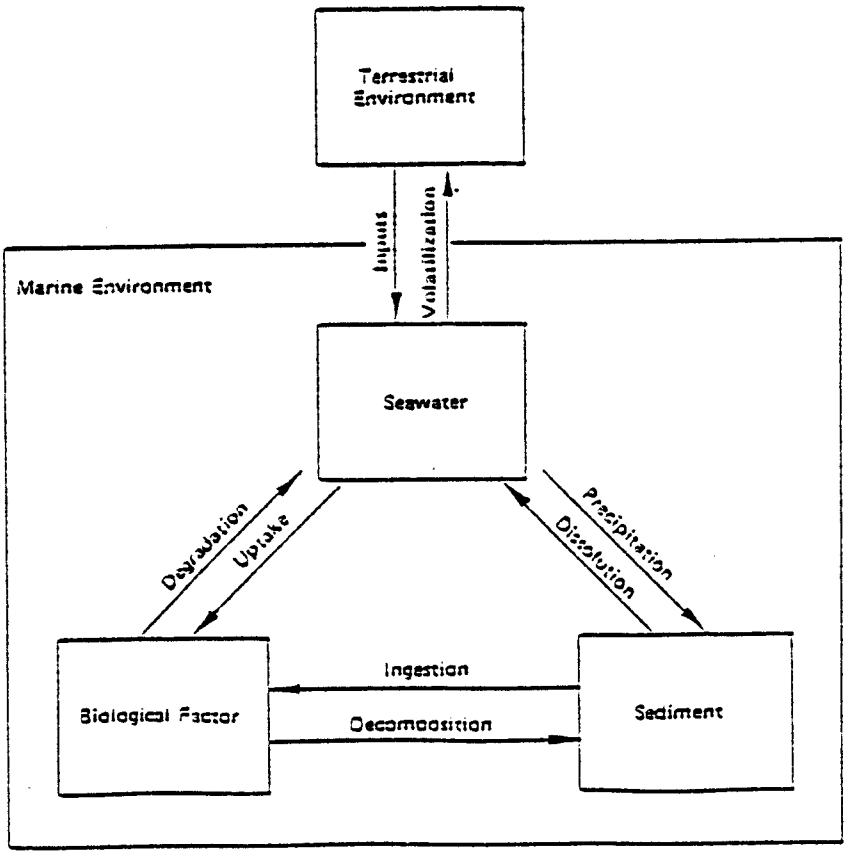


Fig. 1 A general presentation of processes influencing the distribution of contaminants in the environment

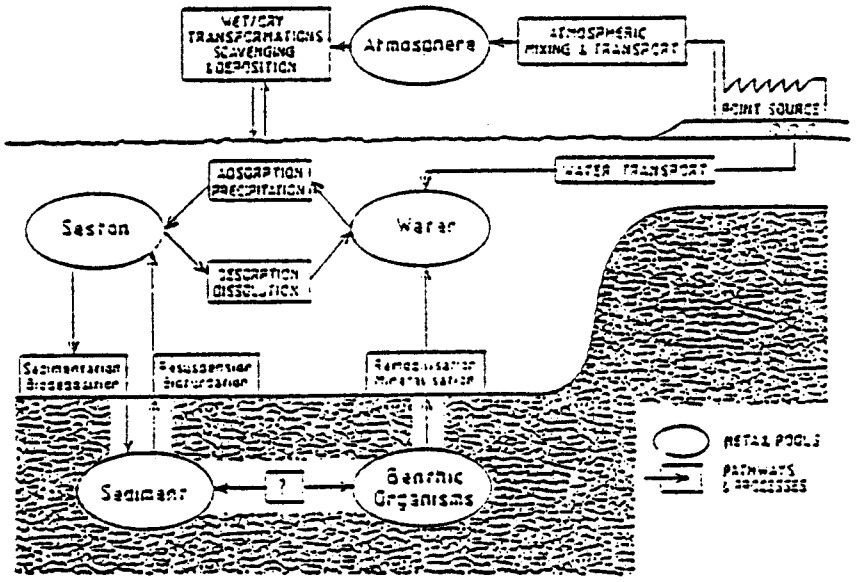


Fig. 2 A more detailed presentation of processes influencing the distribution of contaminants in the environment

## 4. SOME PROPERTIES OF ORGANIC SUBSTANCES

A short repetition of some well known facts of relevance:

* organic substances range widely in solubility in water (consider such extremes as alcohol and DDT)

* most organic substances do not dissociate in water which, together with the low solubility, make their reactions slow in the water phase

* many organic substances have a high volatility and they may therefore evaporate to a high degree

* most of the organic contaminants of concern for the marine environment are lipophilic which means that they do not dissolve in water but can redily dissolve in lipids or in organic solvents.

## 5. DISTRIBUTION OF ORGANIC SUBSTANCES, ACCUMULATION AND INTERACTION WITH BIOLOGICAL PROCESSES

An organic substance such as ethanol, that immediately dissolves in water, will be quickly mixed into the water and become dispersed and transported in the same way as many inorganic substances. High concentrations in the water phase will therefore be only a temporary phenomenon. Evaporation to the atmosphere can occur if there is a large spill of the substance, but since the dissolution is quick the major part of the discharged amount may still go into the water phase. These organic substances will not enter organisms by dissolution in fatty tissues to any significant degree.

If there is a major spill or discharge of oil (mineral oil or vegetabilic oil), or a substance or mixture of substances behaving like an oil, the physical processes may be of very significant importance for the removal and modification of the discharged material. Evaporation from e.g. a crude mineral oil may actually be, quantitatively, the most important process involved in removing the oil from the sea! After evaporation of the most volatile parts of the oil the remaining part may have a density higher than water and will therefore sink to the bottom and be incorporated in the sediment. Refer to Figure 3 for further details.

The most important properties of the organic contaminants of concern for the marine environment are, from a monitoring perspective, their lipophilicity and the fact that when they undergo chemical modification by various biotic or abiotic reactions the reaction products are often as harmful as the parent substance.

Because of their lipophilicity these substances may easily become adsorbed or absorbed to organic particles such as humic substances or phyto- and zooplankton in the water. Via these particles the contaminants then continue into the food web and into higher organisms (mussels, fish, birds and mammals).

These lipid soluble contaminants accumulate in biota in two ways. Firstly, they enter and accumulate in the fatty tissues of the "first" organism e.g. a mussel. As the organism grows the concentrations of the contaminant increases in the specimen. This is called bioconcentration. Then

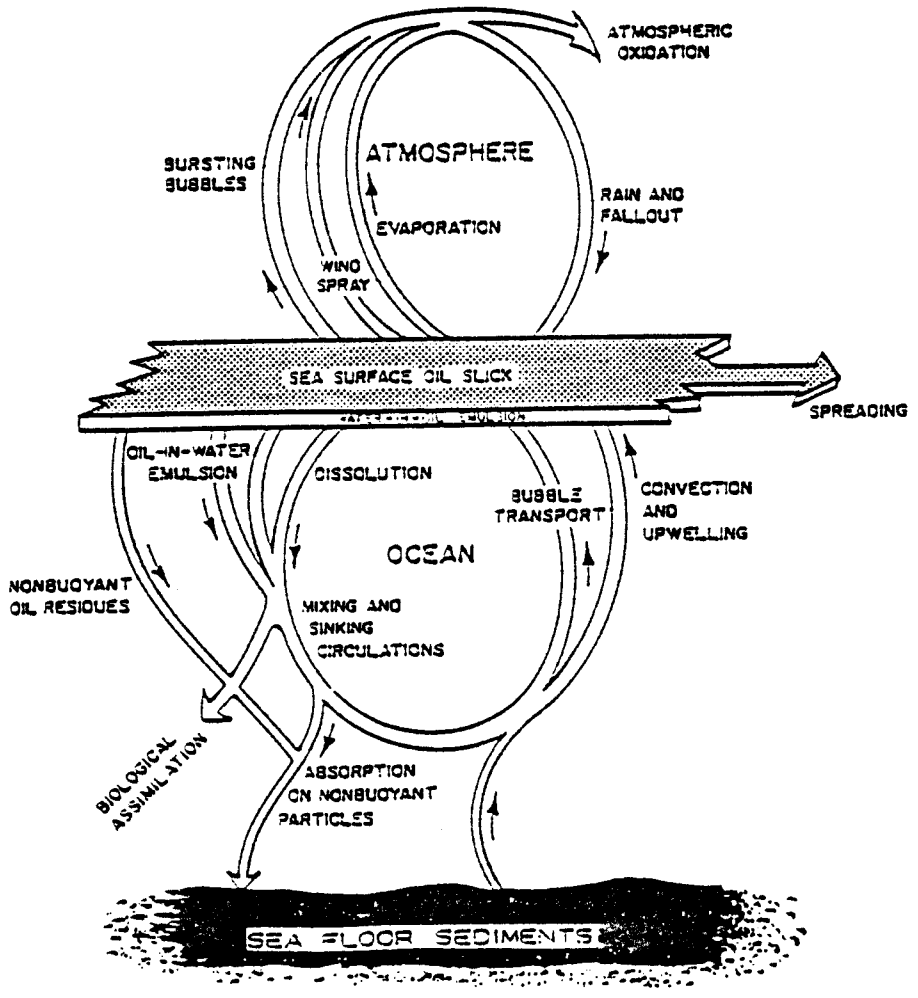


Fig. 3 A general presentation of processes influencing the distribution of mineral oil in the environment.

the mussel is consumed by the "second" organism, which may be a fish. Since a single fish consumes a great number of mussels, all having their content of the contaminant, the fish receives a higher body burden of the contaminant. This way of accumulation through the various levels in the food wed is called biomagnification.

## 6. WHERE TO MONITOR; IN WATER, IN SEDIMENTS OR IN BIOTA?

In principle, the simplest general answer to the question is: go for the best signal-to-noise ratio!

In all quantitative chemical analyses there is a certain signal (noise) from the detector in the measurement instrument as soon as it is ready for operation. If one analyses a sample with a very low concentration of the contaminant it may actually be difficult to quantify - or even to detect the

presence of - the contaminant! This is a low signal-to-noise ratio. Consequently, from a strict analytical standpoint, one should try to focus on analysing those samples where one could expect the highest concentrations of the contaminant. This means samples taken near the source and samples where the contaminant has been concentrating. This does not necessarily mean that the concentrations are high!

Obviously, there are several other factors to consider when deciding on how the contaminant monitoring should be done.

The first thing is to consider the aim of the monitoring! Consider the human health monitoring of DDT or PCB. It would be a waste of resources to make sampling of water and sediments a general part of such a programme. The concentrations of DDT and PCB in the water are extremely low since these substances are strongly lipophilic. They may accumulate in sediments, but since human beings are in very limited contact with sediments this would be of concern in very extreme cases only. Consequently, monitoring of DDT and PCB in edible fish and shellfish is the logical design of the programme.

In general, trend monitoring of contaminants (including nutrients) in the water of open sea areas is very difficult because of the high variability (patchiness) in time and space. For a study of regional distribution at a certain point in time such sampling can be useful, although there are certainly difficulties involved.

Therefore, in general, sediments and biota are the most relevant compartments (matrices) for contaminant monitoring in the marine environment! However, this does not exclude difficulties relating to variability in space and time!

Advantages with sediments are not only that they accumulate but also that the sample, or rather the material, is stationary from one sampling occasion to the next. Therefore repeated sampling for trend studies are possible. Preconditions for this is of course that localities that should be monitored have sufficient accumulation rates. If sediments are resuspended or frequently eroded they are not suitable for monitoring. The same thing is true if there is a high degree of bioperturbation that disturbs the stratification of the sediment.

Advantages with biota sampling is not only their bioconcentration and biomagnification properties but also e.g. that they offer possibilities to study relationship between contaminant concentrations and their biological effects. Disadvantages are i.a. that seasonal biological cycles influence the contaminant concentrations in the specimens. It is therefore essential that sampling is carried out in a consistent way, which means sampling should always be carried out at the same phase of the biological cycle.

There are several other factors in favour of and against sediments and biota. They will not be anaysed here, but rather described in some of the following lectures.

# WHICH CONTAMINANTS DO I NEED TO MONITOR, AND WHERE SHOULD I LOOK FOR THEM ?

by

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## 1. WHICH CONTAMINANTS SHOULD BE MONITORED?

It would probably be rather convenient to base a monitoring programme on a fixed, and not too long, list of contaminants to be monitored. This would make planning and implementation easier, particularly concerning chemical analyses and assessments. However, in the light of increased knowledge regarding various contaminants and the introduction of new substances of concern, priorities change and new entries are added to the lists of substances to be monitored.

Therefore, this lecture cannot, and will not, present a recipe which would tell you exactly what to monitor and where.

The aim of this lecture is to review the work undertaken by various international organizations regarding the selection of the substances to be monitored and where (in which compartments) they should be monitored. The lecture will also present some approaches on how to identify (new) substances that should be monitored. Finally, some advice is provided to help you decide in which compartments of the marine environment one should monitor these substances.

The examples are taken from various international conventions, working groups etc. The material is presented as a suite of annexes. For the benefit of the reader these annexes contain some more detailed material which will not be used for the presentation.

### 1.1 Substances regulated under the Barcelona Convention

A system used in all marine conventions is to regulate the use and/or discharges of certain substances and material that is known, or at least suspected, to be harmful to the marine environment. The usual procedure has been to define a set of criteria on which to base the selection of a number of substances that should be regulated. Typical criteria were: toxicity, persistence and bioaccumulation. Substances showing high toxicity together with high persistence or ability to bioaccumulate were "banned" which means that they should be eliminated from discharges. A list of such substances is normally referred to as the "Black List" although the term is not used in the convention text itself. Other substances of environmental concern are identified although they are considered as being less harmful. The convention text normally allowes these substances to be discharged although their discharges should be minimised. In a similar way as previously mentioned, a list of such substances is usually referred to as the "Grey List."

The regulations in the text of the Barcelona Convention (as it came into force in 1978) are rather general. As an example Article 8 states: "The Contracting Parties shall take all appropriate measures to prevent, abate and combat pollution of the Mediterranean Sea Area caused by discharges from rivers, coastal establishments or outfalls, or emanating from any other land-based sources within their territories." However, together with the Convention there is a set of Protocols. The "Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources" (that came into force in 1983) has text which is somewhat more specific (Articles 5 and 6) and four detailed annexes (two of which appear in Annex 1) where substances are listed.

### 1.2 Substances regulated under the Paris Convention (PARCOM)

The Convention for the Prevention of Marine Pollution from Land-Based Sources, better known as the Paris Convention, came into force in 1978. Here, already in the text of the Convention, we find the type of detailed regulation included in the LBS protocol of the Barcelona Convention. The Articles 4 and 5, along with Annex A, contain the necessary specifications. (Please refer to Annex 2 of this lecture). As can be seen, the selection of substances is rather similar to that under the Barcelona Convention, but the Paris Convention has defined more stringent criteria for the identification of substances to be included in the lists.

However, as requirements change the Conventions are revised. The Paris Convention has been re-negotiated and the new "Convention for the Protection of the Marine Environment of the North-East Atlantic" was signed in October 1992. The Convention is expected to come into force within the next four to five years. Already now preparations are under way to change the monitoring and assessment systems in order to adjust to the new requirements.

Some examples of this are given in Annex 3. Here, one can see the relevant regulations in Articles 3, 4 and 5 of the Convention, its Annex I and its Appendix 2. The important difference, from a monitoring perspective, is that the text is now more of a framework than a specific list. *It specifies rather few substances but concentrates on groups of substances.* Furthermore, *it specifies a much wider set of criteria for the selection of substances to regulate* and requires the Contracting Parties to take these into consideration when they design their programmes and measures for the protection of the marine environment!

### 1.3 Substances regulated under the Helsinki Convention (HELCOM)

Also this Convention has been re-negotiated. The Convention for the Protection of the Environment of the Baltic Sea Area was signed in April 1992 and it is expected to come into force within the next few years.

As can be seen in Annex 4 to this paper there are large similarities with the new Paris Convention. The extended lists of criteria for the identification of substances (or groups of substances) to regulate are rather similar in the two conventions. It should be noted, however, that due to the concern for the very vulnerable environment of the Baltic Sea the new Convention has a list of substances that are actually banned, as well as a list of pesticides the use of which should be minimised.

## 2. HOW CAN WE IDENTIFY WHICH SUBSTANCES TO MONITOR?

As presented in a previous lecture there are long lists of contaminants monitored by the PARCOM, the North Sea Task Force and the HELCOM. In view of the principles laid down by the revised conventions the lists could possibly be made even longer, since there is an obvious need for managers and the general public to be able to see that programmes for the reduction of harmful substances in discharges are really effective. However, inclusion of a substance in the monitoring programme must be defendable by the aims for the programme!

In this context it must be underlined that rather few of the existing substances have been tested and studied in relation to the criteria laid down in the conventions. A large number of new substances, mainly organic ones, are synthesized every year and very little is known about their hazardous properties. Some examples of substances that are being discussed for inclusion in monitoring programmes are brominated flame retardants, simazine, atrazine and the planar chlorobiphenyls (planar CBs), chlordanes and polychlorinated camphenes (PCC).

However, as monitoring of contaminants is very resource demanding there is a clearer need for a good procedure for the identification and selection of the substances that are most likely to cause harm to the environment and, therefore, should be given priority in monitoring programmes. This is further described below.

The International Council for the Exploration of the Sea (ICES), in its role as scientific advisor to HELCOM, responded to a request to recommend a procedure on how to identify "new" contaminants presenting particular hazards to the environment. The term "new" contaminant could be understood in several ways in this context. It can refer to contaminants not currently controlled under the various conventions, newly synthesized substances, substances showing increases in production or changes of use, existing substances which have undergone revised hazard assessments, or substances that recently came to our attention (e.g. intentional or unintentional by-products of industrial processes).

During a couple of years the ICES Working Group on Environmental Assessments and Monitoring Strategies developed its advice. The deliberations of the Group are contained in Annexes 5 and 6.

## 3. EXAMPLES RELATED TO IN WHICH MEDIA VARIOUS CONTAMINANTS SHOULD BE MONITORED

The ICES, in its role as scientific advisor to PARCOM and partner with PARCOM in the North Sea Task Force (NSTF), responded to a request to recommend to PARCOM and NSTF a scheme to describe in which media the different contaminants or hazardous substances preferably should be monitored. The scheme should preferably also assign priorities between different media for sampling in order to make the monitoring more cost effective. The advice given is included here as Annex 7.

## ANNEX 1

### Selected regulations under the Barcelona Convention

**This annex contains selected material from the "Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources" of the Barcelona Convention**

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Article 5

1. The Parties undertake to eliminate pollution of the Protocol Area from land-based sources by substances listed in annex I to this Protocol.

2. To this end they shall elaborate and implement, jointly or individually, as appropriate, the necessary programmes and measures.

3. These programmes and measures shall include, in particular, common emission standards and standards for use.

4. The standards and the time-tables for the implementation of the programmes and measures aimed at eliminating pollution from land-based sources shall be fixed by the Parties and periodically reviewed, if necessary every two years, for each of the substances listed in annex I, in accordance with the provisions of article 15 of this Protocol.

Article 6

1. The Parties shall strictly limit pollution from land-based sources in the Protocol Area by substances or sources listed in annex II to this Protocol.

2. To this end they shall elaborate and implement, jointly or individually, as appropriate, suitable programmes and measures.

3. Discharges shall be strictly subject to the issue, by the competent national authorities, of an authorization taking due account of the provisions of annex III to this Protocol.

## ANNEX I

### A

The following substances, families and groups of substances are listed, not in order of priority, for the purposes of article 5 of this Protocol. They have been selected mainly on the basis of their:

Toxicity;
Persistence;
Bioaccumulation.

1. Organohalogen compounds and substances which may form such compounds in the marine environment.[1]

2. Organophosphorus compounds and substances which may form such compounds in the marine environment.[1]

3. Organotin compounds and substances which may form such compounds in the marine environment.[1]

4. Mercury and mercury compounds.

5. Cadmium and cadmium compounds.

6. Used lubricating oils.

7. Persistent synthetic materials which may float, sink or remain in suspension and which may interfere with any legitimate use of the sea.

8. Substances having proven carcinogenic, teratogenic or mutagenic properties in or through the marine environment.

9. Radioactive substances, including their wastes, when their discharges do not comply with the principles of radiation protection as defined by the competent international organizations, taking into account the protection of the marine environment.

### B

The present annex does not apply to discharges which contain substances listed in section A that are below the limits defined jointly by the Parties.

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[1] With the exception of those which are biologically harmless or which are rapidly converted into biologically harmless substances.

## ANNEX II

### A

The following substances, families and groups of substances, or sources of pollution, listed not in order of priority for the purposes of article 6 of this Protocol, have been selected mainly on the basis of criteria used for annex I, while taking into account the fact that they are generally less noxious or are more readily rendered harmless by natural processes and therefore generally affect more limited coastal areas.

1. The following elements and their compounds:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1. | zinc | 6. | selenium | 11. | tin | 16. | vanadium |
| 2. | copper | 7. | arsenic | 12. | barium | 17. | cobalt |
| 3. | nickel | 8. | antimony | 13. | beryllium | 18. | thallium |
| 4. | chromium | 9. | molybdenum | 14. | boron | 19. | tellurium |
| 5. | lead | 10. | titanium | 15. | uranium | 20. | silver |

2. Biocides and their derivatives not covered in annex I.

3. Crude oils and hydrocarbons of any origin.

4. Cyanides and fluorides.

5. Non-biodegradable detergents and other surface-active substances.

6. Inorganic compounds of phosphorus and elemental phosphorus.

7. Pathogenic micro-organisms.

8. Thermal discharges.

9. Substances which have a deleterious effect on the taste and/or smell of products for human consumption derived from the aquatic environment, and compounds liable to give rise to such substances in the marine environment.

10. Substances which have, directly or indirectly, an adverse effect on the oxygen content of the marine environment, especially those which may cause eutrophication.

11. Acid or alkaline compounds of such composition and in such quantity that they may impair the quality of sea-water.

12. Substances which, though of a non-toxic nature, may become harmful to the marine environment or may interfere with any legitimate use of the sea owing to the quantities in which they are discharged.

### B

The control and strict limitation of the discharge of substances referred to in section A above must be implemented in accordance with annex III.

## ANNEX 2

### Selected regulations under the Oslo and Paris Conventions

**This annex contains selected material from "The Convention for the Prevention of Marine Pollution from Land-Based Sources"**

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ARTICLE 4

1. The Contracting Parties undertake:

a. to eliminate, if necessary by stages, pollution of the maritime area from land-based sources by substances listed in Part I of Annex A to the present Convention;

b. to limit strictly pollution of the maritime area from land-based sources by substances listed in Part II of Annex A to the present Convention.

2. In order to carry out the undertaking sin paragraph 1 of this Article, the Contracting Parties, jointly or individually as appropriate, shall implement programmes and measures:

a. for the elimination, as a matter of urgency, of pollution of the maritime area from land-based sources by substances listed in Part I of Annex A to the present Convention;

b. for the reduction or, as appropriate, elimination of pollution of the maritime area from land-based sources by substances listed in Part II of Annex A to the present Convention. These substances shall be discharged only after approval has been granted by the appropriate authorities within each Contracting State. Such approval shall be periodically reviewed.

3. The programmes and measures adopted under paragraph 2 of this Article shall include, as appropriate, specific regulations or standards governing the quality of the environment, discharges into the maritime area, such discharges into watercourses and emissions into the atmosphere as affect the maritime are, and the composition and use of substances and products. These programmes and measures shall take into account the latest technical developments.

The programmes shall contain time-limits for their completion.

4. The Contracting Parties may, furthermore, jointly or individually as appropriate, implement programmes or measures to forestall, reduce or eliminate pollution of the maritime area from land-based sources by a substance not then listed in Annex A to the present Convention, if scientific evidence has established that a serious hazard may be created in the maritime area by that substance and if urgent action is necessary.

ARTICLE 5

1. The Contracting Parties undertake to adopt measures to forestall and, as appropriate, eliminate pollution of the maritime area from land-based sources by radio-active substances refereed to in Part III of Annex A of the present Convention.

2. Without prejudice to their obligations under other treaties and conventions, in implementing this undertaking the Contracting Parties shall:

a. take full account of the recommendations of the appropriate international organizations and agencies;

b. take account of the monitoring procedures recommended by theses international organizations and agencies;

c. coordinate their monitoring and study of radio-active substances in accordance with Articles 10 and 11 of the present Convention.

## ANNEX A

The allocation of substances to Parts I, II and III below takes account of the following criteria:

a. persistences;

b. toxicity or other noxious properties;

c. tendency to bio-accumulation;

These criteria are not necessarily of equal importance for a particular substance or group of substances, and other factors, such as the location and quantities of the discharge, may need to be considered.

## PART I

The following substances are included in this Part

i. because they are not readily degradable or rendered harmless by natural processes; and

ii. because they may either:

a. give rise to dangerous accumulation of harmful material in the food chain, or

b. endanger the welfare of living organisms causing undesirable change sin the marine eco-systems, or

c. interfere seriously with the harvesting of sea foods or with other legitimate uses of the sea; and

iii. because it is considered that pollution by these substances necessitates urgent action:

1. Organohalogen compounds and substances which may form such compounds in the marine environment, excluding those which are biologically harmless, or which are rapidly converted in the sea into substances which are biologically harmless.

2. Mercury and mercury compounds.

3. Cadmium and cadmium compounds.

4. Persistent synthetic materials which may float, remain in suspension or sink, and which may seriously interfere with any legitimate use of the sea.

5. Persistent oils and hydrocarbons of petroleum origin.

## PART II

The following substances are included in this Part because, although exhibiting similar characteristics to the substances in Part I and requiring strict control, they seem less noxious or are more readily rendered harmless by natural processes:

1. Organic compounds of phosphorus, silicon and tin, and substances which may form such compounds in the marine environment, excluding those which are biologically harmless, or which are rapidly converted in the sea into substances which are biologically harmless.

2. Elemental phosphorus.

3. Non-persistent oils and hydrocarbons of petroleum origin.

4. The following elements and their compounds:
   Arsenic Lead
   Chromium Nickel
   Copper Zinc

5. Substances which have been agreed by the Commission as having a deleterious effect on the taste and/or smell of products derived from the marine environment for human consumption.

## PART III

The following substances are include din this Part because, although they display characteristics similar to those of substances listed in Part I and should be subject to stringent controls with the aim of preventing and, as appropriate, eliminating the pollution which they cause, they are already the subject of research, recommendations and, in some cases, measures under the auspices of several international organizations and institutions; those substances are subject to the provisions of Article 5:

- Radioactive substances, including wastes.

## ANNEX 3

**Selected regulations under the new Paris Convention**

**This annex contains selected material from "The Convention for the Protection of the Marine Environment of the North-East Atlantic."**

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### ARTICLE 3
### POLLUTION FROM LAND-BASED SOURCES

The Contracting Parties shall take, individually and jointly, all possible steps to prevent and eliminate pollution from land-based sources in accordance with the provisions of the Convention, in particular as provided for in Annex I.

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### ARTICLE 4
### POLLUTION BY DUMPING OR INCINERATION

The Contracting Parties shall take, individually and jointly, all possible steps to prevent and eliminate pollution by dumping or incineration of wastes or other matter in accordance with the provisions of the Convention, in particular as provided for in Annex II.

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### ARTICLE 5
### POLLUTION FROM OFFSHORE SOURCES

The Contracting Parties shall take, individually and jointly, all possible steps to prevent and eliminate pollution from offshore sources in accordance with the provisions of the Convention, in particular as provided for in Annex III.

**ANNEX I**
ON THE PREVENTION AND ELIMINATION OF POLLUTION
FROM LAND-BASED SOURCES

ARTICLE 1

1. When adopting programmes and measures for the purpose of this Annex, the Contracting Parties shall require, either individually or jointly, the use of

- best available techniques for point sources
- best environmental practice for point and diffuse sources

including, where appropriate, clean technology.

2. When setting priorities and in assessing the nature and extent of the programmes and measures and their time scales, the Contracting Parties shall use the criteria given in Appendix 2.

3. The Contracting Parties shall take preventive measures to minimise the risk of pollution caused by accidents.

4. When adopting programmes and measures in relation to radioactive substances, including waste, the Contracting Parties shall also take account of:

(a) the recommendations of the other appropriate international organisations and agencies;

(b) the monitoring procedures recommended by these international organisations and agencies.

ARTICLE 2

1. Point source discharges to the maritime area, and releases into water *or air* which reach and may affect the maritime area, shall be strictly subject to authorisation or regulation by the competent authorities of the Contracting Parties. Such authorisation or regulation shall, in particular, implement relevant decisions of the Commission which bind the relevant Contracting Party.

2. The Contracting Parties shall provide for a system off regular monitoring and inspection by their competent authorities to assess compliance with authorisations and regulations of releases into water *or air*.

ARTICLE 3

For the purpose of this Annex, it shall, inter alia, be the duty of the Commission to draw up:

(a) plans for the reduction and phasing out of substances that are toxic, persistent and liable to bioaccumulate arising from land-based sources;

(b) when appropriate, programmes and measures for the reduction of inputs of nutrients from urban, municipal, industrial, agricultural and other sources.

## APPENDIX 2
## CRITERIA MENTIONED IN PARAGRAPH 2 OF ARTICLE 1 OF ANNEX I AND IN PARAGRAPH 2 OF ARTICLE 2 OF ANNEX III

1. When setting priorities and in assessing the nature and extent of the programmes and measures and their time scales, the Contracting Parties shall use the criteria given below:

(a) persistency;
(b) toxicity or other noxious properties;
(c) tendency to bioaccumulation;
(d) radioactivity;
(e) the ratio between observed or (where the results of observations are not yet available) predicted concentrations and no observed effect concentrations;
(f) anthropogenically caused risk of eutrophication;
(g) transboundary significance;
(h) risk of undesirable changes in the marine ecosystem and irreversibility or durability of effects;
(i) interference with harvesting of sea-foods or with other legitimate uses of the sea;
(j) effects on the taste and/or smell of products for human consumption form the sea, or effects on smell, colour, transparency or other characteristics of the water in the marine environment;
(k) distribution pattern (i.e., quantities involved, use pattern and liability to reach the marine environment);
(l) non-fulfillment of environmental quality objectives.

2. These criteria are not necessarily of equal importance for the consideration of a particular substance or group of substances.

3. The above criteria indicate that substances which shall be subject to programmes and measures include:

(a) heavy metals and their compounds;
(b) organohalogen compounds (and substances which may form such compounds in the marine environment);
(c) organic compounds of phosphorus and silicon;
(d) biocides such as pesticides, fungicides, herbicides, insecticides, slimicides and chemicals used, *inter alia*, for the preservation of wood, timber, wood pulp, cellulose, paper, hides and textiles;
(e) oils and hydrocarbons of petroleum origin;
(f) nitrogen and phosphorus compounds;
(g) radioactive substances, including wastes;
(h) persistent synthetic materials which may float, remain in suspension or sink.

## ANNEX 4

## Selected regulations under the new Helsinki Convention

**This annex contains selected material from "The Convention on the Protection of the Marine Environment of the Baltic Sea Area, 1992."**

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### Article 5
### Harmful substances

The Contracting Parties undertake to prevent and eliminate pollution of the marine environment of the Baltic Sea Area caused by harmful substances from all sources, according to the provisions of this Convention and, to this end, to implement the procedures and measures of Annex I.

### ANNEX I
### Harmful substances

PART I GENERAL PRINCIPLES

1.0 Introduction

In order to fulfil the requirements of relevant parts of this Convention the following procedure shall be used by the Contracting Parties in identifying and evaluating harmful substances, as defined in Article 2, paragraph 7.

1.1 Criteria on the allocation of substances

The identification and evaluation of substances shall be based on the intrinsic properties of substances, namely:

- persistency;
- toxicity or other noxious properties;
- tendency to bio-accumulation,

as well as on characteristics liable to cause pollution, such as

- the ratio between observed concentrations and concentrations having no observed effect;
- anthropogenically or long-range significance;
- transboundary or long-range significance;
- risk of undesirable changes in the marine ecosystem and irreversibility or durability of effects;
- radioactivity;
- serious interference with harvesting of sea-foods or with other legitimate uses of the sea;
- distribution pattern (i.e. quantities involved, use pattern and liability to reach the marine environment);
- proven carcinogenic, teratogenic or mutagenic properties in or through the marine environment.

These characteristics are not necessarily of equal importance for the identification and evaluation of a particular substance or group of substances.

1.2 Priority groups of harmful substances

The Contracting Parties shall, in their preventive measures, give priority to the following groups of substances which are generally recognised as harmful substances:

a) heavy metals and their compounds;
b) organohalogen compounds;
c) organic compounds of phosphorus and tin;
d) pesticides, such as fungicides, herbicides, insecticides, slimicides and chemicals used for the preservation of wood, timber, wood pulp, cellulose, paper, hides and textiles;
e) oils and hydrocarbons of petroleum origin;
f) other organic compounds especially harmful to the marine environment;
g) nitrogen and phosphorus compounds;
h) radioactive substances, including wastes;
i) persistent materials which may float, remain in suspension or sink;
j) substances which cause serious effects on taste and/or smell of products for human consumption from the sea, or effects on taste, smell, colour, transparency or other characteristics of the water.

PART 2 BANNED SUBSTANCES

In order to protect the Baltic Sea Area from hazardous substances, the Contracting Parties shall prohibit, totally or partially, the use of the following substances or groups of substances in the Baltic Sea Area and its catchment area:

2.1 Substances banned for all final uses, except for drugs

DDT (1,1,1-trichloro-2,2-bis-(chlorophenyl)-ethane) and its derivatives DDE and DDD;

2.2 Substances banned for all uses, except in existing closed system equipment until the end of service life or for research, development and analytical purposes

a) PCB's (polychlorinated biphenyls);
b) PCT's (polychlorinated terphenyls).

2.3 Substances banned for certain applications

Organotin compounds for antifouling paints for pleasure craft under 25 m and fish net cages.

PART 3 PESTICIDES

In order to protect the Baltic Sea Area from hazardous substances, the Contracting Parties shall endeavour to minimize and, whenever possible, to ban the use of the following substances as pesticides in the Baltic Sea Area and its catchment area:

| | CAS-number |
|---|---|
| Acrylonitrile | 107131 |
| Aldrin | 309002 |
| Aramite | 140578 |
| Cadmium-compounds | - |
| Chlordane | 57749 |
| Chlordecone | 143500 |
| Chlordimeform | 6164983 |
| Chloroform | 67663 |
| 1,2-Dibromoethane | 106934 |
| Dieldrin | 60571 |
| Endrin | 72208 |
| Fluoroacetic acid and derivatives | 7664393, 144490 |
| Heptachlor | 76448 |
| Isobenzane | 297789 |
| Isodrin | 465736 |
| Kelevan | 4234791 |
| Lead-compounds | - |
| Mercury-compounds | - |
| Morfamquat | 4636833 |
| Nitrophen | 1836755 |
| Pentachlorophenol | 87865 |
| Polychlorinated terpenes | 8001501 |
| Quintozene | 82688 |
| Selenium-compounds | - |
| 2,4,5-T | 93765 |
| Toxaphene | 8001352 |

## ANNEX 5

### Identification of "new" contaminants; Part I

**This annex contains selected material from the Report of the (ICES) Working Group on Environmental Assessments and Monitoring Strategies (1990).**

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6. CONSIDER AND REPORT ON SYSTEMATIC PROCEDURES TO ASSESS THE HAZARDS OF POTENTIALLY TOXIC SUBSTANCES AS A MEANS OF IDENTIFYING PRIORITY MARINE CONTAMINANTS (IN THE CONTEXT OF ACMP's ENVIRONMENTAL MANAGEMENT PRINCIPLES), TAKING INTO ACCOUNT THE MCWG COMMENTS AND RELEVANT NORTH SEA TASK FORCE PAPERS

This item was introduced as a continuation of the 1989 meeting discussion on mechanisms for the identification of "new" contaminants presenting particular hazards to the environment. In addition, ICES had been asked by HELCOM for assistance in developing a general scheme for the identification of chemical substances with possible harmful properties towards the marine environment based on toxicity, chemical properties, etc., and to provide guidance for its use relevant to the Baltic Sea.

Denmark is the lead country for the work on identification of "new" contaminants within HELCOM. Therefore, Dr. Tonny Nillonen of the Environmental Protection Agency in Denmark was invited to take part in the work under this agenda time.

It was noted that, as ICES covers a larger area than HELCOM, the Working Group should feel free to take a wider view than that corresponding to the present needs of HELCOM.

Discussion centered upon methods for identifying "new" contaminants of significance to the sea. There was normally a long chain of events from the design of a new product or substance, through testing, licensing, etc. to production and use. It would be preferable to intervene in this system at an early enough stage, if it appears that significant environmental risks would be involved.

The term "new" contaminant could be understood in several ways; referring to contaminants not currently controlled under the various conventions, newly synthesized substances, substances showing increases in production or changes of use, existing substances which have undergone revised hazard assessments, or substances which newly came to our attention(eg., intentional or unintentional by-products of industrial processes).

Ian Davies presented the UK Department of the Environment Red List Selection Scheme. This scheme had been designed to identify a limited range of substances which presented the greatest threat to the aquatic environment and which, therefore, would be proposed for priority control through the EC. The selection of priority substances was based upon considerations of toxicity, persistence, bioaccumulation potential, and "input" in a semi-quantitative way on various time scales through a series of decision trees. It was noted that the scheme as used was applied to individual substances (or occasionally classes of chemicals), and not to complex mixed wastes. It was further noted

that the scheme relied upon some knowledge of the values of the initial parameters (which may not be immediately available for some compounds of interest), and that the number of substances selected would depend upon the numerical limits set to the classification of the values of each parameter as high, medium, or low.

Cindy de Wit reviewed the quantitative structure activity relationship (QSAR) approach to modelling the biological properties of compounds from molecular structural information. Commonly, multivariate statistical procedures were used in this modelling. In order to design the model, it is necessary to know or calculate the values of the properties of interest for a certain number of members of a class of compounds. If the model is found to satisfactorily describe the variability in the properties, the model may be used in a predictive sense for other members of the same class of compounds.

Similar procedures have been widely used over the pat few years in the design of pharmaceuticals, and have been used to predict environmentally significant properties, e.g., $K_{o/w}$ (partition coefficient between octanol and water as a description of the fat solubility of the substance in question), or the mutagenicity of hydrazines.

In the discussion, it was noted that QSAR dealt with individual substances, as did the Red List selection scheme. QSAR might be used to estimate the values of parameters needed for such selection schemes where relevant data were not available from direct measurements. Caution was expressed over the use of QSAR on its own, and particularly over the prediction of toxicity, as this could vary greatly between and within species. The use of QSAR methods, although promising if the same good experience could be demonstrated for environmental science as for pharmacological science, could not yet be regarded as a complete substitute for direct measurements. For some compounds and properties of interest, the models were not yet sufficiently sophisticated.

It was generally felt that the hazard posed by individual compounds was more rapidly assessed than that of complex mixtures. Inventories of chemical usage by individual factories could give information on possible major components of wastes. National licensing or, to some extent, patenting schemes could give leads on the types of new compounds which may be released to the aquatic environment. If the nature of the substances is known, chemical and biological testing, QSAR studies, and risk assessment can be undertaken.

Assessment of the risk associated with mixed complex wastes of indeterminate composition cannot be approached in the same way. Examples were given, including the occurrence of haemolytic anemia in salmon in a Scottish river, and various effects outside some Swedish pulp mills. In both cases, the investigations had been generated by observations of biological effects. The effects stimulated research to identify chemically the biologically active components of the waste giving rise to the effects. This suggested two general approaches that might be adopted. Firstly, it might be possible to characterize, in a fairly complete way, the chemical composition of a complex contaminant source (e.g., effluent or river), or the suite of chemicals accumulated by some animals at a high trophic level. These approaches would place different emphases on such properties as persistence or bioaccumulation.

An alternative approach might be through exploring the toxicity of effluent streams, rivers, sediment extracts, etc., and subsequently attempting to identify the chemical components responsible for any observed biological effect. This approach might allow regulatory action to be taken to limit the impact before the precise chemical cause has been identified. There were proposals in hand to test the toxicity of water and sediment extracts from the Skagerrak and the Rhine to bacterial strains (e.g., Mictroton system). The association of a biological effect with a particular effluent stream may be sufficient to call for controls. It was noted that there was a general tendency in several countries for regulatory authorities to require dischargers to characterize their wastes more completely in both chemical and biological senses. Biological effluent treatment was becoming more common, although it was noted that particularly significant components resistant to biological decomposition might be unaffected by this system. It was noted that, e.g, in France, dischargers had to make payments related to the solides content and toxicity to Daphnia magna of their effluent, and this encouraged the adoption of improved waste control or the best available technology (known as BAT in the work of the various commissions for the marine environment).

The Working Group agreed that the following general recommendations could be made:

a) There is a need for the optimization of testing based on sublethal effects to supplement acute toxicity tests. The tests should cover organisms with short life-times and rapid replication rates, as well as those with much slower reproduction rates.

b) QSAR methods should be developed to predict specific important ecological properties of substances, such as bioaccumulation potential, persistence and toxicity.

c) Resources should be allocated for developing new approaches to searching for biologically active contaminants already present in the environment.

The Working Group report of 1989, the 1989 ACMP report, and the GESAMP document noted at this meeting all contained guidance on the critical properties of substances which governed their biological impact. The Working Group also had before it information on three schemes for selecting priority compounds. HELCOM had indicated its need for advice on standardized approaches to the selection. The Working Group agreed with HELCOM that it was necessary to assess the properties, criteria, and selection schemes that had been used in relation to the principles outlined by ACMP and elsewhere.

It was, therefore, agreed to establish a small group to work intersessionally on the comparison of the various approaches that had been taken to the selection of priority potential marine contaminants. The group would consist of Ian Davies (coordinator), Michel Joanny, Frank van der Valk, and Cindy de Wit. If time permitted, the Group would also attempt to address the other questions on identification of contaminants raised by HELCOM.

It was noted that within the work of HELCOM, and in parallel with the Paris Commission's TWG (Technical Working Group), efforts were being made to address the control of complex mixed wastes through consideration of each industry in a generic manner. For example, within HELCOM the iron and steel industries had

been catalogued and assessed, and attention was now moving to the chemical industries. The latter was proving more complicated, and needed to be treated in various sub-divisions according to the nature of the operations being carried out. Other possible generic classifications were petrochemicals, textiles, sewage, etc.

The Working Group recommended in relation to complex discharges that:

a) Work to prepare inventories of chemicals used by various industries should continue.

b) Biological tests in mesocosms should be encouraged, in order to identify the potential biological effects of complex wastes, and fractions of complex wastes.

c) Efforts should be made to chemically characterize the substances causing effects noted in b) above.

d) There should be intensification of filed monitoring and research programmes to address the appropriate effects and chemicals identified in b) and c), above. These programmes should be undertaken in areas where impacts would be predicted, and these may include both coastal and off-shore waters.

e) A system of biological specimen banking should be established to allow retrospective analyses, for example, of new contaminants.

## ANNEX 6

## Identification of "new" contaminants; Part II

**This annex contains selected material from the Report of the (ICES) Working Group on Environmental Assessments and Monitoring Strategies (1991).**

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### REVIEW OF SOME APPROACHES TO THE SELECTION OF SUBSTANCES OF PARTICULAR HAZARD TO THE ENVIRONMENT

#### A. Introduction

This short review paper has been prepared in response to the task defined at the 1990 meeting of WGEAMS, and describes some systematic procedures which have been employed for the assessment of the hazards of potentially toxic substances, as an approach to the identification of priority contaminants for control measures. A recent GESAMP report (No. 45, Global Strategies for Marine Environmental Protection) clearly points out the need for defined goals in environmental protection, and emphasizes the need for key scientific elements in a comprehensive strategy. These elements include information on such factors as sources, transport, transformation, and fate of contaminants, nd of their effects on man, living resources and amenities. A primary goal of this work is hazard assessment of activities and chemicals, and subsequently risk assessment and management. The development of the schemes that are described below mainly has had as an objective the ability to identify substances posing particularly high risk in the marine environment using relatively simple and available data, and thus to focus subsequent management actions.

Selection schemes for the identification of priority aquatic pollutants have recently been extensively reviewed by Jackson and Peterson in a report to the EC on study contract B 6612/290/89. They noted that selection schemes are mainly based either on decision/logic trees (as in the UK scheme described below) or on a system of ranking based on values or scores for selected parameters.

The logic tree schemes require the selection of trigger values for various parameters to determine the progression of compounds through the tree. The selection of these values is of critical importance to the final decision on any compound. Similarly, the scoring schemes sued in ranking systems control the relative weight given to particular properties of the substances under consideration, and therefore can be used to reflect the relative importance of properties as assessed by the designers of the scheme.

A fundamental problem with selection schemes is that to be useful they must be able to operate on the relatively limited data that are available for many compounds of potential interest to regulators and, at the same time, produce lists of priority substances that are not unmanageably long. This means that the schemes must eliminate large numbers of substances to arrive at a short final list, but in doing so must be reliable. Users of the scheme must have some confidence that hazardous substances have not inadvertently been

eliminated during the selection process. There may, therefore, be a conflict between simplicity and universality of operation with reliability of selection (avoiding false non-selection).

B. Types of Selection Schemes

Examples of various types of schemes will now be described.

1. A simple scheme based on trigger values for a limited number of properties. This type of scheme may be particularly useful for the initial screening of large numbers of compounds, for which very detailed information is not available.

In 1990, GESAMP published report No. 42, "Review of potentially harmful substances: Choosing priority organochlorines for marine hazard assessment". This document sets out to develop a scheme for the identification of those members of the broad group of chlorinated hydrocarbons which warranted particularly stringent controls on their use and disposal. The assessment scheme is shown in Appendix 1.

The group recognised that the limited amount of data available for many of the compounds made it necessary to adopt a very simple screening procedure. The initial analysis excluded all pesticides, PCBs, dioxins and furans, but considered about 720 other compounds. The group considered that the most critical parameters were the octanol/water partition coefficient (log $K_{o/w}$) (as an index of likely bioaccumulation), persistence, and toxicity. Trigger values were as follows:

| Parameter | Trigger value | Comments |
|---|---|---|
| log $K_{o/w}$ | >3 | From literature or calculated from the molecular structure (QSAR) |
| Persistence | >1 week | In water |
| Toxicity (LC50, EC50) | <10 mg/l | |

A fourth factor, the production and use of the chemical, was also sometimes considered.

The scheme considered a chemical to have been selected if it met two or more of the trigger values (or was very similar in structure to others which did so). Chemicals were not selected if they did not meet any of the trigger values, and other chemicals could not be assessed through lack of data.

The scheme identified 77 compounds which were considered to be in need of further more detailed assessment to ascertain the true level of risk. It therefore can be considered a preliminary screening scheme as a precursor to more rigorous assessment. The scheme was not applied to pesticides, PCBs, dioxins or furans because it was clear that they would all meet at least one of the criteria, and that a different form of assessment would be needed.

The list of compounds selected is shown in Appendix 2.

In applying this scheme, the authors found that the data were limited for many substances. Environmental criteria are needed for an ever-increasing number of chemicals, and it was necessary to adopt a QSAR approach to the problem. The authors do not describe in detail the QSAR methods that they used, but in employing the method they recognised that QSAR assessments can provide at least a guide to the likely physico-chemical and biological properties of a wide range of substances.

2. A logic tree scheme, which seeks to avoid making judgements on the relative importance of, say, toxicity and carcinogenicity, by assessing types of impact through different logic trees.

A systematic procedure has been adopted by the UK as a contribution to the deliberations of the International Conferences on the Protection of the North Sea. The initial candidate substances were derived from candidate List 1 substances (from the EC Dangerous Substances Directive) and form substances included in the Third North Sea Conference reference list. This priority-setting scheme was used by the UK to develop a "Red List" of priority substances, and would be applicable to other chemicals in the future as necessary, provided that the required physical, chemical, and biological data (or most of them) are available.

The scheme is described in detail in Appendix 2, and consists of four decision trees, which address the possibilities of acute aquatic toxicity, chronic toxicity, bioaccumulation, and carcinogenicity. It attempts to assess the relative hazard through each of these routes, taking into account the likelihood that the substance being assessed will enter the aquatic environment. For a substance to be recognised as a priority substance, and therefore be included on the Red List, it was necessary for it to qualify through one or more of the decision trees. It did not have to qualify through all trees, and therefore the scheme did not seek to define the relative importance of, say, bioaccumulation versus acute toxicity.

3. As an example of a ranking or scoring system, a scheme developed by the Ontario Ministry of the Environment is described. The description is taken from Jackson and Peterson, and is presented in Appendix 3.

C. Discussion

There are, therefore, a number of approaches that have been taken to priority chemical selection, and to the details of individual schemes within these approaches. As has been noted above, the choices of both the assessment parameters and the trigger values or scorings associated with them can greatly influence the final list of chemicals. Jackson and Peterson tabulated the parameters that appear in 14 selection schemes (see Table 1). It can be seen that the complexity of the schemes varies greatly, with schemes using between 2 and 11 parameters, out of a range of 16. The most commonly used parameters are acute aquatic toxicity, persistence, and bioaccumulation potential ($K_{o/w}$). In a second group are carcinogenicity, acute mammalian toxicity, and physico-chemical properties.

Surprisingly little use is made of chronic toxicity data (possibly reflecting their relative scarcity) and production or use tonnage (which must influence, but not control, the amount of the substance entering the marine environment).

There is similarly great variability in the trigger values (see Table 2) used in different schemes. In most parameters, there seems to be a range of about 100 in the selected values of many of the triggers. For example, triggers on acute toxicity vary from 0.1 to 10 mg/l, and bioaccumulation factors from 2100 to 15,000.

The IEE, IEA and EIW organised a seminar on "The setting of a common selection scheme of dangerous substances", with reference to EC Directive 76/464 (Dangerous Substances) and the North Sa Declaration (March 1990) in July 1990, in Como, Italy. The conclusions of the seminar are reproduced as Appendix 4. The purpose of the seminar was to develop a framework within which a selection scheme should be developed. The seminar did not come to a conclusion as to the type of scheme which should be adopted, but indicated that trigger values, etc., should reflect standards and other values already applied in EC Directives, bearing in mind the Precautionary Principle where necessary, and that any scheme should be thoroughly tested using substances for which good data were available.

It can be concluded that there is some measure of agreement as to the possible structures of the more detailed selection schemes, and that they may be classified as scoring/ranking schemes and logic tree schemes, and that some parameters which describe either the exposure of an organism to a compound or the response of that organism should be included. These basic parameters include toxicity, persistence, bioaccumulation potential, and preferably parameters concerned with input to and behaviour in the environment. However, there is as yet no clear agreement on the range of parameters that should be used, or on the scorings/trigger values that should be applied.

KEY TO TABLES 1 AND 2 (from Jackson and Peterson, 1989)

| | |
|---|---|
| United Kingdom: | Hedgecott and Copper, 1991<br>Dept of the Environment, 1988<br>Dept of the Environment, 1989<br>Byrne, C.D., 1988<br>Agy and Zable, 1990<br>Zabel and Jennings, 1987 |
| Italy: | Sampaolo and Bientti, 1984, 1989 |
| SRI: | Stamford Research Institute, 1980 |
| XI/85/001: | CEC, 1985 |
| XI/730/89: | CEC, 1989 |
| North Sea: | Second International Conference on Protection of the North Sea, 1987<br>Third International Conference on Protection of the North Sea, 1989, 1990 |
| Netherlands: | Working Group on Criteria for the assessment of motor pollution (BCW) 1989 |
| CEFIC: | CEFIC, 1987 |
| ICI: | ICI water and liquid effluent panel, 1988 |
| Korte et al.: | Korte et al., 1986 |
| BMU: | Advisory Council to the Federal Ministry for the Environment, 1987 |
| GDCL BUA: | Gesellschaft Deutscher Chemipa, 1989a, 1989b |
| France: | Commission D'Evaluation de l'ecotoxicité des substances chimiques, 1986 |
| Ontario: | Environment Ontario, 1989 |

Table 1

Use of parameters.

| | United Kingdom | Italy | # SRI | XI/85/001 | # XI/730/89 | North Sea | Netherlands | CEFIC | ICI | Korte et al | BMU | GDCh BUA | France | Ontario |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ranking/scoring | | * | * | | | | | | | | * | * | | * |
| Logic tree | * | | | | | * | * | * | * | * | | | | |
| Appearance on other lists | (*) | | (*) | | | | | (*) | (*) | | | * | (*) | |
| Teratogenicity | | * | | | | | | | | | | | | * |
| Mutagenicity | | * | | | | * | | | | | | * | | * |
| Carcinogenicity | * | * | | | | * | * | | * | | | * | * | * |
| Chronic mammal toxicity | | * | * | | | | | | | | | | * | |
| Acute mammal toxicity | * | * | * | * | | | | | | | * | * | | * |
| Chronic aquatic toxicity | * | | * | | | | * | | | | | | | * |
| Acute aquatic toxicity | * | * | * | * | * | * | * | * | * | | * | * | * | * |
| Models | | | * | * | * | | | | | * | | | | |
| Bioaccumulation/$K_{ow}$ | * | * | * | * | | * | | * | * | * | (*) | * | * | * |
| Persistence | * | * | * | * | | * | * | * | * | * | (*) | * | * | * |
| Persistence | * | * | * | * | * | | | | | * | (*) | | * | * |
| Point/Diffuse | * | | * | | | | | | | | | | | |
| Presence in environment | | | | | | * | * | | | | | * | | |
| Use tonnage | * | | * | | | * | | | | | | | | |
| Production tonnage | * | * | * | | | * | * | | | | | (*) | | |

Italy - Sampaolo and Binetti
France - Mission du Contrôle

Note - The way in which parameters are used in different schemes may not be comparable
# model-based exposure assessments
(*) may not be integral part of priority setting

## Table 2

### Comparison of Trigger Values in Different Schemes.

| Scheme/ Parameter | U.K. | ICI | CEFIC | Netherlands | Mission du Contrôle France | Scoring system GDCh Germany | 6th amendment CEC XI/85/001 | Scoring system Canada | Scoring system Italy | North Sea |
|---|---|---|---|---|---|---|---|---|---|---|
| Acute toxicity mg/l fish 96h, $LC_{50}$ | **<1.0** **>100** | **<1.0** >10 | **<0.1** >1.0 | **<0.01** | **<0.1** >1.0 | **<1.0** >100 | **<0.1** >10 | **<0.1** | **<10** >1,000 | **<1.0** (>100) |
| Chronic toxicity mg/l | **<0.01** >1.0 | | | **<0.01** + <60% degradation in four days | | | | | | |
| Persistence half life, days | **>100** 2.10 | **>100** <10 | **>100** <30 | Degradation <10% in 28d | **>40** | Included | Not readily degradable | **>100** | **>30** <1 hour | $\frac{BOD}{COD}$ **<0.5** |
| Bioaccumulation factor (Log $K_{ow}$) | **>1,000(>3.5)** <100(<2) | **>1,000** | **>1,000** | Not yet included | **>100(>3)** | **>100** <100 | **>300(>3.5)** | **>15,000** | **(>3)** (<0) | **>100** |
| Higher organism $LD_{50}$ mg/kg | **<50** >500 | | | | | **<25** | **<25** | **<0.5** | | |
| Production and use, tonnes/year | **>10,000** <1,000 | | | **>100** | | | | | **>100,000** <1 | |
| Solubility in water mg/l | **>1,000** <1 | | | | | | | | | |
| Volatility, Pascals | **>0.133** <0.000133 | | | | 1½ <1 hour | | | | **>500** <10 | |
| Carcinogenicity | Yes | Yes | | Yes (1½ >1 day) | Yes | Yes | | Yes | Yes | |
| Occurrence, mg/l | | | | In Rhine | | **>1** | | Yes | | |

Note - these figures are not directly comparable - see text
Bold type indicates high value
Normal type indicates low value

APPENDIX 1
(from GESAMP, 1990)

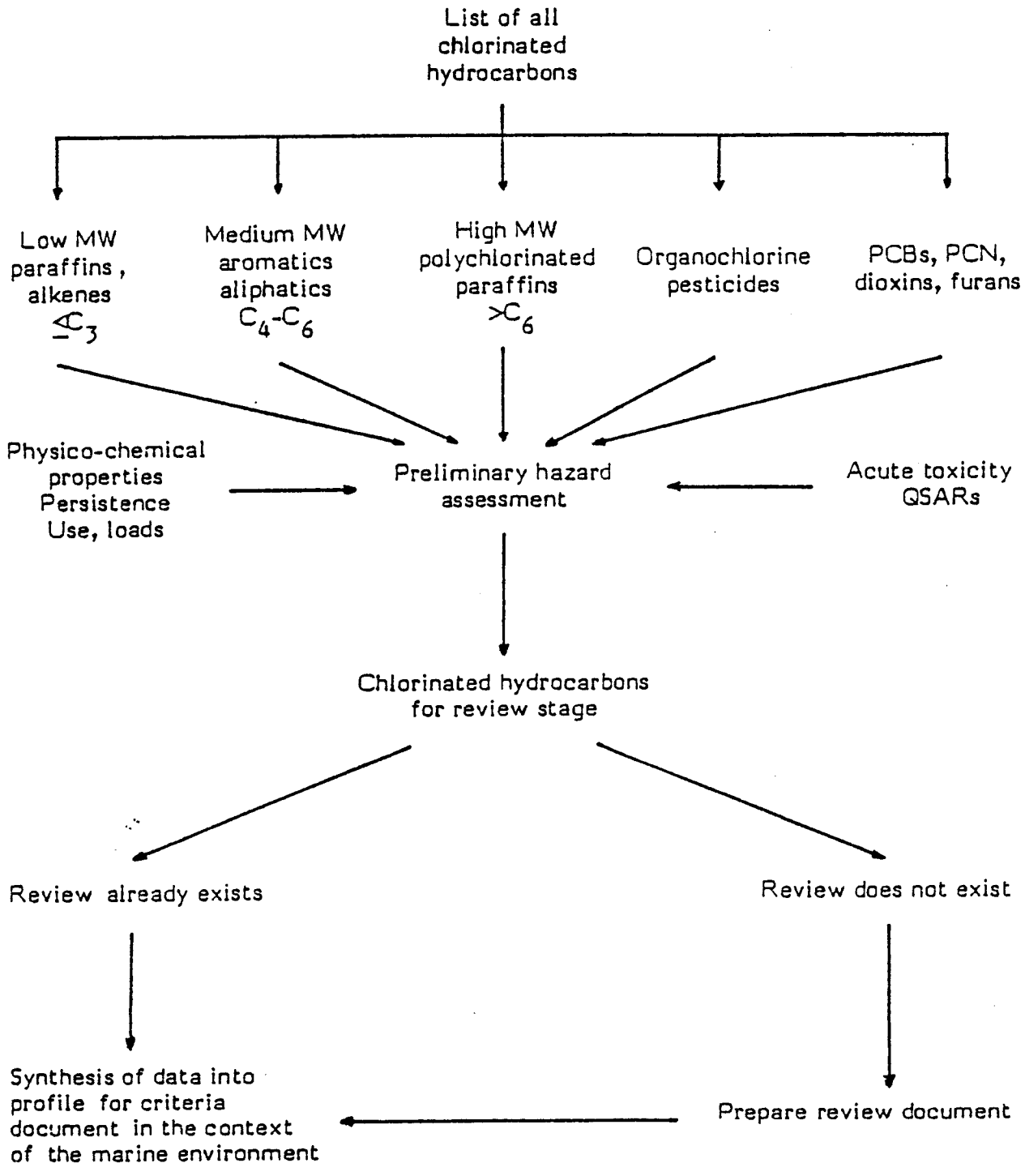


Figure 1. Recommended procedure for reviewing organochlorine compounds

**List of Potentially Harmful Organochlorine Substances Selected on the Basis of Data Examined**

Substances of Group 1: $C_1$ to $C_3$ Compounds

1,1,2,2-Tetrachloroethane
1,12,2-Tetrachloroethylene
1,1,2-Trichloroethane
1,1,2-Trichloroethylene
Chloroform
Dichloromethane
Epichlorohydrin
Hexachloroethane
Vinylchloride
Methylchloride
Pentachloroethane
Tetrachloroethene
Tetrachloromethane

Substances of Group 2: $C_4$ to $C_6$ Compounds

1,1,2,3,4,4-Hexachloro-1,3-butadiene
1,2,3,4-Tetrachlorobenzene
1,2,3,5-Tetrachlorobenzene
1,2,3-Trichlorobenzene
1,2,4,5-Tetrachlorobenzene
1,2,4-Trichlorobenzene
1,3,5-Trichlorobenzene
1-Chloro-2-nitrobenzene
1-Chloro-3-nitrobenzene
1-Chloro-4-nitrobenzene
1-Chlorobutane
2,3,4,5-Tetrachlorophenol
2,3,4,6-Tetrachlorophenol
2,3,5,6-Tetrachlorophenol
2,3-Dichlorophenol
2,5-Dichlorophenol
2,4-Dichlorophenol
2-Chloro-1,3-butadiene
3,4,6-Trichlorocatechol
Chlorobenzene
Hexachlorobenzene
Hexachlorocyclohexane
Hexachlorocyclopentadiene
3-hlorophenol
1,3-Dichlorobenzene
2-Chlorophenol
1,2-Dichlorobenzene
4-Chloroaniline
4-Chlorophenol
1,4-Dichlorobenzene
Pentachlorobenzene
Pentachlorophenol
Pentachloropyridine
Tetrachlorocatechol

Annex I: continued

Substances of Group 3: Compounds with more than $C_6$

DL-3-(a-Acetonyl-p-chloro-benzyl)-benzylchloride
1-(o-Chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane (o,p'-DDD)
1,1'(Dichloroethylidene)-bis[4-Chlorobenzene] (p,p'-DDE)
1-(o-Chlorophenyl)-1-(p-chlorophenyl)-2,2,2-Trichloroethane (p,p'-DDT)
2,4,5-Trichlorophenoxy acetic acid
2,4,5-Trichlorotoluene
2,4-Dichloroacetophenone
2,6-Dichlorobenzonitrile
2-Chloro-4-nitrotoluene
2-Chlorotoluene
3-Chlorotoluene
4-Chlorotoluene
Benzotrichloride
Octachlorostyrene
2,3-Dichlorotoluene
2,4-Dichlorotoluene
2,5-Dichlorotoluene
2,6-Dichlorotoluene
3,4-Dichlorotoluene
Tetrachloroguaiacol
3,4,5-Trichloroguaiacol
4,5,6-Trichloroguaiacol
4-Chlorostyrene
α,α,2,6-Tetrachlorotoluene

Group 4: PCBs, PCDD/PCDF

Total of 209 PCB isomers

Commercial mixtures of PCBs: Aroclors, Kaneclors, Chlophens, etc.

Total of 210 PCDD/PCDF isomers
of these 17 "toxic" isomers (=2,3,7,8-Cl substituted PCDD/PCDF)

APPENDIX 2
(from Hedgecott and Cooper, 1991)

## SECTION 4 - LOGIC FOR THE SELECTION PROCEDURE

### 4.1 SCENARIOS FOR PRIORITY CANDIDATE SELECTION

The selection procedure consists of four decision trees, or scenarios, which will select those substances which pose the greatest hazard to the aquatic environment on the basis of a combination of their toxicity, bioaccumulation, carcinogenicity, persistence and likelihood of entering water. The four scenarios are summarised below and in Figures 1-4. Where reference is made in the Figures to 'high' or 'low' values, such as 'high' acute toxicity, the definition of 'high' and 'low' can be obtained from the threshold values presented in Table 6. These threshold values have been selected by the DoE, but they may be changed for any particular run as described under Item 7 of Section 5.2.

Table 6

Numerical values for high and low thresholds for chemical property or toxic end points.

| Property | Toxic end point or parameter | Unit | High | Low | Insignificant |
|---|---|---|---|---|---|
| Acute aquatic toxicity | 96 hr LC50 (fish) or 48 hr EC50/LC50 (invert) | mg/l | <1.0 | >100 | - |
| Sub chronic or chronic aquatic toxicity | NOEC | mg/l | <0.01 | >1 | - |
| Persistence in the aquatic environment | Half life | days | >100 | <10 | <2 |
| Bioaccumulation potential | Bioconcentration factor<br>Log Kow | -<br>- | >1000<br>>3.5 | <100<br><2 | -<br>- |
| Toxicity to higher organisms | Significant toxic effect or oral LD50 | mg/kg | <50 | >500 | - |
| Production and use | | tonnes/ year | >10000 | <1000 | - |
| Solubility in water | | mg/l | >1000 | <1 | - |
| Volatility | | Pascals | >0.133 | <0.000133 | - |

4.1.1 The short-term scenario

This considers whether the substance is likely to reach a concentration in water at which acute toxic effects have been reported.

The decision is based on the substance's acute toxicity to aquatic organisms, its persistence in surface waters and its estimated level of input. Substances may be considered as priority candidates if they meet the criteria outlined in Figure 1.

4.1.2 The long-term scenario

This considers whether the substance is likely to reach a concentration in water at which chronic toxic effects have been reported.

The decision is based on the substance's chronic toxicity to aquatic organisms, its persistence in surface waters and its estimated level of input. Substances may be considered as priority candidates if they meet the criteria outlined in Figure 2. It should be noted that there is much variability in the duration and toxic endpoints of chronic toxicity tests for fish and invertebrates. Therefore the selection procedure is restricted to chronic toxicity data relating to survival, reproduction and growth and odes not consider, for example, behavioural or biochemical responses. In addition the duration and endpoint are included in the database for each reported chronic toxicity value, so that the user can exercise his or her professional judgement as to its relevance.

4.1.3 The food chain scenario

This considers whether the substance is likely to reach a level at which toxicity will occur in higher organisms as a result of bioconcentration through the food chain.

The decision is based on the substance's estimated level of input, its persistence in surface waters, its bioaccumulation in aquatic organisms and its toxicity to higher organisms (mammals). Substances may be considered as priority candidates if they meet the criteria outlined in Figure 3. It is recognised that the use of a rat (or other mammalian) acute oral LD50 as a toxic endpoint for higher organisms is less than ideal, but no other more suitable value is consistently reported in the literature.

4.1.4 The carcinogenicity scenario

This considers whether the substance's carcinogenic, mutagenic or teratogenic properties may result in an increased risk to human health following exposure in or through water.

The decision is based on the substance's: EC carcinogenicity, mutagenicity or teratogenicity classification; the exposure risk route; and the estimated level of input to water. Substances may be considered as priority candidates if they meet the criteria outlined in Figure 4. Those that are effective solely by the inhalation route are not considered because the priority selection procedure is only concerned with substances in water.

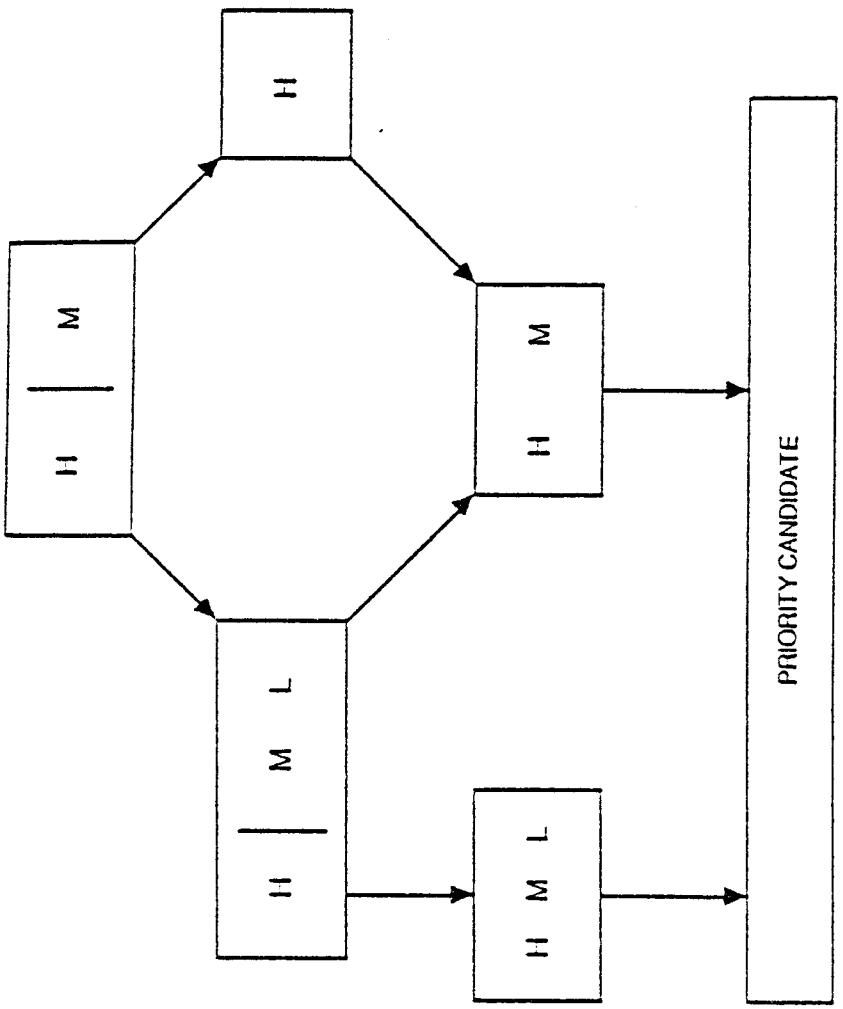


Figure 1 Short Term Scenario

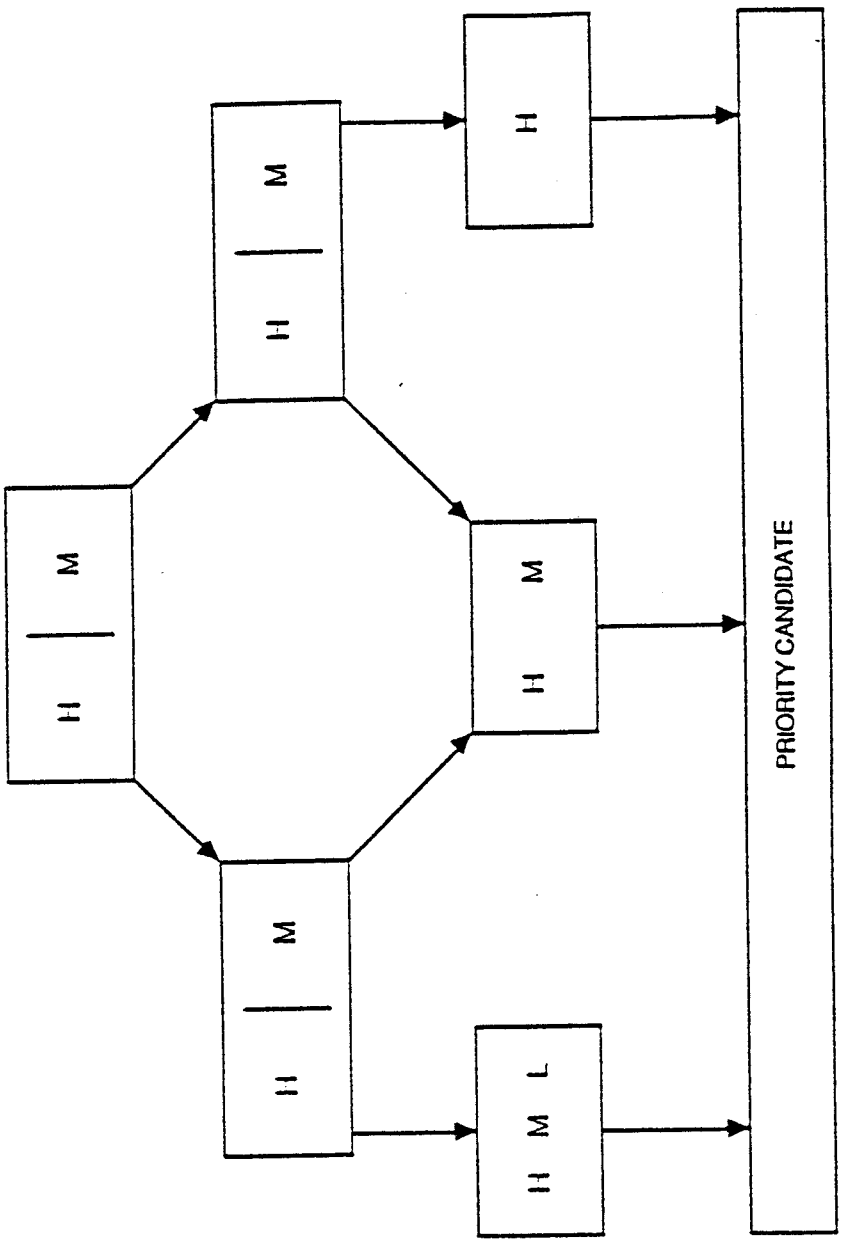


Figure 2 Longer Term Scenario

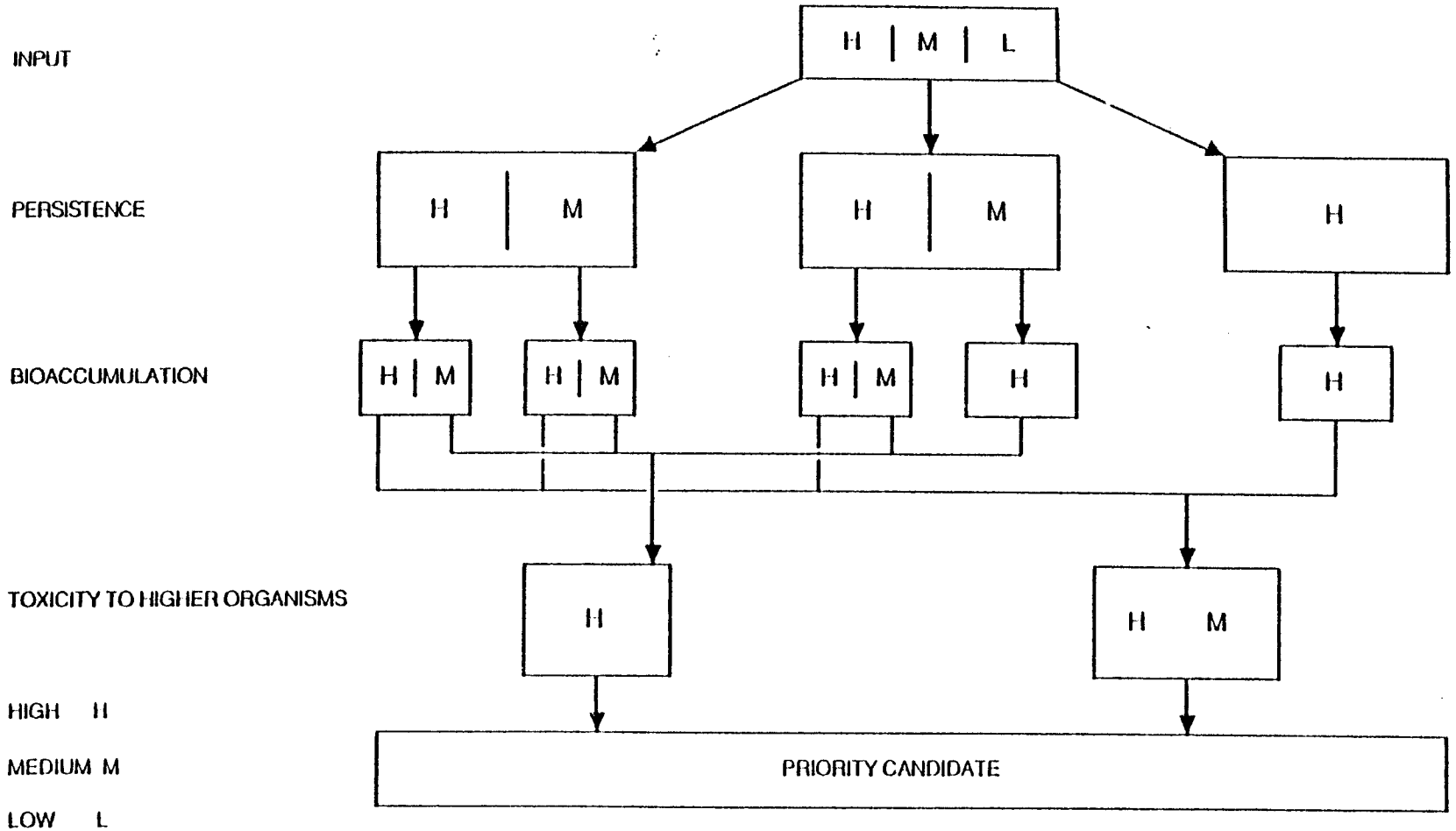


Figure 3 Food Chain Scenario

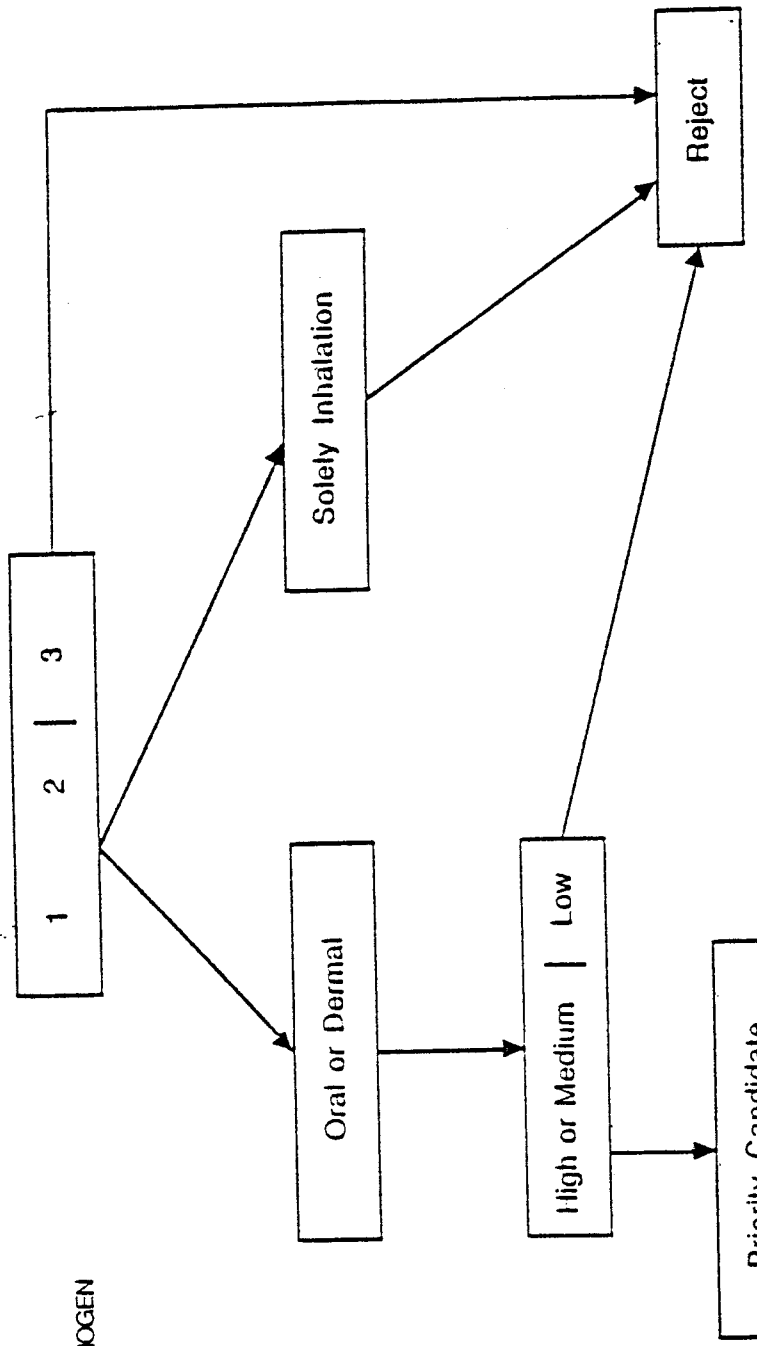


Figure 4 Carcinogenicity Scenario

## 4.2 ESTIMATING INPUT INTO WATER

As part of the risk assessment procedure it is necessary to have an estimate of the extent of a substance's release to the aquatic environment. The scenario outlining this is presented in Figure 5

A substance's input is estimated from its sources (ie point or diffuse), 'escapability' (ie aqueous solubility divided by volatility) and production volume. Substances having predominantly diffuse sources, such as pesticides, are considered to have a higher hazard potential because it is harder to control their releases and they often have a higher percentage release than substances with point sources.

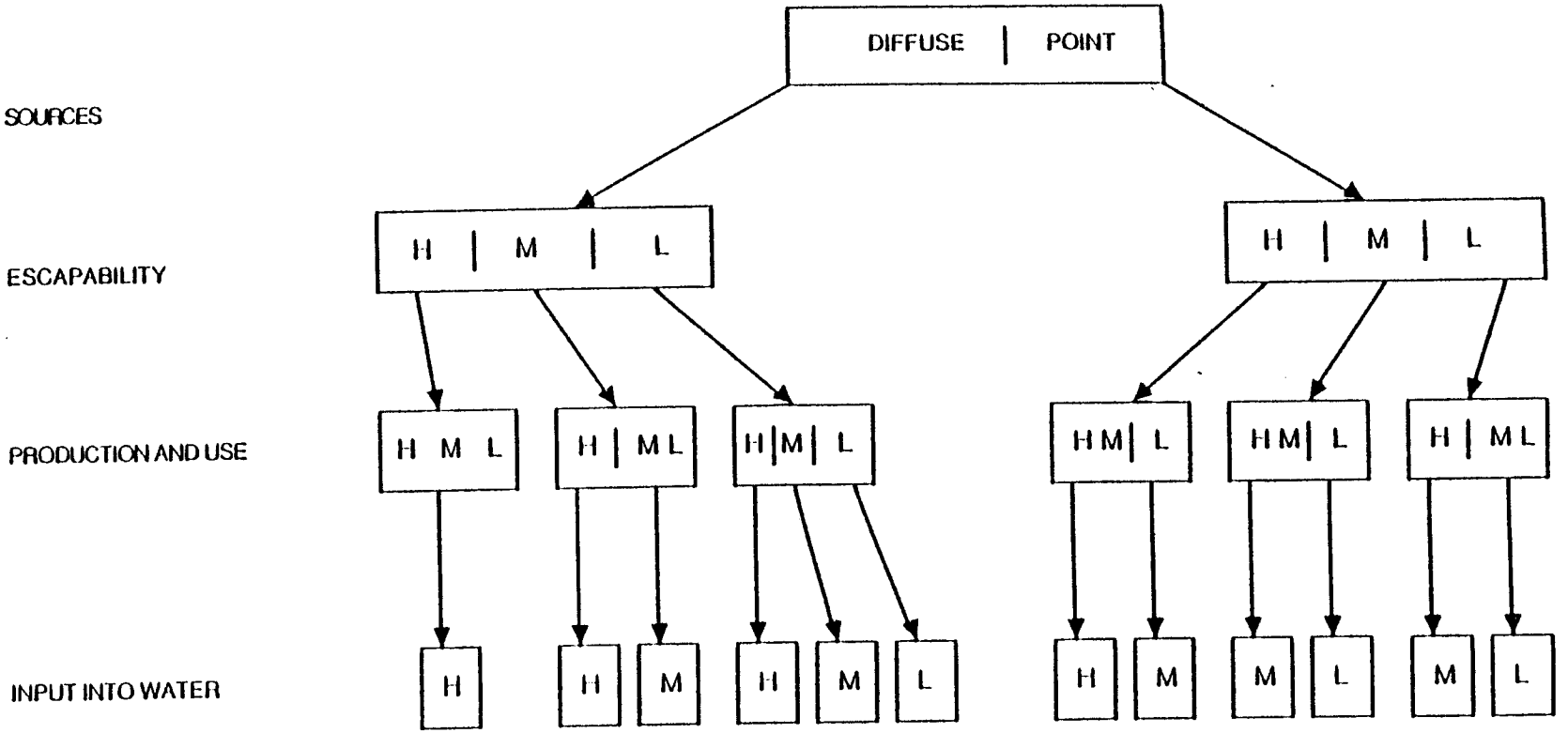


Figure 5 Input into Water

APPENDIX 3
(from Jackson and Peterson, 1989)

10. Canada

Ontario Ministry of the Environment scoring system

Summary

A scoring system which gives score values to effects parameters. The series of scores is considered by expert judgement and if any one exceeds guideline limits, the substance is subject to exposure assessment. Substances of high exposure are then priority listed for more information and monitoring.

Advantages:
- guidelines for scores relating to fixed parameter values
- easy to use and flexible - uses available data
- inclusion of carcinogenicity, teratogenicity, genotoxicity
- potential inclusion of both human and environmental impacts

Disadvantages:
- Priority listing may be by one characteristic only and so different individual characteristics may lead to listing of different substances

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*Ontario Ministry of the Environment*

The Municipal/Industrial Strategy for Abatement (MISA) of the Canadian Ontario Ministry of the Environment uses an Effluent Monitoring Priority Pollutants List (EMPPL) of substances which require more extensive information and monitoring because of their hazard to the environment (Environment Ontario 1989). The listing process is based upon the substance's environmental persistence, bioaccumulation potential, acute and sublethal toxicity to biota and presence in discharges to surface waters.

The first stage of the process is chemical identification - the development of a comprehensive list of candidate substances which are placed in one of two groups .The primary group consists of chemicals detected in effluents and surface waters and the secondary group of chemicals which have not yet been detected but which would be of concern if they were found. Chemicals are chosen from these groups by the MISA office (for the 1988 update, chemicals from organic chemical, iron and steel, mining, electric power generation and pulp paper industries) which are then subject to a preliminary hazard assessment process.

The hazard assessment process uses an effects and fate scoring system with exposure assessment criteria in order to place chemicals on the EMPPL. The scoring system (Environment Ontario undated) gives scores for environmental behaviour parameters:

a. Environmental transport
b. Environmental persistence
c. Bioaccumulation

and for toxicity parameters:

a. Acute lethality
b. Sub-lethal effects on non-mammals
c. Sub-lethal effects on plants
d. Sub-lethal effects on mammals
e. Teratogenicity
f. Genotoxicity
g. Carcinogenicity

Values for each of these parameters are given score values on a scale of 0-10 according to predetermined criteria (Table 1).

Tags are added to scores to indicate availability or reliability of data. Worst-case scenario data and values estimated from models.

Environmental transport examines partitioning between media which can be of importance in evaluating hazard. This data may be obtained from field studies or microcosm work but is more likely to come from a modelling technique such as the Mackay and Paterson Fugacity Level I model (Mackay and Paterson 1981). This model requires data for physicochemical properties and gives partition estimates for air, water, soil, sediment and aquatic biota. Higher scores are assigned to those substances more evenly distributed between compartments but the scores are not taken into account in placing chemicals on the EMPPL - they are for information purposes in assessing which data are most relevant in later stages of the scoring system. Persistence is expressed as half-life (t*) and may be estimated from environmental data, shaker flask studies, Quantitative Structure-activity Relationships (QSARs) or biodegradability tests (in order of preference). t* values of greater than 50 days are of concern but will only be sufficient to place substances on the EMPPL if other substance characteristics are also of concern. Bioaccumulation is usually the bioconcentration factor, preferably measured but often predicted using the octano-water partition coefficient and a value of more than 500 (BCF) is of sufficient concern to place on the EMPPL.

Toxicity data is carefully screened for the relevance of routes of exposure and applicability of tests - such factors as methodology, duration, dosage. Default values are estimated from the best available information and the validity of data is regularly assessed.

Acute lethality may be taken from several test types - mammalian, oral, dermal or inhalation $LD_{50}$ or $LC_{50}$ or aquatic 96 hour $LC_{50}$. Listing would occur for oral $LD_{50}$ <50 mg/kg or aquatic $LC_{50}$ <10 mg/l. Sublethal toxicity may also be assessed from a range of tests. For aquatic animals and plants, values of $EC_{50}$, maximum aquatic toxic concentration (MATC) or no observed adverse effect concentration (MATC) or no observed adverse effect concentration (NOAEC) are given as trigger values for scores which indicate concern. Mammal chronic toxicity uses no observed effect levels (NOEL). The data used for non-mammalian sublethal toxicity for aquatic environments will preferably be fish, *Daphnia* or another more sensitive species, preferably using full or partial life-cycle tests which include reproductive effects.

Teratogenicity, genotoxicity/mutagenicity and carcinogenicity are scored separately. Teratogenicity uses the EPA definition of "frank developmental malformations detrimental to the survival, future development or well-being of newborn", not including secondary effects of toxicity to embryo, foetus or mother. Behavioural teratology is also briefly considered and the possibility of reversible teratogenicity (e.g. delayed development). Teratogenicity is given scores on the basis of the threshold value at which the effect occurs and the effects themselves left to expert judgement for consideration.

Genotoxicity/mutagenicity is scored according to effects on DNA directly (highest score) or upon DNA synthesis and repair. Various tests and assays are recommended for gene mutation or chromosomal aberrations (see later section for details) and QSARs may be used if data is treated with appropriate caution and tags appended to the score.

Carcinogenic properties are indicated by tumour formation and while the initiation of tumours may be dose-related, the development is dose-independent. The scoring is based upon type of carcinogenicity, the highest scores being assigned to direct-acting carcinogens which interact with genetic material.

All of the scores assigned, particularly for the parameters with narrative scoring (teratogenicity, mutagenicity, carcinogenicity), must be assigned with some degree of expert judgement.

The scores are not combined into an index but left as a series of numbers with tags. If scores for several subparameters are available, for examples oral, dermal and aquatic acute toxicity, the highest will be taken as the score.

If any one of the series of scores exceeds predetermined limits for any parameter (Table 3), the substance is examined for exposure assessment criteria (Table 2). If the substance is detected in effluent or in the environment or if its presence is probable in discharge from a plant where the substance is used or manufactured, the substance is then placed on the Effluent monitoring priority pollutants list.

The substance, once on the EMPPL, is a priority for monitoring and for detailed hazard assessment which will give details of case studies and more detailed toxicological use and disposal information. Details of preliminary and detailed hazard assessments are contained in the Ontario Ministry of the Environment CESARS database.

Table 1 Ontario

EMPPL Effects Assessment Criteria

Bold text indicate concern levels

| PARAMETER | UNITS | 0 | 4 | 7 | 10 |
|---|---|---|---|---|---|
| Environmental Transport* | Percent partitioning measured or predicted | Any single medium contains >95% of the total amount released | Any single medium contains >90-95% of the total amount released | Any single medium contains >80-90% of the total amount released | No single medium contains >80% of the total amount released |
| Environmental Persistence | 1½ (days) | ≤10 | >10 to 50 | **>50 to 100** | **>100** |
| Bio-accumulation | BCF<br>Log $K_{OW}$ | ≤20<br>≤2.0 | >20 to 500<br>>2.0 to 4.0 | **>500 to 15,000**<br>**>4.0 to 6.0** | **>15,000**<br>**>6.0** |

* The Environmental Transport parameter is for information purposes only, and does not influence the promotion of a chemical to the EMPPL

## Table 1 Ontario

### EMPPL Effects Assessment Criteria contd.

### Bold text indicates concern levels

| PARAMETER | UNITS | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|---|
| Acute Lethality | oral LD50 mg/kg | >5,000 | >500-5,000 | >50-500 | **>5-50** | **>0.5-5** | **≤0.5** |
| | aquatic LC50 mg/L | >1,000 | >100-1,000 | >10-100 | **>1-10** | **>0.1-1** | **≤0.1** |
| **Sublethal Effects Non-Mammals** | **aquatic EC50 mg/L** | **≥20** | **2-<20** | **0.2-<2** | **0.02-<0.2** | **<0.02*** | **<0.02*** |
| Sublethal Effects Plants | aquatic EC50 mg/L | >100 | >10-100 | >1-10 | **>0.1-1** | **0.01-0.1** | **<0.01** |
| Sublethal Effects Mammals | oral NOEL mg/kg | >1,000 | >100-1,000 | >10-100 | **>1-10** | **>0.1-1** | **≤0.1** |
| Teratogenicity | narrative mg/kg/day | no terata, or terata only at >1,000 | **terata or developmental anomalies at >50-1,000** | **terata or developmental anomalies at >10-50** | **terata or developmental anomalies at >1-10** | **terata at >0.01-1, without overt maternal toxicity** | **terata at ≤0.1, without overt maternal toxicity** |
| Genotoxicity Mutagenicity | narrative | no evidence of genotoxicity or mutagenicity with adequate testing | positive results in vitro only | genotoxic/ mutagenic in prokaryotic systems only | **effects on DNA, but no direct interactions with DNA** | **clastogenic effects but no direct interactions with DNA** | **genotoxic/ mutagenic usually with direct interactions with DNA** |
| Carcinogenicity | narrative, human and animal bioassay data | no tumours in adequate studies on at least two species, and does not interact with genetic material | **tumours in only one animal species, negative results in others** | **causes benign tumours in more than one species, and does not interact with genetic material; promotor only; or causes cell transformation in vitro only (negative evidence in vivo)** | **tumourigenic in bioassays at doses causing metabolic enzyme saturation, or associated with lesions that predispose to tumours. No interaction with genetic material** | **indirect-acting (epigenetic) carcinogen, no interaction with genetic material** | **direct-acting carcinogen that interacts with genetic material** |

* The Environmental Transport parameter is for information purposes only, and does not influence the promotion of a chemical to the EMPPL

Table 2 Ontario

EMPPL Exposure Assessment Criteria*

| Classification | Criterion |
|---|---|
| A | Almost always present, based on >50% detection and minimum of 5 data points |
| B | Potentially present in Ontario, based on: detection in effluent or reported as being discharged: *confirmed Ontario use/manufacture and probable presence in a process aqueous discharge;* or detected in the natural environment and probable Ontario use/manufacture |
| C | Inferred to be present in a process aqueous discharge and probable Ontario use/manufacture |
| D | Possibly present, based on use/manufacturing information only |
| E | Not present, based on analytical data and/or inference from the literature |

* Amendment to 1987 EMPPL exposure assessment criteria is indicated by italic type

Table 3 Ontario

Parameter Scores Leading to Promotion to the EMPPL (Concern Levels)

| Parameter | Promote if score Equals or Exceeds |
|---|---|
| Persistence | 7* |
| Bioaccumulation | 7 |
| Acute Lethality | 6 |
| Sublethal Toxicity, non-mammals | 6 |
| Sublethal Toxicity, plants | 6 |
| Sublethal Toxicity, mammals | 6 |
| Mutagenicity/Genotoxicity | 6 |
| Teratogenicity | 2 |
| Carcinogenicity | 2 |

* A persistence score of ≥7 alone does <u>not</u> cause promotion to the EMPPL but may support promotion based on other parameters

# ANNEX 7

## Contaminants monitoring in different media

**This annex contains selected material from the Report of the (ICES) Working Group on Environmental Assessments and Monitoring Strategies (1990).**

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### OPTIMISATION OF MONITORING MARINE CONTAMINANTS WITH RESPECT TO MARINE MATRIX

ICES has been requested to supplement its advice on appropriate marine matrices for monitoring contaminants for the purposes of the Joint Monitoring Programme (JMP) by the addition of a number of new contaminants identified by the North Sea Task Force in its Monitoring Master Plan, which is intended as a supplement to the JMP in the North Sea. This document contains the information supplied by the Working Group on Environmental Assessments and Monitoring Strategies in its 1989 report, with the addition of material covering the new contaminants.

The matrices considered included sea water, sediments, and biota, as are included in the current JMP. The matrices were selected as those most appropriate for the provision of the greatest information in relation to each monitoring purpose. They were selected on scientific grounds, and did not take any account of relative costs or convenience of the alternative choices.

In some cases, no matrix has been recommended, either because the monitoring of a particular contaminant was not appropriate to the monitoring purpose, or because advice could not be given for technical reasons. More complete explanations of individual cases are given below.

In many cases, primary and secondary choices of matrix are given and, in some cases, tertiary choices. These choices should be viewed as alternatives, or complementary choices, but the Working Group considered that, if circumstances permitted, a primary matrix should be preferentially selected for analysis, as this would provide the greatest amount of information relevant to the particular monitoring purpose. The Working Group recognised that suitable primary matrices may not be available in all monitoring locations, and in such cases, secondary or tertiary matrices should be used. It was fully appreciated that, in some cases (particularly in relation to Purpose c), the assessment of the existing level of marine pollution) a more comprehensive assessment might be obtained by the analysis of the contaminant in all matrices. However, the priority selections of matrices were made with the aim of providing the most useful scientific information for assessing distributions of contaminants, and focussing attention of those matrices that might enable the most consistent picture of distributions over wide areas to be obtained through the collective efforts of a number of laboratories and countries.

It was also recognised that in some cases matrices will be chosen on the basis of pre-existing local information and on-going monitoring programmes. The advice in the following sections should not be taken as denigrating the continuation of existing monitoring programmes designed in the context of local conditions that are yielding useful information, even if they do not wholly match the selections advocated here.

The Working Group wished to remind JMG that, in all circumstances, the reliability of the information from a monitoring programme, and its consequent value, is dependent upon the attention paid to quality assurance at all stages of the measurement programme (sample collection, storage, preparation, pre-concentration, analysis, standardisation and interpretation). Participating laboratories should be required to adopt appropriate procedures in this area.

Purpose A: The assessment of possible hazards to human health (Table 1)

The Working Group recognised that, in the generality of the area covered by JMG, none of the contaminants considered presented a widespread serious hazard to human health through the consumption of marine foodstuffs. In some cases (e.g., copper, zinc, arsenic, chromium, and nickel), the contaminants were not normally of concern with respect to fisheries products. Equally, the monitoring of contaminants in sea water or sediment would not have any direct applicability to human health risk, and these considerations are reflected in Matrix Table 1. This table, therefore, provides advice on the contaminants and matrices that should be included in a regional or wider scale survey to assess the possible hazards to human health presented by the presence of selected contaminants in marine foodstuffs. In several cases, primary and secondary choices of matrix are given.

The Working Group also recognised that areas of contamination could exist which could give rise to localised increases of concentration in foodstuffs. Such situations were unlikely to be detected or adequately described by large-scale surveys, and were better approached through specially designed and targeted monitoring exercises by national or local authorities. In such circumstances, the relevant authorities should assess the most important exposure pathway by which the contaminant reached the public through marine foodstuffs. The monitoring programme should be directed at that pathway, and not be constrained by the advice given in Table 1 in relation to broader scale surveys. For example, in some areas there may be concern over the concentrations of CBs in the muscle of lipid-rich fish species, such as herring or mackerel, and in such circumstance sit would be appropriate to analyse herring or mackerel muscle.

Purpose C: The assessment of the existing level of marine pollution (Table 2)

Water

In designing Table 2 (and Table 3), the Working Group took note of the JMG recommendation (JMG 14/15/1, Annex 8) that "seawater analysis should not, as a rule, be used for purpose (d) - trend monitoring (OSPAR 10/11/1, § 2.12). Although the Commissions agreed that seawater analyses were not the most appropriate compartment for detecting true statistical trends in time (purpose (d)), the Commissions nevertheless acknowledged that the monitoring of seawater at a more regular frequency than once every five years could be justified:

1) in areas with enhanced levels of contaminants; and

2) in areas where changes could be expected as a result, for example, of known reduction in inputs (OSPAR 10/11/1, § 2.13).

In discussing sea water analysis, the Working Group drew a distinction between near-shore waters, in which marked salinity gradients may be found and which are more likely to be influenced by riverine or land-based inputs of contaminants, and off-shore waters where gradients are normally substantially less marked, and which are more remote from the above-mentioned inputs of contaminants.

The use of water analysis to reflect current levels of marine contamination is attractive in that it concerns the important aqueous phase, the environment in which both biota and sediment exist. However, the Working Group recognised the considerable efforts being made by the Marine Chemistry Working Group to improve the comparability of analytical performance among laboratories engaged in sea water analysis in member states. The requirements for precision and accuracy of analysis at low concentrations limit the number of determinants that could be considered in off-shore waters to mercury, cadmium copper, zinc and lead, all at secondary matrix level. Even in these cases, it would be essential for each laboratory to establish in-house quality control procedures, and for rigorous assessments to be made to establish comparability between laboratories, with particular attention to lead.

In near-shore waters, concentrations may be somewhat more variable and subject to anthropogenic influences, and chromium and nickel analyses might also be considered. The same quality assurance precautions would be needed. In near-shore waters, it is necessary to take account of any correlation between contaminant concentrations and salinity, and of the influence of the concentration and composition of suspended matter on the dissolved contaminants.

Sea water is not a matrix of choice for CBs, as the octano: water partition coefficients indicate that the compounds would be predominantly associated with sediment or biota.

The concentrations of arsenic naturally present in sea water make the discrimination of anthropogenic influences from natural processes difficult and, therefore, sea water is not indicated as an appropriate matrix.

The Working Group recognised that some sea areas (usually small and isolated) existed in which the inputs of contaminants are sufficiently large to cause marked elevations of contaminant concentrations in sea water, or in which changes in concentrations could be expected. As agreed by the commissions, in such areas it might be appropriate for national authorities to give more prominence to water analysis in monitoring programmes.

Sediments

There is very considerable emphasis laid on the use of surficial sediments as a primary matrix for most of the contaminants. Participating laboratories should take full account of the most recent advice on the selection of sampling locations and methods (see, e.g., Section 15, Coop.Res.Rep. No. 142 (1987); Annex 2, Coop.Res.Rep. No. 124 (1983); Annex 2,

Coop.Res.Rep. No. 132 (1984)). Areas of high sedimentation and low bioturbation rates are particularly favourable. It is also necessary to subject the samples or data to appropriate normalisation procedures to allow, particularly, for grain size variations.

Biota

Both sediment and shellfish are indicated as primary monitoring matrices for TBT. Whilst the main area of concern over TBT is its effects on shellfish, particularly molluscs (oyster, dogwhelk, etc.), these organisms are by nature of limited geographical distribution. TBT, and its derivatives DBT and MBT, can be found in sediment, especially near shipyards, harbours and areas of extensive shipping and mariculture, and the monitoring of sediment should allow the use of a single matrix in a wider range of environments (e.g., into low salinity areas of estuaries) than would be possible using one mollusc species.

In preparing advice in relation to this monitoring purpose, the Working Group interpreted the purpose as referring to marine contamination, rather than marine pollution (as stated in the purpose). It must be emphasised that this advice has no relation to effects of contaminants on biota. Biological effects monitoring is, in the view of the Working Group, covered by JMG Purpose (b). The Working Group envisaged that once biological effects monitoring was established, it would be accompanied by appropriate chemical measurements of the active contaminant or contaminants. It may be possible subsequently to make inferences of the likely extent and intensity of biological effects from the results of Purpose (c) monitoring, by application of correlations between effects and contaminant concentrations derived from Purpose (b) monitoring.

Purpose D: Assessment of the effectiveness of measures taken for the reduction of marine pollution with the framework of the conventions (Table 3)

Measures taken within the framework of the Conventions to reduce the level of marine pollution are primarily directed at the control and reduction of inputs of contaminants. The main inputs are from riverine sources, land-based discharges, the atmosphere, and by direct dumping. The most efficient way to assess the effectiveness of the measures taken to reduce inputs is therefore to monitor the inputs, and JMG should take note of efforts already being made within the Commissions to assess the levels and trends of inputs. JMG may wish to take note of the comments in the 1988 ACMP report on the estimation of gross and net riverine inputs, and on atmospheric inputs. The monitoring of inputs can give detailed information on the effects of control measures on individual or localised groups of contaminant sources, and can therefore be particularly useful in regulatory procedures. It is likely that more and larger responses will be obtained when monitoring is conducted closer to the sources being regulated. Thus, for example, for land discharges, rivers and streams will generally yield higher signal-to-noise ratios than the marine environment.

It is also necessary to assess the effectiveness of the control measures in improving the quality of the marine environment. It is this aspect of trend monitoring that is covered by the Working Group advice in Table 3.

The Working Group noted that monitoring for the assessment of temporal trends of contaminants in the marine environment is very much less developed than monitoring for Purposes (a) and (c). There is an ICES Working Group on Statistical Aspects of Trend Monitoring (WGSATM) which is primarily addressing questions in this area. The advice in Table 3 represents the combinations of matrices and contaminants which WGEAMS feels have so far demonstrated the potential to display temporal trends, or which (e.g., shellfish) are likely to be usable in the near future. The table, therefore, represents a statement of the current "state of the art", and JMG should be aware that, as the subject is developed, additional combinations may become appropriate. With these considerations in mind, most of the recommendations are indicated as primary matrices, to reflect that they are very much alternatives.

When considering monitoring for temporal trends, it is necessary to consider the likely length of time which may elapse before any change in input may be reflected in the monitoring matrix This length of time will be a complex function of environmental factors and processes, the magnitude and rate of changes in inputs, analytical factors, and data analysis procedures, with particular emphasis on the variance of each of the contributory media and processes. This may have particular importance in relation to the frequency with which JMG may wish to assess the effectiveness of measures taken by the Commissions, or the frequency of regional assessment exercises (e.g., in the North Sea area).

The WGSATM has conducted a simplistic assessment of trend monitoring data on the mercury content of fish muscle and liver made available to ICES, and estimated from these data that fish muscle analyses could detect (with at least 0.95 probability) changes of at least 30% over a period of 10 years, whilst fish liver analyses could only detect changes of 50% or more. Such observations should be taken into account by JMG when assessing the potential usefulness of temporal trend monitoring, bearing in mind that the data set analysed, whilst selected as representing the "best available case" in terms of data quality and quantity, was limited in respect to both of these.

Biota

In relation to the use of biota in trend monitoring, the WGSATM pointed out that the detection of trends in contaminant concentrations in biota may not necessarily imply that environmental levels or inputs have changed. Circumstances are quire conceivable in which other environmental factors, for example leading to a change in type or availability of prey species, could give rise to changes in the degree of exposure of the predator species to the contaminants concerned.

Sediments

Table 3 particularly emphasises the potential of down-core analysis of sediments in trend monitoring for a wide range of contaminants. As noted with respect to Purpose (c), and in footnote 5 to Table 3, it is particularly important to pay attention to the site selection and data normalisation procedures discussed in other ICES documents. Arsenic and chromium analyses are not recommended as it is as yet unclear how the distribution of these elements may be affected by changes in redox potential in anoxic sediments.

The JMG should take note of comments in the 1989 report of the Working Group on Marine Sediments in Relation to Pollution on the influence of sedimentation rate and bioturbation intensity on the ability of sediment core samples to reflect changes in input to the sediment. It is also likely that sediment core analyses will reflect general basin conditions, rather than changes in single sources or types of input.

Water

Water analysis is not recommended for trend monitoring (except for lindane). However, in circumstances of marked contamination and where changes are expected, as discussed for Purpose (c), contaminant monitoring in sea water may be appropriate, provided that statistical considerations indicate that such analyses could reliably reflect the effects of control measures.

The comments above on the relationship between contaminant monitoring and biological effects monitoring apply equally to monitoring for Purpose (d).

**Clarification of contaminants included in the following Matrix tables:**

A) Chlorinated biphenyls (CBs).

CBs on an individual basis: CB nos. 28, 31, 52, 101, 105, 118, 138, 153, 156 and 180. This list is the same as that of the ICES/IOC/OSPARCOM intercalibration exercise and involves all three mono-ortho-Cl substituted CBs considered to be of environmental concern

B) Chlordanes

Cis-chlordane, trans-nonachlor, trans-chlordane, oxychlordane

C) Planar chlorobiphenyls: CBs-77, 126, and 169

D) Polychlorianted dibenzodioxins and dibenzofurans (PCDD/PCDF)

All seventeen 2,3,7,8-polychlorinated dibenzo-*para*-dioxins and dibenzofurans

E) DDT

Only p,p'-DDT, p,p'-DDE and p,p'-DDD (TDE)

F) Polycyclic aromatic hydrocarbons

PAHs selected: Naphthalene and C1-, C2- and C3-alkyl derivatives; Phenanthrene and C1- and C2-alkyl derivatives; Anthracene; Dibenzothiophene and C1- and C2-alkyl derivatives; Fluoranthene; Pyrene; Benz[*a*]anthracene; Chrysene (+ triphenylene); Benzofluoranthenes; Benzo[*e*]pyrene, Benzo[*a*]pyrene; Perylene; Benzo[*ghi*]perylene; Indeno[1,2,3-*cd*]pyrene and Dibenz[*a*,*h*]anthracene

G) Polychlorianted camphenes (PCC)

For quantification purposes it has to be borne in mind that this extremely complex mixture can only be quantified on the basis of technical mixture equivalents (e.g., toxaphene).

H) Triazines

Simazine and Atrazine are the major compounds of importance

I) Polybrominated diphenyl ethers (PBDE)

2,4,2',4'-tetrabromodiphenylether and 2,4,5,2',4'-pentabromodiphenylether as representatives of the brominated fire retardants

J) Polybrominated biphenyls (PBB)

# Matrix Table 1

## In relation to the assessment of possible hazards to human health (JMP Purpose a)

| Matrix \ Contaminant | CBs | γ-HCH | Hg[5] | Cd | Cu[3] | Zn[3] | As[4] | Cr[3] | Ni[3] | Pb | MeHg | TBT[3] | Chlor-dane[3] | Planar CB | PCDD/PCDF | DDT[3] | Diel-drin | PAH | PCC | Tri-azines[3] | PBDE[6] | PBB[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shellfish | P | P | P | P | | | | | | P | P | P | | P | P | | P | P | P | | | P |
| Fish muscle | | | P | | | | | | | | P | | | | | | | | | | | |
| Fish liver | S[2] | S[2] | | S[1] | | | | | | | | | | S[2] | S[2] | | S[2] | | S[2] | | | S[2] |

P: primary matrix
S: secondary matrix

Notes and Qualifications:

1. If fish liver is not a consumed fisheries product, no analysis is needed.
2. If fish liver is not a consumed fisheries product and there remain human health concerns, transfer attention to fish muscle.
3. These contaminants are not normally of concern in respect to the consumption of fisheries products.
4. Arsenic is present in seafood in measurable concentrations,but its chemical form makes it of little concern with respect to human health.
5. Hg should be understood to include methyl-mercury compounds. In countries where public health regulations refer to methyl-mercury rather than total mercury, samples may be analysed for methylmercury.
6. Too little is known about the toxicity to assess potential hazard.

# Matrix Table 2

## In relation to the assessment of the existing level of marine pollution (i.e., contamination) (JMP Purpose c)

| Matrix \ Contaminant | CBs | γ-HCH | Hg | Cd | Cu | Zn | As | Cr | Ni | Pb | TBT | MeHg | Chlor-dane | Planar CB | PCDD/PCDF | DDT | Diel-drin | PAH | PCC | Tri-azines | PBDE | PBB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nearshore water | | P | P[1] | P[1] | P[1] | P[1] | | P[1] | P[1] | P[1] | S[1] | | | | | | | | | P | | |
| Offshore water | | S | S[1] | S[1] | S[1] | S[1] | | | | S[1] | | | | | | | | | | | | |
| Surficial sediments[2] | P | | P | P | P | P | P[5] | P | P | P | P | P | P | P | P | P | P | P | P | | P | P |
| Shellfish | S[3] | S[3] | S[1] | S[1] | | S[1] | | | | S[1] | P | P | S[3] | S[3] | S[3] | S[3] | S[3] | S[3] | S[3] | | S[3] | S[3] |
| Fish muscle | | | T[1,4] | | | | S[1,4] | | | | | S[4] | | | | | | | | | | |
| Fish liver | S[4] | | | T[1,4] | | | | | | T[1,4] | | S[4] | S[4] | S[4] | S[4] | S[4] | S[4] | | S[4] | | S[4] | S[4] |

P: primary matrix
S: secondary matrix
T: tertiary matrix



Notes and Qualifications:

1. Potential addition/alternative to sediment measurements in areas where sediment conditions are not wholly favourable.

2. Should be accompanied by total organic carbon measurements, size fractionation (<63 μm), and description oft he sediment type. Sampling should be carried out following current ICES guidelines.

3. Could be carried out on an opportunistic basis, as may provide additional information on distribution.

4. Sedentary species only (e.g., flatfish).

5. The signal-to-noise ratio for discriminating between anthropogenic and natural influences is extremely low.

# In relation to the assessment of the effectiveness of measures taken for the reduction of marine pollution (i.e., contamination) in the framework of the Conventions (JMP Purpose a)

| Matrix \ Contaminant | CBs | γ-HCH | Hg | Cd | Cu[4] | Zn | As[6] | Cr[7] | Ni[4] | Pb | TBT | MeHg | Chlordane | Planar CB | PCDD/PCDF | DDT | Dieldrin | PAH | PCC[7] | Triazines | PBDE | PBB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Water | | P[2] | | | | | | | | | P | | | | | | | | | P | | |
| Sediment profiles[5,8] | P | P | P | P | P | P | | | P | P | | | P | P | P | P | P | | | | P | P |
| Shellfish | | | P | P | P | P | | | | P | P | P | | | | | | P | | | | |
| Fish muscle | | | P[1,3] | | | | | | | | | P[1,3] | | | | | | | | | | |
| Fish liver | P[1,3] | | | | | | | | | | | | P[1,3] | P[1,3] | P[1,3] | P[1,3] | P[1,3] | | | | P[1,3] | P[1,3] |

P: primary matrix
S: secondary matrix

Notes and Qualifications:

1. Considerable care has to be taken with species selection and availability, sampling protocol, and statistical aspects of data analysis.

2. Considerably greater effort is required, in respect to sampling and analytical frequency, if measurements are made in water, but the potential signal-to-noise ratio for trends is greater than that in sediments.

3. Sedentary species should be selected.

4. Highly unlikely that any trend signal related to anthropogenic influences will be detected.

5. Care should be taken in selecting favourable areas of high sedimentation rate, and limited bioturbation, following the latest ICES Guidelines for monitoring contaminants in sediments, including organic carbon measurements and appropriate normalisation procedures. Care should be taken to avoid contamination of subsurface sediment during dampling.

6. No recommendation can yet be made, except that As should not be measured in sediment profiles.

7. No recommendation can yet be made.

8. Care should be taken to avoid sub-surface contamination of sediment profiles.

# CORRECT SAMPLING - WHAT IS THAT ?

by

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(preliminary version)

A B S T R A C T

The material presented in this paper illustrates the influence of natural biological cycles in organisms on the monitoring of contaminants. The concentration in e.g. fish muscle or fish liver from one and the same specimen can vary quite considerably depending on when during the annual cycle the sample is taken.

This paper is a compilation of material mainly based on the four publications listed below.

1. Anonymous: "Influence of the sampling period on results from bio-indicator based monitoring". (Presented at the meeting with the Joint Monitoring Group, January 1993).

2. Anonymous: "Spatial and seasonal differences in the PCB content of the mussel Mytilus edulis". (Presented at the meeting with the Joint Monitoring Group, January 1993).

3. Anonymous: "Trends in pollutants in blue mussel Mytilus edulis and the flounder Platichthys flesus from two Dutch estuaries". (Presented at the meeting with the Joint Monitoring Group, January 1993).

4. Bignert et al., 1993: "The need for adequate biological sampling in ecotoxicological investigations: a retrospective study of twenty years pollution monitoring". The Science of the Total Environment, 128(1993):121-139.

**AGENDA ITEM 3B**

OSLO AND PARIS CONVENTIONS FOR THE PREVENTION OF MARINE POLLUTION
EIGHTEENTH MEETING OF THE JOINT MONITORING GROUP
THE HAGUE: 25-29 JANUARY 1993

# INFLUENCE OF THE SAMPLING PERIOD ON RESULTS FROM BIO-INDICATOR BASED MONITORING

Presented by FRANCE

## INTRODUCTION

The use of bio-indicators to monitor contamination of the marine environment has become common practice over the past twenty years, as direct measurement of trace pollutants in the water relies on sophisticated and costly techniques of sampling and analysis. Furthermore, due the high variability of the marine environment, punctual measurements in the water fail to provide an adequate representativity of the chronic condition of this environment. Marine organisms have the capacity to store the contaminants present in the water. This phenomenon of bio-accumulation and its reversal, i.e. decontamination, are very slow processes. Consequently, the pollutant contents measured in marine organisms reflect much more effectively a chronic condition of their environment.

Monitoring programs relying on marine organisms as bio-indicators have been developed in many countries. The term of "Mussel Watch" has become the consecrated designation for monitoring networks using mussels as indicators. However, a great many other organisms are used commonly as well: oysters and other bivalves, fish, sea urchins, algae, etc.

Within the framework of the Oslo and Paris Conventions, the Joint Monitoring Group has set up a monitoring program for the Norther Atlantic area, based on inputs from each contracting parties.

## SEASONAL VARIATIONS IN MUSSELS

One of the major problems encountered in the use of bio-indicators is that of seasonal variations. An abundant literature is devoted to this problem and to the possibilities of limiting its impact on monitoring results. All of these prior studies concur on the existence of a maximum value in winter or spring and of a minimum value in summer or fall. Most authors regard these seasonal variations as being related to the shellfish reproduction cycle. Conversely, a number of authors feel that the variations are due to a great extent to the bioavailability of contaminants in the marine environment.

Various strategies may be used to reduce the effect of seasonal variations on monitoring results. In the case of the JMG, the guidelines recommend a single yearly sampling, prior to the spawning period. The results provided by the various contracting parties have shown that this guideline sometimes proves difficult to comply with. On the basis of this observation, the JMG has requested the French delegation to prepare a report regarding the effects of the sampling period on monitoring results.

Since 1979, the French "Mussel Watch" (RNO) has been relying on four annual samplings. Consequently, the resulting extensive set of data provides the opportunity of conducting a detailed study of seasonal variations in conjunction with breeding cycles, whenever these cycles are known.

An initial approach, illustrated on Figure 1, reveals the obvious existence of such variations in the case of one metal contaminant (Cd) and of one organic contaminant (PAH) in mussels from two separate sites along the French coastline (Atlantic and English Channel).

A closer analysis of this Figure leads to two comments. In the case of cadmium, first of all, the lack of sampling regularity prior to 1984 prevents any determination of seasonal variations. Secondly, a comparison of both diagrams reveals a time lag between the maximum values. The maximum values for cadmium are always observed in the second sample of the year (in May), and in the first sample (February) for PAHs. It should be noted however that the RNO sampling schedule prescribes specific sampling periods and that the actual maximum values may in fact occur between two of them.

Sampling normally takes place in February, May, August and November. Although some allowance is authorized to exceed these periods for reasons fo convenience, the great majority fo samples is nevertheless collected during the recommended months. However, the considerable number of samples collected since 1979 enables the calculation of monthly averages since each month is represented a sufficient number of times, particularly when a larger area is considered rather than a restricted site.

Figures 2 to 6 show the results derived from this approach for several pollutants along the Atlantic and English Channel shores of the French coastline. The number of samples included to calculate the respective means is shown next to each dot.

An analysis of these figures clearly reveals that the sampling period can influence the recorded concentrations by a considerable factor (e.g. 1 to 3 for cadmium and PAHs). In the case of a monitoring program based on a single yearly sampling, this could have significant consequences when evaluating the degree fo contamination fo an area (base line). Furthermore, this phenomenon can seriously jeopardize the assessment of long-term trends if the sampling period is not identical throughout the study.

For purposes of illustration, we simulated on the basis of RNO data, what would result from a cadmium monitoring program relying on one yearly sampling conducted in March-April or in September-October at a site along he Loire River between 1979 and 1991:

| | March-April | September-October |
|---|---|---|
| Mean | 2.13 | 1.11 |
| Sdv | 0.64 | 0.41 |
| Observations | 30 | 25 |

In accordance with the thresholds defined by the Oslo and Paris Conventions, the Loire River would thus be classified in the first case as a moderately contaminated area (mean >2 mg $kg^{-1}$ dry weight), and as a low contaminated area in the second case (mean <2 mg $kg^{-1}$ d.w.).

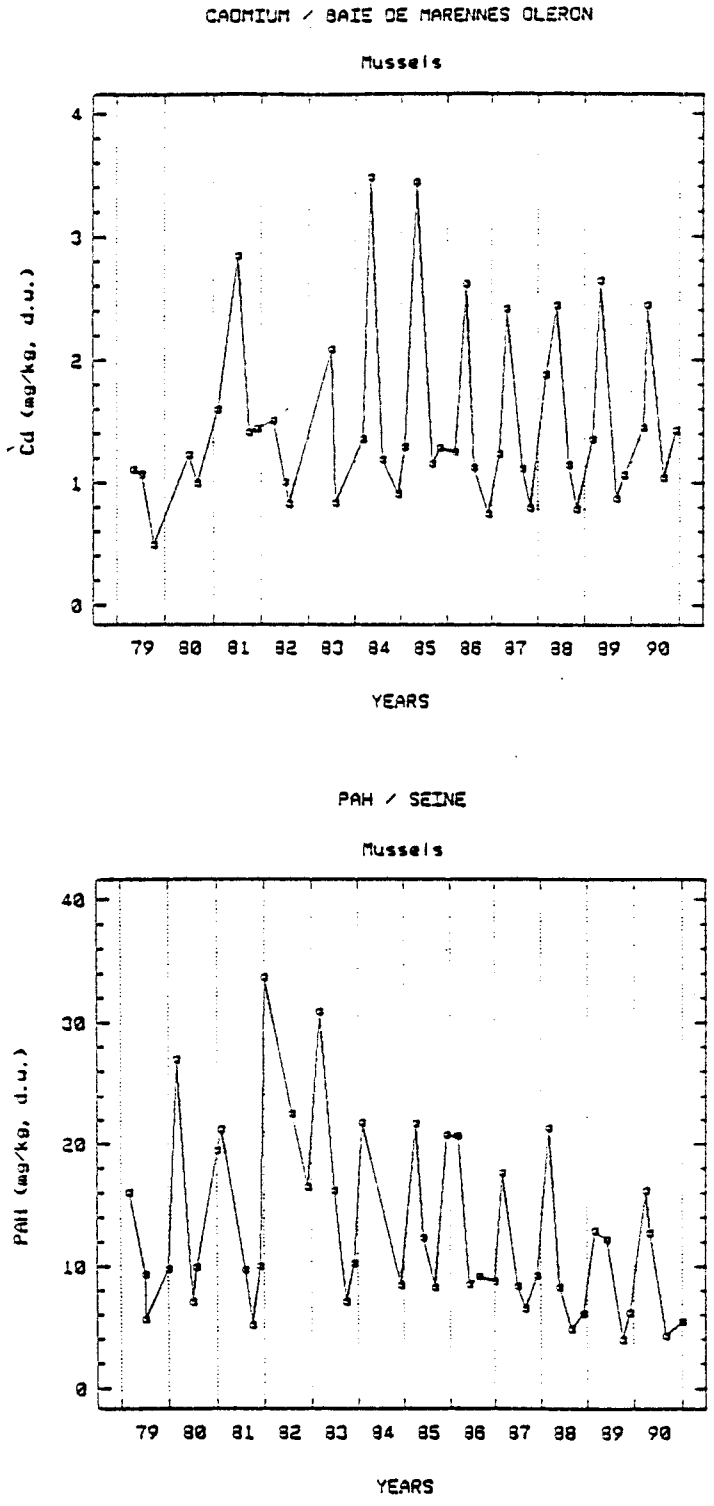


Fig. 1 Seasonal variations in mussels for cadmium in the bay of Marennes-Oléron (Atlantic coast) and for PAH in the Seine estuary (Channel coast)

The same simulation applied to the Seine estuary results in a mean value of 6.15 mg $kg^{-1}$ d.w. for the months of March-April and of 1.96 mg $kg^{-1}$ d.w. for the months of September-October, i.e. a ratio of 3.14. Hence, this discrepancy between the recorded pollutant contents would lead to an even more serious change in the classification of the area under study (from high to low level).

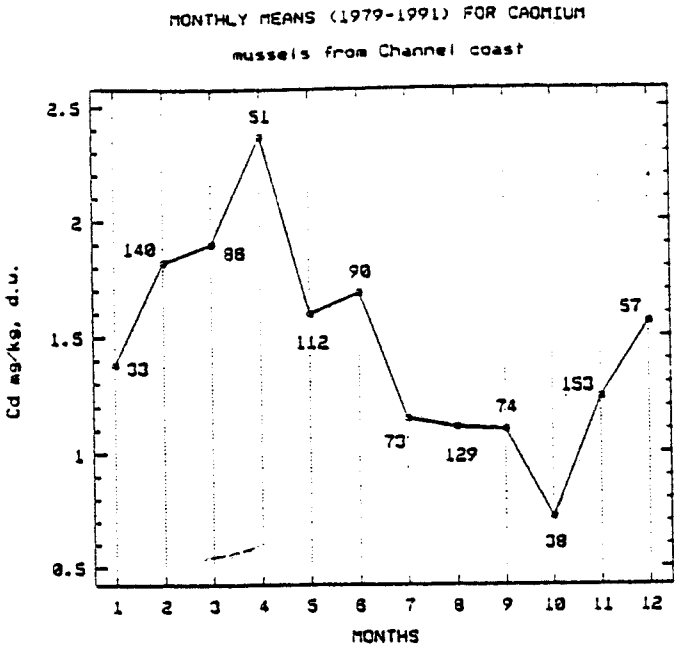


Fig. 2

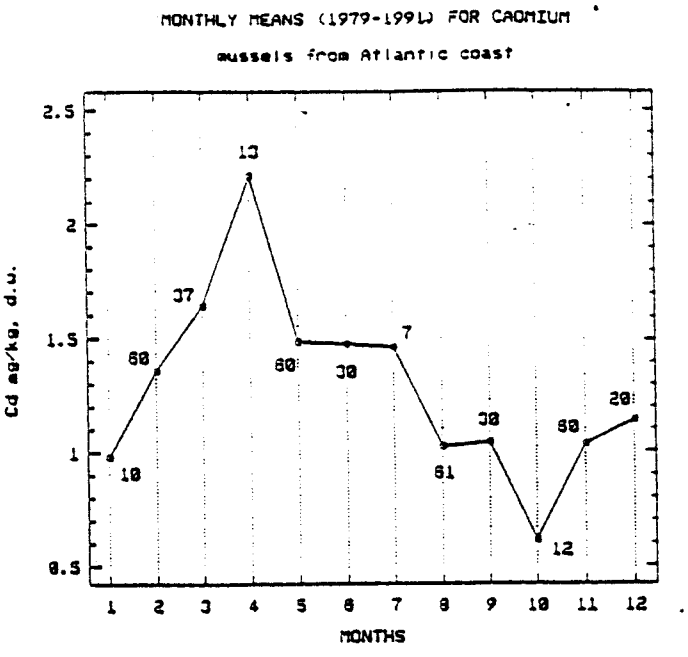


Fig. 3

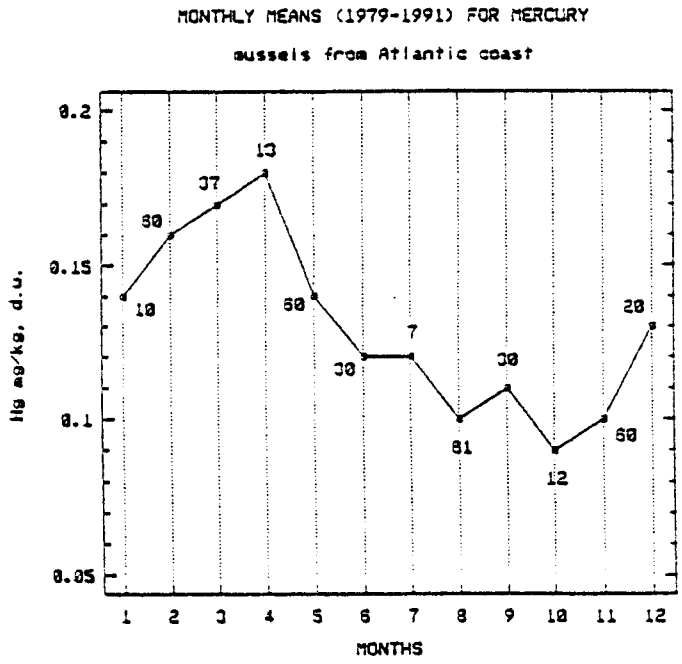
MONTHLY MEANS (1979-1991) FOR MERCURY
mussels from Atlantic coast
µg kg/kg, d.w.
0.2
0.15
0.1
0.05
10
60
97
13
60
30
7
81
30
12
60
20
1 2 3 4 5 6 7 8 9 10 11 12
MONTHS

Fig. 4

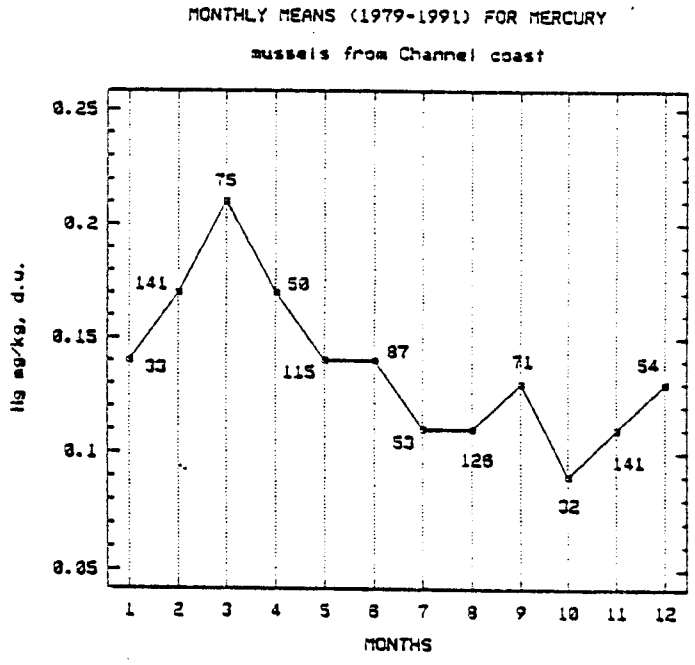
MONTHLY MEANS (1979-1991) FOR MERCURY
mussels from Channel coast
µg kg/kg, d.w.
0.25
0.2
0.15
0.1
0.05
33
141
75
50
115
87
53
128
71
32
141
54
1 2 3 4 5 6 7 8 9 10 11 12
MONTHS

Fig. 5

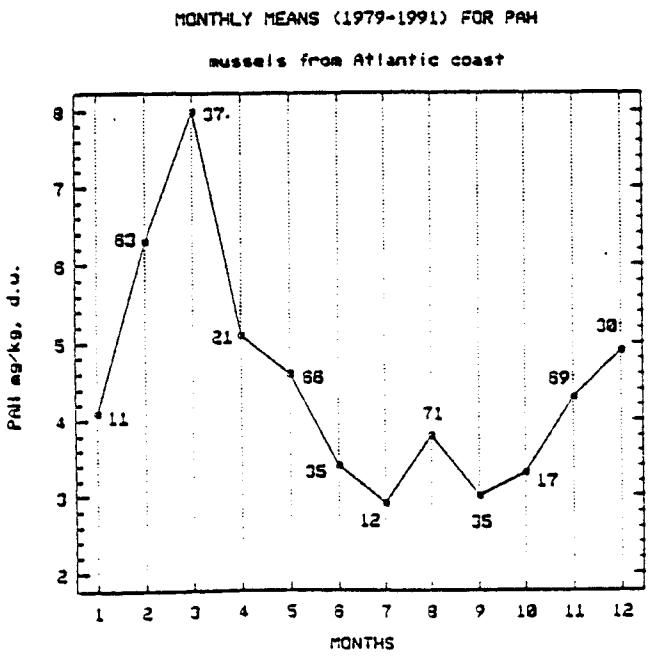


Fig. 6

Table 1 summarizes the results from this simulation, showing all the parameters measured in English Channel mussels under the RNO monitoring program.

Table 1

| | Hg | Cd | Pb | Cu | Zn | PCB | DDT | GHCH | PAH |
|---|---|---|---|---|---|---|---|---|---|
| Average months 3&4 | 0.18 | 1.99 | 2.83 | 8.0 | 117.9 | 1135 | 12.6 | 7.3 | 7.27 |
| Average months 9&10 | 0.12 | 1.00 | 1.85 | 6.9 | 91.0 | 856 | 11.0 | 4.9 | 3.31 |
| Ratio | 1.5 | 2.0 | 1.5 | 1.2 | 1.3 | 1.3 | 1.1 | 1.5 | 2.2 |

Obviously, the values presented in this table include results recorded at several sites along the entire English Channel coastline over two months. On a shoreline as extensive as this, there may be a certain time lag between maximum seasonal values among the various sites. The ratios expressed in this table should therefore be regarded as minimum values. In practice, they may be much higher for any given single site. The cases of he Loire and Seine Rivers illustrated above constitute good examples of the phenomenon.

**It would thus appear that the sampling period can induce a slant in monitoring results. The amplitude of such distortion is not negligible and can alter an assessment of the degree of contamination of a given area.**

## CORRELATION WITH THE BREEDING CYCLES

Along the French Atlantic coast, the gametogenesis of mussels starts in late September and spawning occurs primarily in February, March or April. However, the spawning period may sometimes extend until late June. In summertime, the mussel accumulate stores of glycogens (see Annex).

Figure 7 compares the mussel reproduction cycle described above with the monthly means recorded by the RNO for cadmium and PAHs along the French Atlantic coastline. The parallel reveals a good correlation.

It is generally acknowledged that spawning in mussels occurs when the water temperature ranges between 10 and 19°C, with an optimum range of 10°C to 12°C. Clearly, the spawning period necessarily varies from one area to another, and a fortiori from one country to another depending on the latitude.

However, there still remains the question of the potential correspondence between the cycles observed and the cycles which determine the inputs into the environment, i.e. hydrodynamics,weather conditions, etc. and which may be merged with the reproduction cycles.

## CONCLUSION

The influence of the breeding cycle on monitoring results is far from negligible and may, in certain cases, lead to significant discrepancies in the assessment of the degree of contamination of an area.

A monitoring program based on four yearly samplings and working on annual means could help reduce this impact. However, such a sampling schedule is costly and laborious, and sometimes difficult to apply in the framework of broad international programs.

In the case of monitoring based on a single yearly sampling, several factors should be considered:

1. It should be well understood that, depending upon the sampling period, the resulting picture of the actual contamination of a site will be either worsened or mitigated. This aspect should be kept in mind when assessing he degree of contamination.

2. Regardless of the recommended sampling period, a rigorous execution fo the sampling schedule requires a thorough knowledge of the sexual condition of the bivalves under study. The observation of this condition on the subjects themselves is inapplicable in a routine monitoring program. Consequently, it is essential to have a thorough knowledge of the breeding cycles of the animal used as bio-indicator at each of the monitored sites.

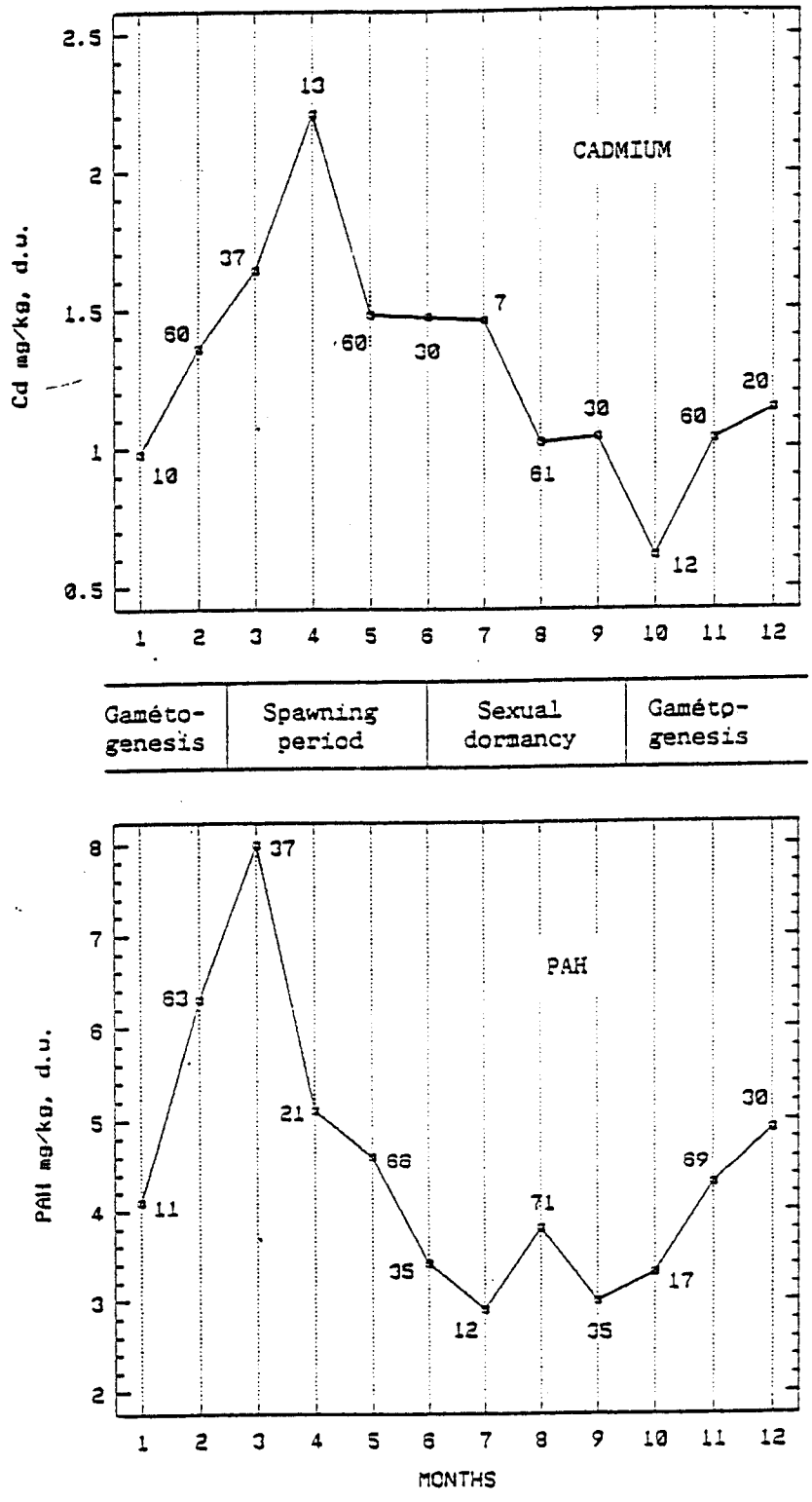
CADMIUM
Cd mg/kg, d.u.
2.5
2
1.5
1
0.5
13
37
60
10
60
30
7
61
30
60
12
20
1 2 3 4 5 6 7 8 9 10 11 12
Gaméto-genesis
Spawning period
Sexual dormancy
Gaméto-genesis
PAH
PAH mg/kg, d.u.
8
7
6
5
4
3
2
37
63
21
11
66
35
12
71
35
17
69
30
1 2 3 4 5 6 7 8 9 10 11 12
MONTHS

Fig. 7

## ANNEXE

# ACTIVITE REPRODUCTRICE DE DEUX PRINCIPALES ESPECES DE BIVALVES CRASSOSTREA GIGAS ET MYTILUS EDULIS, CULTIVEES DANS LE BASSIN DE MARENNES-OLERON

Nicole FAURY, Jacqueline RATISKOL
IFREMER, DEL, B.P. 133, F-17390 LA TREMBLADE

Le bassin de Marennes-Oléron est le plus important centre conchylicole français. Le stock ostréicole (100000 tonnes) le place au premier rang national, et au deuxième rang de la production de naissain. Cette activité cohabite avec la mytiliculture considérée marginale comparée à l'ostréiculture.

## Reproduction des huîtres

Le suivi de la reproduction de l'espèce Crassostrea gigas existe depuis 1971, suite à son introduction en France consécutive á l'épizootie ayant provoqué la disparition de l'espèce Crassostrea angulata. Elles sont largement cultivées sur l'estran et se développent également sur des bancs naturels.

La période de reproduction de l'huître en milieu naturel dépend de la conjugaison de nombreux facteurs principalement hydrologiques et météorologiques (hérale et al., 1986). La gamétogénèse débute à des températures de l'eau voisines de 10°C, les salinités doivent être comprises entre 25 et 35% (Auger, 1976).

Cinq stades d'évolution sont généralement décrits (Le Dantec, 1968).

A Marennes-Oléron, les stades I et II apparaissent à la faveur du réchauffement printanier des eaux en Mars ou Avril.

Le stade III correspondant au développement maximum des gonades est observé entre Juin et Août. Durant cette période, lorsque les conditions sont réunies, l'émission des produits génitaux correspondant au stade IV, intervient. Elle peut se poursuivre jusqu'en Septembre.

Au cours de l'été, pontes et nouvelles gamétogénèses peuvent se succéder si les conditions le permettent. Le stade V (déplétion de la glande) se confond avec le stade 0.

Le repos sexuel se déroule donc principalement d'Octobre à Février.

## Reproduction des moules

A Marennes-Oléron, l'espèce rencontrée est Mytilus edulis. Elle est cultivée en zone intertidale selon la technique des bouchots. Cette activité représente un stock de 3500 tonnes, dont environ 2000 tonnes commercialisées chaque année.

Il est couramment admis que l'émission des gamètes intervient lorsque la température est supérieure à 10°C, et se poursuit jusqu'à des températures maxima de 19°C. La fourchette préférentielle se situe toutefois entre 10 et

12°C, conditions que l'on rencontre dans le bassin de Marennes-Oléron en Février, Mars, Avril. Toutefois, selon les années, des larves ont pu être détectées de Février à début Juillet.

Une étude menée à IFREMER La Tremblade par Boromthanarat en 1983 et 1984 décrit les différents stades de gonades et leur période de formation.

Ainsi la gamétogénèse débute par le stade I (manteau épais, mais les gamètes ne sont pas observables) à la fin de l'été (fin Septembre). Elle se poursuit tout l'automne et conduit au stade III de maturation, vers Décembre.

La ponte n'intervient que lorsque les conditions de milieu deviennent favorables (température et salinité) et peuvent ainsi commencer dès Février.

Le stade 0 correspondant au repos sexuel (manteau mince, absence de gamète) apparaît fin Juin.

Durant l'été l'animal constitue des réserves de glycogènes en quantité importante, celles-ci reflètent l'absence d'activité sexuelle.

**Conclusion**

L'activité reproductrice est différente selon les bivalves et selon leur implantation géographique.

Dans le bassin de Marennes-Oléron la gamétogénèse des huîtres Crassostrea gigas se déroule de Mars à fin Septembre, la ponte ayant lieu de Juin à Septembre. La période de reproduction des moules Mytilus edulis s'etend de fin Septembre à fin Juin, la ponte se déroulant de Février à Juin.

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**AGENDA ITEM 3B**

OSLO AND PARIS CONVENTIONS FOR THE PREVENTION OF MARINE POLLUTION
EIGHTEENTH MEETING OF THE JOINT MONITORING GROUP
THE HAGUE: 25-29 JANUARY 1993

# SPATIAL AND SEASONAL DIFFERENCES IN THE PCB CONTENT OF THE MUSSEL MYTILUS EDULIS

Presented by the NETHERLANDS


## ABSTRACT

Seasonal and spatial variation in the concentration of PCBs, fats (non-polar lipids) and total lipids and the condition of the mussel Mytilus edulis were assessed in three differing water bodies of he Dutch delta area. Highest concentrations of PCBs in the mussel were found in the Westerschelde estuary, with much lower concentrations in the Oosterschelde and the brackish lake Grevelingenmeer. Spatial differences were strongly related to salinity; lower concentrations were found at the more saline stations, pointing to freshwater inputs as being the origin of the PCBs in mussels. The PCB concentration in mussels, on the basis of dry or total weight, in general increased during summer, autumn and winter and decreased strongly during spring. The strong decrease is related to the spawning of gametes. Seasonal changes in the PCB concentration on the basis of the dry weight were not related to changes in the fat content or the condition of the animals. Only PCBs on a fat basis were negatively related to fat content, indicating a dilution of PCBs during seasonal fat accumulation and concentration of PCBs during fat utilization, in such a way that the total PCB concentration in the animal remains the same. It seems that, besides reproduction, equilibrium partitioning is the most probable mechanism that determines the PCB content of mussels.


## INTRODUCTION

The concentration of PCBs in marine invertebrates is said to be best explained by equilibrium partitioning between body lipids and the ambient water (Duinker et al., 1983). Therefore, the concentration of PCBs in the tissues of an animal such as the mussel Mytilus edulis should reflect the PCB concentration in its environment. PCBs partition primarily into the non-polar part of the lipids, the fats and therefore the PCB concentration is often expressed on a fat basis (Boon and Duinker, 1986; Duursma et al., 1986). Because of their lipophilicity, changes in the PCB concentration might also be related to changes in the lipid or fat content (Boon and Duinker, 1986). The concentrations of lipids and fats change throughout the seasons in relation to animal condition and reproduction (Gabbott and Bayne, 1973; Zandee et al., 1980; Bayne et al., 1982; Gabbot, 1983).

The aim of this study was to assess the seasonal changes in PCB concentration in relation to changes in lipid or fat content and condition of the edible mussel M. edulis from three areas in the dutch Delta area differing strongly with regard to their tidal dynamics and degree of contamination.

## MATERIALS AND METHODS

Mussels, Mytilus edulis, of 4-6 cm length were sampled quarterly from the low water level at 10 stations in the Delta area in the south-western part of the Netherlands (Fig. 1). Four stations were situated in the brackish and saline part of the Westerschelde estuary, which is heavily polluted. Three stations were situated in the Oosterschelde, a former estuary, cut off from its riverine input and with a low level of contamination. Three stations were located in the brackish Lake Grevelingenmeer, which has no tidal cycle and a low level of contamination.

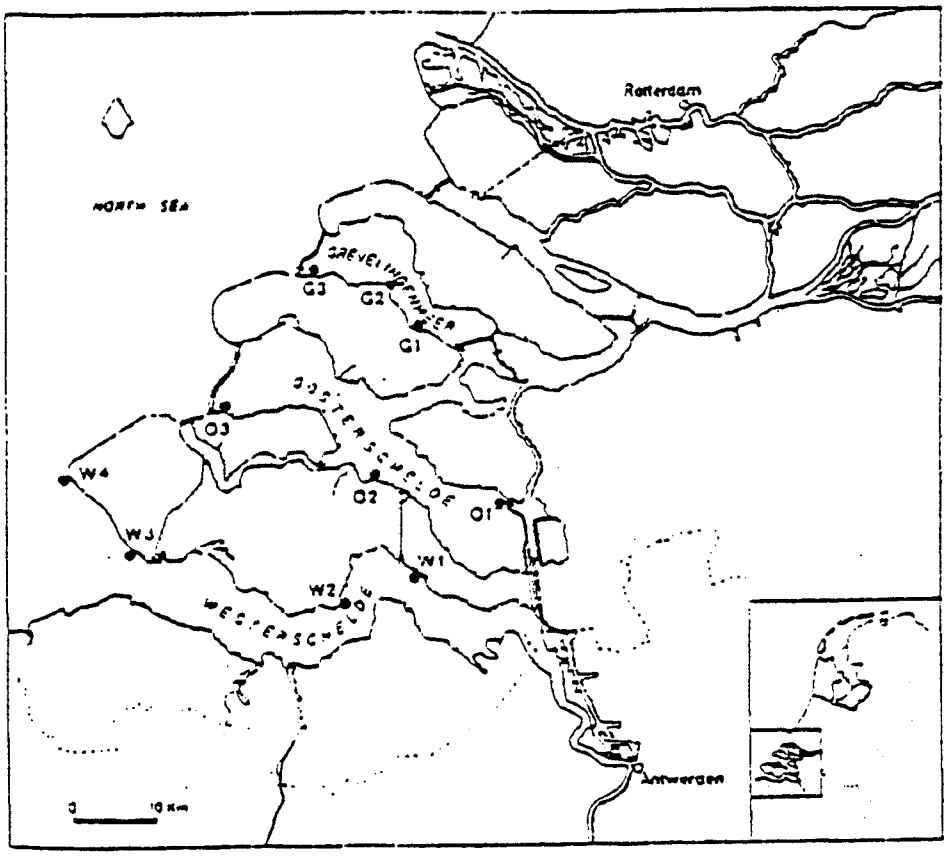


Fig. 1 Location of the sampling stations in the Dutch delta area

On each occasion, 10 mussels per station were sampled for PCB and fat analyses and 10 for the analyses of lipids and condition. The 10 mussels for PCB and fat analyses were dissected with pre-cleaned stainless steel knives, pooled, weighed and ground in an agate grinder after 3 days freeze-drying. The mussel-powder was extracted with n-pentane in a micro-Soxhlet. The extracts were evaporated to constant weight to weigh the extractable lipids, hereafter referred to as fats, and treated according to Duursma et al. (1986) for a final PCB gas chromatography analysis on a Packard Instrument 438A with ECD. Results for six replicate samples have a relative standard deviation of an average 4.72 (range 2.8-7.0).

The other 10 mussels were dissected, freeze-dried for 3 days and weighed. The condition was calculated as the average dry weight per unit volume; the average length to the third power was used as an index of the volume. The lipid concentration was measured in a chloroform-methanol mixture according to the method of Blight and Dyer (1959).

The fat content extracted by means of n-pentane will be less than the lipid fraction extracted by means of the chloroform-methanol mixture. n-Pentane extracts mainly non-polar material, excluding polar compounds such as phospholipids, whereas chloroform-methanol also extracts the latter fraction (schneider, 1982; Boon et al., 1984; Duursma et al., 1986).

## RESULTS

The congener composition of the PCB mixture in the mussel was very constant (Fig. 2). Therefore the contribution of the most referred congeners 28, 52, 101, 118, 153, 138, 180 to the total PCB concentration was fairly constant; on average 48% (SD = 4, n = 40).

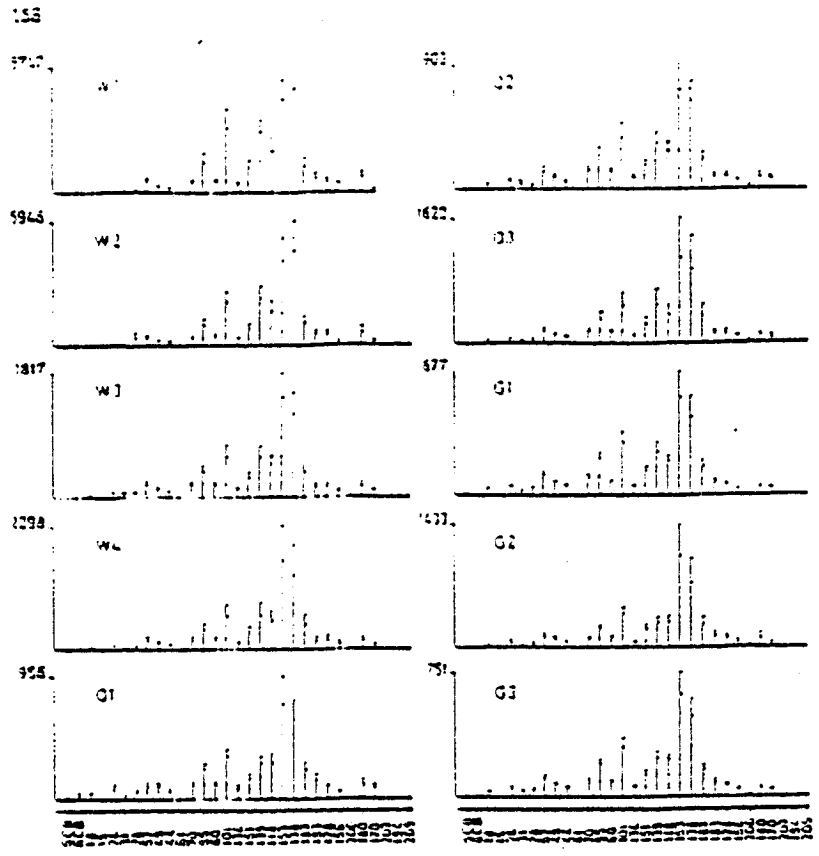

Fig. 2 The average PCB composition of mussels at the 10 sampling stations. The concentrations of the individual congeners are normalized on the basis of the congener with the highest concentration. The congeners are numbered according to Ballschmiter and Zell (1980) and given in the order of elution from the GC column. The standard error is given between horizontal bars. On the left the mean concentration of the congener with the highest concentration is given in ppb (on a fat basis)

The highest PCB concentrations were found in the upstream, eastern, stations of the Westerschelde estuary (Fig. 3a,b). These stations have the lowest salinity (Fig. 3c). From this a significant negative relation was found between PCB concentrations in mussels and salinity (Table 1). he PCB concentrations of the mussels from all stations of he tidal Oosterschelde and non-tidal (stagnant) Grevelingenmeer were low and comparable. Seasonal fluctuations in the Oosterschelde, especially at the stations nearer to the North Sea, were stronger than in the Grevelingenmeer.

On a dry weight basis the PCB concentrations in general increased during winter, dropped to low levels in spring and increased again during summer (Fig. 3a). However, on a fat basis the PCB concentration mostly showed a continuous decrease from autumn or winter to summer (Fig. 3b). This decrease coincided with an increase in the fat content (Fig. 3d), although a small drop in the fat content occurred during spring at some stations. These changes in the PCB and fat concentration showed a significant (negative) correlation (Table 1, Fig. 4). This indicates that animals with increasing fat content did not concentrate more PCBs in their body, but 'diluted' their PCB content (on a fat basis).

In contrast to the fat content no consistent seasonal changes were found for the lipid content (Fig. 3d). Therefore, the ratio of fats to lipids changed throughout the year, being on average 0.4 during autumn, 0.6 during winter, 0.5 during spring and 0.8 during summer. Similar values and variations for this ratio were found for Macoma balthica (Beukeman and de Bruin, 1977) and M. edulis (Zandee et al., 1980).

For the condition, a slight seasonal trend was found: i.e. during winter and spring the condition was lower than during summer and autumn (Fig. 3f). The slight seasonal changes in the lipid content or in condition cannot be related to the more marked changes in the PCB content (compare Fig. 3a or b with 3e and 3f for each station). Remarkably, on the basis of all data a relation was found between the PCB and the lipid content, whereas no such relation was found between the PCB and the fat content. Since seasonal changes in lipid content did not contribute to this relation, spatial differences with similar trends have to be the cause. Indeed, as for PCBs (Fig. 3a,b) the lipid content was highest at stations in the Westerschelde and lowest at stations in the Oosterschelde and Grevelingenmeer (Fig. 3e).

## DISCUSSION

The concentrations of PCBs in mussels (2-48 ppm on a fat basis) are in the range of those reported by Duursma et al. (1986) (3-53 ppm). The mussels from the Westerschelde estuary showed the highest PCB concentrations and the strongest fluctuations, possibly because of continuous input of contaminated fresh water from the Scheldt river. There is clearly a relation between the salinity and PCB content, as was also found by Duursma et al. (1986). These authors also stated that this relation was to be expected because the PCBs in the Delta area originate from fresh water inputs, such as the Rhine, Meuse and Scheldt. In relation to salinity the PCBs demonstrate conservative behaviour. In the mussels from the Oosterschelde the PCB concentrations were much lower, and the seasonal fluctuations were less considerable, especially in the vicinity of the mainland, probably because this water body is still tidal but isolated from its riverine input. Similarly, in the completely isolated Grevelingenmeer, low concentrations and the smallest seasonal fluctuations occurred.

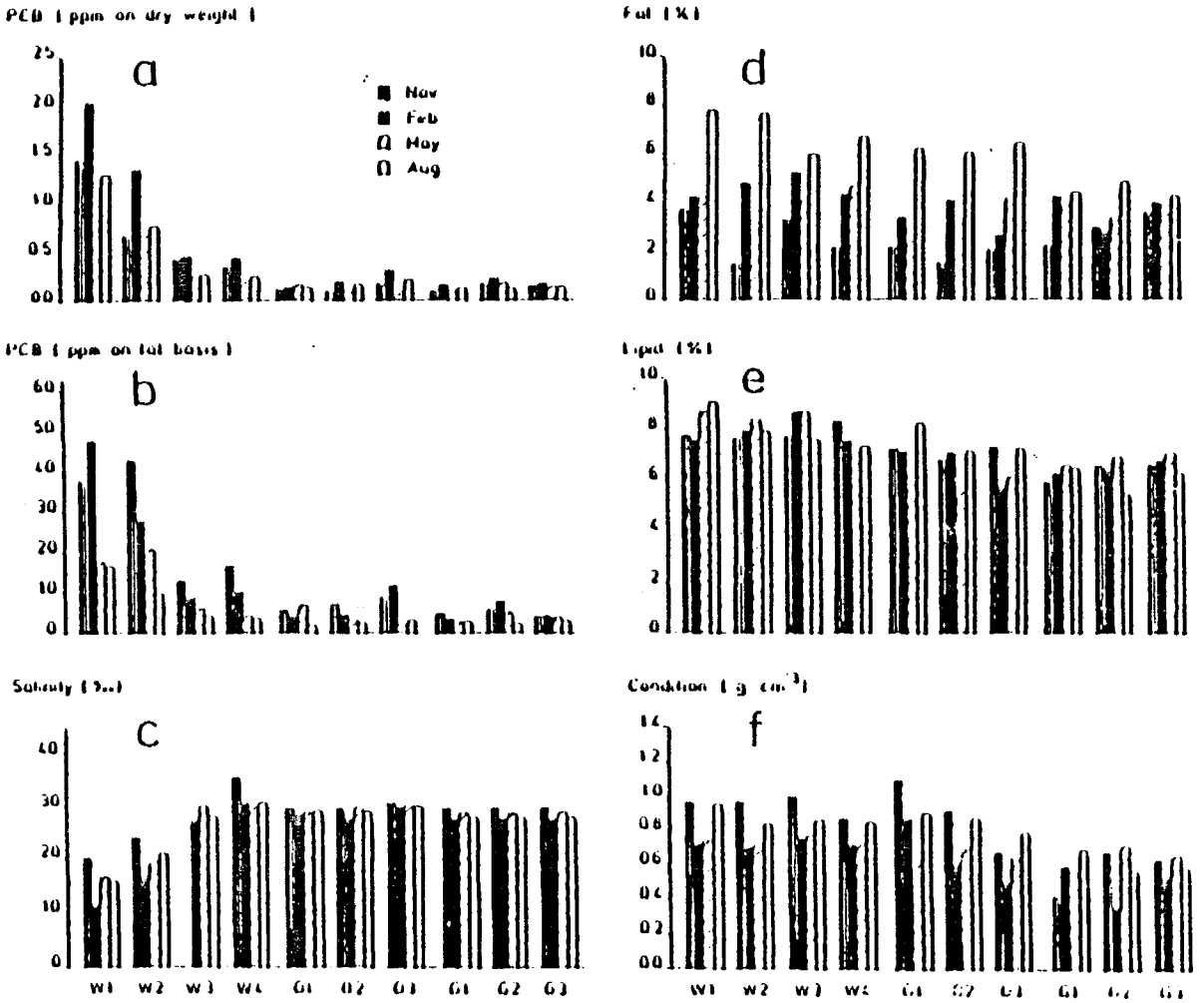


Fig. 3 The concentration of PCBs in mussels on the basis of (a) dry weight and (b) fat in mussels; (c) the salinity of the ambient water; (d) the fat, and (e) lipid content and (f) condition of the mussels at the 10 sampling stations

Table 1

Significance* of the linear regression analyses between the PCB concentration of the mussel Mytilus edulis and its fat or lipid content, its condition and the salinity of the ambient water. The correlation coefficient is given tin parentheses (n=40).

| | Salinity | Fat | Lipid | Condition |
|---|---|---|---|---|
| Original data | | | | |
| PCB (on a dry weight basis) | ***(-0.87) | - | ***(-0.51) | - |
| PCB (on a fat basis) | ***(-0.70) | - | **(-0.40) | - |
| Indexed data* (seasonal changes) | | | | |
| PCB (on a dry weight basis) | - | - | - | - |
| PCB (on a fat basis) | - | ***(-0.79) | - | - |

*** p<0.01; *** p<0.001; - not significant (p>0.05)
* To unravel relations between seasonal changes in the different parameters measured, the original data were indexed per station and per parameter as a proportion of their annual average (e.g. Station W1:PCB on fat (ppm): original data are: November, 38.2; February, 47.9; May, 17.8; August, 16.5; average, 30.1; the indexes are thus: Nov. 1.3. Feb. 1.5. May 0.6 Aug. 0.9

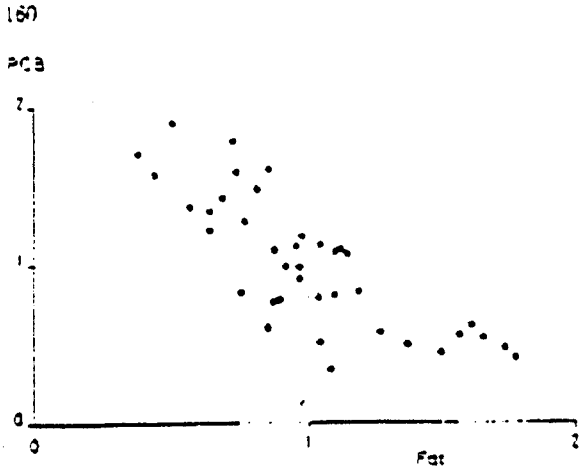


Fig. 4 Relation between the seasonal changes in the indexes of the PCB (on a fat basis) and fat content of mussels. The data for PCBs and fat were indexed per station on the basis of their annual averages (see example in Table 1). (Regression analysis: y=1.94-0.94x, n=40, r=-0.79, p<0.001)

Seasonal changes inchlorinity, lipids or condition were not related to changes in the PCB concentration, but there was a strong negative relation between fat and PCB on a fat basis. This negative relation suggested a 'dilution' by seasonal fat accumulation, or concentration of PCBs due to fat utilization, in such a way that the total concentration of PCBs (in an animal on a dry weight basis) remains constant. From this, it can be concluded that the PCB content of the total animal is not determined by the fat content, but mainly by other factors. Equilibrium partitioning between the organism and the ambient water will be such a factor (Duinker et al., 1983; Duursma et al., 1986). The relation between PCB content and salinity is a result of this equilibrium partitioning. The positive relation between lipids and PCBs could indicate that some lipids, other than fats, may play a role. However, this is not yet known, and the relation might also be indirect and non-causal.

Another strong influencing factor, namely reproduction, appears from this study. In the area studied, mussels spawn in the months of April and May (Hummel et al., 1988). Gametes contain a relatively high concentration of lipids, consisting primarily of neutral lipids (fats) into which PCBs mainly partition (Bayne et al., 1975; Sastry, 1979; Zandee et al., 1980; Gabbott, 1983). During gametogenesis in autumn and winter, food an body reserves are converted into lipids in the gametes. With the transfer of lipids to the gametes, PCBs might be concentrated in the gametes also, as was found for mussels by Hummel et al. (1989). Moreover, these authors found, over a period of 4 months, no substantial decrease in the PCB content of mussels transplanted from polluted station W2 to the less polluted station O2. Therefore, it is most likely that the decrease in PCB content during spring in mussels is due to the spawning of gametes. A similar mechanism of accumulation of PCBs in gametes and elimination by spawning has been observed for rainbow trout (Guiney et al., 1979) and the copepod Acartia tonsa (McManus et al., 1983). To estimate the magnitude of the decrease in PCB concentration by spawning, the total PCB content per individual has been calculated (thus not on the basis of fat or dry weight, but total dry body weight) (Fig. 5). From this it is concluded that mussels might "lose" one-half to two-thirds of their PCB content by means of reproductive output. It therefore remains a crucial question: to what extent do these maternally transposed PCBs influence the fitness of mussel larvae?

From this study it is also concluded that expressing the PCB content on a fat basis only does not yield precise ecologically relevant information; it obscures the possible role of the reproductive output of adults.

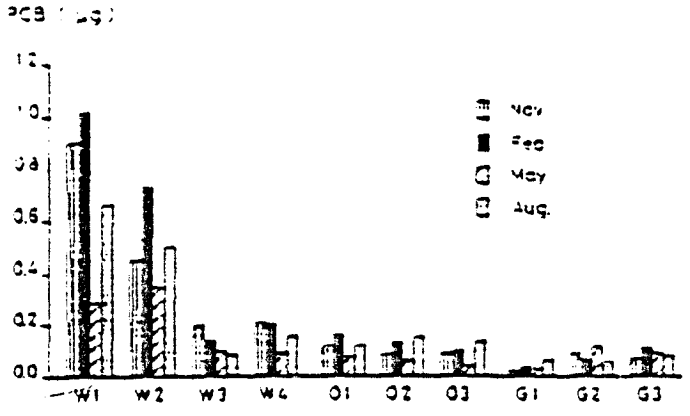


Fig. 5 The PCB content of an individual mussel at the 10 sampling stations, obtained by multiplying the PCB content (on a dry weight basis) by the average dry weight of a mussel per station and per season.

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**AGENDA ITEM 3C**

OSLO AND PARIS CONVENTIONS FOR THE PREVENTION OF MARINE POLLUTION
EIGHTEENTH MEETING OF THE JOINT MONITORING GROUP
THE HAGUE: 25-29 JANUARY 1993

# TRENDS IN POLLUTANTS IN BLUE MUSSEL MYTILUS EDULIS AND FLOUNDER PLATICHTHYS FLESUS FROM TWO DUTCH ESTUARIES 1985 - 1990

Presented by the NETHERLANDS

Temporal trends and length-concentration relationships are presented for data from the Joint Monitoring Programme for microcontaminants in the blue mussel Mytilus edulis and flounder Platichthys flesus from the Ems Dollard and Western Scheldt estuaries in the Netherlands over the period 1985 to 1990. Statistical analyses were carried out in conformity with ICES procedures.

The analyses show only a decrease in levels of dieldrin in mussels from the Western Scheldt. Cadmium concentrations in flounders from the Ems Dollard have increased. No linear trend had been detected in either estuary for the other contaminants (PCB, PAH, pp'DDE, lindane, Hg, As, Cu, Cr, Zn, P, Ni). The importance of taking the fat weight into account in analysing PCB trend and length relationship is illustrated.

Concentrations of PCB, PAH, chromium and nickel in mussels and PCBV in livers of flounders decrease with increasing size. Mercury concentrations in flounders increase with length.

In this paper a statistical analysis of temporal trends is presented for the data of microcontaminants in the blue mussel Mytilus edulis and flounder Platichthys flesus from two Dutch estuaries over the period 1985 to 1990. These data have been collected as the Dutch contribution to the Joint Monitoring Programme (JMP) of the Oslo and Paris Commissions (e.g. Akkerman & Van Zwol, 1990).

The North Sea Conferences (London, 1987; The Hague, 1990) produced international agreement on reduction of micropollutant loads in the period 1985-95. In fact the ultimate goal is the reduction of contaminant levels in the environment, especially in organisms. For two fringe areas of the North Sea the trends presented here may serve as one of the interim assessment of these goals. General conclusions on environmental contaminant levels in the two estuaries can only be drawn with knowledge of chemical processes within the estuarine environment, physiological and biological processes within the organisms and information on trends in other compartments (Misra et al., 1989; ICES, in prep.). To improve the reliability of identified trends samples were taken in a length-stratified manner because this biological parameter might affect the concentration of microcontaminants (Moriarty et al., 1984). For this reason, length-concentration relation-ships are discussed in this paper. A correlation between concentration and length may point to weight- or age-related uptake processes.

## MATERIALS AND METHODS

Samples of natural mussels and flounders were collected from the Ems Dollard and the Western Scheldt (Fig. 1) each year in October over the period 1985-90. The water quality in the Western Scheldt is mainly determined by the input from the river Scheldt and in the Ems Dollard by industrial activities along the estuary. In the Western Scheldt mussels were sampled in the middle section and flounders in the mouth of the estuary. In the Ems Dollard both species were collected in the mouth of the estuary. At the sampling site for mussels the salinity in the Ems Dollard is higher than in the Western Scheldt (approximately 29‰ and 24‰ respectively).

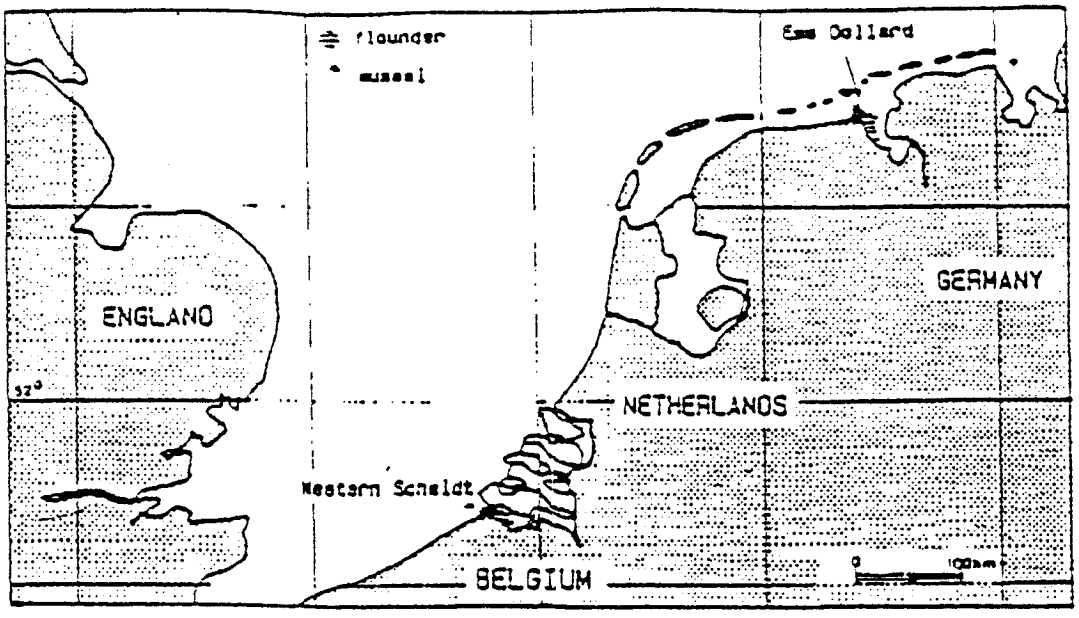


Fig. 1 Sampling locations in the Western Scheldt (JMP code 25.2.2 (mussel) and 25.2.2. (flounder)) and Ems Dollard (JMP code 22.2.2 (mussel) and 21.1.4 (flounder))

Mussels were sampled in five logarithmically equi-distant shell length classes: 25-30mm. 31-37 mm. 38-46 mm. 47-56 mm. and 57-70 mm. Each class contains approximately 50 mussels which are pooled prior to analysis. Flounders were sampled in five logarithmically equidistant length classes: 200-225 mm. 225-250 mm. 250-280 mm. 280-315 mm. and 315-350 mm. each containing five organisms. All flounders were analysed individually. In 1985 the programme on flounders in the Ems Dollard was not completed, because all length classes were not represented in the sample and in the same year no data for PCBs in flounders from the Western Scheldt are available. In both cases the data for 1984 were used instead.

Chemical analyses were performed for the JMP by the laboratory of the Food Analysis Institute at the Netherlands Organization for Applied Scientific Research according to standard operating procedures (Luten et al., 1986). In mussels concentrations of trace metals (Cd, Cu, Cr, Zn, Pb, Ni, As, Hg) and organic microcontaminants (PCB, PAH, pp'DDE, lindane, dieldrin) were

identified in the soft tissue. In flounders mercury was measured in muscle tissue and cadmium and PCBs in liver tissue. Metal concentrations were considered on a wet wt basis and a fat wt (FW) basis. Cadmium in flounder livers was considered on a wet wt basis only, as few dry wt analyses have been performed on livers because of insufficient material.

The statistical analyses for pp'DDE, lindane and dieldrin in mussels were only performed on the Western Scheldt data, because in many cases the concentrations in the Ems Dollard were at the detection limits. In the case of polyaromatic hydrocarbons the six of Borneff were totalled. For PCBs the congener IUPAC nr. 138 was used and in the case of mussels also the sum of 7 congeners (28, 52, 101, 118, 138, 153, and 180). Because mussels within one length class are pooled before chemical analysis, this results in only one observation per year per parameter per length class. The average length of the mussels in each length class weighted by number has been chosen as he length variable.

Statistical analyses were carried out in conformity with ICES procedures (Nicholson, 1985; ICES, 1986). It is common practice to transform concentrations to natural logarithms of concentration to obtain residuals which show a normal distribution. Experience has also indicated that the relationship between log concentration of contaminant sin fish tissue and length X is linear (ICES, 1986). The following statistical models were used to analyse the length-effect and for linear trend assessment in each area:

$$\log_e C_{it} = \mu_t + \alpha_t * X \qquad (1)$$
$$\log_e C_{it} = \mu_t + \alpha * X \qquad (2)$$
$$\log_e C_{it} = \mu + \alpha * X \qquad (3)$$
$$\log_e C_{it} = \mu + \alpha * X + b * Y \qquad (4)$$

where:

$\log_e C_{it}$ = natural logarithm of concentration in i'th length class in year t
X = length
Y = year
$\mu_t$ = different intercept in each year
$\mu$ = common intercept in all years
$\alpha_t$ = different regression coefficient of length in each year
$\alpha$ = common regression coefficient of length
ß = regression coefficient of year

If model 1 is significantly better than model 2 the length-concentration relationship differs from year to year and no general conclusion can be drawn. If there is no year-length interaction then model 2 is valid. When $\alpha$ is significant there is length effect. If model 2 is significantly better than model 3 there are yearly variations. If model 2 is significantly better than model 4 there are variations in the concentrations that cannot be explained by a linear trend.

T-tests were carried out to determine differences between both sampling sites. The statistical analyses were performed with the statistical package SYSTAT (Wilkinson, 1990) and all tests were carried out at the 5% significance level. Observations with a studentized residual greater than 3.5 or less than - 3.5 were considered as outliers. Concentrations of mercury,

cadmium and PCB-138 in flounders are presented in box and whisker plots. In these plots the edges of the box represent the first and third quartiles and the median as a line inside the box. The whiskers extend to a maximum distance from the edges which is calculated as 1.5 times the difference between the first and third quartiles. Points deviating by more than 2.7 standard deviations from the median are marked by asterisks or circles.

## RESULTS

The yearly geometric mean concentrations in mussels in all classes are shown in Figs. 2, 3. Box and whisker plots of mercury, cadmium and PCB-138 concentrations in all flounders are presented in Fig. 4.

The average concentrations and number of analyses per compound over the whole period are presented in Table 1. The mussel data contain four outliers in the Western Scheldt which have been removed (Hg class 1, 1989; Zn and As class 1, 1987; Cu class 4, 1988). The flounders data of the Western Scheldt contain four outliers (PCB-138 in 1989 (1 mg $kg^{-1}$ WW) and 1990 (1.7 mg $kg^{-1}$ WW); Hg in 1990 (0.32 and 0.38 mg $kg^{-1}$ WW) and five percentages of fat wt in livers from Ems Dollard have been removed.

The results of analysis on linear length effect and linear trend are summarized in Table 2. All residuals show a normal distribution. Except for a few cases, the results for metal concentrations are comparable when expressed on a dry wt and wet wt basis, just as for organic pollutants on a fat wt and wet wt basis. In all cases except copper in mussels from the Ems Dollard and nickel in mussels from the Western Scheldt there are significant between-year variations.

In the Ems Dollard there is a significant increase in cadmium concentrations in flounder livers over the period 1985-90. A significant decrease only occurs for dieldrin in mussels from the Western Scheldt. Statistical analyses of PCB concentrations in flounder livers from the Ems Dollard also suggest a significant decrease when concentrations are expressed as wet wt (Fig. 5). The influence of fat wt changes the conclusions dramatically because there is a trend in the percentage fat wt in livers. The concentrations of PCB in liver fat therefore give no indication of an improvement in PCB levels (Fig. 4).

Figure 6 illustrates the significant length effects in mussels. In the case of chromium, nickel, PCB and PAH there is a significant decrease with length. Cadmium, copper, mercury and lead in mussel tissue show variable results between the estuaries. Only in the case of cadmium in mussel from the Western Scheldt is there an increase with length. There appeared to be no linear length-effect for zinc, arsenic, dieldrin, lindane, and pp'DDE. The concentrations of mercury in the muscle tissue of flounders from the Ems Dollard show a positive correlation with length (Table 3). This is also found in the Western Scheldt but no general relationship can be derived due to year-length interaction. The concentration of cadmium in the liver of flounders is generally not correlated with length, but there is a significant positive correlation in the Western Scheldt in 1985, 1987, and 1988. PCB concentrations in flounder livers, when expressed on a wet wt basis, show a significant increase with length due to an increased percentage fat wt with length (Table 3). So by contrast, PCB concentrations show a decrease with length when the concentration is expressed on a fat wt basis (Table 3).

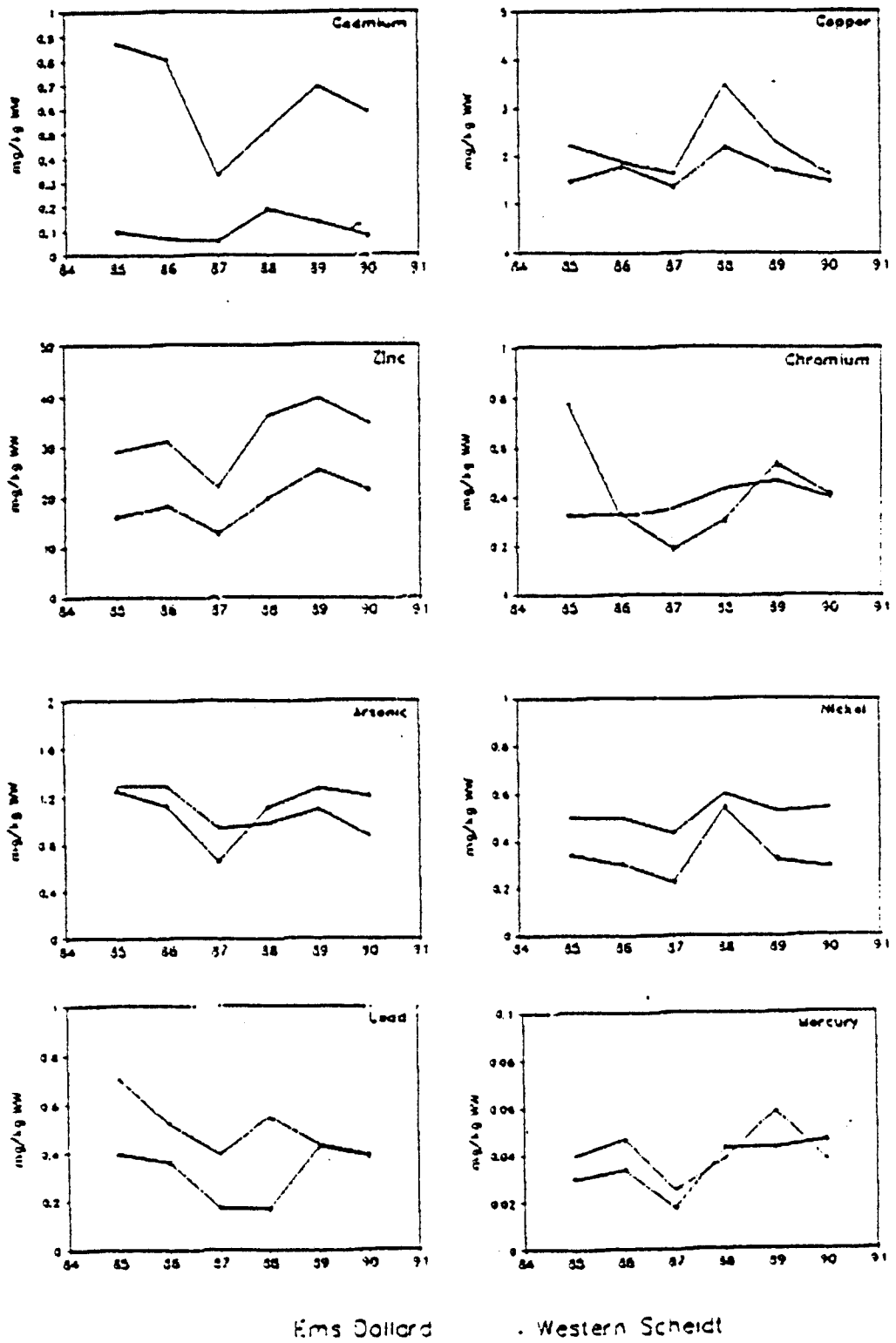


Fig. 2 Yearly geometric mean concentration of trace metals in mussels from Ems Dollard and Western Scheldt, 1985-90

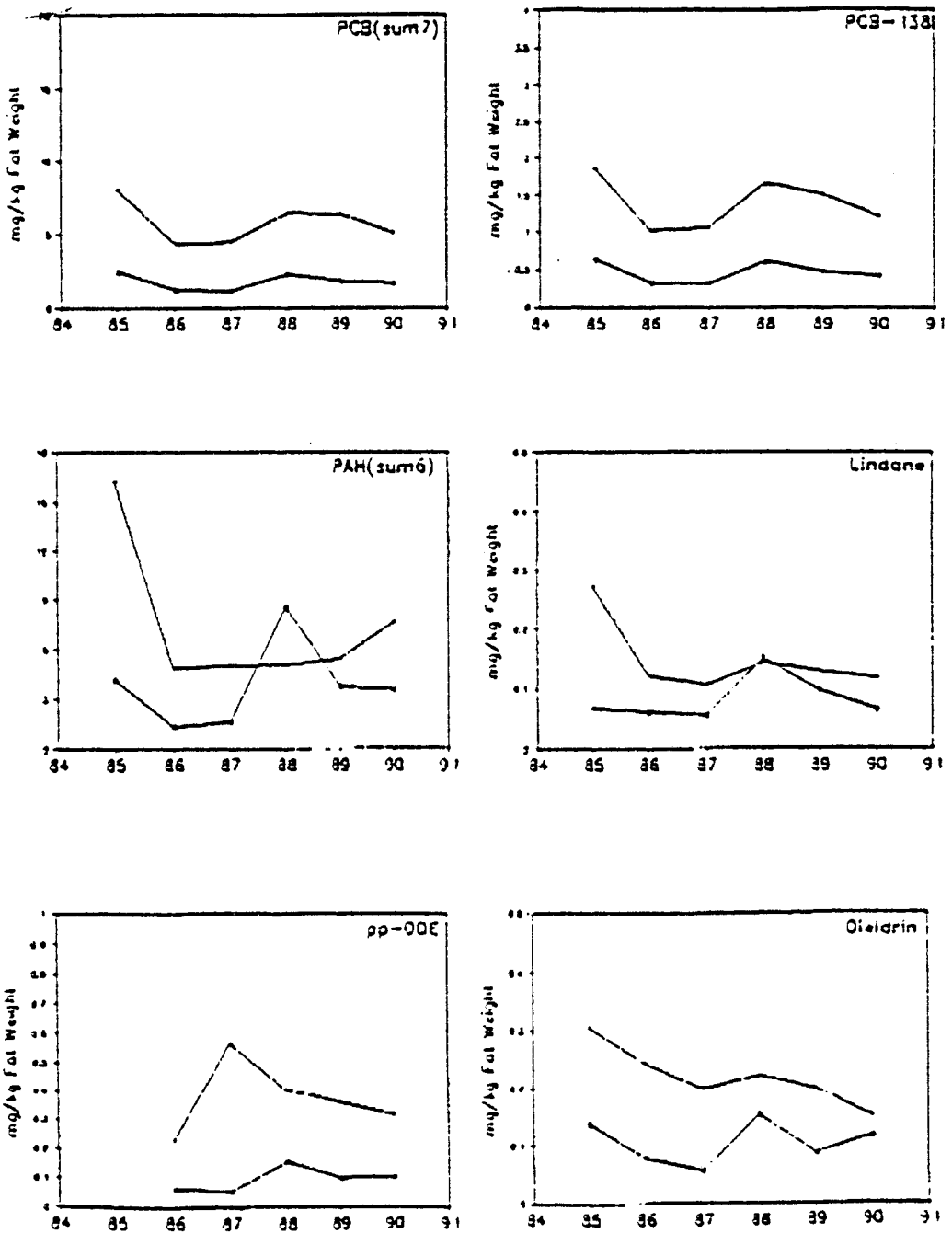


Fig. 3 Yearly geometric mean concentration of organic microcontaminants in mussels from Ems Dollard and Western Scheldt, 1985-90

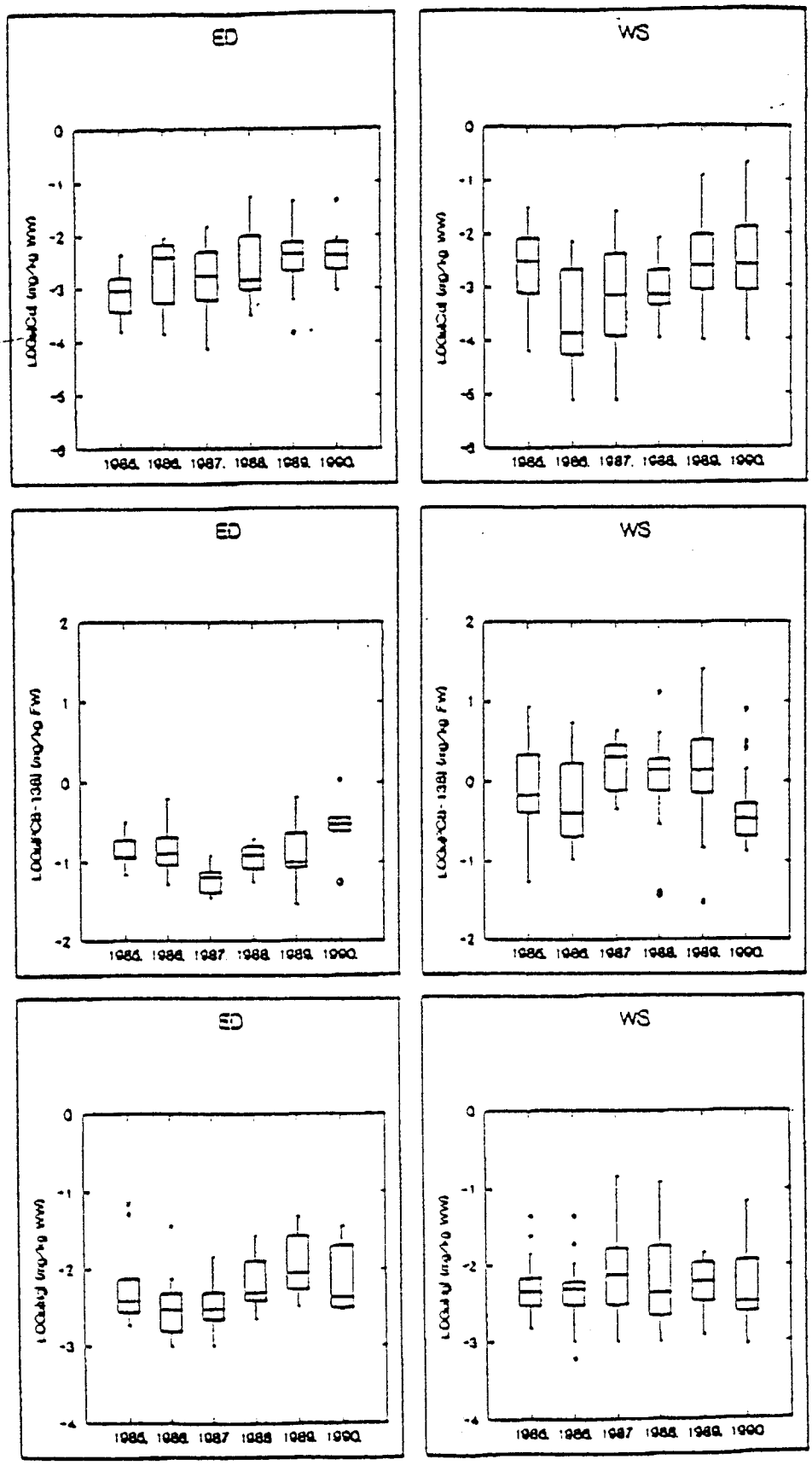


Fig. 4 Box and whisker plots of cadmium and PCB-138 concentration in liver and mercury concentration in muscle tissue of flounders from the Ems Dollard (ED) and Western Scheldt (WS), 1985-90

Table 1

Geometric mean concentrations $x_x$ of metals and organic contaminants (mg $kg^{-1}$ WW), wet wt and fat wt (%) in Mytilus edulis and Platichthys flesus from Ems Dollard and Western scheldt, 1985-90 (n = number of observations).

| | Ems Dollard | | Western Scheldt | |
|---|---|---|---|---|
| | $X_x$ | n | $X_x$ | n |
| Mytilus edulis | | | | |
| Cd | 0.10 | 28 | 0.60 | 29 |
| Cu | 1.62 | 29 | 2.11 | 28 |
| Zn | 18.19 | 29 | 31.53 | 28 |
| Cr | 0.34 | 29 | 0.43 | 29 |
| As | 1.09 | 28 | 1.04 | 28 |
| Ni | 0.33 | 28 | 0.52 | 29 |
| Pb | 0.30 | 28 | 0.49 | 28 |
| Hg | 0.035 | 24 | 0.038 | 24 |
| PCB (sum 7) | 0.022 | 25 | 0.092 | 27 |
| PCB-138 | 0.006 | 25 | 0.022 | 27 |
| PAH (sum 6) | 0.045 | 24 | 0.100 | 28 |
| Dieldrin | 0.001 | 24 | 0.003 | 27 |
| Lindane | 0.001 | 24 | 0.002 | 27 |
| pp'DDE | 0.001 | 24 | 0.006 | 23 |
| Wet wt | 83.07 | 29 | 81.68 | 29 |
| Fat wt | 1.36 | 25 | 1.67 | 28 |
| Platichthys flesus | | | | |
| Hg | 0.107 | 99 | 0.106 | 159 |
| Wet wt muscle | 76.08 | 99 | 77.68 | 159 |
| tissue | 250 | 99 | 281 | 159 |
| Length (mm) | 0.07 | 118 | 0.06 | 151 |
| Cd | 0.052 | 85 | 0.116 | 157 |
| PCB-138 | 67.92 | 118 | 64.57 | 151 |
| Wet wt liver | 15.34 | 85 | 15.98 | 157 |
| Fat wt liver | | | | |

The average concentrations in mussels from the Western Scheldt are higher than those from the Ems Dollard except for arsenic and mercury (Table 1). Although there is a significantly higher fat content in mussels from the Western Scheldt it does not explain the differences in the levels of the organic contaminants. The average cadmium and mercury concentration in flounders from both areas do not differ, but PCB levels in the Western Scheldt are significantly higher.

## DISCUSSION

The probability that a certain change in time will be detected depends on the magnitude of the change as well as the pattern of change. Nicholson and Fryer (1990) pointed out that a linear change in load is less likely to be detected than a single stepwise change in load, for example. For an analysis over a short period as in this study a linear approach still seems best.

Table 2

Summary of the statistical analyses on linear length effect and linear trend of microcontaminants in Mytilus edulis and Platichthys flesus from Ems Dollard and Western Scheldt, 1985-90.

| | Ems Dollard | | Western Scheldt | |
|---|---|---|---|---|
| | Length | | Length | |
| | effect | Trend | effect | Trend |
| Mytilus edulis | | | | |
| Cd | - | - | + | - |
| Cu | - | - | + | - |
| Zn | - | - | - | - |
| Cr | + | - | + | - |
| As | - | - | - | - |
| Ni | + | - | + | - |
| Pb | + | - | - | - |
| Hg* | +** | - | - | - |
| PCB (sum 7) | + | - | +o | - |
| PCB-138 | + | - | +o | - |
| PAH (sum 6) | ++ | - | + | - |
| Dieldrin | | | - | +§ |
| Lindane | | | - | - |
| pp'DDE* | | | - | - |
| Platichthys flesus | | | | |
| Cd | - | +♯ | ++ | - |
| Hg | - | - | ++ | - |
| PCB-138 | + | -• | + | - |

+ - Significant
+ + - Length effect with interaction between length and year
* Excluding data from 1985
** Not significant when concentrations are expressed on a dry wt basis
+o Not significant when concentrations are expressed on a wet wt basis
§ Decrease
♯ Increase
• Significant decrease when concentrations are expressed on a wet wt basis

During the period 1985-90 there only appeared to be a significant linear decrease in dieldrin levels in mussels from the Western Scheldt while cadmium levels in flounders from the Ems Dollard increased. No trends were detected in either estuary for the other contaminants. PCB is one of these contaminants and poses a severe threat to the marine environment (Ministry of Transport, Public Works and Water management, 1989). Although there is international agreement on banning these compounds the statistical analyses give no indications of an improvement in levels of PCBs in biota of either estuary. In the North Sea PCB levels in the livers of Atlantic Cod also remained constant over the period 1979-87 (de Boer, 1988). So despite the international concern of the marine environment there has not been a recent and general improvement with regard to contaminant levels in biota.

Table 3

Linear regression between concentrations of mercury and PCB-138 and percentage fat wt versus the length L (mm) of the fish, according to the model y=a+b*L (ED = Ems Dollard, WS = Western Scheldt).

| y | Tissue | Unit | Area | a | b | $R^2$ | n |
|---|---|---|---|---|---|---|---|
| $\log_e$[Hg] | muscle | mg $kg^{-1}$ WW | ED | -3.084 | 0.006 | 0.20 | 99 |
| $\log_e$[PCB-138] | liver | mg $kg^{-1}$ WW | ED | -4.215 | 0.005 | 0.12 | 89 |
| | | | WS | -3.041 | 0.004 | 0.10 | 158 |
| $\log_e$[PCB-138] | liver | mg $kg^{-1}$ FW | ED | -0.147 | -0.003 | 0.11 | 85 |
| | | | WS | 1.094 | -0.004 | 0.10 | 157 |
| Fat wt | liver | % | ED | -12.2 | 0.11 | 0.18 | 89 |
| | | | WS | -15.0 | 0.11 | 0.28 | 158 |

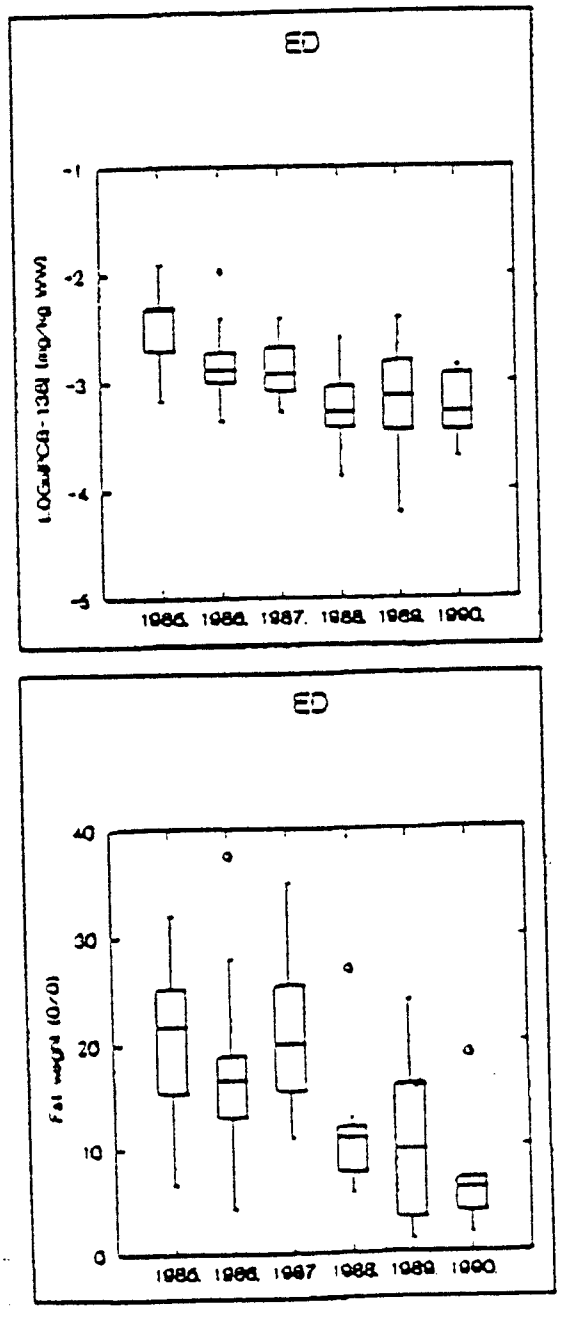


Fig. 5 Box and whisker plots of PCB-138 concentrations on a wet wt basis and percentage fat wt in the livers of flounders from the Ems Dollard, 1985-90

Luten et al. (1986) reported earlier JMP data on cadmium mercury, lead, zinc and copper in mussels form the Dutch coastal area during the period 1979-83. The average concentrations of cadmium and mercury in mussels from the Western Scheldt indicate a decrease over the period 1985-90 in comparison with levels in 1979-83. As a result mercury concentrations in mussel and flounder from both estuaries no longer differ. Cadmium levels in mussels from the Western Scheldt are still higher than in the Ems Dollard, but those in the

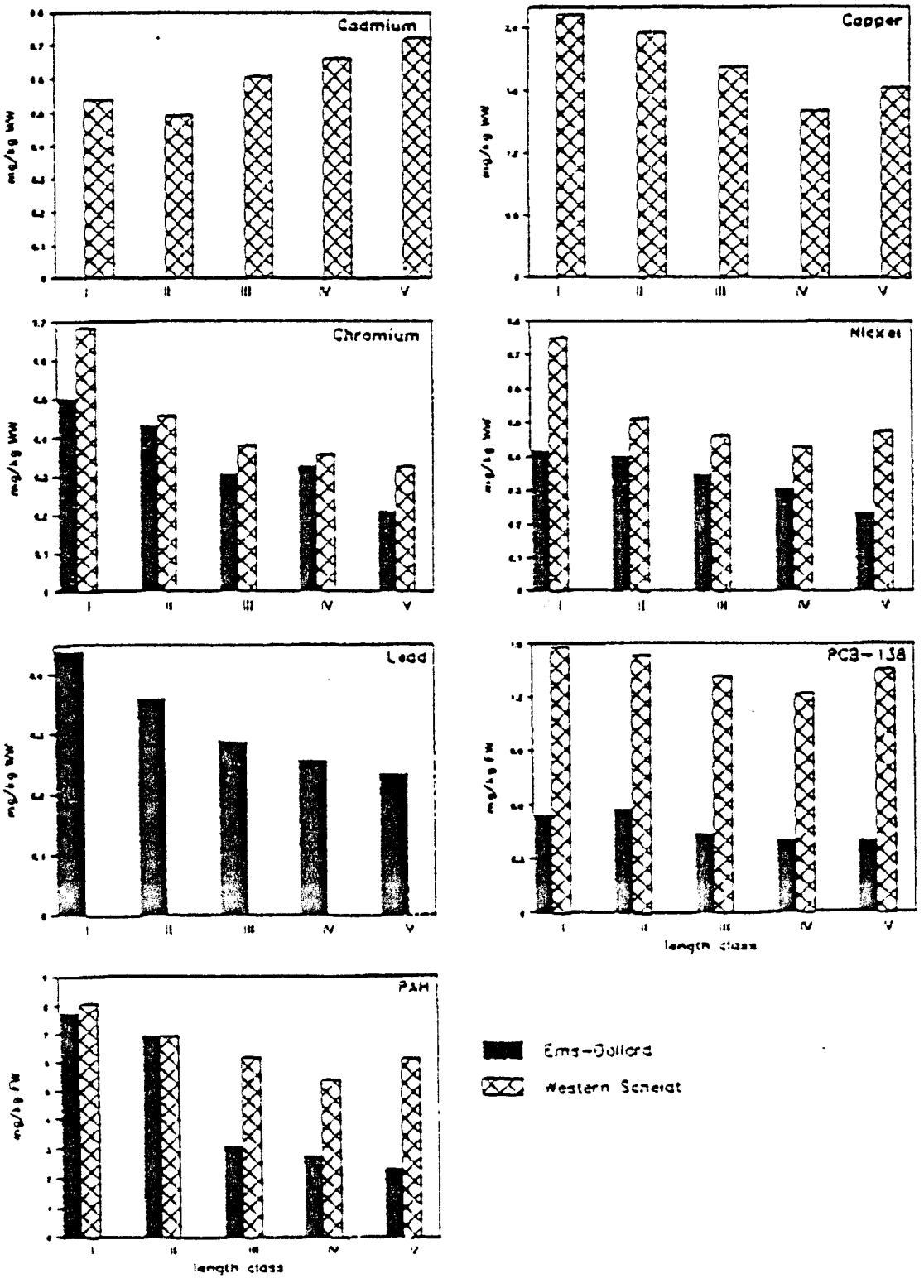


Fig. 6 Geometric mean concentrations of cadmium, copper zinc, chromium, arsenic, nickel, lead and mercury per length class mussels from ems Dollard and Western Scheldt (I=25-30mm; II=31-37mm; III=38-46mm; IV=47-56mm; V=57-70mm)

liver tissue of flounders in both estuaries are comparable. The high PCB levels in flounder livers and mussels from the Western Scheldt reflect the serious pollution of the river Scheldt (Hupkes, 1990). The higher contaminant levels in mussels from the Western Scheldt in comparison with the Ems Dollard may also be due to the fact that the sampling site is more affected by river discharge.

The influence of size of an aquatic organism on uptake and release of toxicants from water is described in a bioaccumulation model presented by Roberts et al. (1979). As larger individuals tend to pump less water through their body per unit of body weight the uptake is lower than in smaller individuals. The pollutant is also more diluted due to growth. In agreement with this idea and with field observations of mussels by Cossa et al. (1980) and Olafsson (1986) the data presented here show a decrease in chromium and nickel concentrations with increasing size in both estuaries, and also for mercury and lead levels in mussels from the Ems Dollard and copper concentrations in mussels from the Western Scheldt. A decrease with length was also found for PAH in mussels. cadmium content in mussels from the Western Scheldt however show an increase with length.

Kuwabara et al. (1986) reported an increase in PCB concentrations in mussel on a wet wt basis. In this study a minor decrease in PCB concentrations on a fat wt basis was found. This is consistent with the correlation found for PCB in the livers of flounders. The increase in mercury concentrations in the muscle tissue with the length of flounder found in this study is a well known phenomenon (Mance, 1985; Luten et al., 1987). Mercury in fish is mainly present as methylmercury, which has a very high retention rate in flounder (a half-life of approximately 1000 days: Clarkson, 1972). In the case of constant exposure this compounds therefore tends to increase with age or length.

In the case of cadmium in the liver tissue of flounder no clear effect of length was found. Other covariables like liver wt may be more clearly related to contaminant burden in liver tissue (Nicholson et al., 1991).

Finally, the concentrations in fish and shellfish presented in this paper were compared with Dutch consumption standards (mercury in fish and shellfish: 1 mg $kg^{-1}$ WW; PCB-138 in fish liver; 1.5 mg $kg^{-1}$ WW; cadmium and lead in shellfish 1 and 2 mg $kg^{-1}$ WW, respectively). However it should be pointed out that no mussels are caught for consumption in the area around the sampling sites. A few maximum concentrations do approach or exceed the standards: PCB-138 in liver of a flounder from the Western Scheldt (1 mg $kg^{-1}$), mercury in a flounder from the Ems Dollard (0.9 mg $kg^{-1}$ WW) and cadmium in mussels from the Western Scheldt (1.2 mg $kg^{-1}$ WW). In all cases the yearly average concentrations in fish and shellfish from both estuaries meet the standards.

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# The need for adequate biological sampling in ecotoxicological investigations: a retrospective study of twenty years pollution monitoring

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## ABSTRACT

The measurement of concentrations of persistent bioaccumulating chemicals in ecological samples is an important tool in ecotoxicological science. It is important to consider the need for both chemical and biological precision when designing research programmes for studying environmental pollution. The balance between chemical and biological precision is discussed on the basis of a database from the Swedish Contaminant Programme covering the period 1969-1989. The negative consequences of the analysis of pooled samples instead of individual specimens are demonstrated. The importance of various biological parameters such as sampling sites, content of fat and age of the specimens analyzed is shown. The prerequisite of a sufficiently long lime-span and frequency of sampling in monitoring studies is shown.

Key words: monitoring methods; pooled samples; organochlorines; mercury; fat; toxicology.

## INTRODUCTION

The major reason for concern regarding persistent and bioaccumulating chemicals in the environment is their potential effect on biota. High concentrations of persistent chemicals in sediment, soil, air and water do not in themselves pose an important problem unless they are, or will be, bioavailable and bioaccumulate. Consequently, it can be argued that the major efforts in studies of spatial distribution and temporal trend monitoring of concentrations of persistent chemicals ought primarily to be devoted to the analysis of biota so that the bioavailable fractions of the pollution can be assessed. In any attempt to find the transport mechanisms, it is imperative to undertake complementary studies of concentrations in the surrounding media.

For all kinds of material from the ecosystem there is a set of factors which causes variations in the concentrations of contaminants. How can we interpret data if we do not know the variation and the explanatory factors behind the variations?

Initially, there are three major reasons for determining the concentration of a contaminant.

(i) To elucidate whether a chemical is a potential threat to biota it is necessary to study the bioaccumulation and/or biomagnification of the chemical by determining the concentration in representative parts of the nourishment web.

(ii) In order to disclose where in the environment serious implications and threats to biota from bioaccumulating, persistent chemicals occur, representative biological samples from the areas and ecosystems under study must be selected.

(iii) To evaluate whether the threat to biota by a pollutant increases, decreases or is stable. For a society which has to determine the priority of measures against environmental pollution it is also imperative to ascertain that the measures taken have been adequate. Here, too, comparable and representative biological samples must be selected for chemical analysis.

When contaminant concentrations have been determined, risk evaluations can be made, conclusions drawn and legislative measures taken by society.

In order to show the importance of considering biological variation before any conclusions are drawn, we use earlier published data on DDT, PCB and mercury concentrations in wildlife samples. Time-series of individually analysed specimens from the Swedish Contaminant Monitoring Programme over a period of 20 years serve as the data base. From these data it is possible to study retrospectively the consequences of: the analysis of pooled samples instead of individual specimens, the disregard of various biological parameters affecting the estimated concentrations, too short time series and infrequent sampling in monitoring studies.

*How to improve biological precision and to select an adequate sampling population?*

When sampling was begun in 1969, a number of herring (Clupea harengus) were collected from different catches in various areas of the Baltic. During 1969 nd 1970, before legislative steps were taken to prohibit the use of DDT, 276 herring specimens in all were collected from 16 catches in the area of the southern Baltic indicated in Fig. 1 (Jensen et al., 1972). They were analysed individually for the presence of DDT and PCB in muscle tissue according to the analytical method described by Jensen et al. (1972). The mean concentration of DDT substances (DDT + DDD + DDE = sDDT) was 40 mg/kg extractable lipid. This value corresponds to the concentration that would result from all the individual samples being pooled (Fig. 2a). The range is, however, considerable (6-250 ppm). Furthermore, the distribution is severely skewed and thus the appropriateness of using the arithmetic mean can be questioned. If the mean concentrations of the 16 separate catches are presented instead (the mean

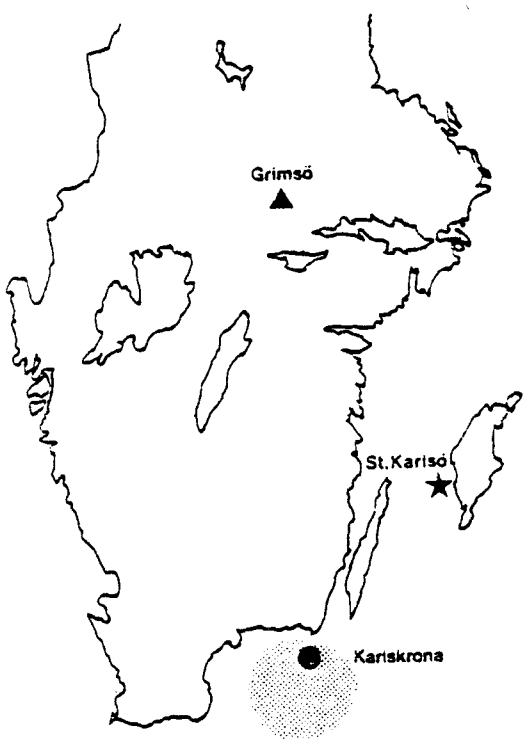


Fig. 1 Sampling areas of starlings (▲), guillemots (★) and herrings in the southern Baltic (▒) as well as in the Karlskrona archipelago (●)

values thus simulate the analysis of one pooled sample from each catch), the uncertainty of the predictions from single pooled samples becomes evident (Fig. 2b).

If we then consider the sampling season and separate the samples into herring collected during the period July-March (non-breeding period) and April-June (breeding season), the variation within the two samples is still substantial and no significant difference is found (Fig. 2c,d). From the literature it is known, however, that herring migrate. This is true especially for older fast-growing specimens, whereas younger ones stay in a certain area (for a review, see Parmanne, 1990). We therefore separated each of the two seasonal samples in tow age classes: less than 4 years and older. Figures 2e-h, show that the variation within the two subsamples of young herring decreases and leads to a significant difference in concentrations between the seasons for young specimens but not for old ones.

Perttilä et al. (1982) have reported a correlation between age and concentrations of sDDT and PCB in Baltic herring. Their conclusions were drawn from the analysis of pooled samples from different age classes. In an earlier report based on individually analysed specimens, Jensen et al. (1972) also found higher mean values in older fish than in younger ones. In the discussion it was claimed that this increase by age was not solely the effect of longer exposure time but was laos due to differences in migration habits between various age classes.

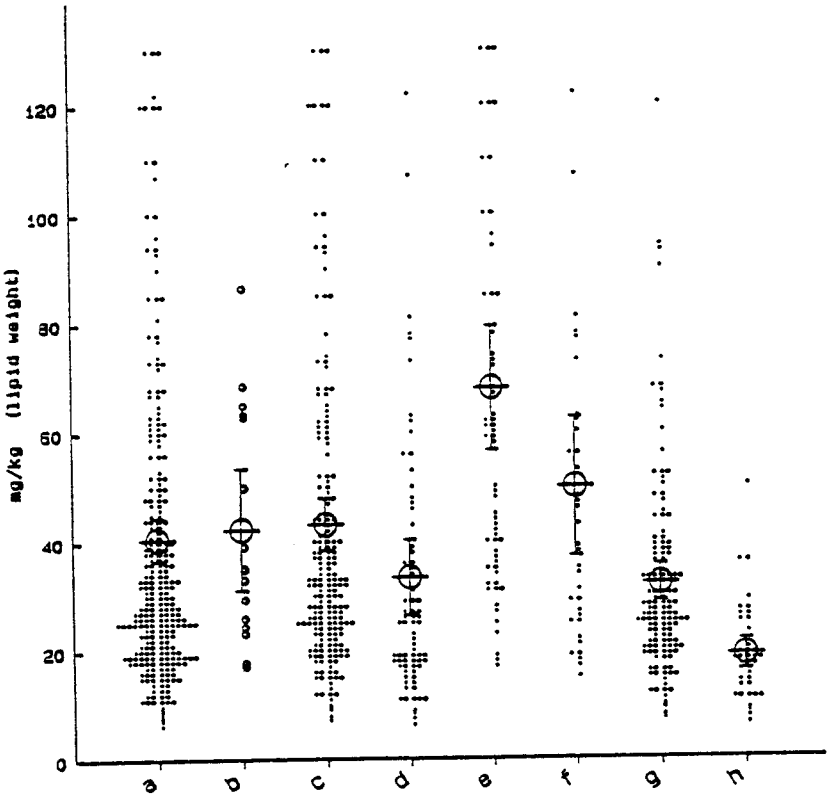


Fig. 2 Concentrations of DDT compounds (mg/kg lipid weight) in herrings collected during 1969-1970 in the southern Baltic. (a) individual concentrations of all herrings; (b) pooled samples of each catch; (c) the breeding season; (d) the non-breeding period; (e) breeding season, older than 3 years old; (f) non-breeding period, older than 3 years old; (g) breeding season, 1-3 years old; (h) non-breeding period, 1-3 years old. ⊖, arithmetic mean values. Vertical bars indicate 95% confidence interval of mean. (Five values greater than 140 mg/kg are excluded in Fig. 2 but are included in the estimation of the confidence intervals)

The herring samples presented in Fig. 2 have been divided into separate age classes in Fig. 3 and results that would have been obtained from pooled samples are simulated by using the arithmetic mean of the analysed individuals for each age class. As in the material presented by Perttilä et al. (1982), a regression analysis using the mean concentration (pooled sample) versus age on both the seasonal samples gives significant correlations. The correlation coefficients from the material collected during the breeding period and the non-breeding period were $r=0.82$, $P<0.01$ and $r=0.84$, $P<0.05$, respectively. A study based on pooled samples only would probably be interpreted as an age dependence of the sDDT concentrations and claim that the exposure time (age) explains the dependence.

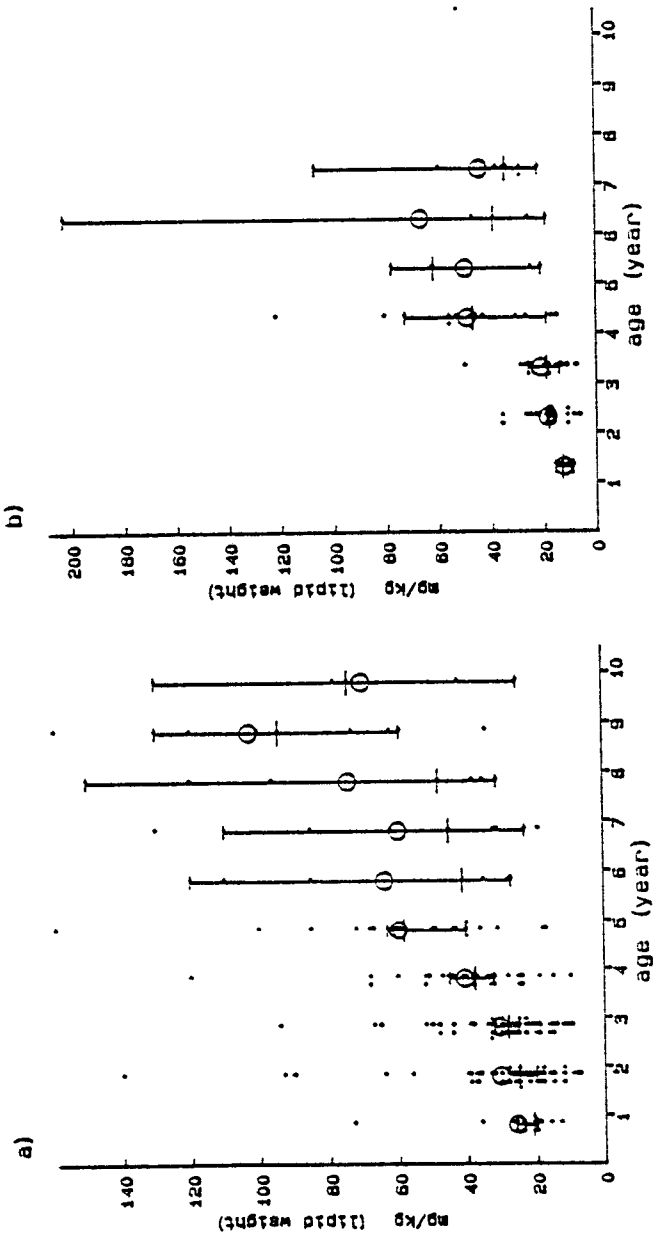


Fig. 3 Concentrations of DDT compounds (mg/kg lipid weight) in herrings collected in the southern Baltic during (a) the breeding season; (b) the non-breeding period. The material is divided into gage classes. O, arithmetic mean, → median. Vertical bars indicate 95% confidence interval of median. (Two values greater than 160 mg/kg are excluded in Fig. 3a but are included in the estimation of the confidence intervals)

The concentration of a chemical in a pooled sample will always represent a weighted arithmetic mean of its constituent individual parts. If the distribution of the individual concentrations is severely skewed the result obtained from the pooled sample will not be the best measure of the central value; the median, for example, would be more appropriate.

In Fig. 3, the median and its 95% confidence interval are therefore also presented. There is no evident difference in concentrations between the age classes up to 2-3 years of age in the two seasonal materials. Over the age of 4 years, the confidence intervals increase substantially.

According to Parmanne (1990), younger age classes are less migratory than older ones. One possible explanation of the increased variance in older age classes is that the older fish do not represent the sampling area only, but also many different areas from which they have migrated. If so, one would expect that the older age classes would show greater variance than the younger ones. The correlation between concentrations of DDT and age is thus possibly not caused by the length of the exposure but by variations in the migration routes.

In order to avoid greater variability in older age classes, we have endeavoured to use herring younger than 4 years in the following trend monitoring study.

*What is a time-trend and what is short term fluctuation by chance?*

In order to demonstrate the need for long series to establish a proper time-trend, we have used our data on PCB concentrations in muscle tissue of young herring collected in the same area as above. For the time-trend study, 25 specimens less than 4 years old were annually collected in late April to early June at one specific site off the Karlskrona archipelago. The material covers the period 1972-1988, except for the years 1977 and 1982, for which figures are lacking due to irregularities in the collection programme.

In Fig. 4, the material is separated into two periods of equal duration, the first covering 1972-1979 and the second, 1980-1988. If we scrutinize the two time-series separately, no trend with respect to concentrations of PCB can be seen. If we combine the two periods, an interpretation becomes easier, especially since it is based on individual analysis and thus possible to take the within-year variation into consideration (Fig. 5). If the study had been based on the analysis of single annual pooled samples we would not be aware of the uncertainty of the annual estimate.

During the period studied most governments took measures to prohibit the use of PCB. It would have been reasonable to expect that this would have provided an opportunity to measure a real decrease. However, due to the inevitable short-term chance fluctuation that takes place, it is still difficult to prove a trend in a period of less than 8 years. Based on data from 17 years we can state that concentrations have decreased by more than 50%.

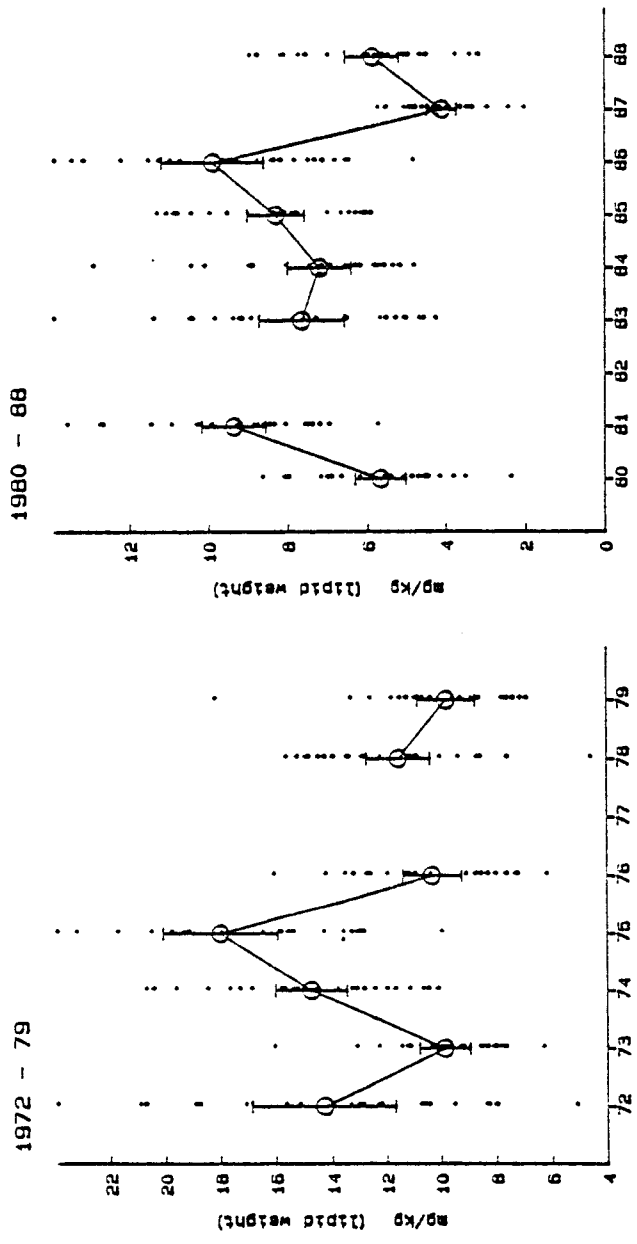


Fig. 4 Concentration of total PCB (mg/kg lipid weight) in young herrings collected during the breeding season in the Karlskrona archipelago. The material is divided into two periods of equal duration: (a) 1972-1979, (b) 1980-1988. O, arithmetic mean. Vertical bars indicate 95% confidence interval of mean

*Is there a need for annual sampling or can sampling be done less frequently?*

In harsh times with decreasing budgets, monitoring programs are subjected to economics of various kinds. One method often discussed is to sample less frequently.

On the basis of the annual collected data in Fig. 5, we simulate a sampling each third year, starting in 1972, 1973 or 1974, respectively. Furthermore, we simulate, utilizing the mean values, time-series based on the analysis of pooled samples (Fig. 6). Are any of these three presentations adequate to demonstrate the trend over the period? Again we can observe the influence of short-term fluctuation by chance and the necessity to consider this in all monitoring programmes, whether the objective is to describe temporal, spatial or species variation in environmental pollution.

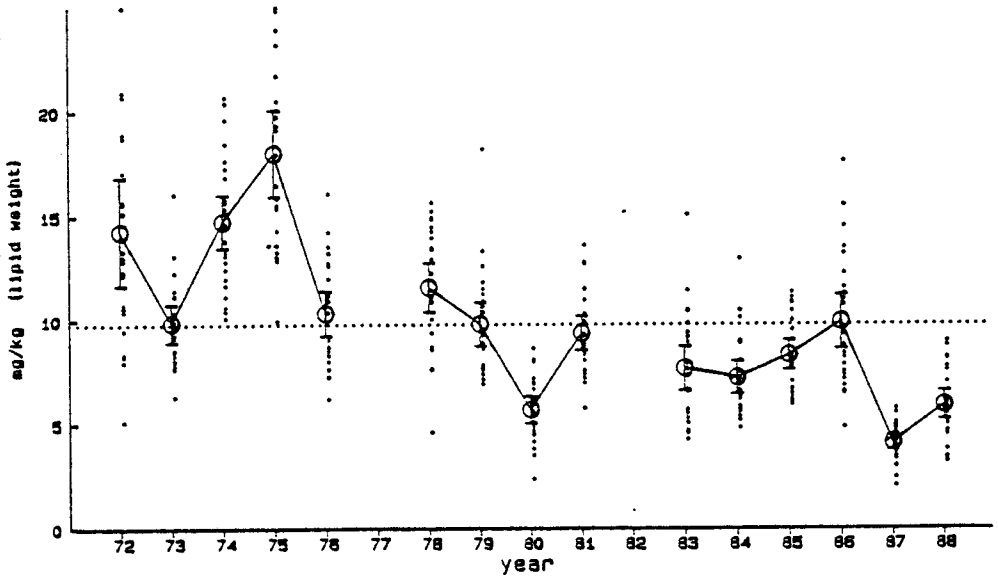


Fig. 5 Concentration of total PCB (mg/kg lipid weight) in young herrings collected during the breeding season 1972-1988 in the Karlskrona archipelago. O, arithmetic mean. Vertical bars indicate 95% confidence interval of mean

*Should concentrations of halogenated organic compounds be expressed on a fresh weight or on a fat weight basis?*

Many of the halogenated organic compounds present in the environment are lipophilic and thus dissolved in the fatty tissues of the organisms. From the consumer's point of view it seems logical to determine the concentrations on a fresh weight basis. If, however, we want to use biological samples to answer specific questions concerning the pollution load in the environment with regard to spatial variation or time-trend analysis, it seems more logical to determine the concentrations in the compartment or tissue where it is enriched. If the fat amount of the organism or tissue analysed is not assessed, we are restricted to using concentrations expressed on a fresh weight basis. We must then keep in mind that we are working with an undefined mixture of water, proteins and lipids. The relevance of knowing the fat concentration in the individual specimens is demonstrated below.

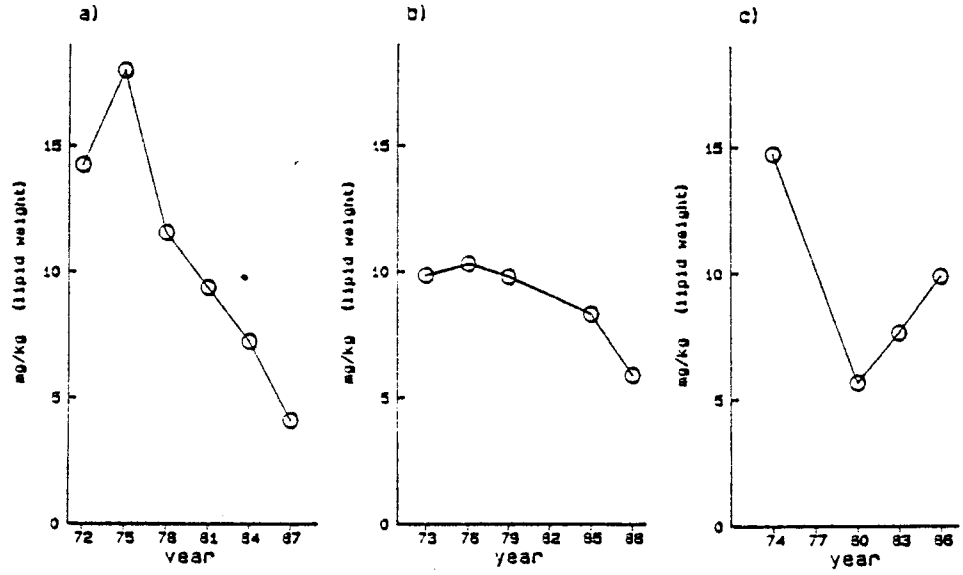


Fig. 6 Annual mean concentration of total PCB (mg/kg lipid weight) in young herrings collected during the breeding season in the archipelago of Karlskrona. The three examples demonstrate the time-series that would be obtained if sampling were performed every 3 years starting n 1972, 1973 and 1974, respectively

In the herring samples from the time-trend study in the Karlskrona archipelago, no obvious trend or variations in concentrations of PCB, measured on a wet weight basis, occurs between 1983 and 1988. When correlating the logarithmic values of the concentrations of PCB in extractable fat with the logarithmic values for the fat percentage of the individual herring specimens, a strong correlation is found ($r = -0.83$, $b = -0.75$, $n = 149$, Fig. 7). This material represents the breeding season, when the fat percentage is low. In comparison, we correlate similar data for the same years but use a material consisting of annual samples of muscle tissue from 20 herrings collected at the same sampling site but during the autumn (Sep-Nov) and analyzed individually. The analysis was performed at the same laboratory using the same method as for the material collected during he breeding season. The herrings collected in autumn are fatter and the correlation and slope is considerably smaller ($r = -0.39$, $b = -.028$, $n = 120$). Thus, at higher fat percentage (>4%) during the non-breeding period, the PCB concentration measured on a fat-weight basis is more or less uninfluenced by an alteration in the fat content (Fig. 7). During this period, higher fat content in the tissue results in higher PCB concentrations on a fresh weight basis. The steep slope of the regression line between concentration of sPCB in lipids and the fat content in samples collected during the breeding season increases variability in concentrations and uncertainty in the evaluation of the analytical results. Thus, sampling during the non-breeding period is preferable. In order to maintain long time series already started, however, comparable samples from the breeding season must still be collected, but should then be adjusted for this known correlation.

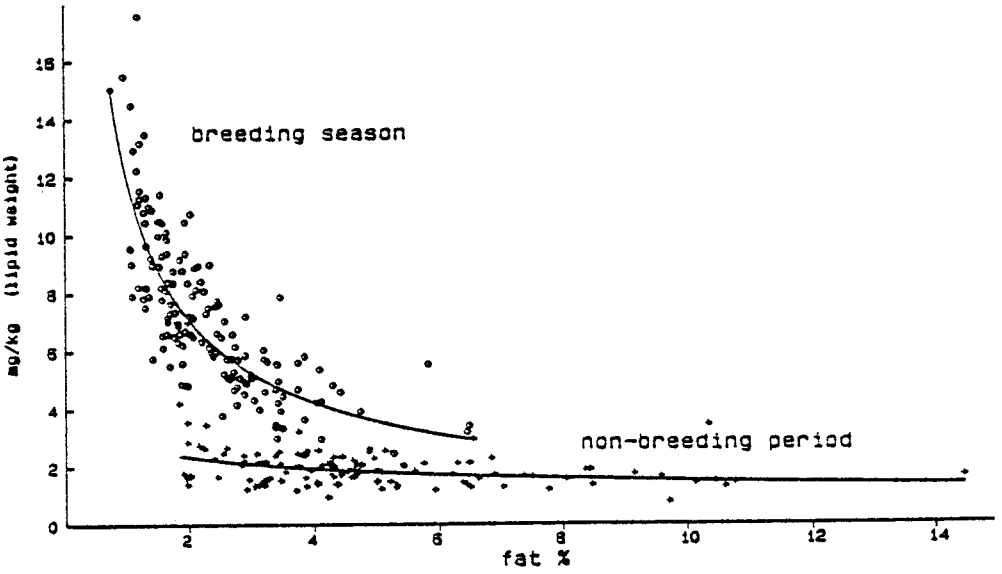


Fig. 7 Concentration of total PCB (mg/kg lipid weight) versus fat percent in young herrings collected during the breeding season (O) and the autumn (+) in the Karlskrona archipelago during the period 1983-1988. The regression lines are based on logarithmic values for the concentration of PCB and fat percent

We have at present no firm explanation for the log-linear correlation in PCB concentrations expressed on a lipid weight basis, versus percentage of fat in the tissue. The point of maximum curvature of the curves is found between 2% and 4% fat, which is low for herring, and occurs mostly during the spawning season and immediately thereafter. One possible explanation is that a partition equilibrium between water and fat, due to gill ventilation, is established only during the non-breeding period, when the fish are in a physiologically stable phase. During the spawning season, on the other hand, the lipid content of the fish decreases rapidly due to high energy consumption during the spawning period. If the fat metabolism is too fast the partition equilibrium between body-fat and water is not established, and the concentration of PCB consequently increases in the remaining lipids. Thus the explanation of the variation in contaminant concentrations (lipid weight) at low fat percentages in herring could be fast rate of metabolism. Another explanation or a complementary factor is a possible change in fat composition during starvation, altering the partition coefficients between fish body-fat and water.

The time-trend of PCB covering the period 1972-1988, shown in Fig. 5 can be improved if we consider the log-log-linear correlation found and adjust for fat content (Fig. 8). The adjustment is done by fitting regression lines for the logged concentrations versus the logged fat percentages for each year separately and estimating the pooled slope (weighted mean slope) for all years (Sokal and Rohlf, 1981). The adjusted mean concentration $Y$ for year $t$ is estimated by using the model:

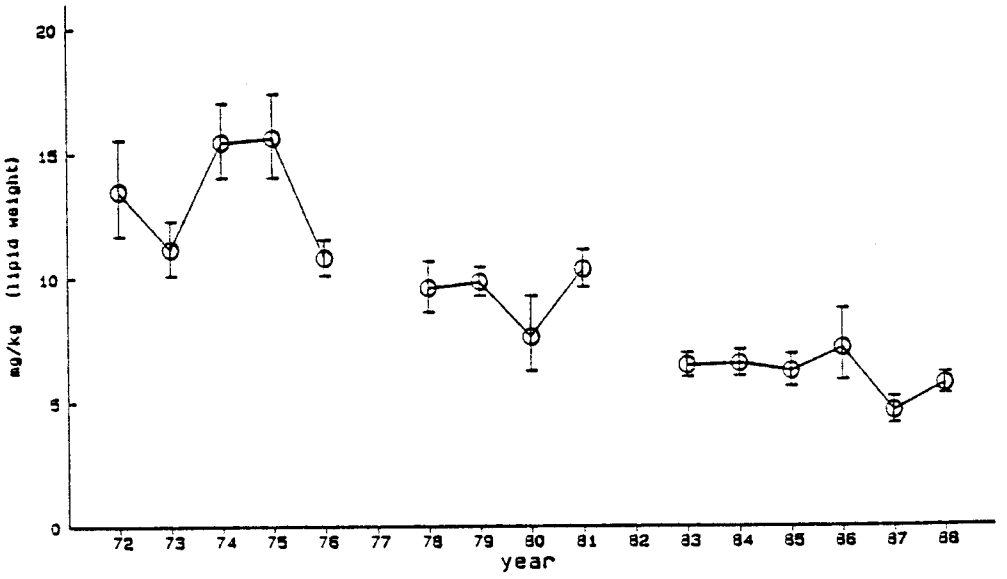


Fig. 8 Concentration of total PCB (mg/kg lipid weight) adjusted as described in the text, in young herrings collected during the breeding season 1972-1988 in the Karlskrona archipelago. O, arithmetic mean. Vertical bars indicate 95% confidence interval of mean

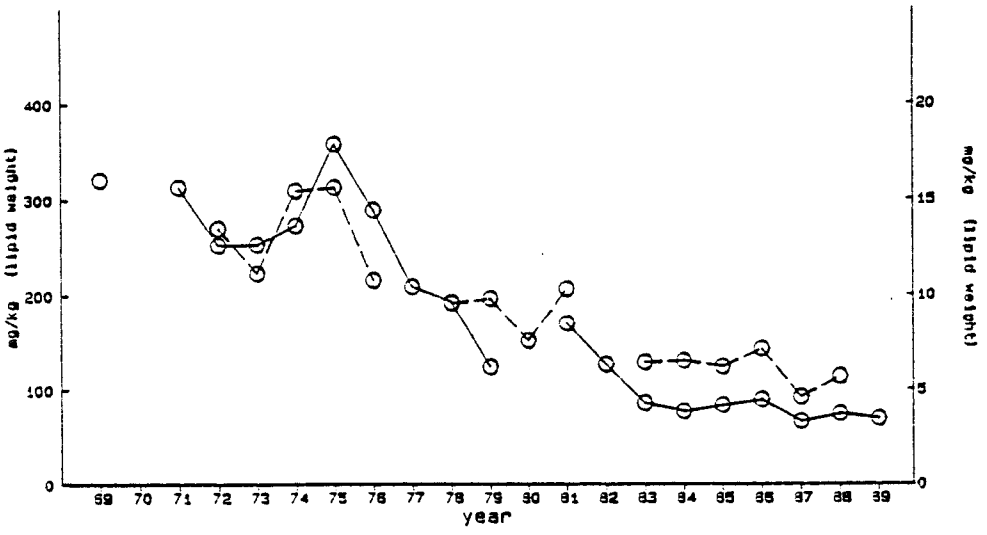


Fig. 9 Concentration of total PCB (mg/kg lipid weight) in herrings (---) and guillemot eggs (——) from the southern parts of the Baltic. The PCB concentration in herrings is adjusted as in Fig. 8. O, arithmetic mean

$$\log(Y) = \alpha_t + b \log (X)$$

where $\alpha_t$ is the intercept for year $t$, $b$ is the common slope of all years and $X$ is the mean logged fat percent for all years.

The trend after adjustment is smooth and agrees surprisingly well with results from a time-trend study of about 10 annually sampled eggs of guillemot (Uria algae) breeding in the central baltic (Fig. 9). The data have partly been presented earlier (Olsson and Reutergårdh, 1986) and a report including more recent material is under preparation (Bignert et al., unpublished). All the eggs used in this preparation were laid in May. The example shows that the identification of explanatory factors improves the possibility of making a proper assessment.

*How important is spatial variation?*

The following example will show that a proper definition of the sample site is essential for the evaluation of recorded data. The aim of the study was to follow the temporal variation in mercury pollution in a forest district in central Sweden. Fledglings of starlings (Sturnus vulgaris), one from each nesting box, were annually collected and pectoral muscle analysed. The analytical methods used have been published earlier (Lindstedt and Skare, 1971; May and Stoeppler, 1984). In the forest district concerned starlings were collected at four different sites, each located in the vicinity of small isolated hamlets surrounded by pastures. The four sites were all assumed to be locally unpolluted reference areas with regard to mercury. They were all located within a radius of 3 km.

The fledglings were regarded as belonging to the same population (Fig. 10a). The temporal trend appears to reveal a substantial decrease in mercury concentrations over time if annually pooled samples are analysed (simulated in the graph as annual mean values). When analysing the individual specimens, we found, however, a large variation in the annual data which could be explained by considerably diverging concentrations in fledglings from one site. These results were therefore separated form those of the other sampling sites (Fig. 10b). The difference between the two materials is obvious. The highest concentrations are found in fledglings from a breeding site close to two lakes. Our attempts to find indication so previous local mercury pollution in the area gave no positive result. The difference might be caused by different feeding habits. Young starlings are fed on imagos and the larval stages of a variety of insects (Diptera, Lepidoptera, Coleoptera, Hymenoptera, Heteroptera) as well as other vertebrates. The larval stages of some of these insects are aquatic, a circumstance which may influence the bioaccumulation of mercury. It has been shown that birds of prey feeding mainly on food from aquatic food webs generally have higher concentrations of mercury than those with a predominantly terrestrial diet (Odsjö and Olsson, 1975; Broo and Odsjö, 1981; Lindberg and Odsjö, 1983).

The individual analysis as well as the definition of sampling site for specimens of starling made it possible afterwards to make a more valid interpretation of the data.

## CONCLUSIONS AND DISCUSSION

In all kinds of scientific work, proper and comparable results are imperative for reliable evaluations, which will otherwise be mere guesswork. Decisions and expensive undertakings can be rendered inadequate and even worthless.

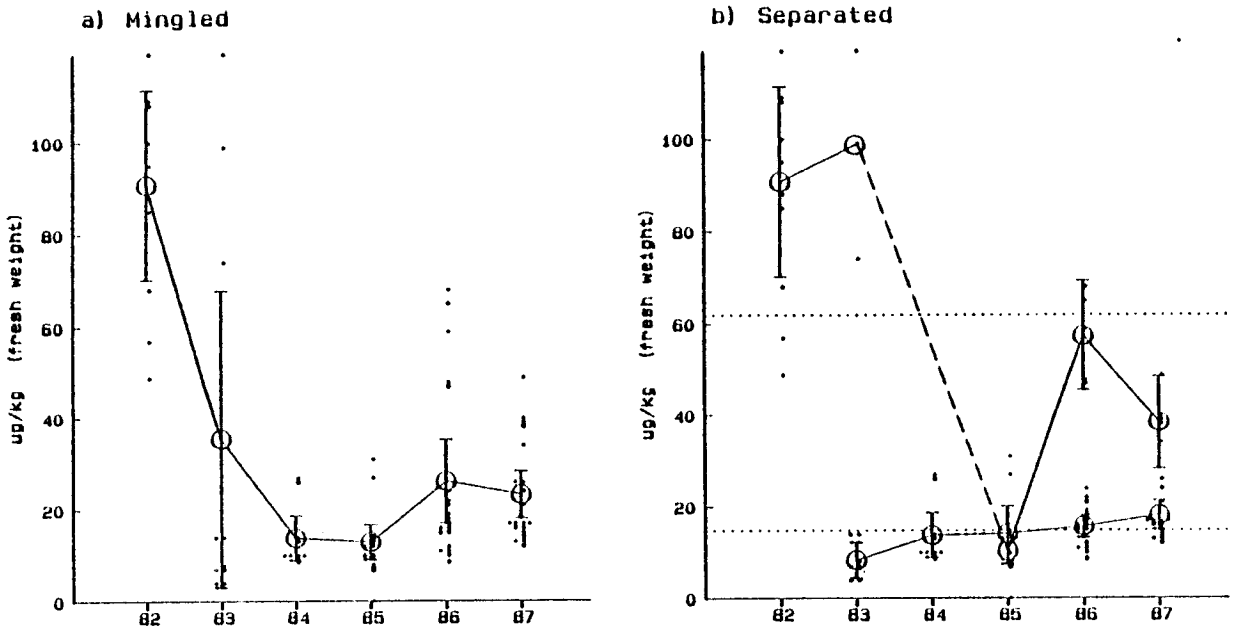


Fig. 10 Concentration of mercury (µg/kg fresh weight basis) in the fledglings of starlings collected at 4 sites in a forest district in central Sweden. (a) the time-series of all 4 sites, (b) time-series representing the fledglings of the diverging site in contrast to the other sites. See text. A dotted line indicates the mean value during the investigated period

It might be tempting to explain a difference in the concentrations of a pollutant from the analysis of two samples of, e.g. herring collected at two different sites. However, with more samples from each local population we often find a considerable variation in concentration within the population, which means that we do not know whether this difference actually exists. If, with the aid of applied statistics, we establish a difference, the results may serve as a base for conclusions on the pollution burden at the two sites. A significant difference in concentrations in the two herring populations does not, however, say that the difference obtained is explained by a difference in pollution load at the two sites. To draw correct conclusions from the results we have to know whether biological parameters such as sex, age, size, physiological status and collection season affect the results. In such cases we can either compensate for the influence or use only samples that in all known aspects are comparable. To use the concentration of a substance in a species as the single criteria when judging trends is rarely enough.

The pooling of several individual specimens into pooled samples may lead to more reliable estimates of contaminant concentrations, as the pooled sample represents a greater part of the population investigated. To get an estimate of the variation, an appropriate number of pooled samples has to be analysed If the cost of sampling additional individuals is small in relation to the cost of extra chemical analysis, this method should be considered (van der Meer, 1990). The risk of loosing valuable information when pooling is, however, always considerable. A prerequisite for comparing any results is a knowledge of the sample variance. When analysing pooled samples, the variation due to biological factors is obscured. Individual specimen analysis offers an opportunity to reveal the cause of variation. If the factors explaining the variance are taken into consideration and pooling is used due to low chemical-analytical capacity, the number of analyses must still be sufficient to estimate the variance and determine an adequate confidence interval. To draw conclusions from comparisons between single pooled samples is most certainly a waste of resources.

Pooling often implies that future freedom in evaluation is severely restricted.

To reach fundamental knowledge about the dependence between contaminant concentrations and various ecological factors, e.g. age, length, weight, sampling season etc., individual analyses are required. The information they provide is essential and will govern the formation of a sampling strategy and the choice of what population to sample.

The distribution and variance of contaminant concentrations within the sample are informative in themselves. They might disclose an unexpected heterogeneity in the sample otherwise hidden in the pooled sample. An increased variance is often an indication of ongoing contamination before it is possible to verify a difference in mean values. Another implication is that single outliers will influence the mean but they are not possible to identify unless analysed separately. Concentrations, non-normally distributed, found by the analysis of individual specimens can be transformed in order to approach normal distribution and constant variance. The measure of location might be changed, e.g. from an arithmetic to a geometric mean or median, whereas a pooled sample always represents a weighted arithmetic mean of the specimens included. Misra (1987) and Nicholson et al. (1989, 1990) give examples and discuss some of the statistical implications when using pooled samples in time-trend studies.

Contaminant concentrations based on individual analyses can often be adjusted for biological and physiological covariates such as age, length, weight, fat content etc. (see below and Fig. 7). If a covariate is known to be essential, pooled samples should be stratified according to that covariate (Anon., 1986). The structure of dependence is, however, seldom a simple linear univariate relationship and is thus difficult to use as a criterion for stratification. Furthermore, chemical covariates are not detected until the chemical analysis has been carried out and are therefore impossible to use for stratifying pooled samples.

In some situations we want to relate biological effects, e.g. breeding disturbance, injuries of various kinds etc., to contaminant concentrations. This is hardly possible when using pooled samples.

Biological variability usually greatly exceeds chemical analytical precision. When chemical analysis becomes intricate this is, however, not always the case. The pooling of several specimens will only reduce the biological part of the variance. The analytical part has to be estimated by an appropriate number of replicates. It is worrying that, for complicated analyses, where the cost of an additional analysis is high, precision might be coarse. High cost is, however, not a relevant argument for not establishing the analytical precision. Any use of analytical results can be questioned if the reproducibility of the method is not given and constantly checked.

In the literature, including some of our own reports, there are several examples of studies in which only a few pooled samples, or even only one pooled sample, have been analysed in order to describe a certain ecological niche or a spatial variation in pollution. The high costs of analysing dioxins have, as an example, resulted in most of our knowledge of the appearance of these contaminants in the ecosystem being based on the analysis of only a few pooled samples.

But what can be judged from a single analysis and what is a representative sample? Is the information in such reports of any scientific value?

Biological variation such as within and between populations, seasonal variation and short-term fluctuation by chance are often neglected when interpreting data, whether the aim is to study spatial distribution or to study variation of the pollution over time. Even with the most sophisticated chemical analyses, where all kind of analytical errors are minimized, the results of one single or a few biological samples will not provide more information than the samples permit from a biological point of view.

The high costs, both in money and analytical competence, of assessing the many different halogenated organic compounds are a threat to the scientific reliability on which society bases measures against environmental pollution. Chemical analysis and biological sampling are both indispensable tools for studying the impact of chemicals on the environment. It is necessary that chemical research leading to the identification of hitherto unknown contaminants in nature should lead to the development of analytical methods which can be used for series-analysis and be a vehicle for biologists. We will otherwise get a more or less endless list of chemicals occasionally found, but without guidance to determine which are the most important for further studies. Toxicity data for the compounds might be useful if the information

is relevant to the ecosystem studied. Without representative concentrations from nature, toxicity data has only a limited value.

Another purpose of this article is to demonstrate the difficulty of monitoring pollution even when data from long-time series are available and even is these are based on a biologically well-defined material, collected during a period of obvious decreasing pollution. How long will it take before a significant change in the pollution can be established, if we must rely on the analysis of scattered, pooled or poorly defined samples, where the factors governing the variation in concentrations are unknown? It should be kept in mind that the explanatory factors for the variation do not need to be identical or even similar for substances which from a chemical point of view, normally can be regarded as similar. It is necessary that the precision in chemical analysis be optimized for the number of analyses which are required to answer specific biological questions of priority. The optimizing procedure might even imply a lowering of the chemical precision to benefit the biological precision by making a high number of analyses available. Thus it is imperative either to develop less time-consuming analytical techniques or to get a substantial increase of analytical resources to improve precision, both from the chemical and the biological point of view.

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# UPTAKE, STORAGE AND ELIMINATION OF CHEMICAL CONTAMINANTS (METALS, ORGANOCHLORINE COMPOUNDS, RADIONUCLIDES) IN MARINE BIOTA

by

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Chemical contaminants are ubiquitous. Metals occur naturally as ions, compounds and complexes and can be natural components of sea water and sediments. Many of them are extracted, purified and processed for industrial use and then released to the environment again. Therefore metals can build up to high concentrations and may then represent a risk to marine life and human consumers of seafood. Organochlorine compounds do not naturally occur in the environment. These synthetic compounds, particularly pesticides (e.g. DDT) and certain industrial chemicals (e.g. PCBs) which are produced and used in large quantities are now widely distributed in the environment. Being fat-soluble, persistent and largely non-biodegradable, they accumulate in sediments and in the lipids of organisms. Their build-up in top predators, particularly marine mammals and birds, causes damage to them while their presence in seafood can make it toxic for human consumption.

As a result of a general redistribution, living organisms can be exposed to these toxic components, through respiration, tegument contact and consumption. Some of them are among the most widely distributed chemical contaminants of large-scale pollution of the world ocean (Table 1).

The ability of aquatic organisms to concentrate and to integrate chemical contaminants is named *bioaccumulation* or *bioconcentration*. *The concentration factor* is defined by the following ratio:

$$\frac{\text{contaminant concentration within the organism}}{\text{contaminant concentration within ambient water}}$$

When the concentration factor for a given contaminant increases, in a food chain, from the lower trophic levels to the higher tropic levels, this is named *bioamagnification*.

Certain metals are, besides having negative effects in high doses, essential for organisms in small doses. Organisms need at least 11 essential trace elements: iron (Fé), copper (Cu), zinc (Zn), cobalt (Co), manganese (Mn), chromium (Cr), molybdenum (Mo), vanadium (V), selenium (Se) nickel (Ni) and tin (Sn). these are important for enzyme metabolism. Accumulation mechanisms were developed by organisms to enrich these essential elements from the extreme dissolution in seawater. but at the same time, non essential

and extremely toxic elements like mercury, cadmium and lead are also absorbed from seawater by organisms which use the same mechanisms as for essential elements. For instance chromium is necessary but is also poisonous in higher concentrations.

No biochemical reactions are known in which mercury, cadmium and lead play a positive role; therefore, these elements even in very small concentrations are basically toxic: they have always a negative effect on physiological processes.

Table 1

The most widely distributed toxic components of large-scale pollution of the world ocean (Patin, 1982).

| Groups and components of pollution | Priority[a] | Degrees of biological danger[b] | Prevalence |
|---|---|---|---|
| **Radionuclides** | | | |
| $^{90}$Strontium | 1 | | Global |
| $^{137}$Caesium | 1 | | Global |
| $^{238}$Plutonium | - | | Global |
| Tritium | - | | Global |
| $^{144}$Cerium | - | | Global |
| **Organochlorine toxicants** | | | |
| DDT and its metabolites | 2 | ++ | Global |
| Polychlorinated biphenyls | 2 | ++ | Global |
| Aldrin | 2 | ++ | Global |
| Dieldrin | 2 | ++ | Local |
| Lindane | 2 | ++ | Local |
| **Metals** | | | |
| Methyl mercury | 1 | ++ | Global |
| Cadmium | 3 | (+) | Global |
| Mercury | 4 | ++ | Global |
| Lead | 4 | (+) | Global |
| Zinc | - | + | Local |
| Copper | - | + | Regional |
| Arsenic | 6 | (+) | Regional |
| Chromium | - | (+) | Local |
| Iron | - | - | Local |
| Manganese | - | - | Local |
| **Petroleum and petroleum products** | 5 | (+) | Global |
| **Detergents** | - | ? | Regional |

Note: (a) Indicators of priority from the point of view of danger to man are shown in conformity to the recommendations of the United Nations Environment Programme (UNEP, 1974); (b) degree of danger for marine organisms: ++ strong; + considerable; (+) weak; ? not determined; - insignificant (GESAMP, 1973).

That organisms continue to survive in the sea despite these negative effects is due to the fact that they tolerate the toxicity of these trace elements not only at the low concentrations that occur naturally in seawater, but also in the higher concentrations which organisms inevitably accumulate in their tissues. Uptake mechanisms cause more trace elements to be absorbed than the organism needs (Bryan, 1976). If excretion is not sufficient, toxic trace elements may be transformed into a non toxic compounds and stored away in the digestive gland, the kidneys or the shell of molluscs. As long as excretion and storage mechanisms are effective, sensitive tissues like the brain are protected from accumulating too high concentrations of toxic elements which might be harmful to physiological processes (Gerlach, 1981).

In detail, physiological and biochemical strategies for uptake, storage and elimination of toxic elements may differ widely. Various mechanisms are involved in these processes, depending on the element and organism.

It is generally accepted that the first effects of a chemical on an organism will be at the cellular and subcellular level (Fig. 1). If the reactions which occur at that level are damaging enough, they will affect organ function. but if the reactions are adaptive, they will not produce a harmful effect at a higher level of cellular and tissue organization.

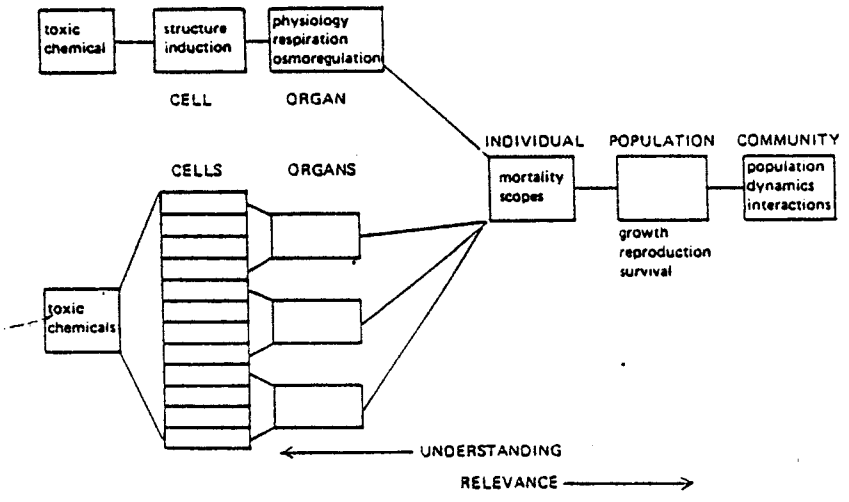
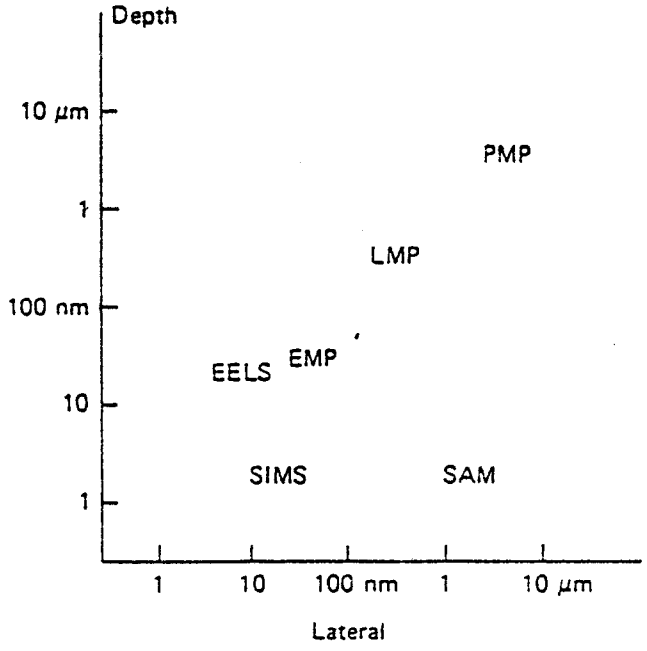



Fig. 1 Progressive effects of pollutants at different levels of biological organization (modified from Haux and Forlin, 1988 in Lloyd, 1991)

Therefore, direct chemical analysis of the metal content of the organelles, cells, organs and tissues, using very sensitive, reproducible and precise microanalytical methods are informative of environmental contamination (Chassard-Bouchaud, 1991). Figure 2 presents the main characteristics of the different methods which can be used.



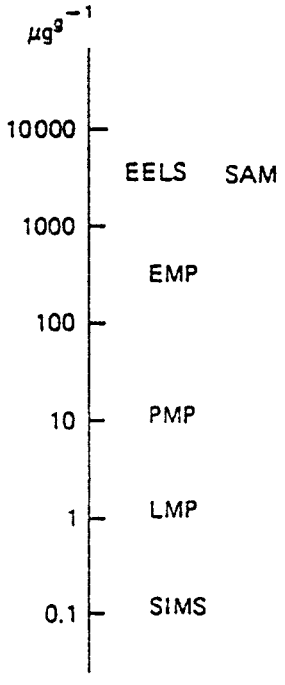




Fig. 2 a. Lateral and depth resolution of microanalytical techniques. Modified after Roomans et al. (1988). EELS: electron energy loss spectrometry. EMP: electron microprobe (=electron probe X-ray microanalysis). LMP: laser microprobe. PMP: proton microprobe. SAM: scanning Auger microanalysis. SIMS: secondary ion mass spectrometry (from Chassard-Bouchaud, 1991)

b. Sensitivity of microanalytical techniques. Modified after Roomans et al. (1988). EELS: electron energy loss spectrometry. EMP: electron microprobe (=electron probe X-ray microanalysis). LMP: laser microprobe. PMP: proton microprobe. SAM: scanning Auger microanalysis. SIMS: secondary ion mass spectrometry (from Chassard-Bouchaud, 1991)

Moreover, using the photon microscope, it is easy to observe if the tissues and organs are damaged and using the transmission electron microscope it is easy to observe the ultrastructural abnormalities.

But what happens at the organism level? Concerning chemical contaminants, the following processes have to be considered: uptake, storage and elimination.

*Uptake* of toxicants from seawater follows several different ways which are partly combined: uptake happens via gills, digestive tract and tegument epithelium. *Elimination* may happen via some of these routes and obligatory through excretion organs. *Storage* happens in a great variety of tissue and organs which are called *target tissue* or *organs* of bioaccumulation: they differ widely depending on the elements, taxonomic groups and even on the species belonging to the same taxonomic group. They may be the following:

- muscles which are the edible parts in fish, crustaceans etc....
- digestive gland of bivalves, crustaceans etc...
- fish liver
- shells and byssal threads of bivalves
- exoskeletons of crustaceans
- reproductive organs
- certain types of cells such as amoebocytes, macrophages...

Moreover, for a given chemical, in a given species and in a given organ, bioaccumulation may differ depending on *several ecological factors:*

- *abiotic factors:*
  - chemical form (valency state....)
  - pH
  - salinity
  - temperature: an example is given with cadmium which is more poisonous at high temperatures and low salinity (Fig. 3).
- *biological factors:*
  - age and size: usually small and young organisms may accumulate at a higher level partly due to a higher metabolism in young than in adults.
  - sex and sexual maturity: in the crab Cancer pagurus, the digestive gland concentrates more plutonium in the female than in the male. Some trace elements such as Zn, Mn, Co, Cu concentrate at a higher level in the ovary than in the testis of Carcinus maenas.
  - moulting and moulting cycle: in crustaceans some elements may be accumulated more before moulting than after (Co). The example of crustaceans will be developed further on.
  - food supply

To summarize, Fig. 4 represents the exchange processes existing between a marine organism and its environment concerning the pathways of uptake, storage and elimination of chemicals.

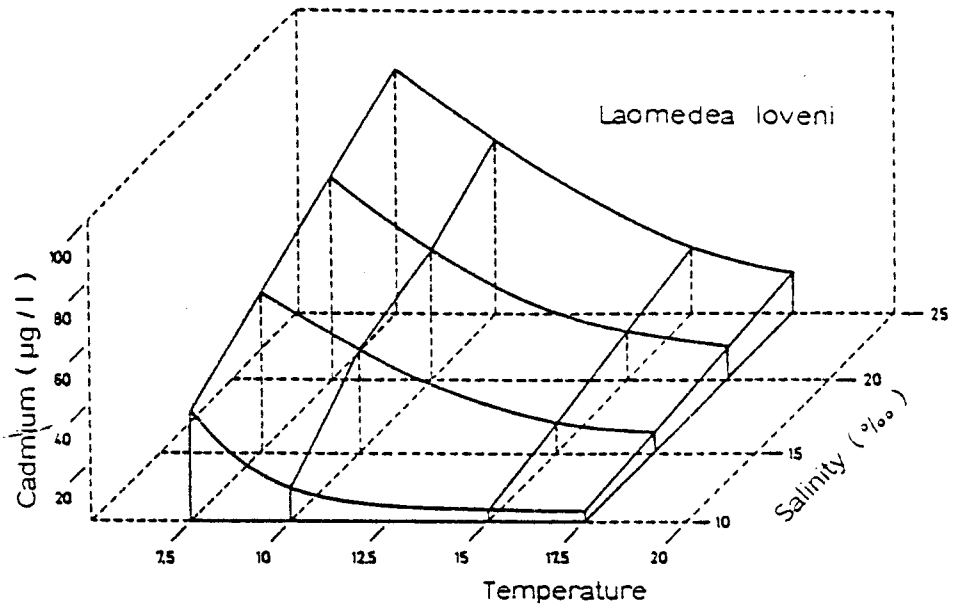



Fig. 3 Experimental animals react differently to poisons, depending on the ecological conditions under which they have been kept. The diagram shows the cadmium concentration at which half the number of polyps in a colony of hydroid polyps (Laomedea loveni) is reduced, after 7 experimental days (7 d $LC_{50}$). At below 20 µg $l^{-1}$, cadmium is most poisonous at high temperatures and low salinity (Theede et al., 1979 in Gerlach, 1981)

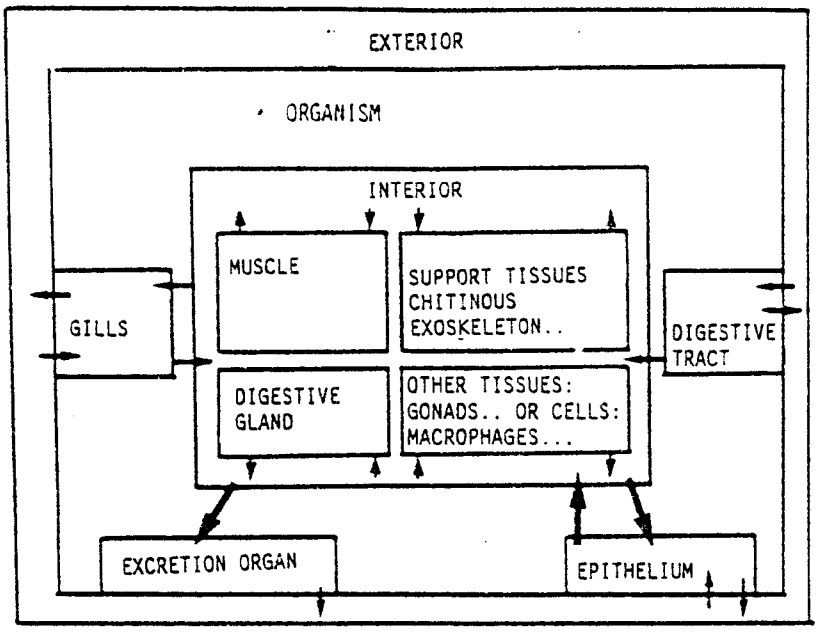



Fig. 4 Figure showing exchange between marine organism and its environment

Conversely, pollutants have several impacts on ecosystems (Fig. 5).

Among chemical contaminants, it is very important to take into account radioactive elements. They enter the sea from both natural and anthropogenic sources at variable concentrations which may pose a threat to marine organisms. The main input from man is from wastes and accidental releases. In normal conditions the largest quantities of radionuclides are derived from nuclear reprocessing plants. Most of the power stations are located alongside freshwaters and their effluents are transported to the sea via river systems.

Since many organisms accumulate metal contaminants from water and sediments into their body, they are called "indicator species": they act as sentinels in providing the chemical composition of their environment: refer to the other paper entitled "Criteria for the selection of organisms for monitoring purposes". These types of indicators belong mainly to bivalve molluscs (particularly to filter feeders), crustaceans and fish.

We present some examples of species belonging to bivalves (the marine common mussel Mytilus sp.), crustaceans (crab: Liocarcinus puber) and very briefly Teleost fish.

## RADIONUCLIDES

Marine organisms at different trophic levels are capable of concentrating radionuclides relative to the amounts present in the water. Fig. 6 presents the concentration factors of plutonium at the different trophic levels: the highest levels are detected in primary producers and then they decrease in primary consumers, secondary consumers and at the end tertiary consumers which exhibit the lowest levels. This is due to the fact that lower organisms feed on sediment which is able to concentrate radioactivity; higher organisms then feed on the lower ones. Some concentration factors for selected radionuclides are given in Table 2. For marine foodstuffs, species other than fish must be considered, because the lower organisms are eaten by man and they may also form part of the food chain to fish.

Table 2

Concentration factors for specified radionuclides
(CEC, 1979 in Galle and Masse, 1982).

| Radionuclides | Marine concentration factors | | | | | Fresh water concentration factors | |
|---|---|---|---|---|---|---|---|
| | Fish | Crustacea | Molluscs | Sediments | Seaweed | Sediments | Fish |
| H-3 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| C-14 | 5000 | 5000 | 5000 | 100 | 4000 | 2000 | 5000 |
| Sr-90 | 1 | 10 | 10 | 500 | 10 | 2000 | 30 |
| I-131 | 10 | 100 | 100 | 100 | 1000 | 200 | 30 |
| Cs-137 | 50 | 30 | 30 | 500 | 30 | 30000 | 1000 |
| Pu-239 | 10 | 100 | 1000 | 50000 | 1000 | 30000 | 10 |
| Am-241 | 10 | 200 | 2000 | 50000 | 2000 | 30000 | 30 |

Concentration factor = $\frac{\text{activity/unit wet weight of edible material/or unit dry weight of sediment}}{\text{activity/unit volume of filtered water (in Bq tonne}^{-1}\text{ per BQ m}^{-3})}$

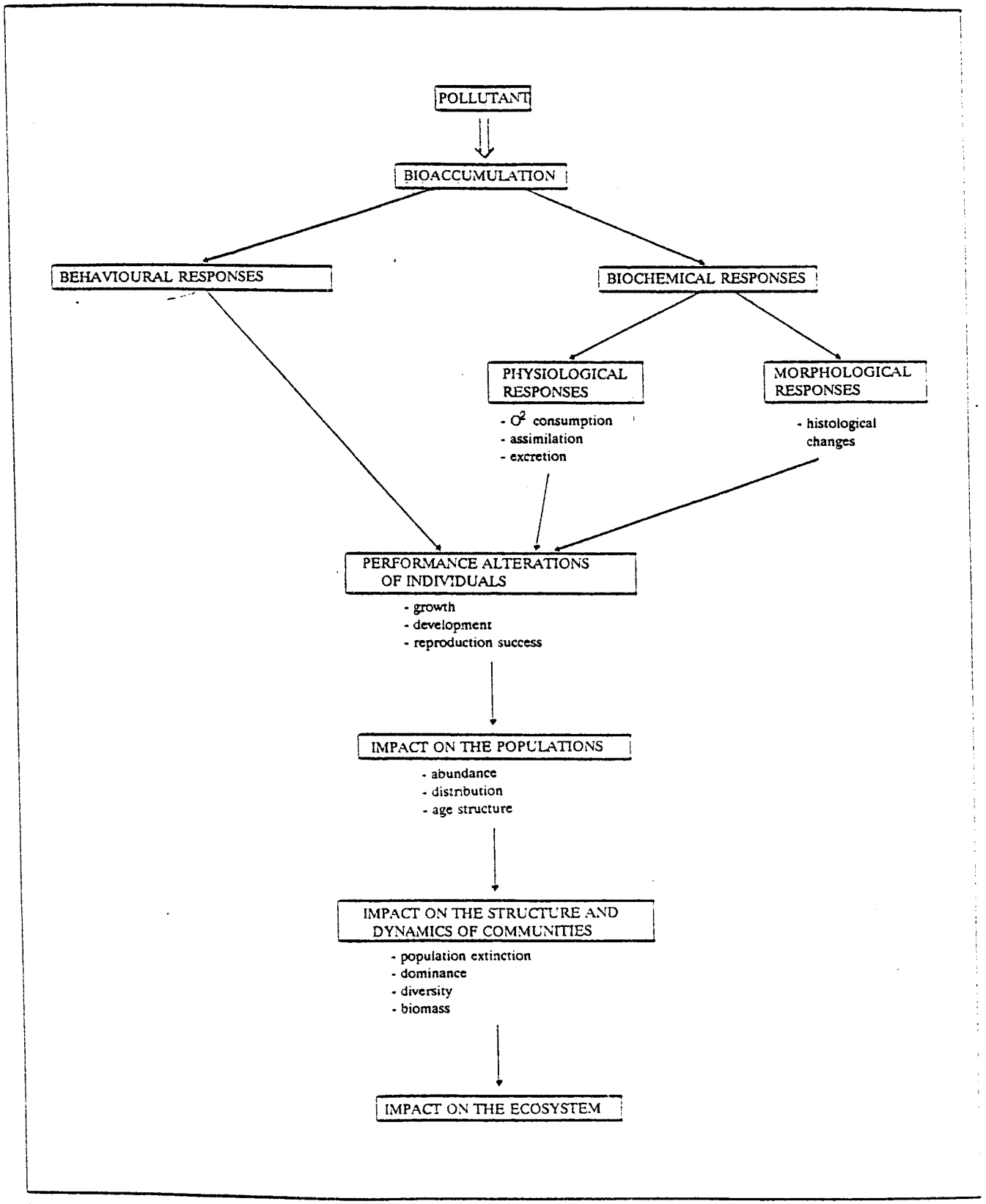



Fig. 5 Sequence of impact induced by a pollutant within an ecosystem (Caquet et al., 1989)

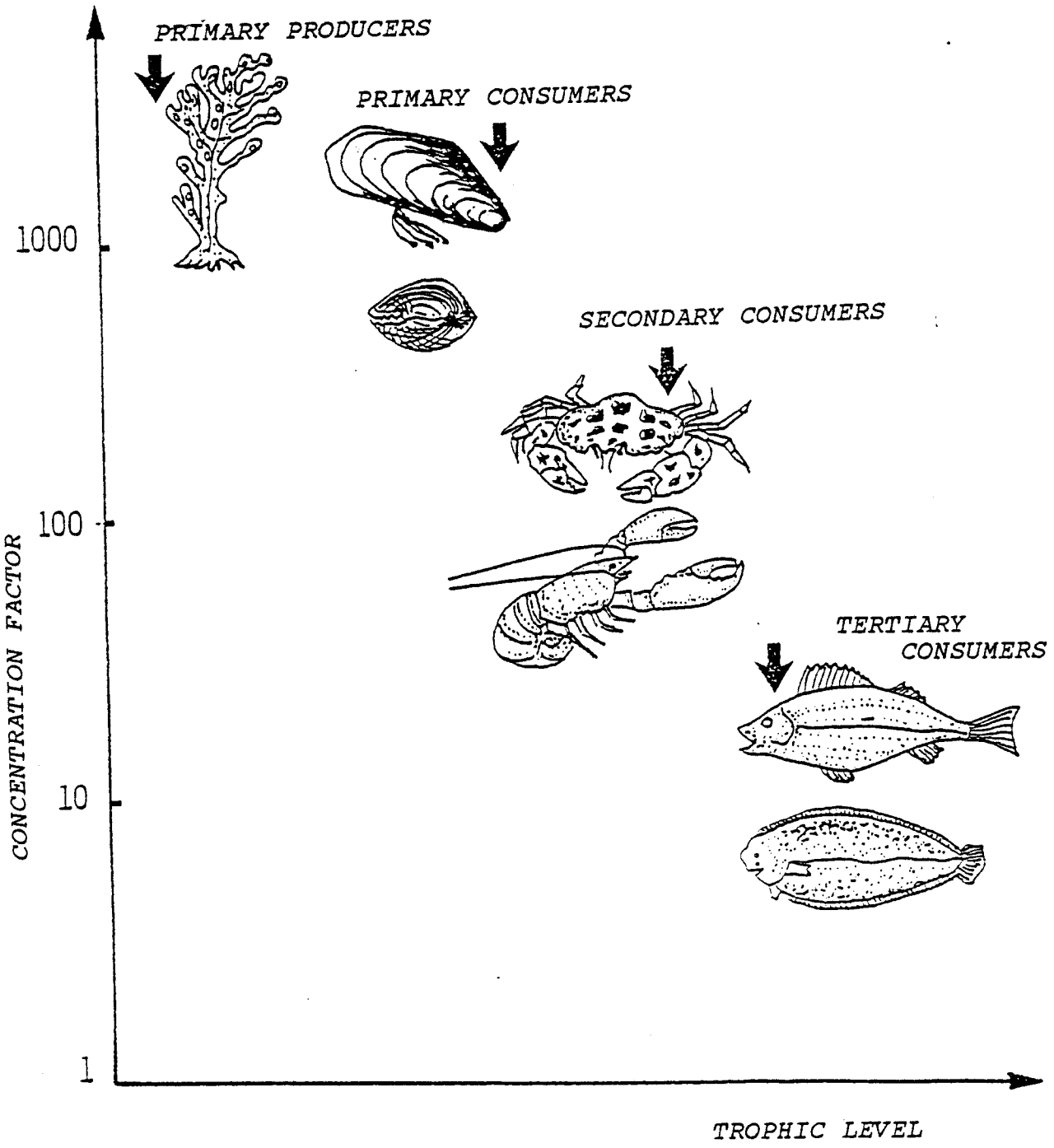



Fig. 6 Concentration factors of plutonium at the different trophic levels

*Uranium bioaccumulation by the mussel Mytilus edulis:*

Uranium uptake takes place via the gills and the digestive tractus, and to a lesser degree via the mantle. Besides the digestive gland, which is the main storage organ of concentration, other tissues bioaccumulate the radionuclide: mantle, intestines, byssal threads, gonads. Excretion happens mainly via the kidney. Figure 7 represents these different routes.

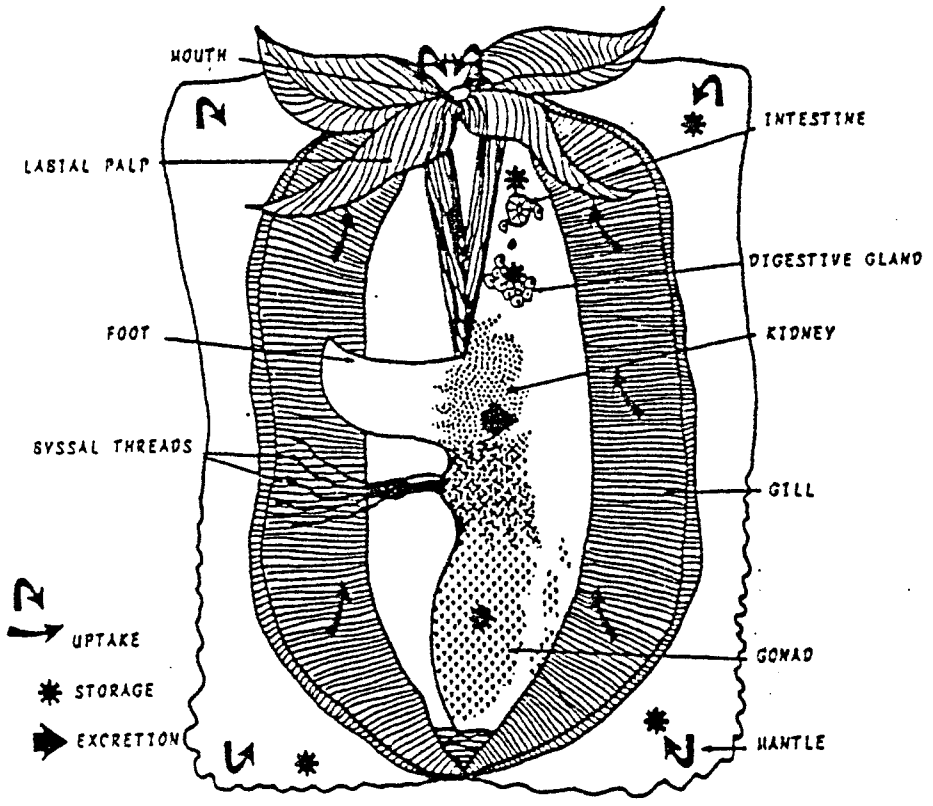



Fig. 7 Uptake, storage and excretion of uranium by Mytilus edulis

*Uranium bioaccumulation by the crab Liocarcinus puber:*

The main concentration organ is the digestive gland and at a lower level, gill and exoskeleton. Crustaceans detoxicate by shedding away their exoskeleton at moulting and by eliminating organelles such as lysosomes (from gill epithelium) and spherocrystals (from digestive gland).

The relative concentrations for cobalt 60, caesium 137 and plutonium 239 in different marine organisms are shown in Fig. 8; it demonstrates that molluscs, fish and crustaceans concentrate nearly equally cobalt 60, fish concentrate preferentially caesium 137 and molluscs concentrate preferentially plutonium 239. These data provide useful information when indicator species have to be chosen for pollution monitoring.

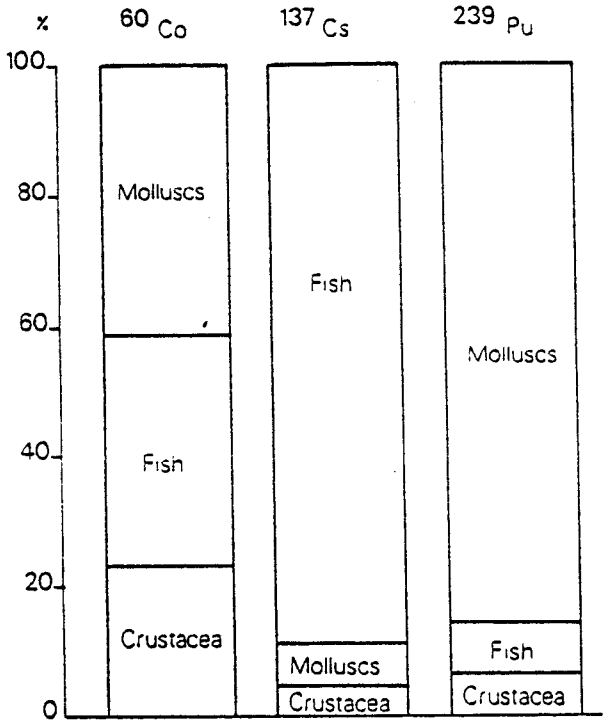



Fig. 8 Percentage contributions of different marine foodstuffs to the 50 year integrated total collective intake for a discharge to the Eastern Irish Sea (CEC, 1979 in Galle and Masse, 1982)

## METALS

Examples are given for chromium which is very toxic and which was investigated in several marine species.

*Chromium bioaccumulation by the mussel Mytilus edulis:*

Fig. 9 shows the tissue distribution of chromium and associated elements: phosphorus and sulphur. It appears that, in this case the main storage tissues are in decreasing order: kidney, gill, muscle, byssal threads, labial palp and at the lowest level, digestive gland.

*Chromium bioaccumulation by the crab Liocarcinus puber:*

The main target tissue, in this case is the muscle and to a lesser degree, the gill. No accumulation in the digestive gland nor in the exoskeleton is observed.

Fig. 10 presents a comparison of the different target bioconcentration sites of two metals (chromium and aluminium), two actinides (uranium and plutonium) and two rare earths or lanthanides (lanthanum and thulium), in molluscs (mussel), crustaceans (crab) and fish (teleost). Data may be summarized as follows:

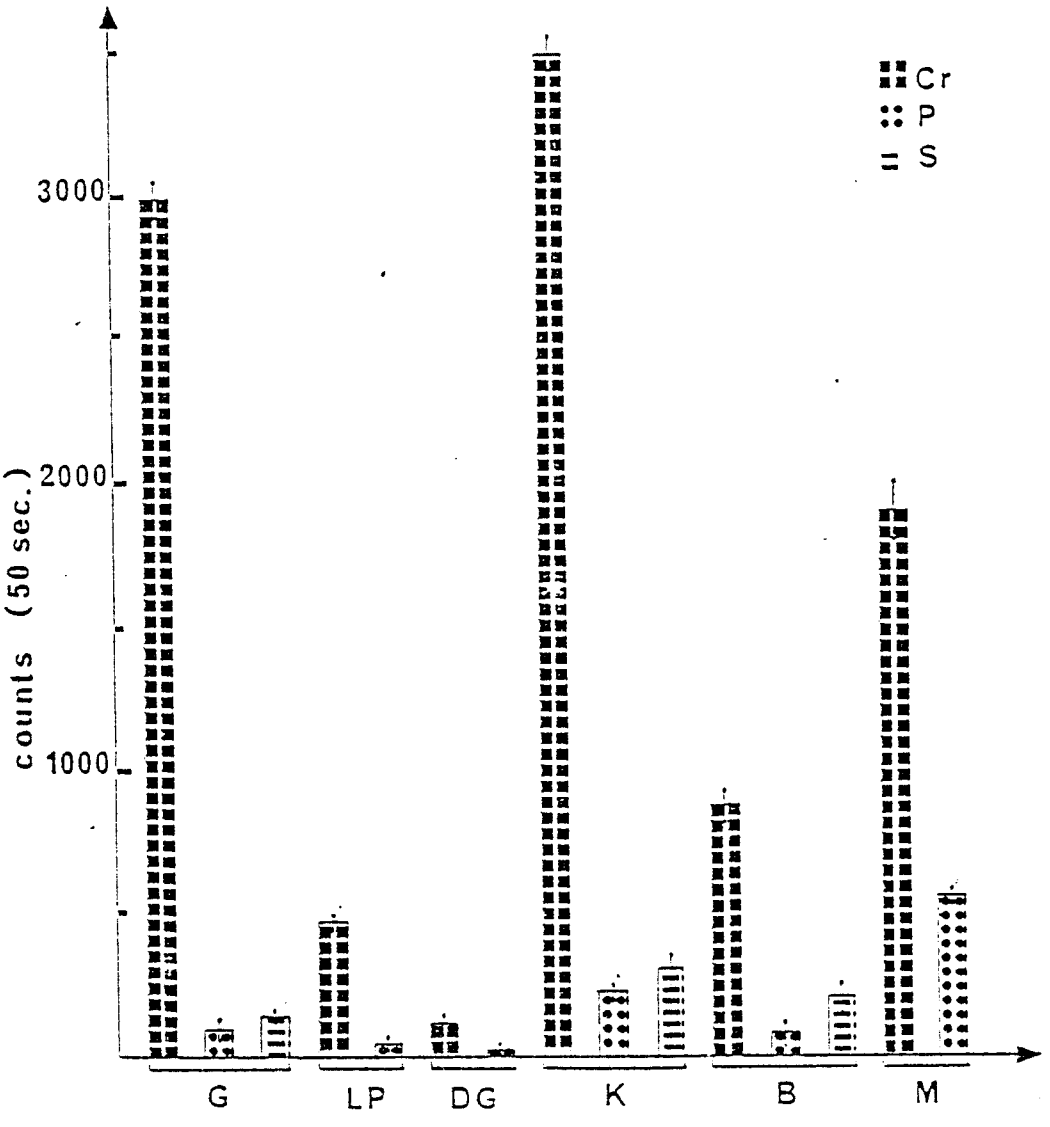



Fig. 9 Mytilus edulis. Cr-exposed mussels. Tissue distribution of chromium (Cr), phosphorus (P) and sulfur (S) obtained by X-ray microanalysis (electron microprobe). Elements were detected from lysosomes of gill (G), labial palp (LP), digestive gland (DG) and kidney (K) and from non-membrane-limited granules of byssus (B) and muscle (M). Bars represent means and standard deviation from measurements on 20 organelles from 10 individuals (in Chassard-Bouchaud, 1993)

- a given element has different concentration sites depending on the organism investigated.
- a given organism has different concentration sites depending on the element investigated.

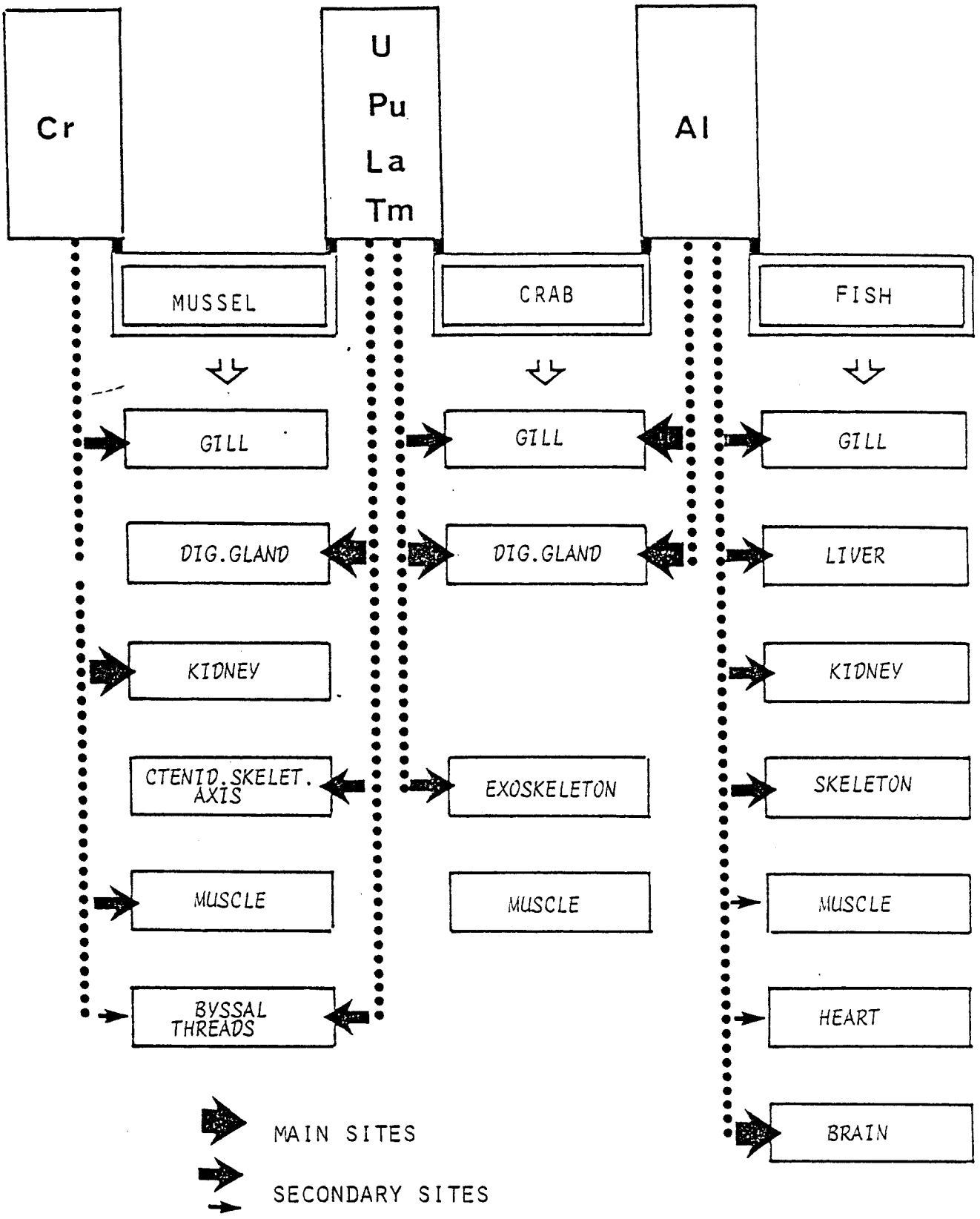



Fig. 10 Comparison of target bioconcentration sites (Pu=Plutonium, La=Lanthanum, Tm=Thulium)

- elements belonging to the same column of the table of element classification, such as lanthanides and actinides behave in the same manner. This is also valuable for other elements such as aluminium and indium for instance. This similar behaviour is due to the electron components of the atoms.

- This preference of contaminants to certain organs provides useful information: the main target organ in an indicator species is the one which has to be investigated first.

ORGANOCHLORINE COMPOUNDS

These substances include a variety of constituents (DDT, aldrin, lindane and other chloro-organic poisons, polychlorinated biphenyls: PCBs). They are distinguished by:

- the presence of chlorine-containing groups in the molecule
- a high environmental stability
- an extreme toxicity.

The toxic properties of individual compounds in this group are different and a mutual comparison is difficult. It has to be pointed out that chloro-organic substances are more toxic than any other organic pollutants. Toxicity effects on the principal species of marine organisms begin in the concentration range of between $10^{-5}$ and $10^{-2}$ mg $l^{-1}$. The sensitivity increases in the sequence: molluscs, fish, crustacea. Fig. 11 presents an example of an insecticide, dieldrin, and the mechanism of its bioaccumulation within a marine food chain. The concentration of this toxicant is increasing from the low trophic levels (in phytoplankton a few ppb) up to the upper levels (in birds such as cormorant: more than 1 ppm). This progressive concentration within the different steps of the trophic chain is an example of biomagnification.

Concentrations of these toxicants are particularly high in littoral areas where industrial wastes are discharged by factories. Mytilus edulis collected from a harbour in the Baltic was shown to contain 28,000 ppm of PCBs. The concentration factor of PCBs in the mussel Mytilus galloprovincialis collected from the littoral waters of the Marseille area, was shown to reach 690,000.

Table 3 shows results obtained by several authors concerning the different sites of metal storage. From these data, considering the column "comment", it has to be emphasized that tissues have to be investigated also at the structural and ultrastructural levels. In some of our previous investigations (Chassard-Bouchaud et al., 1992) we were able to point out the important role played by special types of cells such as hemocytes macrophages (named in that table as "wandering cells") and by target organelles of bioaccumulation such as lysosomes and spherocrystals (named in the table as "granules").

In conclusion, this paper demonstrates, using a few examples how the processes involved in the uptake, storage and elimination of chemical contaminants are different from one species to another for a given element, with in addition, variations due to abiotic and biological factors. When the

main target organ of bioaccumulation of a given element has been determined in a given species, it can be recommended to investigate and analyse only this target tissue.

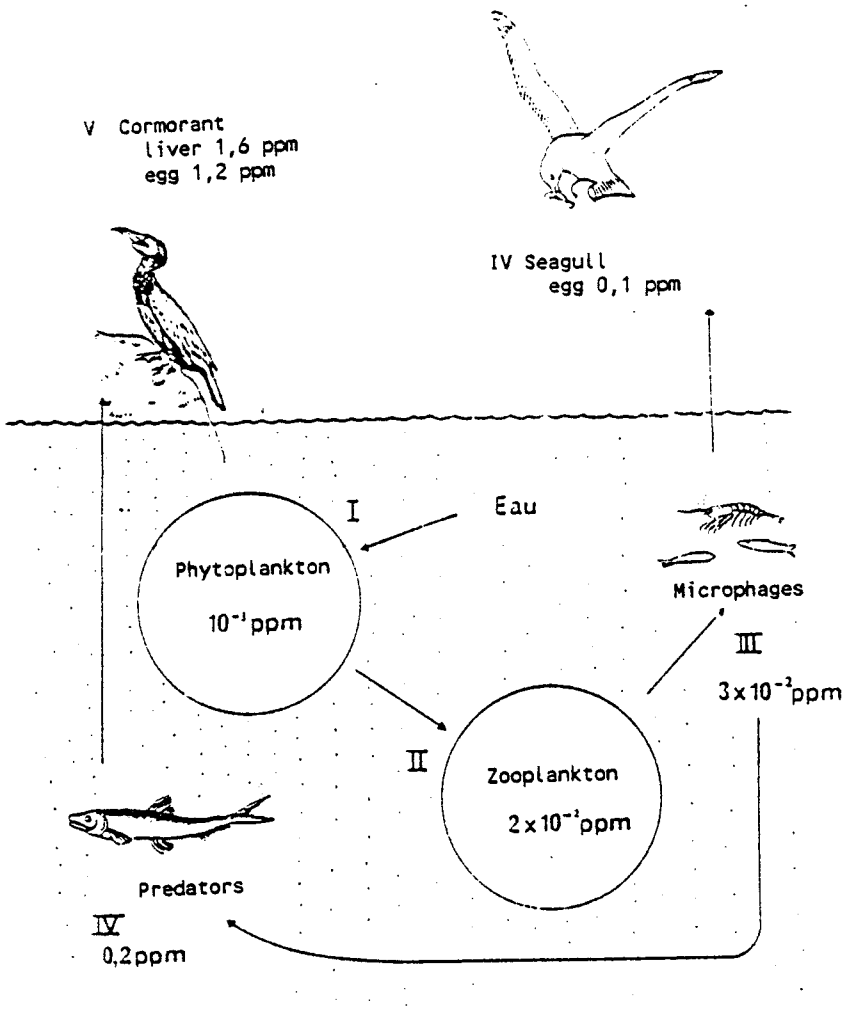



Fig. 11 Mechanisms of progressive concentration (biomagnification) of an organochlorine toxicant, dieldrin in a marine food chain (after Ramade, 1974)

## Table 3

Different sites for metal storage (in Lockwood, 1976).

| Species | Metals | Tissue | Comment | Reference |
|---|---|---|---|---|
| Fish | | | | |
| *Makaira ampla* (Pacific blue marlin) | Hg | Liver, muscle | Low promotion methylmercury-demethylation? | Rivers *et al.* (1972) |
| *Sebastodes caurinus* (rock fish) | Cd | Liver | Induced Cd-metallothionein | Olafson & Thompson (1974) |
| Crustaceans | | | | |
| *Procambarus clarkii* (freshwater crayfish) | Cu, Fe | Hepatopancreas | Large granules in Fe and Cu cells | Ogura (1959) |
| *Homarus vulgaris* (lobster) | Mn | exoskeleton | | Bryan & Ward (1965) |
| *Crangon vulgaris* (shrimp) | Cu | Hepatopancreas | Excess Cu stored as granules* | Djangmah (1970) |
| *Lysmata seticaudata* (shrimp) | Cd | Exoskeleton | 50% total body Cd lost at moult | Fowler & Benayoun (1974) |
| Molluscs | | | | |
| *Biomphalaria glabrata* (freshwater pulmonate) | Cu | Leucocytes | Phagocytosis of excess Cu | Cheng & Sullivan (1974) |
| *Oncomelania formosana* (freshwater prosobranch) | Cu | Connective tissue | Crystals of carbonate deposited by wandering cells* | Winkler & Chi (1967) |
| *Ostrea edulis* (oyster) | Cu, Zn | Leucocytes | Phago- or pinocytosis of excess metals | Boyce & Herdman (1898) |
| *Pecten maximus* (scallop) | Zn, Mn, Pb, Ag, Cd, Cu | Kidney<br>Digestive gland | Large (5µm) granules inside cells<br>May occur in granules* | Bryan (1973)<br>Bryan (1973) |
| *Octopus vulgaris* (octopus) | Hg | Digestive gland | Especially in contaminated conditions | Renzoni *et al.* (1973) |
| Polychaete | | | | |
| *Nereis diversicolor* (ragworm) | Cu, Pb | Epidermis | Fine granules in high-metal worms | Bryan (1974) |

* cf comment in the text

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# CRITERIA FOR THE SELECTION OF ORGANISMS FOR MONITORING PURPOSES

by

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A B S T R A C T

For monitoring chemical contaminants in the sea, marine organisms are commonly used. It is well known that they can concentrate toxicants by uptaking them from water and sediment as dissolved or particulate matter, which enter their organism via gills, digestive tract or tegument epithelia. Toxicants are then stored in various tissues and organs, among which a target is generally determined which will be used then as main indicator. Elimination and excretion happen via several routes.

It is difficult to find the right species for monitoring purposes. Environmental indicators are suitable for the observation of long-term developments in an ecosystem, as well as for planning and controlling effects of anthropogenic activities.

## 1. DEFINITIONS

Figure 1 shows a proposal for the classification of bioindication (Hertz, 1991).

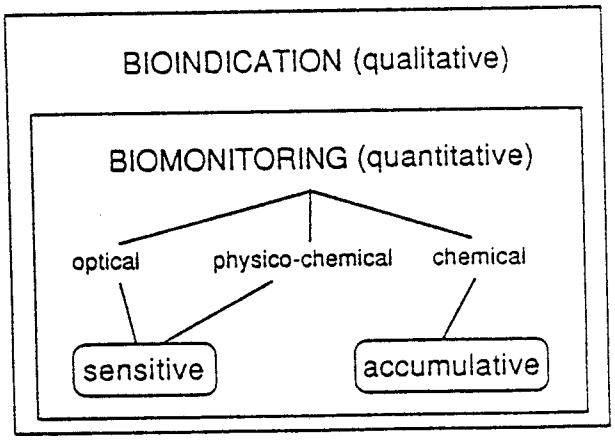


Fig. 1 Classification of bioindication (from Hertz, 1991)

Bioindication means the time-dependent, sensitive response of measurable quantities of biological objects and systems to anthropogenic influences on the environment. In general, a distinction can be made between:

- bioindication as a qualitative method for the detection of the presence of pollutants, and
- biomonitoring as a more quantitative method for the determination of the effects of the pollutants present.

"Biomonitors are organisms which can be used for the recognition and quantitative determination of anthropogenically induced environmental factors" (Bick, 1982). For the detection and recognition of water pollution, biological organisms which respond sensitively and specifically to a given pollutant can be used. In addition, organisms that readily amass the polluting components without changing their chemical nature may be used as accumulators. This classification into sensitive and accumulative biomonitors is now a well-accepted terminology.

## 2. SENSITIVE BIOMONITORS

They are used in aquatic ecosystems as integrator of the pollution stresses caused by contaminants in order to provide early warning systems. They can be divided into two categories:

- ecological surveys
- toxicity testing

### 2.1 Ecological surveys

They may use indicator species or assessments based on the composition of biological communities and numerical diversity. By making comparisons between affected and control areas, ecological surveys can indicate the health of a water body exposed to pollutant loadings.

### 2.2 Toxicity testing

It is used to obtain basic information about the general toxicity of effluents which are expected to be introduced into an ecosystem. A great number of toxicity tests have been performed to answer various questions such as:

- Is the material lethal to the test organism and at what concentration?
- What are the effects on an organism exposed to sublethal concentrations of toxicant for part or all of its life cycle?
- Which organism is most sensitive?
- Under which conditions are contaminants most toxic?
- What are the short-term effects of episodic waste discharges?

Various toxicity tests exist to answer these questions. The investigations can be used for the prediction of environmental effects of a waste, for the comparison of toxicants on animals or for the regulation of effluent discharge.

## 3. SELECTION OF CONTAMINANTS

Among the many possible chemical species which could be considered, the bioaccumulation of heavy metals has been studied most extensively. The chemical substances mostly investigated are given in Table 1. They are important polluting elements in many biological systems. They correspond to trace metals which are the following: arsenic, cadmium, chromium, copper, lead, mercury, nickel, tin and zinc.

Many other chemical substances are measured for monitoring purposes: DDT and other chlorinated pesticides, polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons.

The selection of substances to be monitored should be based on the following considerations:

- the aims of the monitoring programme;
- the findings of the pilot study (which contaminant present at a significant level will justify further study?);
- the ability of the analyst to measure these substances with the required accuracy and precision.

## 4. SELECTION OF ORGANISMS

How to choose the test organisms?

The choice of the test organisms must be guided by several criteria:

- the abundance of the species;
- their geographical range: organisms must be ubiquitous so that the comparisons could be made between areas, countries, continents and possibly hemispheres;
- whether or not they constitute an important link in the food chain;
- the organism accumulates the contaminant without being affected by the levels encountered;
- the organism is sessile and thus representative of the area of collection;
- the organism is sufficiently long-lived, to allow sampling of more than one year class if desired;
- the organism is of a reasonable size, to give adequate tissue for analysis;

Table 1

Chemical substances usually measured in marine organisms for monitoring purposes.

Trace metals

Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni), Tin (Sn) and Zinc (Zn).

DDT and its metabolites

o,p'-DDD, p,p'-DDD, o,p'-DDE, o,p'-DT and p,p'-DDT.

Chlorinated pesticides other than DDT

Aldrin, Alpha-Chlordane, Trans-Nonachlor, Dieldrin, Heptachlor, Heptaclor epoxide, Hexachlorobenzene, Lindane (gamma-BHC) and Mirex.

Polychlorinated biphenyls (PCBs)

Measurements are usually restricted to either a small number of individual compounds (known as congeners) or to the total concentration of PCBs.

Polyaromatic hydrocarbons

These can include:

| | |
|---|---|
| 2-ring compounds | Naphthalene, 1-Methylnaphthalene, 2-Methylnaphthalene, 2,6-Dimethylnaphthalene and Acenaphthene. |
| 3-ring compounds | Fluorene, Phenanthrene, 1-Methylphenanthrene and Anthracene. |
| 4-ring compounds | Fluoranthrene, Pyrene and Benz(a)anthracene |
| 5-ring compounds | Chrysene, Benzo(a)pyrene, Benzo(e)pyrene and Dibenz(a,h)anthracene. |

For the purposes of the Long-term programme for pollution monitoring and research in the Mediterranean sea (MED POL - Phase II) the following chemical contaminants were identified for analysis in marine organisms.

| category I (mandatory) | category II (optional) |
|---|---|
| total mercury | total arsenic |
| organic mercury | radionuclides |
| cadmium | polynuclear aromatic hydrocarbons |
| halogenated hydrocarbons | |

- the organism is easy to sample all the year long;
- the organism is easy to handle in experimental work, robust to survive in the laboratory, allowing investigations on uptake, storage and elimination of contaminants;
- the organism must offer the possibility of working *in situ* on the population level and with native communities;
- the organism exhibits high concentration factors;
- the organism is tolerant of brackish waters, to allow comparisons to be made between estuarine and offshore sites.

## 5. LIST OF SUGGESTED ORGANISMS TO BE USED FOR MONITORING CHEMICAL CONTAMINANTS

### 5.1 MED-POL species

For the purposes of the long-term programme for pollution monitoring in the Mediterranean, the following species, which are nearly all edible and which represent different ecotypes are recommended:

a) Bivalves

*Mutilus galloprovincialis*, or
*Mytilus edulis*, or
*Perna perna*, or
*Donax trunculus*

*M. edulis*, *P. perna* or *D. trunculus* can only be monitored as alternative species if *Mytilus galloprovincialis* does not occur in the area.

b) Demersal fish

*Mullus barbatus*, or
*Mullus surmuletus*, or
*Upeneus molluccensis*

*M. surmuletus* or *U. molluccensis* can only be monitored as alternative species if *Mullus barbatus* does not occur in the area.

c) Pelagic carnivore fish

*Thunnus thynnus*, or
*Thunnus alalunga*, or
*Xiphias gladius*

d) Pelagic plankton feeding fish

*Sardina pilchardus*
Other clupeids should only be monitored as alternative species if *S. pilchardus* does not occur in the area.

e) Crustaceans

Parapenaeus longirostris, or
Nephrops norvegicus, or
Penaeus kerathurus

N. norvegicus or P. kerathurus can only be monitored as alternative species if P. longirostris does not occur in the area.

5.2 Possible organisms for the assessment of contamination in the north Atlantic region

| | Cd | Hg | Cu | Cr | Pb | Zn | HH | PHC |
|---|---|---|---|---|---|---|---|---|
| **Rocky substrate** | | | | | | | | |
| Mytilus edulis (common mussel) | + | + | ? | + | + | + | + | + |
| Littorina littorea (gastropod) | + | | + | ? | + | + | | |
| Patella vulgata (limpet, gastropod) | + | | + | | + | + | | |
| **Muddy substrate** | | | | | | | | |
| Scrobicularia plana (da Costa) (peppery furrow bivalve) | + | + | ? | + | + | + | | |
| Macoma balthica (bivalve) | + | + | ? | + | + | + | | |
| Nereis diversicolor (polychaete) | + | + | + | + | + | + | | |

Key: + = appears to act as good indicator
? = doubt about use as indicator
HH = halogenated hydrocarbons
PHC = petroleum hydrocarbons

NOTES: The organisms listed for muddy substrates are all deposit feeders, whilst those for rocky substrates are filter feeders or herbivores. It is unlikely that contaminant levels in the tissues of the two groups will reflect contaminant levels in the same part of the marine environment.

From the species listed above, it appears that the choice for a good indicator species is becoming more and more restricted....as only Molluscs (bivalves and gastropods) and Polychaete worms remain.

## 5.3 Polychaetes as indicative species

Nereis diversicolor has been extensively investigated as it appears that a simple relationship exists between the heavy metal concentration in the sediment and body tissue: Figure 2 present the results obtained by Bryan (1974).

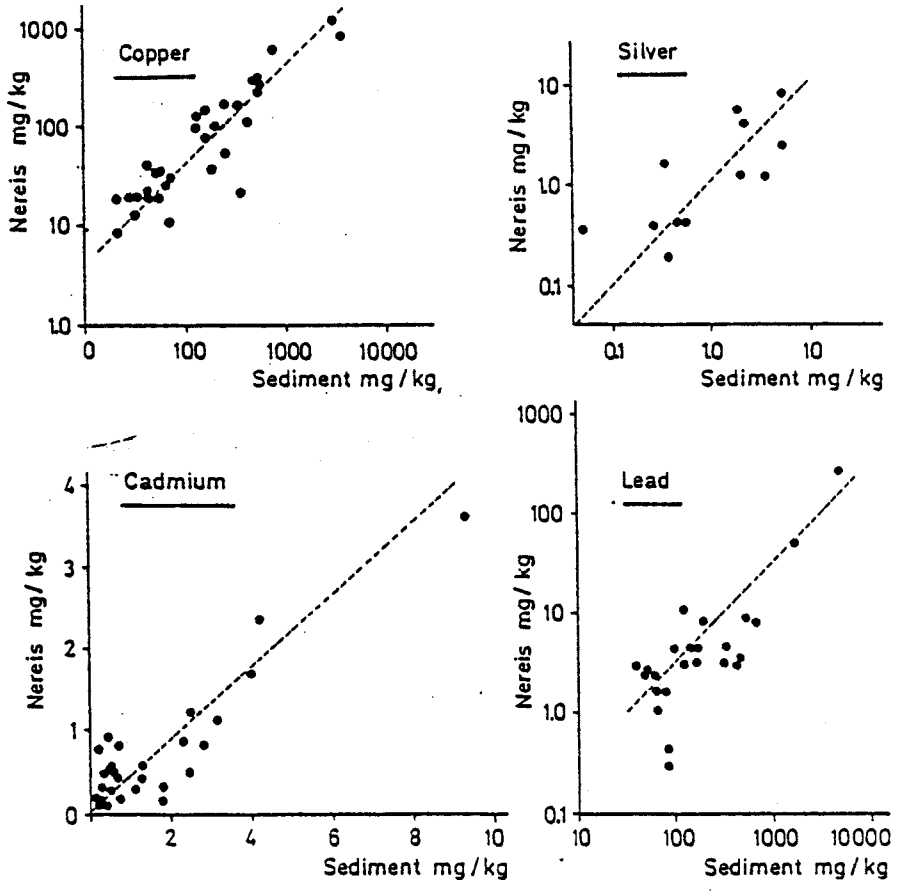


Fig. 2 In Southwest England, sediment with widely differing amounts of heavy metals can be found, depending on geological-mineralogical conditions and waste water relationships of the mines. The heavy metal concentrations in the Nereis diversicolor polychaet differ corresponding to the different locations in which they are found. Simple relationships exist between the heavy metal concentrations in the sediment and body tissue. Concentrations are indicated in mg $kg^{-1}$ dry weight (Bryan, 1974).

## 5.4 Bivalves as indicator species

From the criteria listed in para 4.1 (How to choose the tests organism?), it appears that these characteristics restrict the useful organisms to a range of fairly large, abundant, widespread, intertidal organisms, mainly molluscs. Filter feeding molluscs are more likely to

reflect contaminants in the water column whilst deposit feeders will also be influenced by sediment chemistry. Water chemistry will more rapidly respond to effluent discharges and dispersal conditions at the time of sampling.

Filter-feeders such as mussels are therefore more likely to provide the information required to fulfil the objectives of a monitoring programme concerned with water quality. Common mussels: Mytilus edulis and M. galloprovincialis that are used in global "Mussel Watch" programmes are suitable for spatial and trend monitoring programmes in coastal waters. Table 2 presents concentrations of metals in Mytilus edulis from different areas.

When performing monitoring investigations, several and numerous factors have to be taken into consideration (cf our other paper concerning bioaccumulation processes). Moreover it is necessary to find "hot spots" such as Baie de Seine in France, to collect samples at regular intervals of time (weeks). One has to keep in mind that seasonal variations in food supply and the spawning cycle are known to cause physiological changes inducing variations in contaminant levels in the tissues of some organisms. In order to minimise these variations it is suggested that sampling be undertaken at the prespawning period.

Selection of tissue in the species M. edulis will depend on the chemical contaminant under investigation. As demonstrated in our other paper, the target tissue of chromium concentration is mainly kidney, while for other contaminants it is generally the digestive gland (Chassard-Bouchaud, 1991).

The duration of contamination will of course, induce different effects: Figure 3 demonstrates the poisonous effects of lead.

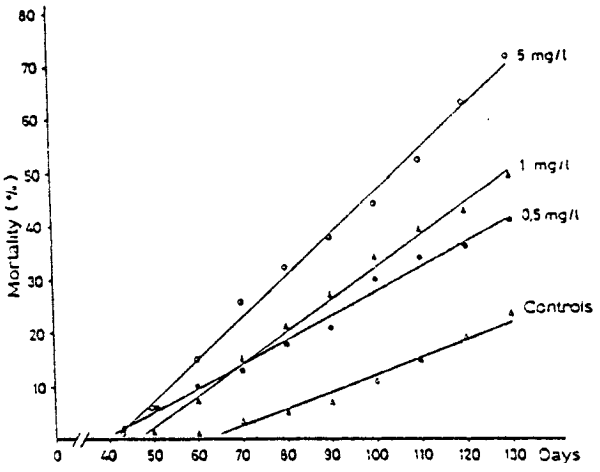


Fig. 3 During the first 40 days, lead is not very poisonous to common mussels (Mytilus edulis) in laboratory experiments. Even at maximum concentrations of approximately 5 mg $l^{-1}$ of lead as lead nitrate corresponding to saturated conditions in seawater, no increase of mortality resulted in the first 40 days of the experimental period. Only in long-term experiments does mortality occur, which increases at higher concentrations of lead. In the diagram, the cumulative mortality is indicated for experiments with 0.5, 1 and 5 mg $l^{-1}$ of lead (Schulz-Baldes, 1972 in Gerlach, 1981)

5.5 The choise of organisms depends on the objective of the programme and the following examples may be proposed:

- filter feeding molluscs (Mytilus edulis) reflect contaminants in the water.
- herbivorous molluscs (Littorina littorea, Patella vulgata) are indicative of contaminants in plants and sea weeds.
- deposit feeders (Scrobicularia plana, Macoma balthica, Nereis diversicolor) reflect contaminants of the water but are also influenced by the ones of sediments.

In conclusion, the mussels such as Mytilus edulis and more generally M. sp. appear as the best indicator species for monitoring chemical contaminants in the sea. But "nobody is perfect"! even the mussel may appear in certain conditions, not sedentary at all! (Fig. 4).

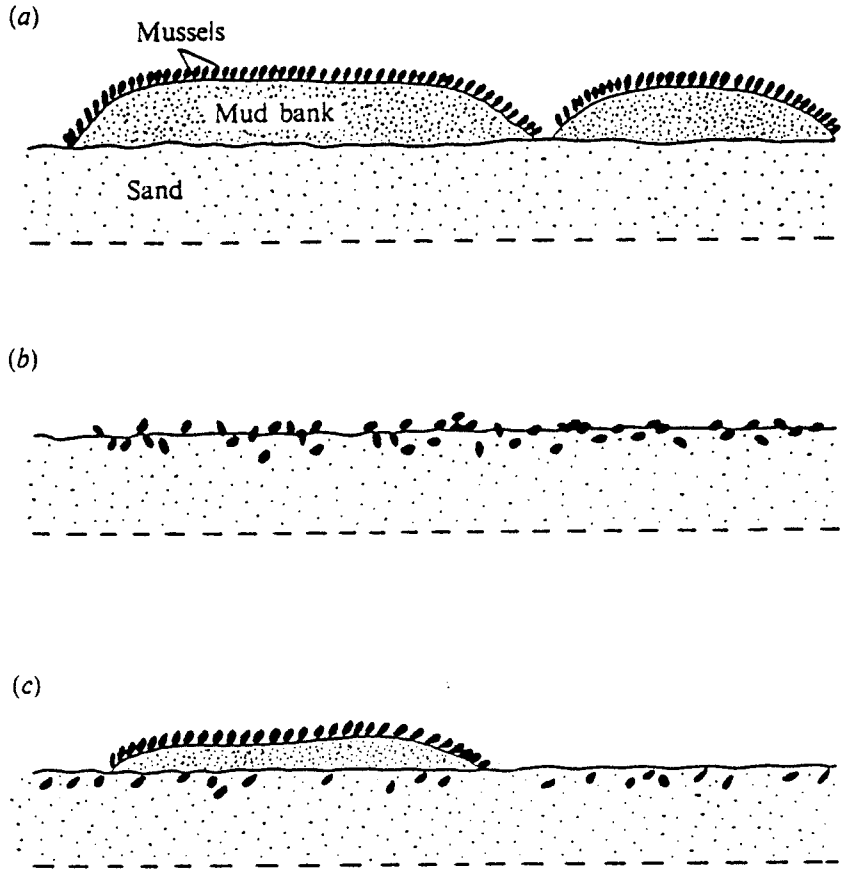


Fig. 4 Diagram of the effects of a storm on mussel banks. (a) Normal state: mussels lying as a carpet covering low banks of mud; (b) Just after the storm; the carpet of mussels has been disrupted and the mussels dissipated over and within the sand bottom, the mud swept away; (c) Some time later; the surviving mussels have gathered in carpets and new med banks are forming, but some individuals have died in the sand. (Redrawn from Thiesen, 1968 in Bayne, 1976)

## Table 2

Concentrations of metals in mussels (*M. edulis*) from different areas (in Bayne, 1976).

| Locality of sampling | Concentration (μg $g^{-1}$ dry wt) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fe | Mn | Co | Ni | Cd | Cu | Pb | Zn | Ag | Cr | Al | V | Mo | Tissue | Author |
| South coast, England | 290 | 3.6 | 0.15 | 2.1 | 0.95 | 2.0 | 0.4 | 0.04 | 0.1 | - | 76.0 | - | - | Shell | Segar *et al.* (1971) |
| South coast, England | 1700 | 3.5 | 1.6 | 3.7 | 5.1 | 9.6 | 9.1 | 91.0 | 0.03 | 1.5 | 1230 | - | - | Soft parts | |
| South coast, England | - | - | - | - | - | 5-26 | - | 60-81 | - | - | - | - | - | Soft parts | B. Brown (personal communication) |
| East coast, England | - | - | - | - | - | 9.50 | - | 167-312 | - | - | - | 5.0 | - | Soft parts | |
| New Zealand | 1960 | 27.0 | - | 7.0 | 10 | 9.0 | 12 | 91.0 | 1.0 | 16.0 | - | - | 0.6 | Soft parts | Brooks and Rumsby (1965) |
| California | - | 6-28 | - | - | 3-7 | 5-11 | 2-8 | 204-341 | 1-1.3 | 2.8 | - | - | - | Soft parts | Graham (1972) |
| California | - | 9.3-45.8 | - | - | 2.5-5.8 | 5.8-8.6 | 9-21.2 | 6.9-14.2 | 4.4-6.3 | 5-7 | - | - | - | Shell | Graham (1972) |
| California | - | 5.9- 7.8 | - | - | 2.0-4.9 | 9.0-303 | 2.2-23.4 | 164-310 | 1-5.5 | 1.5-7.8 | - | - | - | Soft parts | Graham (1972) |
| California | - | 8.4-14.2 | - | - | 2.5-9.2 | 8.1-18.6 | 9-19.4 | 8.6-26.5 | 5.0-7.9 | 5.7-14.2 | - | - | - | Shell | Graham (1972) |

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# THE ROLE OF CONSISTENCY IN THE DATA COLLECTION PROCESS

by

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## An introductory example

The contaminant time series in Figure 1 shows contaminant measurement collected annually over a ten year period. Suppose that the measurements in years 1-5 are from area A and those in years 6-10 from area B. What can we say about the spatial distribution of contaminant levels or about temporal trends in contaminant levels?

The answer is not very much. Contaminant levels in area B between years 6-10 are clearly higher than those in area A between years 1-5. However, we can not infer that contaminant levels in area B are higher than those in area A, because we have never observed contaminant levels in both areas at the same time. Had we sampled both areas throughout the ten year period, we might have observed the contaminant time series in Figure 2 in which contaminant levels in area B are always lower than those in area A. Further, we can not infer that contaminant levels increase over the ten year period, because we have not sampled in the same place throughout the ten years. Had we done so, we might have observed the contaminant time series in Figure 3 in which contaminant levels are decreasing.

The problem with the data in Figure 1 is that they have not been collected consistently - ie different locations have been sampled at different times - so that spatial variation in contaminant levels can not be distinguished from temporal variation in contaminant levels.

This example is clearly contrived, but it does show that if contaminant data are not collected in an appropriate way, it will not be possible to answer important questions about contaminant levels in the environment.

## Consistency

Contaminant levels can vary in many ways. For example, in addition to temporal and spatial variation, measured contaminant levels in biota can depend on the time of year, the size of the organism and the way in which the organism is treated and chemically analysed, etc. The design of a contaminant monitoring programme must take account of all these sources of variation. The generally recognised way of doing so is to remove all the sources of variation that they are not interested in, by "keeping as many things fixed" as possible.

For example, in a temporal monitoring programme, data collection should be the same from year to year, so that any observed changes in contaminant levels can be attributed to temporal variation rather than any

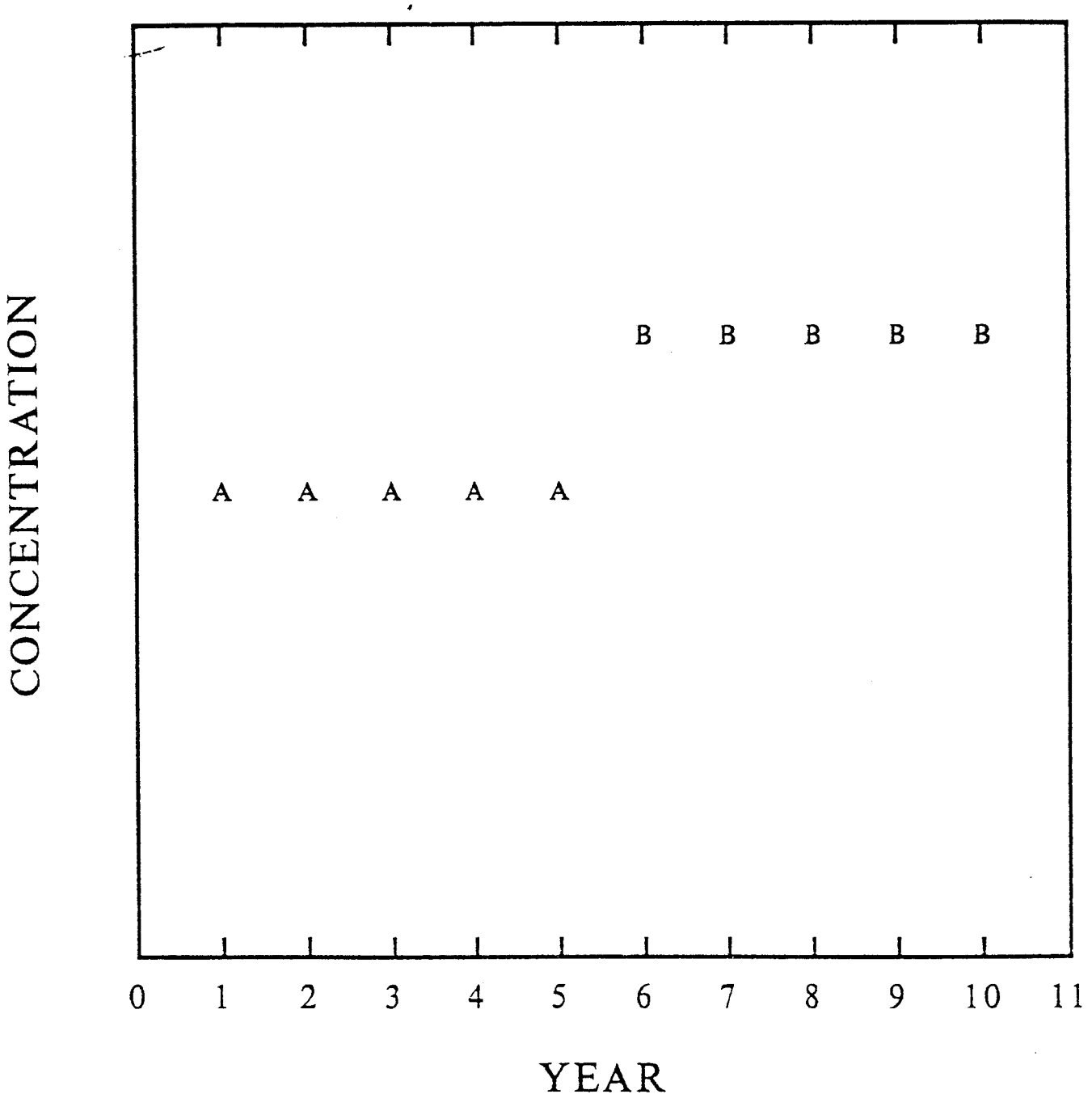


Fig. 1 Hypothetical contaminant time series. Measurements in years 1-5 are from area A and those in years 6-10 are from area B

other source of variation. Similarly, in a spatial monitoring programme, data collection should be the same from area to area, so that any observed changes in contaminant levels can be attributed to spatial variation rather than any other source of variation. In other words, we must sample consistently from year to year in a temporal monitoring programme and from area to area in a spatial monitoring programme.

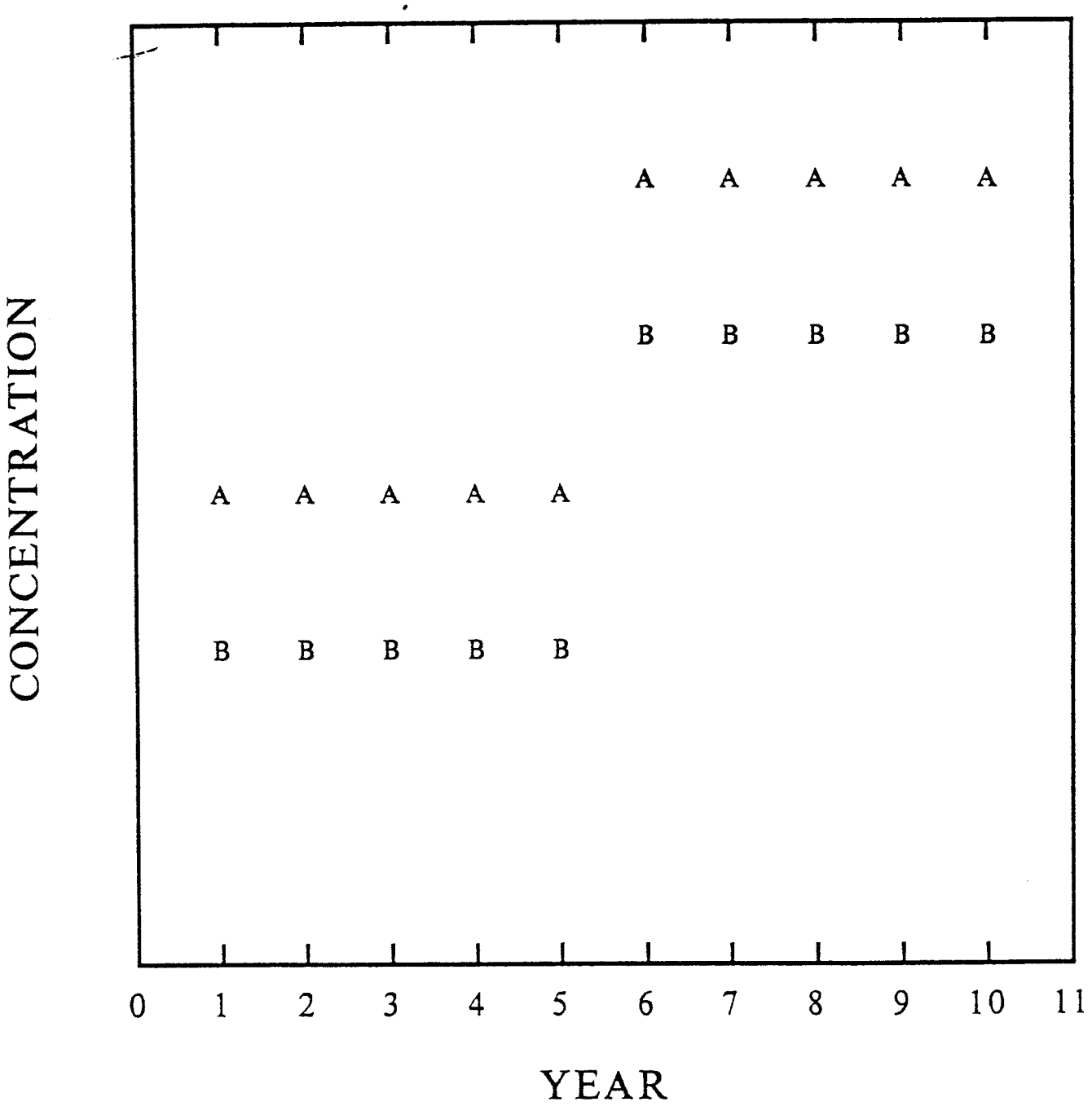


Fig. 2 What might have been observed had areas A and B been sampled throughout the ten year period

**What should be consistent?**

All aspects of the data collection process should be consistent. It is perhaps easiest to demonstrate this with an example. In the temporal monitoring of contaminants in biota, the following are some of the things which must be done consistently from year to year, to obtain a contaminant time series which can be used to investigate temporal trends (see Uthe et al. 1991) for more details).

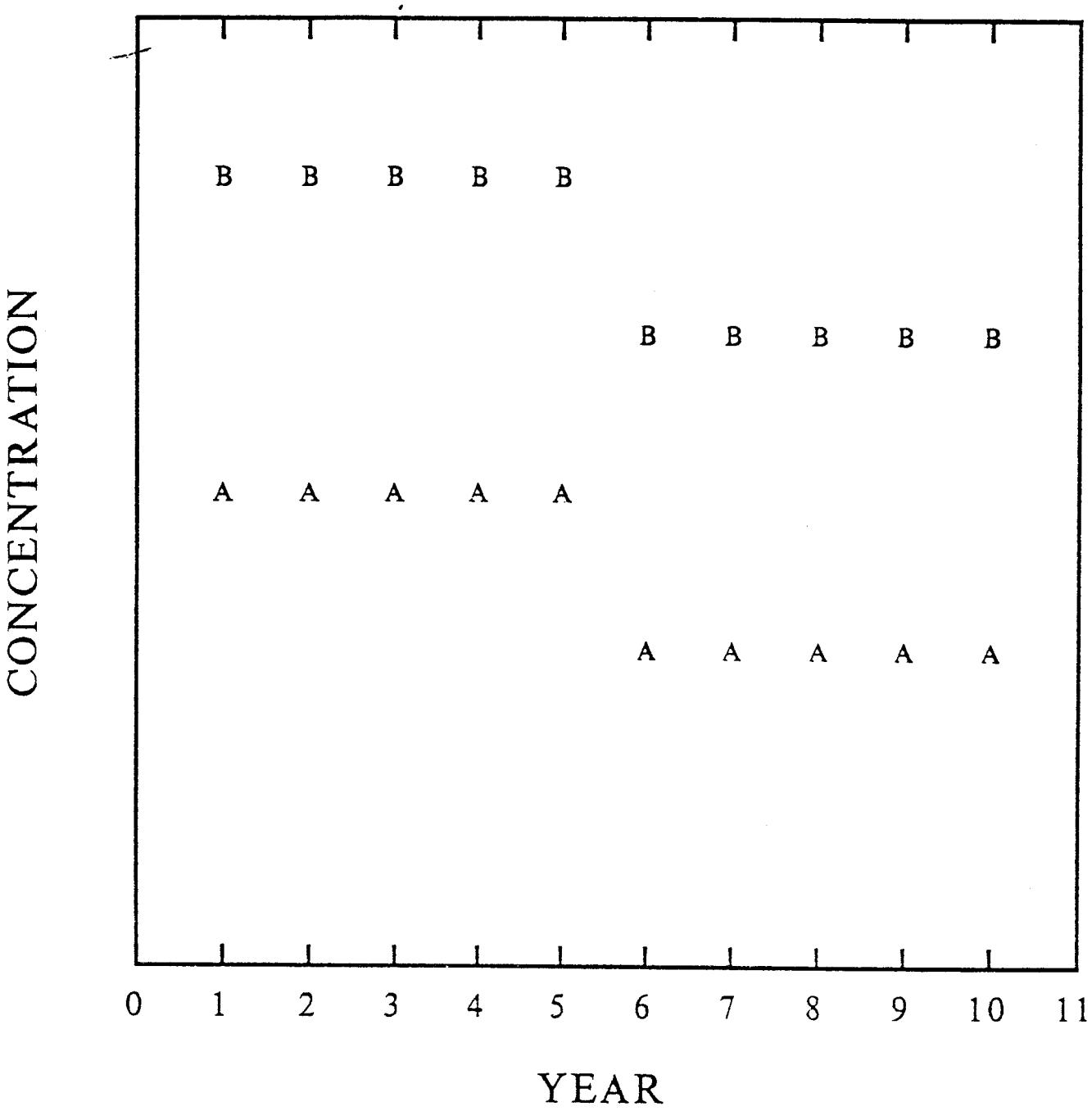


Fig. 3 What might have been observed had areas A and B been sampled throughout the ten year period

Sampling:

- individuals should be taken from the same statistical population each year,
- individuals should be taken from the same location each year,

- individuals should be taken at the same time each year; if there are several sampling occasions each year, then these should be at the same times each year,
- individuals should be of the same species each year,
- the same number of individuals should be taken each year,
- individuals should be handled in the same way each year.

Sample preparation:

- the same tissue type should be used each year,
- the tissue should be prepared for chemical analysis in the same way each year,
- if individuals are pooled for chemical analysis, the same number of individuals should be put in each pool each year,

Chemical analysis:

- the same contaminant should be measured each year,
- the same number of replicate analyses should be made on each individual (or pool) each year,
- the same method of analysis should be used each year, backed up by an on-going analytical quality control programme.

Some of these things are a bit vague. What is meant by the 'same statistical population' and how is the 'same area' defined? These things should be specified at the start of the monitoring programme and should form part of the sampling protocol. Of course, once specified, the sampling protocol should be adhered to.

**What happens if sampling is inconsistent?**

Inconsistent sampling has two main effects:

- it adds noise to the data, making it harder to identify meaningful trends in the data,
- it can lead to spurious trends in the data, which do not truly reflect changes in environmental contaminant levels.

For example, suppose that contaminant levels vary seasonally and that there is a slight downwards trend in contaminant levels over time, as shown in Figure 4. Figures 5, 6, 7 and 8 show what happens if we sample as follows:

a) Fig. 5: Sampling at the same time (ie July 1) each year,

b) Fig. 6: Sampling at random in the same month (ie mid June - mid July) each year,

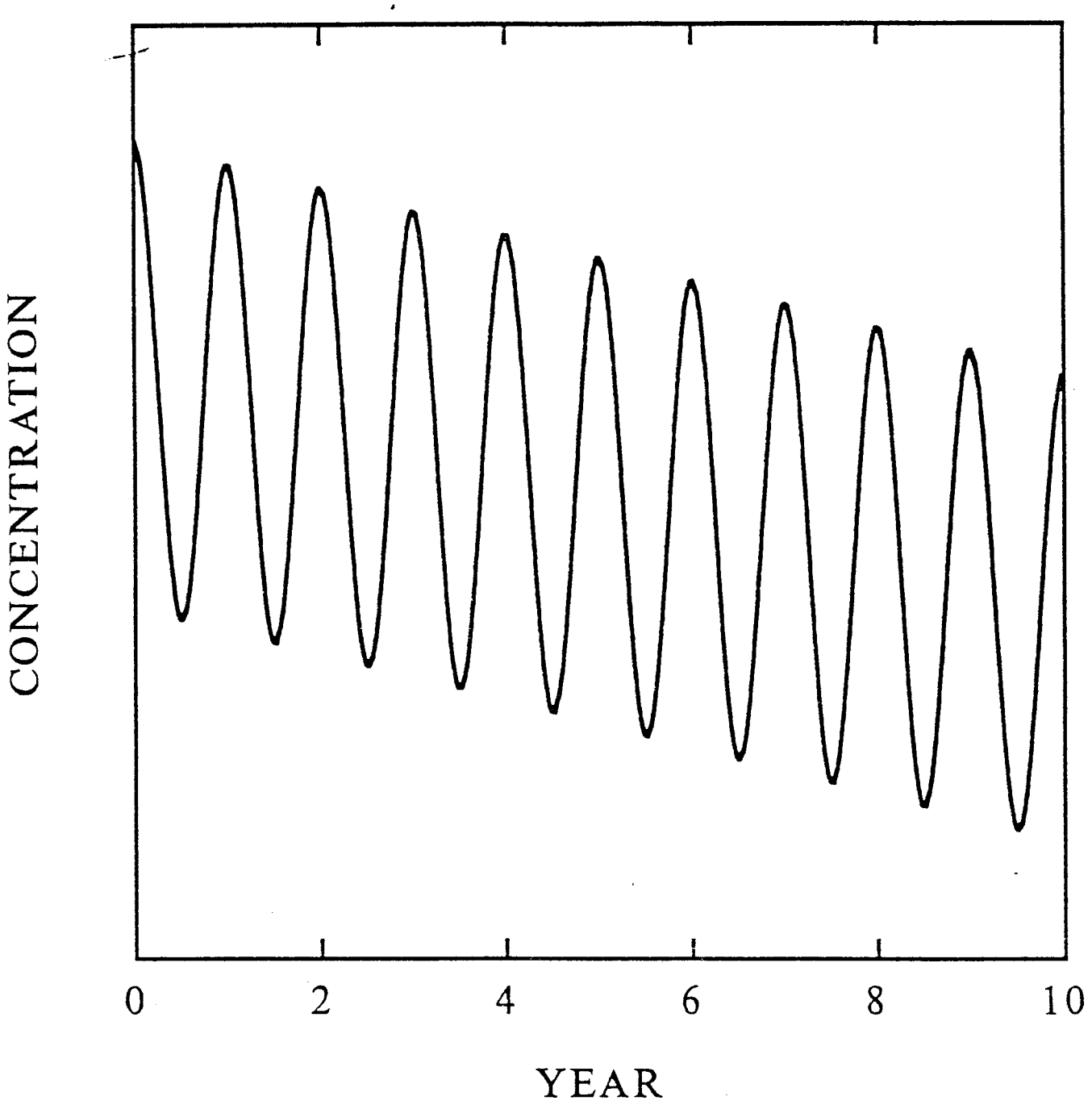


Fig. 4 Contaminant levels showing seasonal variation and a slight downwards trend

c) Fig. 7: Sampling at random at any time of the year,

d) Fig. 8: Sampling progressively a fortnight later each year (ie July 1 in year 1, July 15 in year 2, August 1 in year 3 etc).

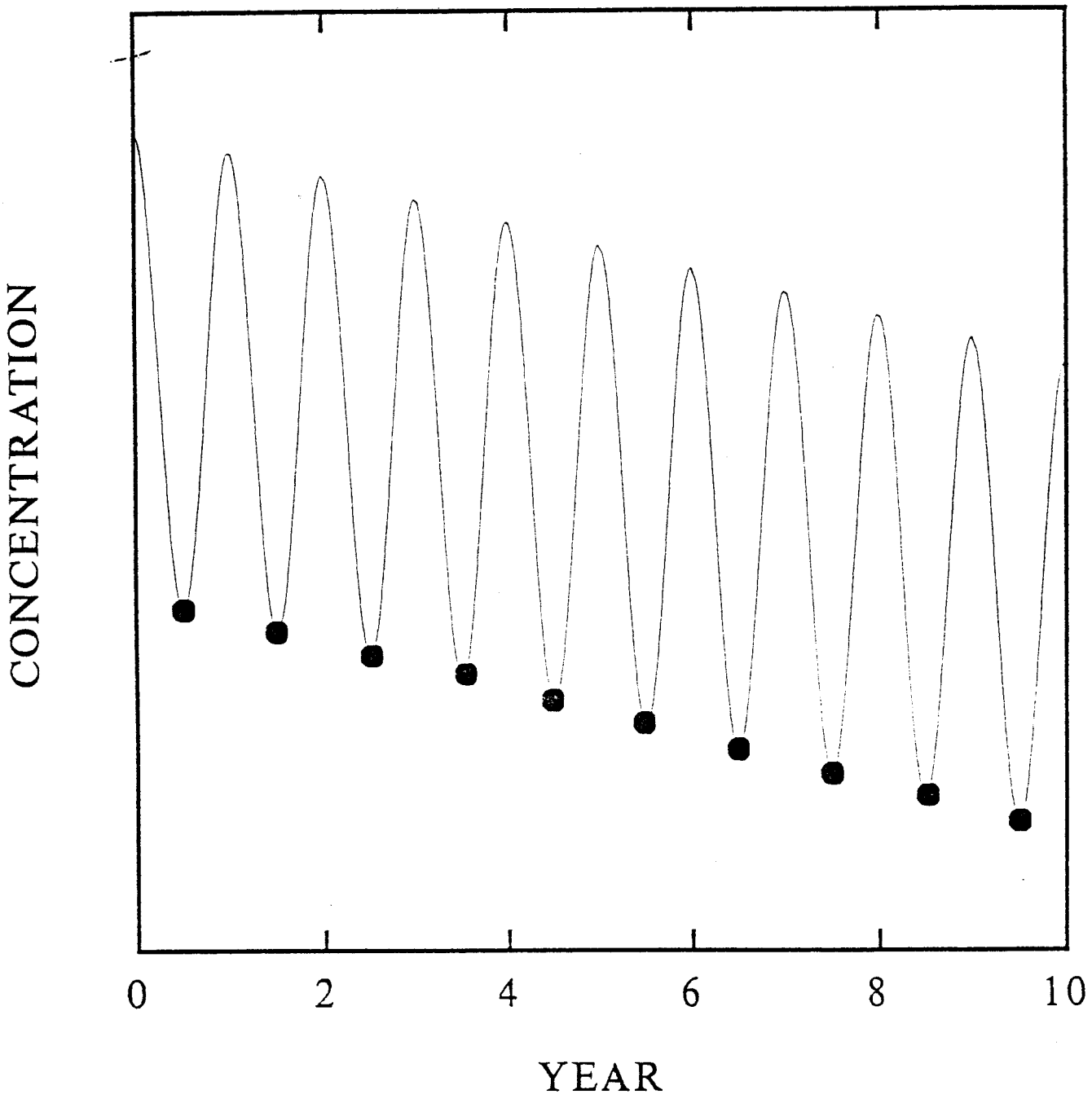


Fig. 5 The effect of sampling at the same time. The observed concentrations are shown by ● and the underlying concentration is indicated by the continuous line

Sampling at the same time each year 'removes' the seasonal variation and the decreasing trend can be clearly seen (Figure 5).

Sampling at random in the same month each year adds a small amount of noise but the decreasing trend can still be clearly seen (Figure 6).

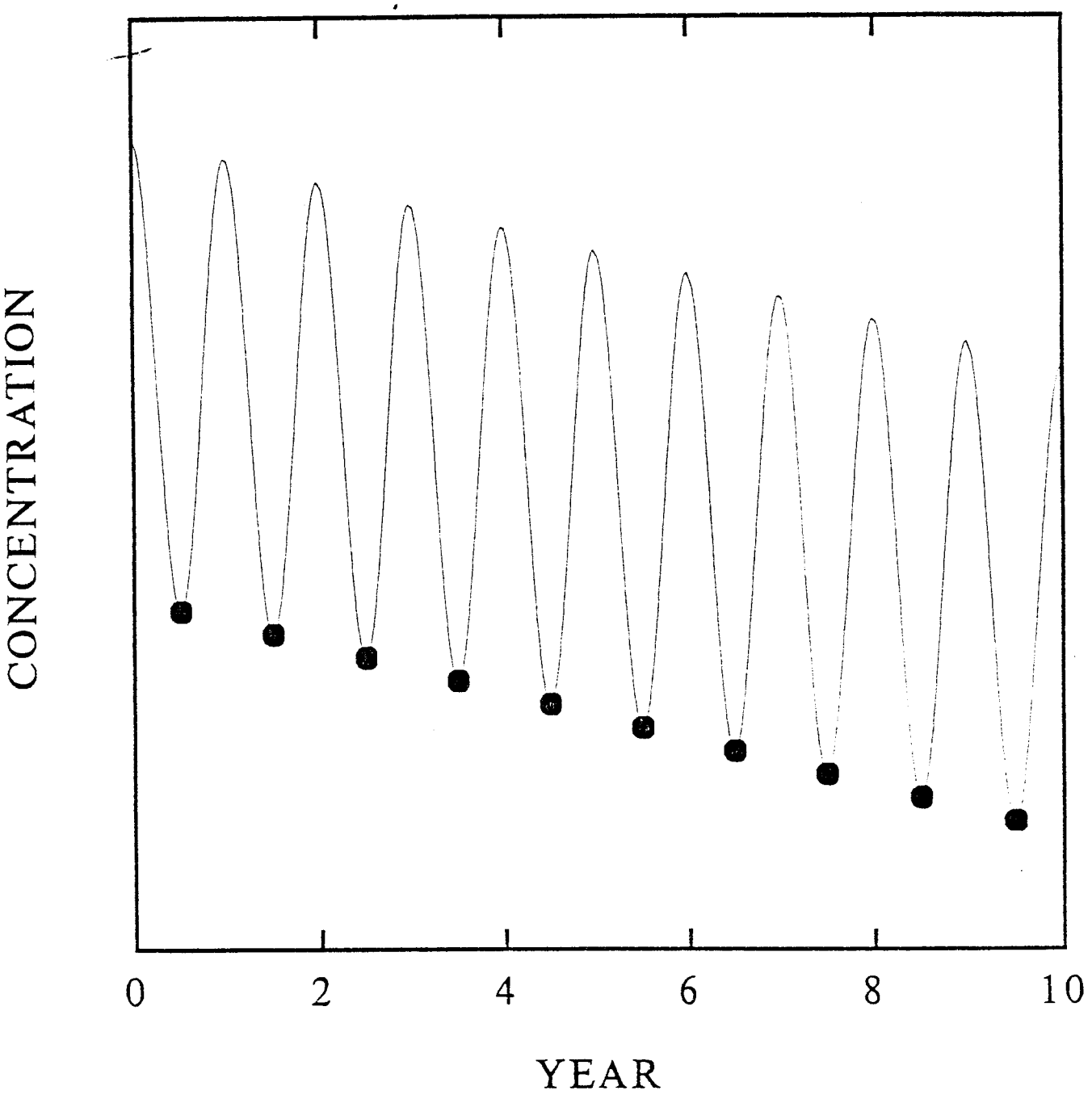


Fig. 6 The effect of sampling at random in the same month. The observed concentrations are shown by ● and the underlying concentration is indicated by the continuous line

Sampling at random at any time of the year adds a lot of noise and no trend can be seen in the data (Figure 7).

Sampling progressively a fortnight later each year induces a spurious upwards trend in contaminant levels (Figure 8).

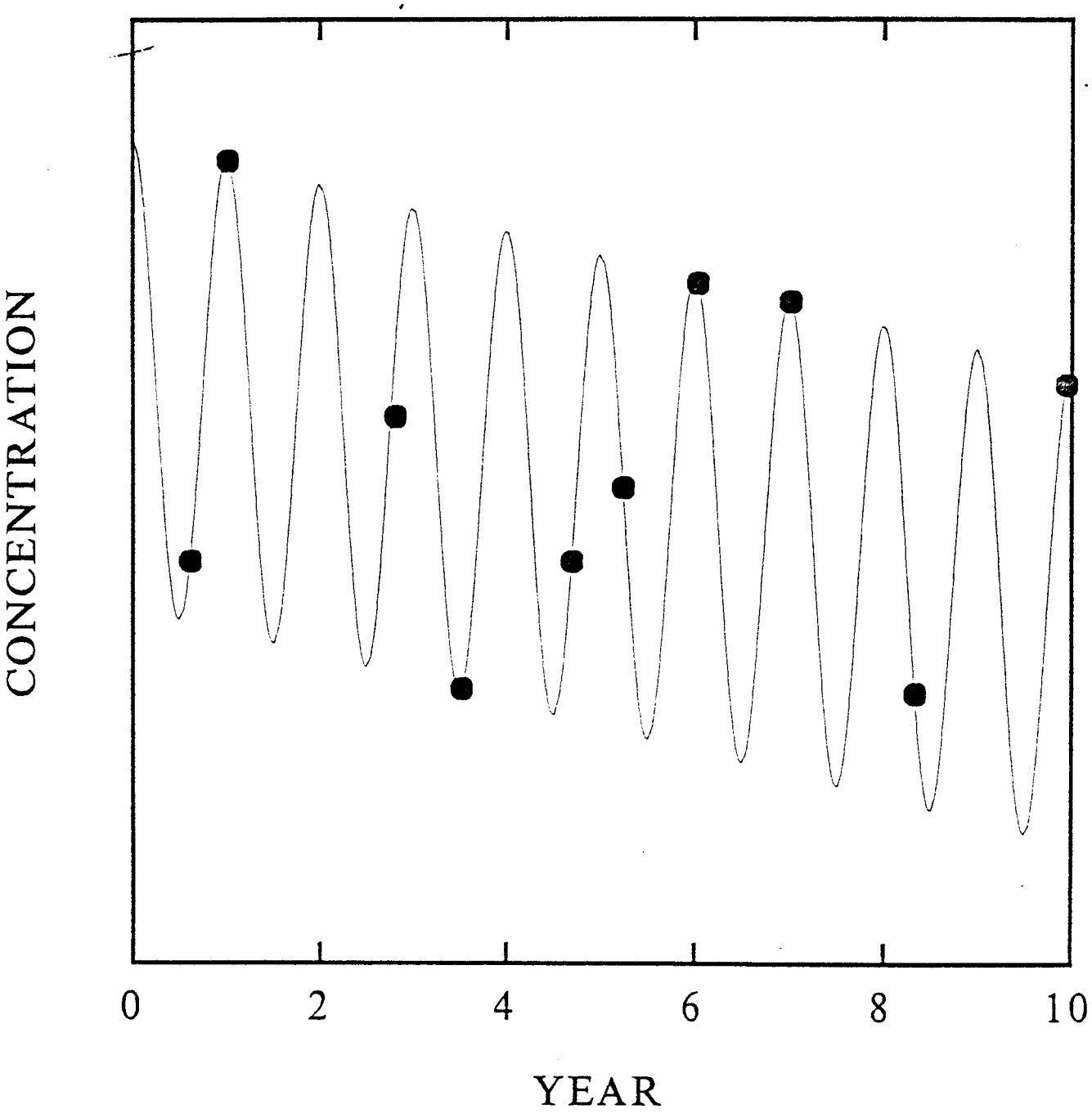


Fig. 7 The effect of sampling at random at any time of the year. The observed concentrations are shown by ● and the underlying concentration is indicated by the continuous line

The example above shows what could happen through sampling at different times each year. Clearly, additional inconsistencies in other parts of the data collection process will merely exacerbate the problem.

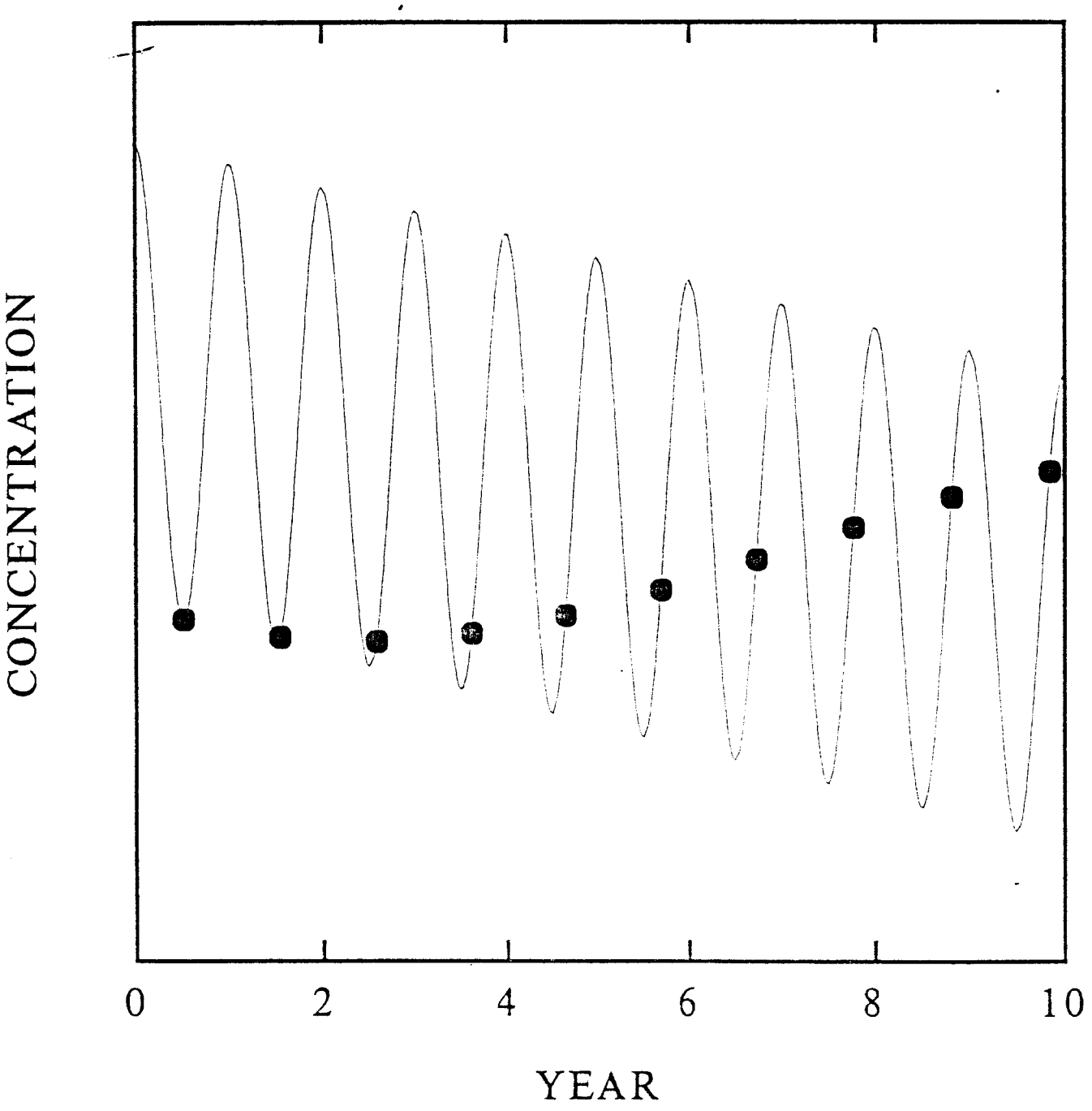


Fig. 8 The effect of sampling progressively a fortnight later each year. The observed concentrations are shown by ● and the underlying concentration is indicated by the continuous line

**Experience with the MED POL data of heavy metals and halogenated hydrocarbons in biota**

Nearly all the MED POL data of heavy metals and halogenated hydrocarbons in biota were collected so inconsistently that it was impossible to use them for temporal trend assessments. For example, samples were

collected at different places, at different times of year, were pooled in different ways, some contaminants were never measured in the same species more than once, etc. The contaminant data can not be used to make inferences about temporal trends in contaminant levels, because the temporal variation can not be distinguished from all the other sources of variation.

## Conclusions

Sampling must be consistent if a monitoring programme is to be of any use, since only then can a monitoring programme address important questions about environmental contaminant levels.

Inconsistent sampling must be avoided because it reduces the effectiveness of a monitoring programme, wastes time and resources, and most seriously of all, can give misleading information about trends in environmental contaminant levels.

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# HOW MANY SAMPLES ARE NECESSARY TO DETECT IMPORTANT TRENDS?

by

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## Introduction

How many samples are necessary to detect important trends? Unfortunately, there is no simple answer. It depends on three main things:

- The type of trend. For example, a temporal monitoring programme will generally require different numbers of samples to detect, eg.

  - a steady increase in contaminant levels,
  - a sudden transient increase in contaminant levels,
  - contaminant levels which exceed some safety threshold.

- The magnitude of the trend. For example, all other things being equal, a monitoring programme designed to detect an increase in contaminant levels of 5% per year over 5 years will require more samples than one designed to detect an increase of 20% per year over 5 years.

- The variability in the data. The more variable the data, the more samples are needed to detect a particular trend.

To choose an appropriate number of samples, we need to consider the statistical power of the monitoring programme. Power studies examine the types and magnitudes of changes that are likely to be detected by the programme. Clearly, power studies depend on the programme's objectives. Here, we shall demonstrate a power study to look at linear trends in contaminant levels over time; full details are given in Fryer and Nicholson (1993). Fryer and Nicholson (1993) also look at the power of a temporal monitoring programme to detect a sudden transient increase in contaminant levels. Nicholson and Fryer (1992) investigate the power of a temporal monitoring programme to detect any between-year variation in contaminant levels. An excellent account of statistical power is given by Cohen (1977).

## A temporal model of contaminant levels

Consider a contaminant monitoring programme in which $R$ samples are taken at the same time in each of $T$ successive years. Let $y_{tr}$ be the log-concentration of the $r$th sample in year $t$ and let

$$y_{tr} = \mu_t + \omega_t + e_{tr},$$

where

- $\mu_t$ is the mean log-concentration in year $t$,

- $\omega_t$ is an error term representing random between-year variation in contaminant levels,

- $\epsilon_{tr}$ is an error term representing random within-year variation in contaminant levels.

Assume that the errors $\epsilon_{tr}$ are independent normal random variables with zero mean and constant variance $\sigma^2$ and that the errors $\omega_t$ are independent normal random variables with zero mean and constant variance $\tau^2$. Further, assume that the $\epsilon_{tr}$ are independent of the $\omega_t$.

Note that:

- Log-concentrations are used, because they are often found to be approximately normally distributed with homogenous within-year variances (eg Anon., 1989). In some situations, alternative transformations might be more appropriate.

- The random within-year variation $\sigma^2$ is the 'natural' variation in contaminant levels found in any population.

- The random between-year variation $\tau^2$ represents between-year variation in contaminant levels in which there is no systematic pattern. This could arise through eg random climatic changes, random fluctuations in discharge, variations in the bias of analytical methods. Investigations of he International Council for the Exploration of the Seas (ICES) Cooperative Monitoring programme (CMP) data on heavy metals in fish muscle revealed considerable random between-year variation (Fryer and Nicholson, 1990).

**Test for a linear trend**

Let

$$\bar{y}_t = \frac{1}{R} \sum_{r=1}^{R} y_{tr}, \qquad t = 1 \ldots T,$$

be the yearly sample mean log-concentrations.

Evidence of a linear trend is assessed by regressing $\bar{y}_t$ on $t$; ie by fitting the models

$$\mathcal{E}[\bar{y}_t] = \mu_1,$$

$$\mathcal{E}[\bar{y}_t] = \mu_1 + b(t-1)$$

giving residual sums of squares $S_1$, $S_2$ respectively, and then comparing the statistic

$$(T-2)\ \frac{S_1-S_2}{S_2}$$

to an F-distribution on 1 and T-2 degrees of freedom (Draper and Smith, 1981).

**Power**

A linear change in log-concentration can be represented by

$$\mu_t = \mu_1 + (t-1)\ \log\left(1 + \frac{q}{100}\right)$$

where $q$ measures the size of the trend; eg $q$ = 5 corresponds to a 5% increase in concentration per year.

The power of the test for linear trend is the probability that a given trend results in a statistically significant test. Power depends on the degrees of freedom

$$\nu = T - 2,$$

and the magnitude of

$$\delta = \left(\log\left\{1 + \frac{q}{100}\right\}\right)^2 \left(\frac{(T-1)\,T(T+1)}{12\,(t^2+\sigma^2/R)}\right) \qquad (1)$$

Strictly, power also depends on the significance level $\alpha$, but only tests at the 5% significance level will be considered here. For a given value of $\nu$, power increases as $\delta$ increases; ie a linear change is more likely to be detected. Thus, the power is positively related to the magnitude of the trend and the number of samples per year $R$ and inversely related to both the within-year and between-year variances $\sigma^2$ and $\tau^2$. In fact, power is also positively related to the number of years $T$, but this relationship is quite complicated because $T$ affects both $\nu$ and $\delta$.

Figure 1 shows power curves (ie the relationship between power and $\delta$) for a range of values of $\nu$. For example, if $q$=5%, $T$=10, $R$=10, $\sigma^2$=0.1, then $\nu$=8, $\delta$=1.79 and the probability of detecting the trend is 0.22. Table 1 shows the effect of $T$ and $R$ on power for different combinations of $\tau^2$ and $\sigma^2$ when $q$=10%.

**How do we use the power curves?**

Suppose that the objective of the monitoring programme is to detect trends of 10% per year or more over a 10 year period. How many samples per year do we need to take?

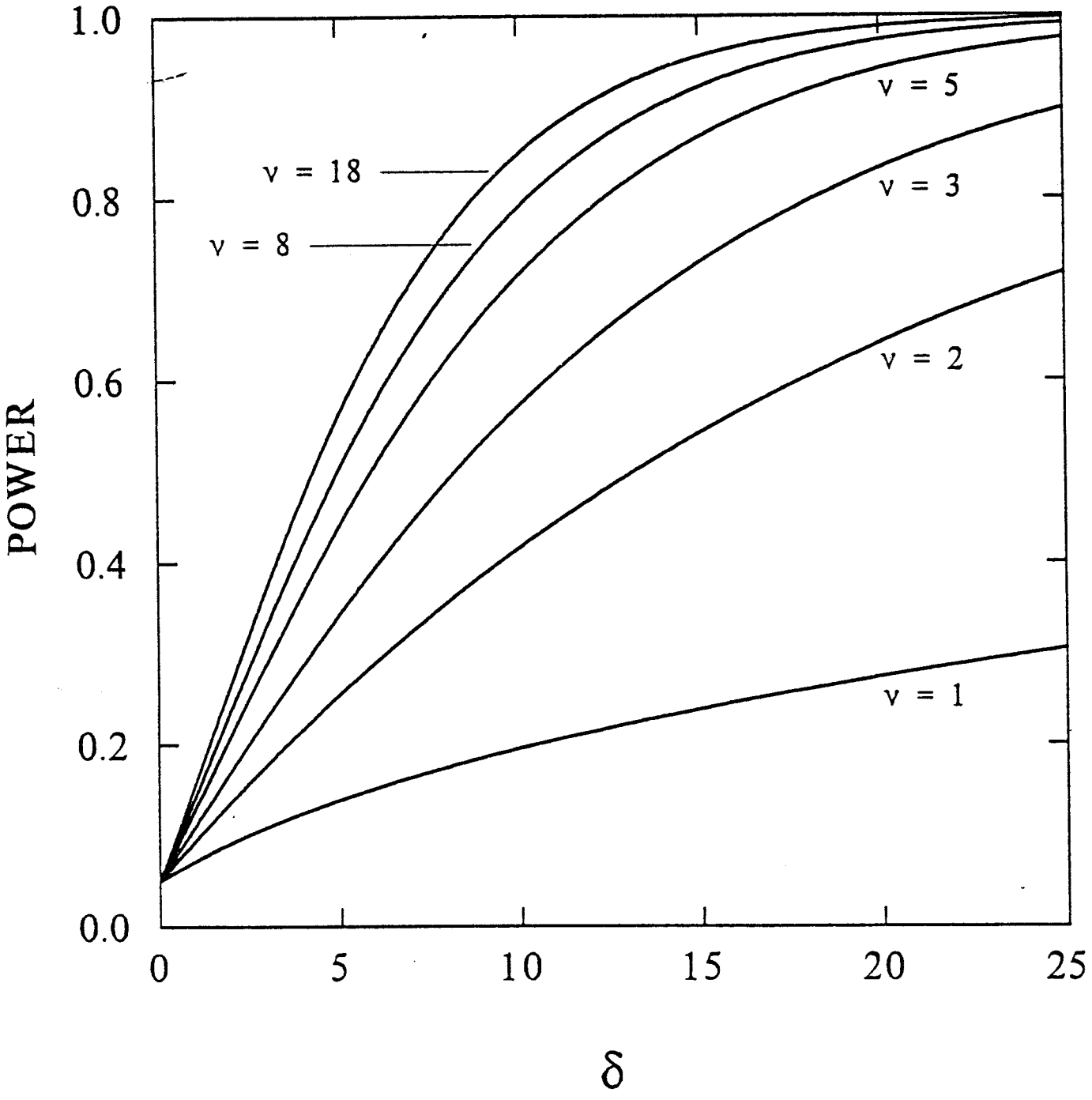


Fig. 1 Power curves for different values of $\nu$

First, we need estimates of the variances $\sigma^2$ and $\tau^2$. An estimate of the within-year variance $\sigma^2$ is easily obtained from a pilot study. However, it is much harder to estimate the between-year variance $\tau^2$, since an appropriate contaminant time series spanning several years is required. One *ad hoc* procedure would be to obtain crude estimates using data from other studies. Fryer and Nicholson (1993) give estimates of both $\sigma^2$ and $\tau^2$ for contaminant levels in cod muscle based on analyses of the ICES CMP data, so lets assume that we are also interested in monitoring contaminant levels in cod muscle.

Table 1

The power to detect a linear trend of $q$=10% for different values of $T$, $R$ and $\tau^2$. Throughout, $\sigma^2$=0.1.

| $\tau^2$ | $T$ | $R$=1 | $R$=5 | $R$=10 | $R$=25 |
|---|---|---|---|---|---|
| 0 | 5 | 0.11 | 0.53 | 0.84 | 0.98 |
| | 10 | 0.67 | 1.00 | 1.00 | 1.00 |
| | 20 | 1.00 | 1.00 | 1.00 | 1.00 |
| 0.025 | 5 | 0.09 | 0.17 | 0.21 | 0.24 |
| | 10 | 0.56 | 0.94 | 0.98 | 0.99 |
| | 20 | 1.00 | 1.00 | 1.00 | 1.00 |
| 0.1 | 5 | 0.08 | 0.10 | 0.10 | 0.10 |
| | 10 | 0.40 | 0.59 | 0.63 | 0.65 |
| | 20 | 1.00 | 1.00 | 1.00 | 1.00 |
| 0.4 | 5 | 0.06 | 0.06 | 0.06 | 0.06 |
| | 10 | 0.19 | 0.22 | 0.22 | 0.22 |
| | 20 | 0.91 | 0.95 | 0.95 | 0.95 |

Suppose that the monitoring programme must detect, with probability greater than 0.9, trends in mercury in cod of 10% per year over 10 years. Estimates of $\sigma^2$ and $\tau^2$ for zinc in cod are 0.102 nd 0.044 respectively. Since $T$=10, we look at the power curve for $\nu$=8 (Figure 1) to find that, for a power greater than 0.9, $\delta$ must be greater than 13.8. Using equation (1) this means that $R$ must be at least 10; ie we need to take 10 samples per year.

Now lets repeat the exercise for nickel, for which $\sigma^2$ and $\tau^2$ are estimated to be 0.068 and 0.11 respectively. Again, $\delta$ must be greater than 13.8 to detect trends in nickel in cod of 10% per year over 10 years with probability greater than 0.9. Unfortunately, no value of R will do this (try it!); there is too much variation in the data to detect these types of trends. So what do we do? One option would be to redefine the objectives of the monitoring programme to detect, say, trends in nickel in cod of 20% per year over 10 years. However, if trends of 10% per year over 10 years are important, it would be necessary to consider an alternative design of monitoring programme or an alternative tissue type / species / compartment which exhibits less random variation in nickel levels (for a fuller discussion, see Fryer and Nicholson, 1993).

**Other considerations**

Although a power study is one way of ascertaining an appropriate sampling level, other considerations must also be taken into account. In particular, it is generally advisable to take replicate samples (N.B. replicate samples, not replicate chemical analyses of the same sample) to guard against eg outside contamination of a sample.

It is important to note that a monitoring programme will usually have several objectives, each requiring a power study. In practice, the number of samples chosen will be some balance of the numbers indicated by the individual power studies.

**Experience with the MED POL data of heavy metals and halogenated hydrocarbons in biota**

In general, the power of the MED POL data of heavy metals and halogenated hydrocarbons in biota for detecting temporal trends was very poor. This was because contaminant time series were not collected over enough years or too few samples were collected each year, or both. For example, even if contaminant levels had increased by 30% per year, most of the MED POL contaminant time series would have been unlikely to have detected these trends.

**Conclusions**

- A monitoring programme should have specific objectives.
- Given estimates of the variability of contaminant data, power studies should be used to help decide the number of samples required to meet the programme's objectives.
- Different programmes will have different objectives and require different power studies.
- Sometimes, it will not be feasible (either financially, practically or statistically) to meet a programme's objectives. This information should be used to either change the programme, redefine the objectives of the programme or cancel the programme.

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# COMPUTERIZATION OF MARINE POLLUTION DATA

by

Adnan AKSEL
United Nations Environment Programme
Mediterranean Action Plan

TO BE DISTRIBUTED AT THE WORKSHOP

REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME NOVEMBER 1990

# *Contaminant monitoring programmes using marine organisms: Quality Assurance and Good Laboratory Practice*

*Reference Methods For Marine Pollution Studies No.57*

Prepared in co-operation with

IOC IAEA FAO

UNEP 1990

NOTE: This document has been prepared jointly by the Intergovernmental Oceanographic Commission (IOC), the International Atomic Energy Agency (IAEA), the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environment Programme (UNEP) under project FP.5102-88-03.

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# PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it. (1), (2)

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources, and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The Methods recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

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International Atomic Energy Agency
International Laboratory of Marine Radioactivity
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which is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

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(1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1 UNEP, 1982.

(2) P. HULM: A Stratery for the Seas. The Regional Seas Programme: Past and Future, UNEP, 1983.

(3) UNEP/IAEA/IOC: Reference Methods and Materials: A programme of comprehensive support for regional and global marine pollution assessments. UNEP 1990.

The present Reference Method provides guidelines for establishing Quality Assurance (QA) and Good Laboratory Practice (GLP) procedures in laboratories involved in the monitoring of contaminants in marine organisms. The guidelines are based on the experience of laboratories participating in large international monitoring programmes including those of UNEP's Regional Seas Programme, the International Council for the Exploration of the Sea (ICES) and the various regional programmes of IOC. Quality Assurance is an essential part of any monitoring programme and is the only means to guarantee comparability of data - without such procedures data reports would be meaningless.

This first edition of the Reference Method for Marine Pollution Studies No. 57 was prepared in cooperation with the Intergovernmental Oceanographic Commission (IOC), the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO). It includes comments received from the joint IOC/UNEP Group of Experts on Methods, Standards and Intercalibration (GEMSI) of GIPME who reviewed the guidelines. The assistance of all those who contributed to the preparation of this reference method is gratefully acknowledged.

CONTENTS


| | | Page |
|---|---|---|
| 1. | Scope and field of application | 207 |
| 2. | References | 207 |
| 3. | Introduction | 208 |
| 4. | Aims of monitoring programmes | 209 |
| 5. | Sampling strategy | 210 |
| 6. | Public health | 211 |
| 7. | Hot spots | 212 |
| 8. | Baseline and trends | 213 |
| 9. | Storage and pre-treatment of samples | 215 |
| 10. | Analytical quality assurance | 217 |
| 11. | Documentation and reporting of data | 219 |
| 12. | Appendix 1 - Selection of Organisms for Monitoring Purposes | 221 |
| 13. | Appendix 2 - Accuracy, Precision and Limit of Detection of Analytical Measurements | 222 |
| 14. | Appendix 3 - Reference Materials (RMs) | 226 |
| 15. | Appendix 4 - Intercomparison Exercises | 229 |
| 16. | Appendix 5 - Analytical Quality Control Charts (AQCC) | 230 |

## 1. SCOPE AND FIELD OF APPLICATION

This publication provides guidelines for obtaining reliable and relevant data during monitoring programmes in which contaminants are measured in marine organisms. It describes the precautions to be taken in each of the procedural steps from planning and sampling to the publication of data reports.

## 2. REFERENCES

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## 3. INTRODUCTION

Many laboratories are actively participating in marine pollution monitoring programmes under the auspices of the United Nations Environment Programme (UNEP), the Intergovernmental Oceanographic Commission (IOC) and the Food and Agriculture Organization of the United Nations (FAO). These organizations have expressed concern about the quality of data arising from such programmes, particularly the accuracy and comparability of data produced by individual laboratories. In order to deal with this problem they have encouraged laboratories to participate in intercomparison exercises, arranged for less experienced laboratories to attend analytical workshops and have arranged for reference analytical methods to be produced and distributed to participants in the various regional seas programmes.

It should be stressed, however, that the acquisition of reliable and relevant data of the appropriate quality, for contaminants in marine samples, is not solely dependant on the production of accurate analytical measurements. The overall quality of data is also dependent on three other factors, which are:

a representative and meaningful sampling programme;

a suitable storage and pre-treatment procedure for samples following collection and prior to analysis; and,

a data assessment procedure.

Unless all four factors are given adequate consideration before and during the monitoring programme, the aims of the programme may not be achieved and valuable time, staff, facilities and other resources may be wasted. The term used to describe the approach to this work is "quality assurance". Quality assurance involves all those steps which are required to guarantee the generation of data of suitable quality to address the defined aims of a monitoring programme.

The purpose of this document is to provide general guidance on quality assurance and to outline the approach that could be taken by laboratories to achieve the specific aim(s) for each marine pollution monitoring programme. Since most laboratories are currently focussing on programmes involving marine organisms, this document will be confined to this aspect.

Since quality assurance work starts from the time when a decision is taken to get involved with marine pollution monitoring it is appropriate to begin by discussing the different aims which laboratories might wish to pursue under this work.

## 4. AIMS OF MONITORING PROGRAMMES

Four main aims can be identified for programmes involving the collection and analysis of marine organisms for the three main groups of contaminants (metals, organochlorine compounds and petroleum hydrocarbons), these are:

(i) The measurement of contaminant levels in edible marine organisms in relation to public health

(ii) The identification of heavily contaminated areas of the sea ("hot spots") where levels of contaminants are at least an order of magnitude higher than levels in clean or uncontaminated areas.

(iii) The establishment of present levels of contaminants in marine organisms (i.e., a "baseline")

(iv) The assessment of changes in concentrations of contaminants in organisms over a period of time (trends).

Each of these programmes places a different demand on the laboratories regarding the method and care to be taken in the collection, storage and analysis of samples. These aspects are considered below in detail in the context of the main aims of each programme.

**It is very important that the investigator should prepare a clear and unambiguous statement on the aims and objectives of the work before embarking on any monitoring programme. Only by doing this will it be clear what type of information will be required, and consequently what criteria should be laid down for the collection, storage and analysis of samples. Time spent on defining aims and objectives and the planning of the various parts of the field and laboratory work will always be rewarded by a more efficient and effective programme.**

Good planning can avoid unnecessary sampling and analyses, i.e,, it is sensible to initially aim for a programme which satisfies essential rather than desirable objectives. It is easy to expand such a programme if the necessary resources are available. Also, if plans are made in advance as to how the data to be collected is to be used, this will assist in the design of field and laboratory work. Finally, there is a need to regularly review the programme of work to assess how well the aims are being met. Such reviews might lead to a reduction of sampling and analyses, and the time gained can be usefully employed elsewhere. Equally it might identify the need to put in more effort; early warning of such extra work can allow time to plan the necessary manpower and other resources.

## 5. SAMPLING STRATEGY

If the sampling strategy is not designed with great care, the data from the measurements may not meet the needs of the programme. On the basis of previous experience considerable effort needs to be devoted to long-term monitoring, so it is important to ensure that this is done in the most efficient and effective way.

Good planning needs considerable time and thought; never be persuaded to rush this aspect of the work and ensure that all disciplines involved are consulted before plans are finalized.

In designing a representative and meaningful sampling programme involving marine organisms there are a number of questions which have to be considered:

a) where should the samples be collected;

b) which organism(s) should be selected for study;

c) when should the sampling be done and what is the appropriate frequency of sample collection;

d) how many individual organisms should be collected on each sampling occasion and which size(s) should be included in each sample; and,

e) which tissue(s) of the organisms(s) should be taken for analysis.

Each of these points will be addressed below for each of the main aims referred to above, i.e., public health, hot spots, baseline and trend studies.

## 6. PUBLIC HEALTH

6.1 Since the aim of the programme is to assess the level of contaminants in edible species then samples should ideally be taken at the point of sale to the general public, i.e., at the fish market. Alternatively the scientist(s) can collect the fish/shellfish from the main fishing areas.

6.2 The selection of organisms will be dictated by the eating patterns of the population. These can be identified by a survey of the species sold at the market, by obtaining information from colleagues in government departments who deal with such matters or in the absence of such information, by distributing a questionnaire to a representative section of the general public.

6.3 Unless there is a seasonal fishing pattern for some species, then samples may be taken at any time of the year. Ideally all species should be sampled at the same time so that a synoptic picture can be obtained for the contaminant levels. A typical monitoring programme might be a survey every 5 years. However, available resources may allow more frequent surveys to be conducted; assuming that the results indicate that this is required (i.e., if concentrations of contaminants in foodstuffs are found to exceed permissable limits). This increased sampling should be confirmed to species "which permissable limits of contaminants are exceeded.

6.4 The size(s) of organisms to be sampled should be based on information collected at the fish market. If small and large sizes are sold to the general public, this should be reflected in the sample. The number of individual organisms in each sample may partly be influenced

by the importance of the species as a foodstuff, by the availability of scientific manpower and by the need to sample sufficient of each species and of each size category to cover the range of values encountered in a typical population or catch. Generally, a sample of 5-10 individuals could be collected for each size range of fish and large shellfish (crabs, lobsters) and 50 individuals for smaller shellfish (e.g. mussels, shrimps).

6.5 Only edible tissue need be analysed for contaminants - usually this means muscle tissue for fish and large crustaceans and whole soft tissue (less vicera) for other shellfish.

6.6 Every opportunity should be taken to collect data on the size (or length) and age of the species. This may assist decision making at a later date if the programme is too focussed on a particular area and/or species.

## 7. HOT SPOTS

7.1 Hot spots are usually found close to estuarine and coastal areas where anthropogenic wastes are discharged. The only offshore areas where hot spots are likely to occur are those which are used for the dumping of wastes from ships (and sites where net deposition of fine material takes place).

7.2 Although one can theoretically select any type of organism to monitor hot spots, in practice the most useful organism for this purpose are the sessile invertebrates. These species, by virtue of their feeding behaviour, can reflect levels of contamination in the soluble phase, in the suspended particulate phase and in the sediment phase (see Appendix 1 for the list of characteristics for organisms to be used in monitoring studies). It should be noted, however, that levels of lipophilic organic compounds (and some inorganic compounds) are most considerably influenced by water: lipid distribution equilibria. This factor may be more important than feeding habits for such compounds.

It should be stated that no single organism can be used to monitor levels in the above three phases. It may be necessary to use a seaweed (macro algae) for the soluble phase, a filter feeder (e.g., mussels) for the particulate phase and a detritus feeder for the sediment phase. The investigator should therefore consult biologists to determine which species are best for the type of waste(s) and phase(s) under examination.

If a single preferred organism had to be nominated, the common mussel (Mytilus edulis or the equivalent local species), would be recommended.

7.3 Seasonal variations in food supply, and the spawning cycle, are known to cause changes in total body weight as well as lipid concentration and composition and consequently contaminant levels in the tissues of invertebrates. In order to minimise these variations it is suggested that sampling be undertaken at the pre-spawning period. Provided sampling covers the area under investigation is a representative manner, one survey is normally sufficient for the identification of hot spots.

7.4 Ideally the number of individuals collected should cover the size range of organisms encountered at the sampling site in order to establish the variations of contaminant levels with size. Although this approach need not be taken at each of the sampling sites, it should be carried out at least at one site in order to allow comparisons to be made with other sites where sampling should be restricted to a small size range. Depending on analytical resources, the organisms from each site can either be analysed individually or bulked. In the latter case, no information will be derived on the variation with size but in terms of the aim of the programme the results can be used to compare data from site to site with some level of confidence (this assumes that a number of replicate analyses are performed on the bulked sample to allow differences to be detected above and beyond those produced by sample variation).

7.5 Whole soft tissue, less vicera, should be taken for analysis. N.B. For the analysis of lipophilic contaminants it is important to measure the lipid content of each sample (e.g., n-hexane extract) in order to ensure that comparisons of data on a lipid basis can be made for different regions and times of sampling (i.e., in relation to the seasonal changes of lipid concentration in organisms).

## 8. BASELINE AND TRENDS

8.1 Sampling sites should include estuarine, coastal and offshore areas to ensure that both clean and contaminated areas are covered in this programme. Sampling at sea should be done by trained personnel operating from research or chartered vessels, rather than by fishermen, to ensure that contamination of the samples during and after collection is kept at an acceptable level.

8.2 Since the aim is to establish the current state of contamination of marine organisms generally, there are no restrictions on the species that can be included in such a study; other than that imposed by the resources available to the investigator. If the laboratory wishes to conduct trend monitoring or compare levels of contaminants at different sites, then it is necessary to include those organisms which will provide the data with which to achieve such aims, e.g. invertebrates.

8.3 Collections should be made over a short interval of time to ensure as synoptic a picture as possible for the baseline survey and to enable comparisons of concentrations of contaminants at different sites. This also ensures that organisms are in the same physiological state (i.e., lipid metabolism, spawning, etc.).

It is appropriate to repeat a baseline study every 5 years unless major changes in discharges occur or are anticipated in the intervening period, which are considered to influence levels of contaminants in organisms over the entire study area. In most cases the effects of increasing or decreasing inputs of contaminants are usually confined to the area in the immediate vicinity of the discharge. More frequent monitoring at these locations would fall under the category of trend studies.

For trend studies the minimum frequency of monitoring for fish and shellfish typically consist of an annual sampling whereas the maximum sampling for invertebrates might be as much as 4-12 times per year. Each laboratory will clearly establish the frequency of sampling which meets the aims of its programme. This frequency will reflect both the changes it wishes to measure over a particular period of time and the resources it has at its' disposal.

8.4 Baseline studies should attempt to cover all size ranges of the species under examination in order to produce a comprehensive picture for the state of the environment. A sample of 5-10 individuals for each size range would be an appropriate number for each fish and major shellfish species. A sample of 25-50 individuals is usually required for smaller shellfish.

Having established the relationship of contaminant levels with size of organisms it is usual to select a particular size or size range for trend studies to reduce variability. The numbers of individuals required for each sample will be determined by the differences in contaminant levels one wishes to be able to differentiate, i.e., the smaller the difference the greater the number of individuals required for each sample. In a recent study of trace metal variability in two populations of Mytilus californianus, through the random sampling of organisms at two

sites, Gordon et. al. found coefficients of variation of 18-40%. They concluded that a sample of 20-100 individuals/site was required to detect a concentration difference of 20% between sites. Differences of 40% could be detected by analysing about 1/3 fewer samples.

8.5 For fish, muscle is the most useful tissue for all purposes. However liver and kidney tissues have been used for both baseline and trend studies. Both hepatopancreas and muscle tissue have been used in studies of large crustaceans. In general, whole soft tissue is taken for smaller shellfish.

## 9. STORAGE AND PRE-TREATMENT OF SAMPLES

### 9.1 Practical guidelines

Guidelines and recommended procedures dealing with the storage and pre-treatment of samples following their collection are given in UNEP Reference Methods for Marine Pollution Studies 7 and 12 (which deal with metals and halogenated hydrocarbons respectively).

The guidelines cover the following:

The types of storage containers to be used to avoid contamination of samples in transit and the recommended temperature for storage prior to dissection and analysis.

The precautions to be taken during the removal of soft tissue from the organism and during the preparation of the sub-sample for analysis to avoid contamination from dissection tools, reagents, laboratory environment and the person carrying out these procedures.

Analysts should ensure that adequate time and effort are allowed for this work since improper storage of samples and poor sample handling prior to analysis will lead to the production of unacceptable data irrespective of how well the subsequent analytical measurements are carried out.

### 9.2 Planning and management

Good planning is essential if this work is to be done efficiently and effectively. The following guidance is given in this respect:

9.2.1 Sampling

- Staff involved in sampling work should be given clear written instructions concerning the methods of collection, particularly the precautions that have to be taken to avoid contamination of samples during the transfer from the site to the storage container.

- Experienced staff should accompany new staff on their first site visit and possibly on later visits to monitor their work.

- A check list of sampling and storage equipment should be compiled for each type of sampling and this should be used before each site visit.

- Adequate time should be allocated for each site visit to ensure that unexpected delays do not cause field staff to give less attention to sampling and storage.

- Log books should be provided to record essential details of samples and any site characteristics that are considered necessary. These logs should be checked on completion of this site visit.

- On arrival at the laboratory samples should be catalogued and securely stored in order to avoid loss of samples.

9.2.2 Dissection of samples

- Ideally dissection of samples should be done on fresh material (cooled but not deep frozen). If these samples cannot be analysed within one or two days they should be deep frozen until they can be analysed.

- If the above procedure cannot be followed, the samples should be deep frozen following collection and only removed for thawing and dissection when the chemical analyses of soft tissue can be done. Repeated freezing and thawing of samples can lead to loss of body fluids and water content. This can not only effect the form and concentration of contaminants in soft tissue but can make the determination of wet to dry weight conversion factors very difficult.

- The dissection of organisms by inexperienced staff, under poorly controlled laboratory conditions, can result in the production of unrepresentative and contaminated samples of soft tissue for analysis. Staff responsible for dissection must be given proper

training in dissection and sub-sampling. They must be issued with the appropriate dissection tools and must work in an area of the laboratory specially kept for this work. It is good practice to assign two people to this work - one to dissect and transfer tissue to the sub-sample container, the other to catalogue the sample and weight of tissue taken for each sub-sample.

N.B. Adequate time must be allowed for this work to avoid staff making errors.

## 10. ANALYTICAL QUALITY ASSURANCE

The earlier section of this document dealt with the collection of representative and meaningful samples of marine organisms and the pre-treatment and storage procedures which ensure that losses and additions of contaminants prior to chemical analysis are kept to a minimum. This section deals with the aspect of quality assurance concerned with the measurement of contaminants in biological tissue namely analytical quality control and assessment.

It is assumed that the analyst has chosen a suitable analytical procedure to provide the appropriate performance characteristics of precision, accuracy and limit of detection (see Appendix 2).

Before an analyst employes an analytical procedure on a regular basis for the measurement of contaminants in a sample of biological tissue it is essential to check its performance characteristics to ensure that the method will produce data of the required accuracy and precision. This applies equally to the Reference Methods for Marine pollution Studies published by UNEP (in cooperation with other UN Agencies) as well as to any methods developed by the laboratory or other published methods used by the laboratory.

This check on accuracy and precision is done by analysing certified reference materials (CRMs) of known matrix and composition. (A list of relevant CRMs is given in Appendix 3). In the absence of such materials the analyst should check the accuracy and precision by the method of "standard addition", i.e., the analysis of samples of the tissue to which known quantities of contaminants of interest have been added (i.e. "spikes"). Total recovery of "spikes" does not however guarantee that the method will produce accurate data for samples since the chemical form of the analyte in the sample and in the spike may not be the same or the concentration/extraction procedure may be inadequate to release the contaminant from its site in the sample. It is advisable, therefore,

whenever possible, to employ CRMs for the evaluation of analytical methods since this is the only true test for accuracy of a method for the sample and analyte(s) under examination. Analysts should choose the CRM which is not only of a similar matrix to the sample but which also has a similar concentration of the analyte(s) under examination. In this respect it is often necessary to analyse two CRMs to cover the upper and lower ranges of concentrations likely to be encountered. This ensures that the method will provide accurate and precise data over the expected range of concentrations.

It must be emphasized, however, that the use of a validated method, suitable instrumentation, experienced personnel, etc. does not guarantee the regular production of reliable data. All analytical work should be done under a system of quality control (the steps taken to minimise errors) and a system of quality assessment (the procedure adopted to verify that the errors are within the acceptable limits).

10.1 Quality Control

The elements of good quality control are as follows:

- Consistent use of reliable qualified personnel, and well-maintained instrumentation.
- Appropriate calibrations and standards.
- Close supervision of all operations by senior personnel.
- Use of CRMs for evaluation methodology.
- Use of reference materials on a regular basis throughout the monitoring programme to check that analytical performance is maintained.
- Participation, encouraged by senior management, in interlaboratory checks of analytical performance (this is the only independent method available to check on a laboratory's analytical capability, see Appendix 4).

10.2 Quality Assessment

The establishment of a system of control charts is the main element of this assessment technique. Control charts are plots of the results of analyses of the same sample over a period of time (see Appendix 5). They allow analysts to check whether their results are falling within the acceptable limits of accuracy and precision.

Data for control charts may consist of the analyses of CRMs and other reference materials. The latter may include homogeneous materials based on real samples which have been prepared by the analyst (known as internal reference materials).

Regular analyses for control chart purposes are essential to avoid waste of valuable time and effort spent on analytical work. Ideally, daily analyses of such internal reference materials should be performed so that only one batch of analytical data is lost if the analysis gets out of control. Many laboratories devote about 10% of their total routine effort to quality control and assessment (see also section 9).

## 11. DOCUMENTATION AND REPORTING OF DATA

The adoption of the following guidelines by a laboratory should provide adequate documentation: to support any data report or decisions on the results of its monitoring programmes, and to allow it to trace samples from the collection stage to the completion of its analyses by providing a record of the appropriate data in logbooks or in computer files.

### 11.1 Documentation

The following documentation is necessary for a laboratory participating in pollutant monitoring activities:

(i) Descriptions of the sampling strategy, methods of collection, procedures of storage, pre-treatment and analytical procedures.

(ii) Sample documentation (description of organisms, numbers or individuals collected for each sample, weights of tissue taken for analysis (individual tissue or homogenate).

(iii) Evidence of successful evaluation and testing of analytical procedures, including details of accuracy, precision and limit of detection.

(iv) The approach used for quality control and quality assessment and evidence that these procedures have been used and have provided acceptable data.

(v) Calibration details, a description of working standards used on each occasion and calculations of results.

(vi) A secure system for the long term storage of data either in logbooks or in computer files is essential. It is therefore advisable to have a duplicate set of records in case one is lost, mislaid or accidently destroyed.

Advice should be sought on the correct method of storing computer tapes and/or discs to ensure the long-term stability of the data files.

11.2 Preliminary assessment, tabulation and storage of data

It has been shown that even the most experienced personnel can make simple arithmetic errors in calculating data. Thus a check should be made for such errors before compiling tables of results. Once this check has been carried out it is appropriate to carry out a preliminary assessment of the quality of the data, prior to its evaluation and publication, to ensure that no erroneous results are included in the data set. This assessment can include a comparison of the results with existing data (i.e., data previously collected by the laboratory or data published in the literature for the study area). Before consigning data to long term storage a final check should be made to ensure that no transcriptional errors have been made in transferring the data (i.e., the re-typing of data sets by typists or data processors can sometimes lead to such errors).

11.3 Publication of data

Data held in logbooks or on a computer which has not been published is effectively lost to the scientific community. Every effort should be made to evaluate and publish these results, even in the simple form of a report. In addition to the results, the contents of a data report would normally include a statement on the aims of the work, a description of the methods used to collect and analyse samples together with a statement on findings and conclusions.

In general it is inadvisable to supply a third party with a copy of your raw data, unless:

(a) it is accompanied by information on its accuracy, precision and limits of determination and details of the sampling record;

or,

(b) it is accompanied by a report from the analyst or principal investigator in which the results have been evaluated.

## APPENDIX 1

### SELECTION OF ORGANISMS FOR MONITORING PURPOSES

The ideal characteristics of organisms for use as indicators of marine contamination are:

- The organism should accumulate the contaminant without being affected by the levels encountered.

- The organism should be sedentary in order to be representative of the area of collection.

- The organism should be abundant in the study region.

- The organism should be sufficiently long-lived to allow sampling of more than one year class if desired.

- The organism should be of a reasonable size, given adequate tissue for analysis.

- The organism should be easy to sample and hardy enough to survive in the laboratory, allowing (if desired) defecation before analysis and laboratory studies of uptake of contaminants.

- The organism should tolerate brackish water.

- The organism should exhibit high concentration factors allowing direct analysis without pre-concentration.

- A simple relationship should exist between contamination residues in the organisms and the average concentration in the surrounding seawater.

These conditions restrict the useful organisms to a range of fairly large, abundant, widespread, inter-tidal organisms, mainly macro-algae and molluscs.

Filter-feeding molluscs are more likely to reflect conditions in the water column, whilst deposit feeders will respond to sediment chemistry. The concentration of contaminants in the water column, however, will largely reflect effluent discharges and dispersal conditions at the moment of sampling. Filter-feeders are therefore more likely to provide the information required to fulfill the objectives of the monitoring programme.

If a single preferred organism had to be nominated, the common mussel, Mytilis edulis, would be recommended. Othe bivalves (particularly mussels and oysters) may also fulfill the criteria indicated. Care must be taken with species identification as concentration factors of some contaminants vary widely between species.

APPENDIX 2

## ACCURACY, PRECISION AND LIMIT OF DETECTION OF ANALYTICAL MEASUREMENTS

### 1. Accuracy

Accuracy may be defined as the closeness of the measured value to the true value. Accuracy is influenced by systematic errors (bias), inherent in the method or caused by some artifact of the measurement, and by random variability (precision) of the method. The concepts of accuracy, in bias and precision are illustrated in the two diagrams below.

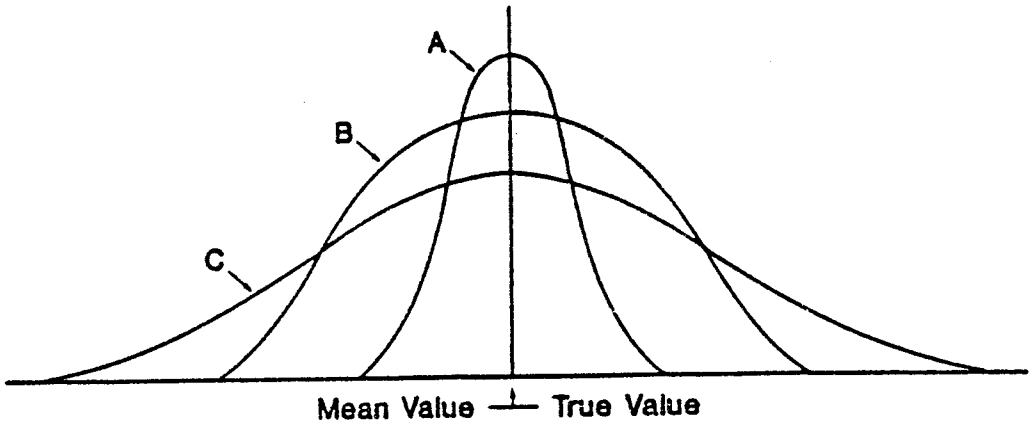


Fig. 1: Examples of Unbiased measurements of high precision (curve A), intermediate precision (curve B), and low or poor precision (curve C).

N.B. Whilst the results from procedure C can provide a mean value which is identical to the true value, by comparison with procedure A, it is a relatively inaccurate procedure.

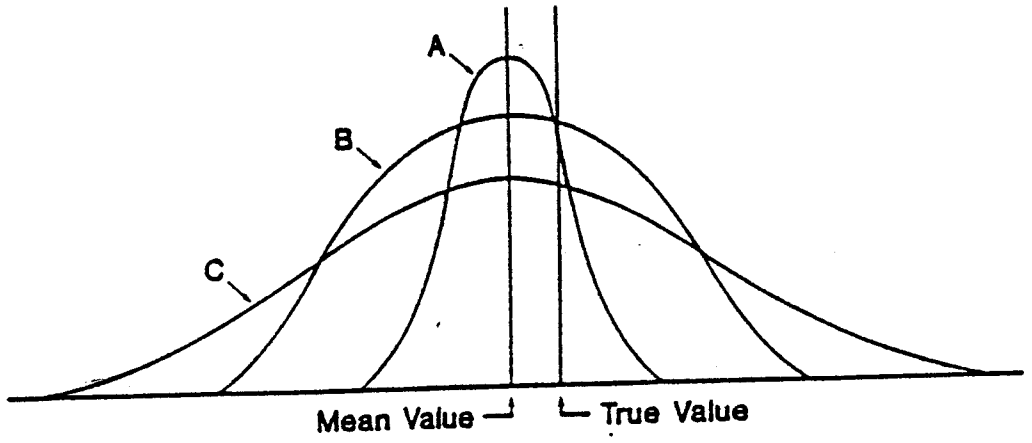


Fig. 2. Biased measurements of different precisions.

All the measurements are biased since the mean values of all three procedures do not coincide with the true value. However, it should be noted that most of the results for measurement "A" will be more accurate than those of "B" or "C", due to precision considerations.

2. Bias

There are four possible sources of bias in analytical measurements:

(i) Inability to determine the appropriate forms of the analyte in the sample.

(ii) The effects of other substances present in the sample (interferences)

These are the two most common sources of bias. However, it may not be possible for a routine analytical laboratory to devote the necessary time and effort to conduct all the tests required to check the sources of variation. In view of this, analysts should minimise these sources of error by adopting analytical procedures which have been recommended by either an expert group or an expert laboratory.

(iii) A biased calibration

Provided that care is taken in preparing any standards required for calibration purposes this source of error should be minimal. The purchase of standards from a reputable company will usually guarantee the accuracy of the stock

standards. The accuracy of working standards (dilutions of the stock standards) will however be determined by the ability of the analyst to carefully carry out the necessary preparatory work, particularly with respect to the cleanliness of operations. Inattention to this aspect can lead to contaminated standards and therefore inaccurate calibrations.

N.B. Remember to double check dilutions of stock standards since simple errors (using the wrong size of pipette or making a mistake in the calculation of the dilution required) can lead to inaccurate standards.

If analytical instruments are not well maintained and serviced regularly the response of them will vary with time thus affecting calibrations.

**(iv) A biased blank correction**

In theory, a blank correction involves the analysis of a sample which contains negligible concentrations of the analytes in a matrix similar to the samples being analysed. This ideal situation is difficult to achieve in practice since "field blanks" are not easily obtained. Whenever possible, however, such blanks should be used and processed in exactly the same way as samples. The reduction or minimisation of a blank value and its variability can be achieved by:

a. ensuring that only high quality reagents and labware are used in the analytical procedure;

and

b. ensuring that all operations are conducted with rigorously cleaned labware and in laboratories which are free from sources of contamination.

3. Precision

Precision, which is defined as the random variability of the measurement procedure, can vary within and between batches of analyses. An estimate of precision from only one batch of analyses can therefore give an over-optimistic estimate of subsequent routine analytical results. Precision should be estimated from a series of analyses, on the same material, conducted over a period of time. This approach enables the total random error to be separated into the error arising from

variations within and between batches of analyses. This information can be useful in identifying the location and magnitude of sources of errors. e.g., a large value for between-batch standard deviation may indicate a changing calibration. Similarly a large value for within - batch standard deviation may inducate a contamination problem.

Since the precision of analytical results often depends on the concentration of the analyte, it is necessary to analyse samples which cover the upper and lower limits of the analytical method. This can be done by choosing the appropriate CRMs (see Appendix 3) to cover the range or by analysing spiked samples of different concentrations.

4. Limit of detection

Limit of detection (LOD) is that concentration at which the analyte can be quantified to a given accuracy with an agreed degree of confidence. The LOD of an analytical procedure can be calculated as follows:

$$St = Sb + \text{constant} \cdot v = Sb + K\,v$$

LOD is based on the relationship between the gross analytical signal (St), the field blank (Sb) and the variability in the field blank (v) measurements.

(Field blanks are samples that contain the analyte(s) at levels below the LOD. These are difficult to obtain so in most instances analysts substitute reagent blanks for them).

In practice most analysts define LOD using a K of 3.

i.e., $LOD = Sb + 3v$

Measurements below 3v should be reported as not detected (nd) with the limit of detection given in parenthesis. In the region 3v - 10v measurements should be reported as detected, again with the LOD being given in parenthesis.

## APPENDIX 3

## REFERENCE MATERIALS (RMs)

A comprehensive list of marine RMs can be found in the latest edition of "Standard and Reference Materials for Marine Science (NOAA Technical Memorandum NOS OMA 51)*" which is produced by the U.S. National Oceanic and Atmospheric Administration in cooperation with the IOC/IAEA/UNEP Group of Experts on Standards and Reference Materials (GESREM). In this document a description is given of each RM (its preparation, its composition and in the case of CRMs the values for the analyte(s) in question) together with the name and address of the organisation from which the RM(s) can be purchased.

At the time of preparing this document there are five major organisations which are actively involved in the production of marine RMs for contaminants in biological tissue. They are:

National Institute of Standards, Science and Technology (NIST), Office of Standard Reference Materials, Gaithersburg, MD 20899 USA.

National Research Council of Canada (NRCC): Institute for Environmental Chemistry, Montreal Rd., Ottawa, Canada K1A OR6; or Institute for Marine Biosciences, 1411 Oxford St., Halifax, NS, Canada, B3H 3Z1.

National Institute for Environmental Studies (NIES), Yatabe-machi, Tsukuba, Ibarake 305, Japan.

International Atomic Energy Agency (IAEA), International Laboratory of Marine Radioactivity, Stade Louis II, 19 Avenue des Castellans, MC 98000, Principality of Monaco.

Community Bureau of Reference (BCR), Community of European Communities, 200 rue de la Loi, B-1049 Brussels, Belgium.

Some commonly used marine RMs are listed below together with the organisation that supply them.

| Material | Matrix | Analyte(s) | Supplier |
|---|---|---|---|
| TORT-1<br>LUTS-1 | Lobster<br>hepatopancreas | Metals (high conc) | NRCC |
| DORM-1 | dogfish muscle | Metals (low conc) | NRCC |

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* Document available from: Intergovernmental Oceanographic Commission (IOC), 7, Place de Fontenoy, 75700 Paris, France.

| Material | Matrix | Analyte(s) | Supplier |
|---|---|---|---|
| DOLT-1 | dogfish liver<br>dogfish liver oil | Metals (intermediate conc)<br>Chlorobiphenyls (CBs) | NRCC<br>NRCC |
| CLB-1-A | Iso octane solution CLB-1-D | CBs standards | NRCC |
| CRM 279 | Sea lettuce | Metals | BCR |
| CRM 349 | Cod liver oil | CBs (IUPAC Nos. 28,52,101,118,138,153,180) | BCR |
| CRM 350 | Mackerel oil | CBs (as above) | BCR |
| MA-A-1/TM | Copepod | Metals | IAEA |
| MA-A-2/TM | Fish Muscle | Metals | IAEA |
| MA-B-3/TM | Baltic fish | Metals | IAEA |
| IAEA-350 | Tuna fish | Metals | IAEA |
| MA-A-1/OC | Copepod | PCBs (1242 and 1254) and selected pesticides | IAEA |
| MA-A-2/OC | Fish Muscle | PCBs (1254 and 1260) and selected pesticides | IAEA |
| MA-M-2/OC | Mussel homogenate | as above | IAEA |
| MA-B-3/OC | Baltic fish | selected pesticides and selected PCB congeners | IAEA |
| IAEA-351 | Tuna fish | as above | IAEA |
| SRM 1566 | Oyster tissue | trace elements | NIST |
| NIES No.6 | Mussel powder | metals | NIES |
| NIES No.9 | Sargassum | metals | NIES |

Analysts who wish to ensure that their analytical data are accurate and precise should purchase one or more of these RMs for use in their evaluation of analytical methods and in their routine quality control work. Although the cost of such materials may appear prohibitive it is sound judgement to allocate funds to the provision of such materials in

order to ensure that time and effort are used constructively and productively. Time devoted to quality assurance work (ca 10% of an analysts time) is time that is well invested.

N.B. Analysts should not use non-marine RMs for validating analytical methods and quality control work associated with marine monitoring programmes. They should choose RM(s) which have matrices which are either identical, or very similar, to the samples under study and which cover the upper and lower range of concentration of contaminants being measured. Further information on the correct use of Reference Materials may be obtained from the literature cited in section 2 of these guidelines.

## APPENDIX 4

## INTERCOMPARISON EXERCISES

These exercises provide analysts with an opportunity to obtain an independent assessment of the quality of analytical data produced by them. They also allow others, including coordinators of multi-laboratory monitoring programmes, to assess the comparability (and in some cases accuracy) of the data being produced by participants in these programmes. They provide a stimulous to improve methodology and encouragement to adopt state-of-the-art methodology. Whenever possible analysts should participate, and be encouraged to participate by their line managers, in such exercises.

The exercises are normally conducted using blind samples - homogenous substances which contain unknown concentrations of the analyte in a matrix identical, or similar, to that normally examined in the associated monitoring programme. Occasionally, analysts receive samples which contain known (but undisclosed to them) concentrations of analyte(s). In all cases these materials are specially prepared for such exercises.

Participants in such exercises are usually asked to analyse the materials by the analytical method(s) used in their normal monitoring work. Coordinators of exercises accept that the results submitted by participants will be the best data produced by them, since experience has shown that greater than usual care is taken by analysts during the analysis of such materials. Well designed exercises will usually include several materials to cover the range of concentrations of analytes and different matrices normally encountered in monitoring programmes in order to assess differences in analytical performance at the upper and lower ranges of concentration. Coordinators of exercises will try to ensure that a sufficient number of experts participate to provide some degree of standardisation or validation for the exercise. The absence of experts automatically means that the results from participants can only be compared rather than assessed for bias.

It is essential that the quality of analytical work is maintained during and in between exercises. Only the analyst can ensure that this actually happens.

For further information on specific exercises, please contact the addresses given on the inside back cover of this document.

## APPENDIX 5

## ANALYTICAL QUALITY CONTROL CHARTS (AQCC)

### 1. Purpose of AQCCs

It has been recommended that a reference material should be analysed periodically to provide a check on the quality of analytical data. The simplist way to assess the results of these analyses is to examine them at the end of the analytical period and decide whether or not they are satisfactory, and thus whether or not the results for samples are acceptable. This approach is very subjective and a much better approach is to plot the results of the analysis of RMs on a simple chart, which contains guide lines that allow an objective decision to be made on the quality of the data. This chart is known as an analytical quality control chart (AQCC).

### 2.1 Construction of an AQCC

Analysts are reminded that before a method is used routinely for samples it must have been rigorously assessed to ensure that it will provide data of the required quality. Assuming that such a method is used the analyst should carry out the following procedure to construct an AQCC, along the lines of that given below in Fig. 1.

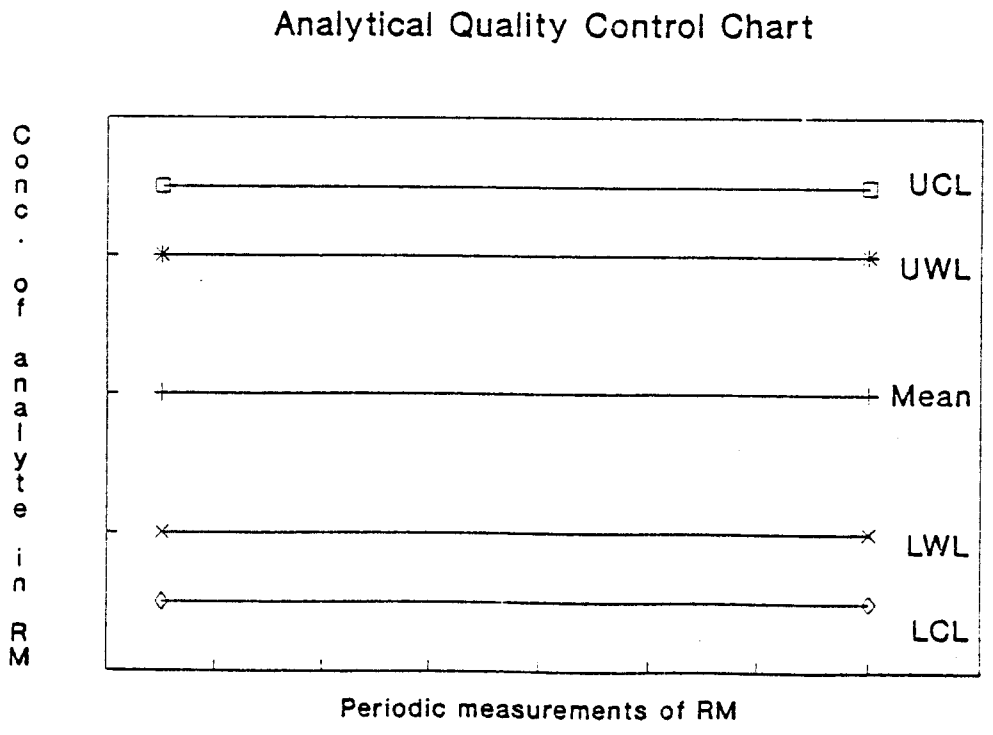


Figure 1. Blank control chart

(i) Select the RM to be analysed with samples on a regular basis.

(ii) Analyse the RM at least 10 times for the analyte(s) under examination. These analyses should not be done on the same day but spread out over a period of time in an attempt to ensure that the full range of random errors within and between batch analyses are covered.

(iii) Calculate the mean value ( $\bar{X}$), and the standard deviation (s) and then plot the following values on a blank control chart: $\bar{X}$, $\bar{X} \pm 2s$ (UWL), $\bar{X} + 3s$ (UCL), $\bar{X} - 2s$ (LWL) and $\bar{X} - 3s$ (LCL).

3. Using an AQCC

Assuming that the analytical measurements for RM(s) follow a normal distribution, 95% of them (19 in every 20) should fall within the area between UWL (upper warning limit) and LWL (lower warning limit). Similarly 99.7% of the results should fall within the area between UCL (upper control limit) and LCL (lower control limit).

The analyst should plot the results of the analysis of RM(s) after each batch of analyses to check where the data lies in relation to these limits. An example of such a plot is given in Fig. 2.

The following guidelines can be used to assess whether the data for the RM(s) and consequently the data for the samples are of acceptable quality, i.e., are the analyses under control.

(a) The mere fact that one result falls outside the warning limits need not require the analyst to doubt the result or take any action provided that the next result falls within the warning limits.

(b) If the results fall outside the warning limits too frequently, particularly if the same warning limit has been crossed more than once on consecutive results, then the analyst needs to assess the source of this systematic error.

(c) If the results on more than 10 successive occasions fall on the same side of the $\bar{X}$ line (either between $\bar{X}$ and UWL or $\bar{X}$ and LWL) then the analyst needs to check the analytical procedure to determine the cause of this error.

(d) If the result falls outside the UCL or LCL lines then the analyst should check the analytical procedure to determine the cause of this source of error.

If any of the above cases occur the analyst should reject the results of the analysis of the particular batch of samples and should not

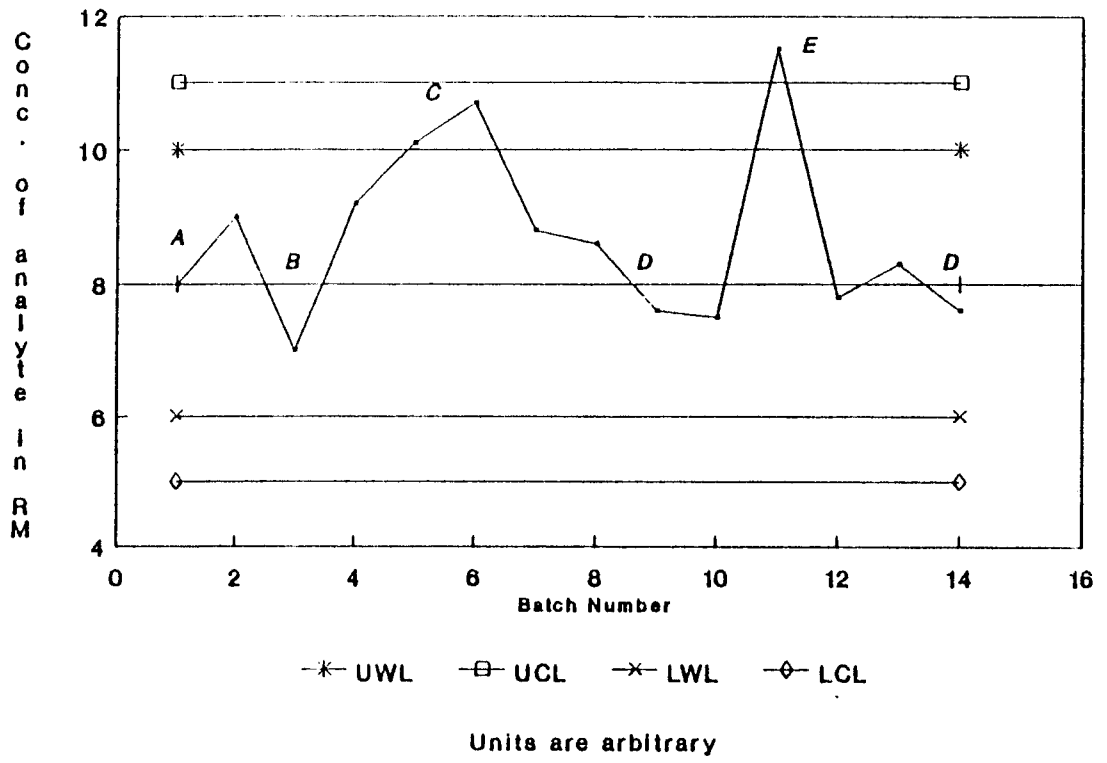


Figure 2. An example of a Quality Control Chart.

ANNOTATIONS

A - A "consensus value" is established by repeated analyses of a RM. Upper and lower warning and control limits are determined statistically from the standard deviation (s) of the n measurements made.

B - The routine measurements of the RM are well within the warning limits. Measurements are under control.

C - Something appears to be contaminating the samples here. Reagents were investigated and a new batch of solvents was found to be at blame and was replaced immediately.

D - The process is back under control.

E - Here, a serious problem was discovered. The previous ten data were rejected and all analyses were discontinued until the fault (dirty glassware this time) was detected and corrected.

carry out any further analysis of samples until the source(s) of the errors have been identified and he/she is satisfied that future analyses will be under control.

4. Use of Internal Reference Materials

The accuracy of a method can only be checked with an SRM or a CRM for which the mean values and standard deviations are well documented. Analysts who choose to use their own specially prepared RM (i.e., an internal RM, IRM) for quality control purposes should note that they are primarily checking the precision of measurements and not their accuracy. These IRMs are very convenient however, for analyses where large quantities of materials are required for each determination (e.g., analyses for organic contaminants) and where the cost of these materials for QC charts would be prohibitive. Full instructions on the preparation and calibration of IRMs will be given in another publication in the present series.

5. Further information

The relevant publications listed in Section 2 of these guidelines contain valuable information on the correct use of reference materials and the application of QC charts. They should form part of every analytical laboratory's reference collection.

# ALTERNATIVE MEANS OF ASSESSING DATA

by

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In contaminant monitoring issues there are usually two main purposes to be addressed: levels of contaminant concentrations and whether these levels are increasing or decreasing. Even though detailed guidelines may exist (a rare occurrence) which may be followed very carefully (even rarer), there is no given method of assessing the results with the possible exception of some spatial and temporal trend evaluation methods recognized by ICES (1988, 1989, 1991). Each data set it would appear to have its own individuality that requires the close attention of the assessor.

## 1. Assumptions

We have to assume that the appropriate guidelines have been followed to meet the monitoring targets in question. Furthermore, it has to be assumed that good laboratory practice (GLP) has been followed and the laboratories involved have participated successfully in appropriate intercalibration exercises. To confirm this, the data should be in such a fashion that it is easy to verify whether or not the guidelines have been followed with regards to, for example, sample count, length size distribution, time of sampling and also, the quality control data should be easily available, especially the results from analyses of certified reference material (CRM) that were carried out during the data series in question.

## 2. Levels

Determining whether a level of a contaminant in a given medium is high or low is of course dependent on which data material one compares with. The 1985 ICES baseline study of contaminants in fish and shellfish of the North Atlantic used **upper quartiles**, among other techniques, to distinguish areas of elevated levels. Box and whisker plots are helpful in this regard. The quartiles were based on the data submitted during one investigation and hence depended on where the samples were collected. For example, the upper 25% of the mercury concentrations in 66 samples of cod liver were above 67 ppb w.w. (0.067 mg $kg^{-1}$ wet weight). The samples were fairly evenly distributed around the British Isles, North Sea and southern Baltic Sea, but these samples were only from a relatively narrow belt of latitude of the convention area (50° to 61°N). Are there significant differences depending on latitude? Would it be wise to compare results for the Mediterranean to these quartiles?

To illustrate, Table 1 compares the concentrations of some metals found in blue mussel (Mytilus edulis) with those of the Mediterranean mussel (Mytilus galloprovincialis). Also shown are different limits to grade the level.

Table 1

Concentrations of metals (ppm d.w.) in the blue mussel (Mytilus edulis) and Mediterranean mussel (Mytilus galloprovincialis). Where necessary, values were converted using a wet/dry weight ratio of 6.

| | Cd | Cu | Hg | Pb | Zn | Reference |
|---|---|---|---|---|---|---|
| Mediterranean: | | | | | | |
| Mg-mean | 0.72 | 7.8 | 1.38 | 4.8 | 162 | Jeftić et al., 1990 |
| Mg-max. | 6.36 | 36 | 42 | 96.6 | 586 | Jeftić et al., 1990 |
| One investigation: | | | | | | |
| Me-85 mean | 1.03 | 7.9 | 0.10 | 1.7 | 95.7 | ICES, 1988 |
| Me-85 $Q_{75}$ | 1.8 | 9.9 | 0.19 | 3.5 | 130 | ICES, 1988 |
| 'Reference values' | | | | | | |
| Me-class 1 | <2 | | <0.2 | <5 | <200 | Knutzen, 1992 |
| Me-class 2 | 2-5 | | 0.2-0.5 | 5-20 | 200-400 | Knutzen, 1992 |
| Me-class 3 | 5-20 | | 0.5-1.5 | 20-50 | 400-1000 | Knutzen, 1992 |
| Me-class 4 | 20-40 | | 1.5-4 | 50-100 | 1000-2500 | Knutzen, 1992 |
| Me-class 5 | >40 | | >4 | >100 | >2500 | Knutzen, 1992 |
| Ecotoxicological: | | | | | | Jonkers & Everts, |
| NL-MRL | 0.07 | | 0.04 | | | 1992 |
| "Health" limits: | | | | | | |
| DK "action limit" | 3 | | 1.8 | 6 | | FAO, 1989 |
| NL | 6 | | 6 | 12 | | JMG, 1992 |
| E "all metals" | 3 | | 3 | 3 | | FAO, 1989 |
| SF | 3 | | 6 | | | PNUN, 1987 |
| I | | | | 12 | | FAO, 1989 |
| GB | | | | 60 | | FAO, 1989 |
| F | | | 3 | | | FAO, 1989 |
| USA | | | 6 | | | FAO, 1989 |
| S | | | 6 | 6 | | PNUN, 1987 |
| "Risk" limits | | | | | | |
| N 0.5 g/d (rural) | 348 | | 408 | 4014 | | Green, 1987 |
| N 1.6 g/d (urban) | 126 | | 132 | 1290 | | Green, 1987 |

Table 1 also illustrates the use of **reference values**. In Norway, the lack of adequate data sets from local areas has enhanced the use of reference values also defined as the *suggested* upper limit to background levels in diffusely polluted areas. These values are based on data from both independent investigations and local measurements. There is of course a subjective element in defining a limit which on the one hand creates uncertainty but on the other hand is a reasonable assessment of the probable levels combining scientific evidence and scientific judgment. As they are used in Norway, the values are under continuous evaluation and some adjustments are made from year to year. This is important in order to compensate for improvements in analytical techniques and probable natural decreasing trends for some contaminants such as PCBs and lead.

Reference values have been frequently used in national assessment work. During the past two years the Norwegian State Pollution Control Authority and the Norwegian Institute for Water Research have worked out a classification system based on **overconcentrations**, the factor by which a contaminant concentration exceeds the reference value. Some reference values proposed by Knutzen (1992) are shown in Table 1.

Another approach is the use of **ecotoxicological reference values.** These are largely based on experimental studies and theoretical coupling to the 'real' environment. Jonkers and Everts (1992) have done some useful work in this area for the Netherlands. Their goal is to establish a concentration of maximum permissible risk level (MRL) of a substance at which 95% of all the species potentially present in the ecosystem plus all 'characteristic species' in certain areas are afforded complete protection in the sense of their chronic No Observed Effect Concentration (NOEC) not being exceeded. It is assumed that the negligible risk level (NRL) of a substance provides complete protection for an ecosystem and it is generally taken as 1% of the MRL. Risk assessment is based on (sub-) chronic toxicity data. However, they often have used an equilibrium partitioning method or extrapolation techniques due to the scarcity of data. The MRLs for cadmium and mercury for mussels are shown in Table 1 and are well below the upper limits for 'Class 1'.

There are not a great number of studies where contaminants alone have been shown to have detrimental effects on the biota. The exceptions being generally cases of extreme contaminant emissions. Soft bottom or benthos surveys have been useful reflecting moderate levels of metals in sediment (Rygg, 1984).

The use of **health limits** is a further method of assessing levels. Risk assessment for the consumption of seafood generally has guidelines quite different from spatial or trend assessment guidelines and often those assessing such data are in a separate governmental body that the other assessors. Hence, the combined assessment of health, spatial distribution and trends is not always feasible. Just the same, it may be useful in cases of extreme contamination to relate the concentrations found to the human consumption standpoint. Table 1 shows examples of various health limits employed by various countries. These are not usually legally binding but represent guidelines on which the respective health authorities can act. Note that the limits are based on a fixed consumption rate and do not reflect local conditions. If we were to calculate the local consumption rates of selected 'rural' and 'urban' communities in Norway the hazardous concentration levels would be considerably higher (cf., Table 1).

Comparison to limits give a rough conception of the levels concerned but the significance of the levels both with regards to overconcentrations or effects have generally been disregarded unfortunately. Furthermore, such comparisons are rarely done on normalized data that is adjusted to variables that are known to correlate to concentrations such as length or sex.

## 3. Temporal trends

So far we have looked at comparing two concentrations, a measured value and a reference value (quartiles, 'background', health) to assess the level. But we cannot progress far without considering statistics in order to determine if two data sets are significantly different. The same principles apply whether one is looking at temporal trends or spatial gradients of contamination. Consider two data sets A and B (Fig. 1). The means may be far apart but the F-test from the analysis of variance (ANOVA) showes that they are not significantly different, because of the spread of data about the mean. This spread is often illustrated by calculating the 95% **confidence intervals** (CI) for the data set (see e.g., Elliot, 1977). *Usually* when the CIs overlap, the two data sets are not significantly different but as shown in Figure 1

there are exceptions. To avoid this, Nicholson (1985) suggests using **confidence bands** where by, if the bands overlap, the two data sets are always not significantly different regardless of variation in sample count and if they do not overlap then you can be certain that they are significantly different if the count in each sample is the same, otherwise you need to test the difference. If the confidence *limits* do not overlap, the samples are always significantly different.

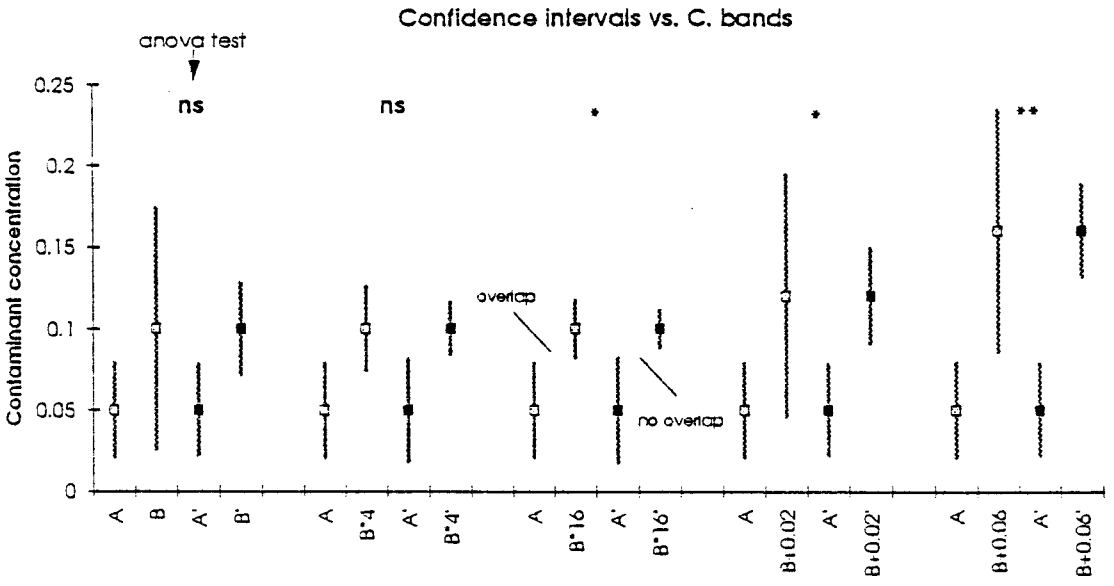



Fig. 1 Examples of methods of comparing means of two data sets (the raw data is in the annex)

When three or more samples (data sets) are involved we are generally concerned whether there is a trend (temporal or spatial). In this case it is difficult t avoid regression analysis. The ICES Working Group on Statistical Aspects of Environmental Monitoring (WGSAEM, former WGSA-Trend-M) have developed techniques for assessing temporal trends. In the Joint Monitoring Programme (JMP) contaminants in fish are monitored in fish muscle (fillet) and liver and in shellfish in whole soft tissue. For contaminants measured in shellfish or fish liver a three model approach has been used (ICES, 1991) and for contaminants in fish fillet a six model approach has been suggested (ICES, 1989). The complexity of the latter is related to the importance of length (age?) as a covariable in the regression. The three model method is simpler because length is not included as a covariable. Both approaches are robust, relatively simple techniques for monitoring trends in biota and good for an initial assessment of the data but they do not preclude the need for further review by the assessors.

## 4. Examples

The Ranfjord is a narrow fjord 70 km long in the north of Norway (14°E, 66.3°N). It has several basins varying in depth between 200-500 m. Undersea landslides and consequent turbidy currents are not uncommon along the steep rim of these basins. Tidal amplitude is normally 1-2 m. The freshwater

discharge into the head of the fjord has an annual mean of about 300 $m^3 s^{-1}$ but often reaches about 600 $m^3 s^{-1}$ during the spring thaw. Surface water (0-5 m) of the inner fjord has a salinity normally about 3-15‰. The underlying seawater salinity varies between 30 and 34‰. Surface temperatures during the winter are near or below freezing and during the summer can vary between 10-15°C.

The major anthropogenic perturbances are contaminant pollution (PAH and metals) and discharge of inert mining trailings (recently about $2x10^6$ tons annually) from mining/smelter industries during the past 3-6 decades. The municipal discharge from about 25000 residents in Moi Rana may enhance eutrophication at the head of the fjord.

The closing of the smelter works and some mining industries by 1989-90 resulted in a 70-90% reduction of contaminant discharge.

Figure 2 shows that there has been an evident reduction in lead concentrations in bladder wrack and blue mussels since 1980 but local influences still persist at station B6. This has to be taken into account when assessing trends.

Although reference stations may be useful in comparing perturbed and unperturbed areas as in the case of Ranosen vs. Saltdalsfjord for lead in fish livers (Fig. 3), this may not be appropriate for all contaminants as indicated for zinc (Fig. 4).

Even when strong gradients are noted in the water it does not necessarily follow that the same proportional gradient will be observed in biota. This has been shown for experimental studies (Table 2) and measurements in the field (Table 3). Where there was a 40 times increase in aromatic hydrocarbons, in the experimental study the increase in mussel was only a factor of 10. In the field the differences were even more marked.

It is apparent that the mussels regulate the intake of metals. Two important mechanisms in this regard are behavioral changes (reduced filter activity) and physiological/biochemical regulation.

Table 2

Overconcentrations (relative to control) of aromatic hydrocarbons (THC in water PAH in mussels) and copper in seawater and blue mussels (from Widdows and Johnson, 1988).

| concentration | seawater | | blue mussels | |
|---|---|---|---|---|
| | THC | Cu | PAH | Cu |
| control | 1 | 1 | 1 | 1 |
| low | 2.1 | 1.6 | 5.6 | 2.2 |
| medium | 10.5 | 10 | 20.7 | 3.7 |
| high | 41.5 | 40 | 9.7 | 8.1 |

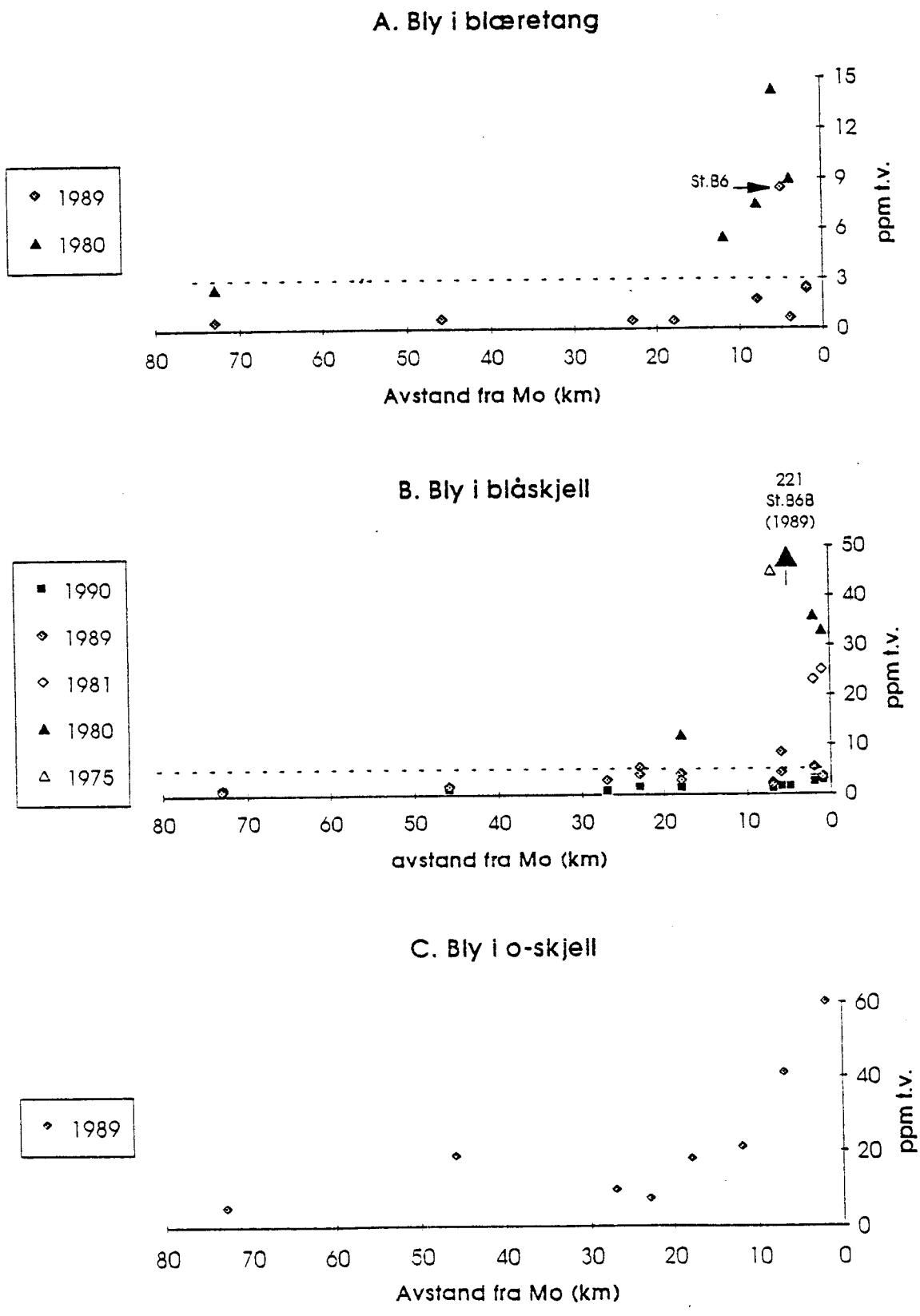



Fig. 2 Variation in lead concentrations (ppm d.w.) in bladder wrack (A), blue mussel (B) and horse mussel (C). The dashed line indicates suggested upper 'background' level

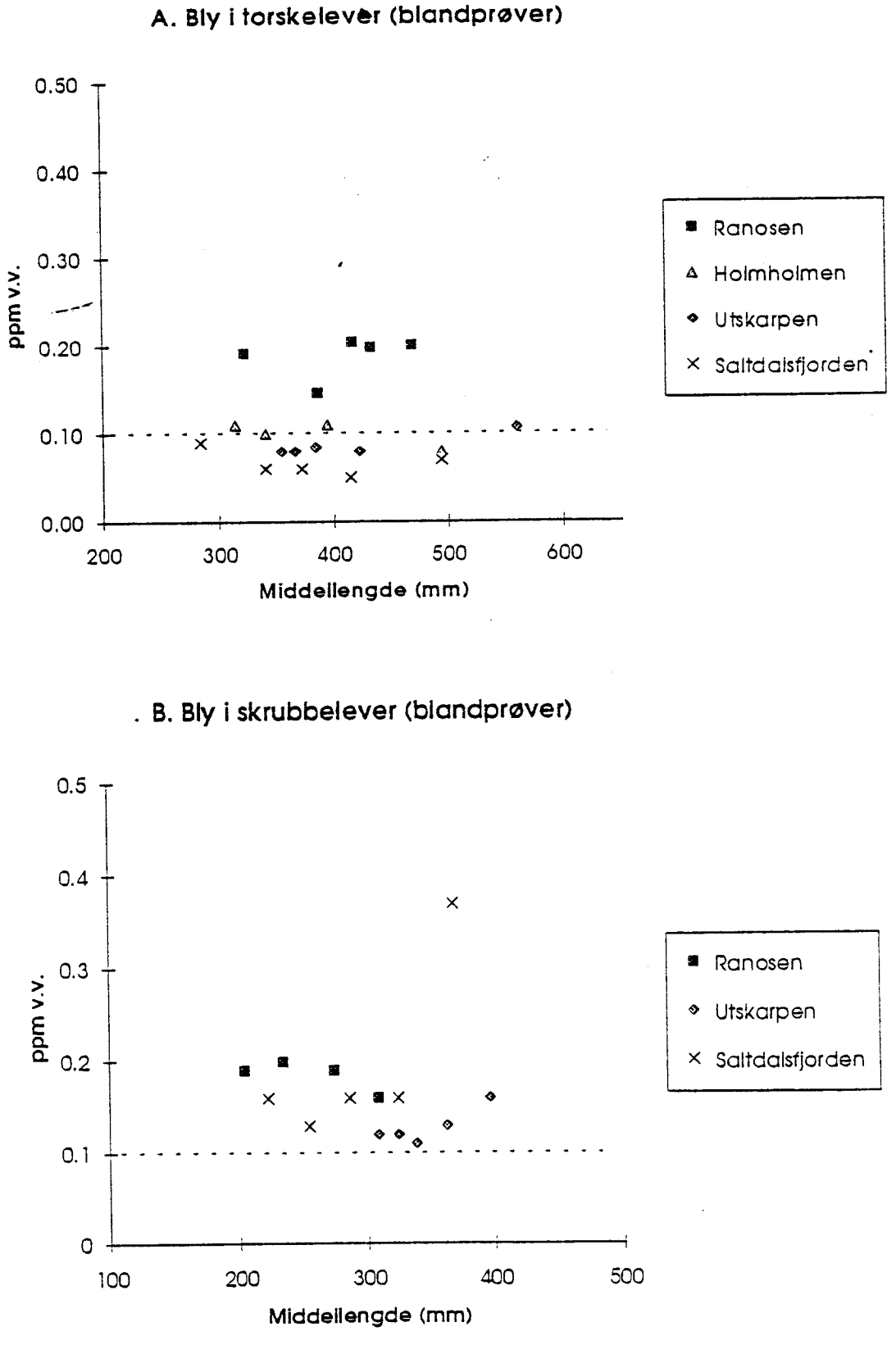
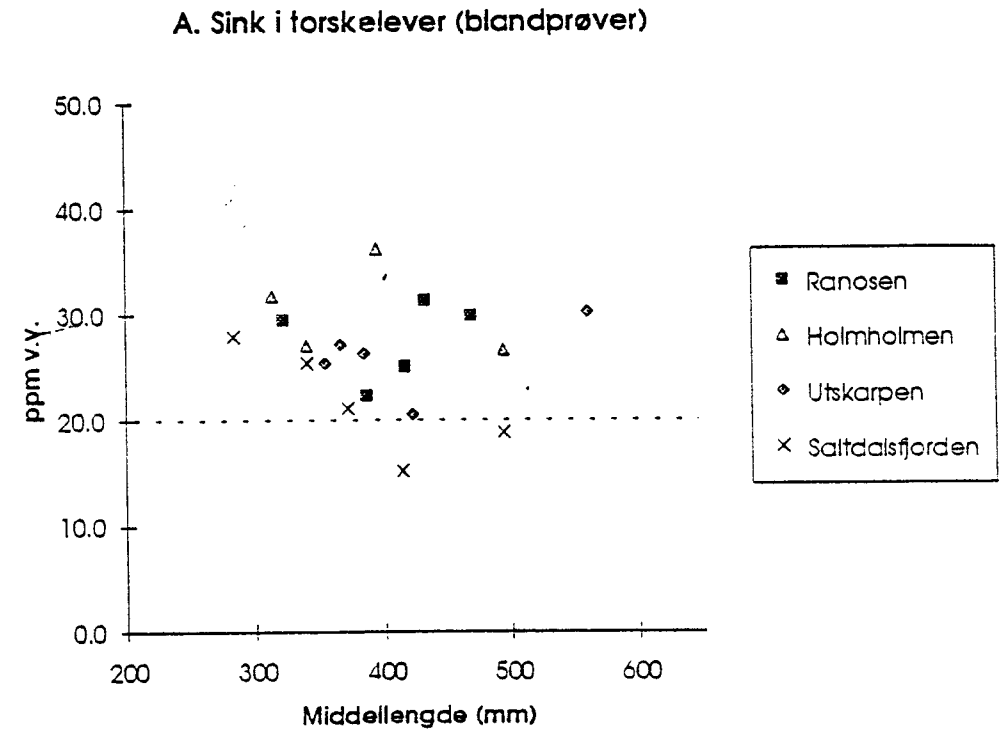



Fig. 3 Variation in lead concentrations (ppm w.w.) in cod and flounder liver (A og B). The dashed line indicates suggested upper 'background' level. The squares were considered the most effected and the X's were samples from a reference fjord



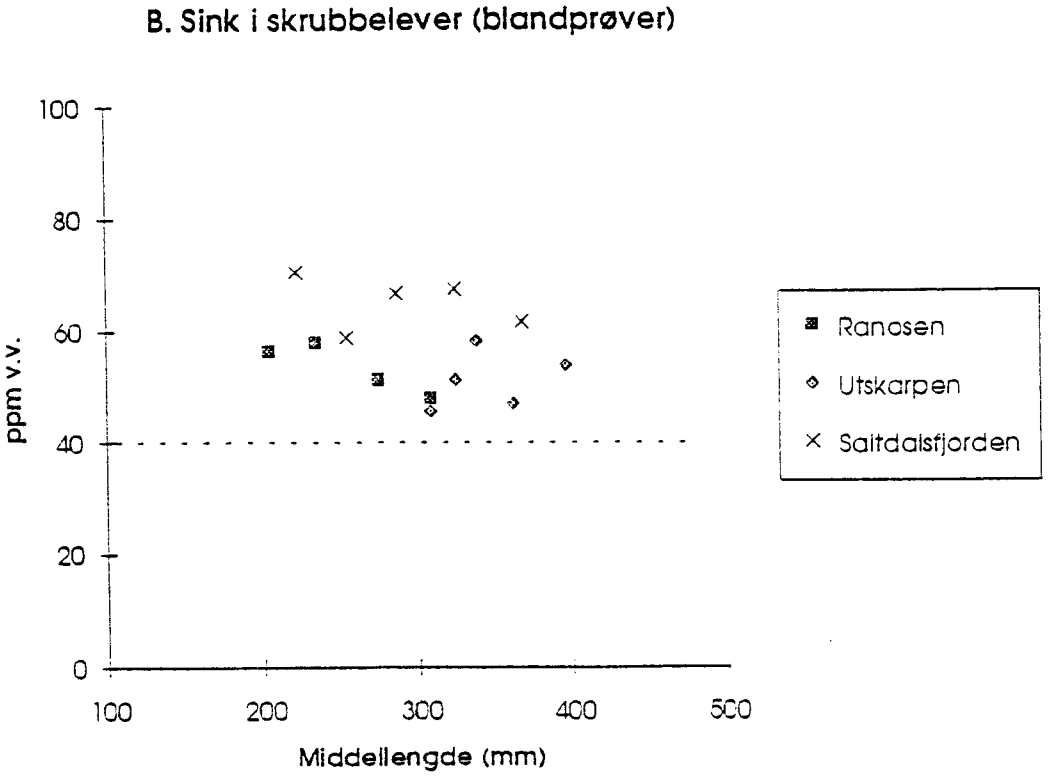




Fig. 4 Variation in zinc concentrations (ppm w.w.) in cod and flounder liver (A og B). The dashed line indicates suggested upper 'background' level. The squares were considered the most effected and the X's were samples from a reference fjord

Table 3

Overconcentrations of metals in seawater (unfiltered) and mussel (from Knutzen and Skei, 1991).

| matrix | station | Hg | Cd | Pb | Zn | Cu |
|---|---|---|---|---|---|---|
| seawater | B2 | 50 | 240 | 260 | 3200 | 50 |
| | B4 | 2 | 22 | 24 | 230 | 9 |
| | B7 | 1.5 | 16 | 14 | 180 | 7 |
| blue mussel | B2 | 7 | 26 | 17 | 3.5 | <1 |
| | B4 | 3.5 | 23 | 11 | 4 | <1 |
| | B7 | 2 | 18 | 7 | 3 | <1 |

## 5. References

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## ANNEX

Example data for illustrating the use of confidence intervals or confidence bands.

| ROW | A | B | X | B*4 | B*16 | B+0.02 | B+0.10 |
|---|---|---|---|---|---|---|---|
| 1 | 0.06 | 0.07 | 0.13 | 0.07 | 0.07 | 0.09 | 0.13 |
| 2 | 0.05 | 0.12 | 0.18 | 0.12 | 0.12 | 0.14 | 0.18 |
| 3 | 0.07 | 0.02 | 0.08 | 0.02 | 0.02 | 0.04 | 0.08 |
| 4 | 0.01 | 0.18 | 0.24 | 0.18 | 0.18 | 0.20 | 0.24 |
| 5 | 0.06 | 0.11 | 0.17 | 0.11 | 0.11 | 0.13 | 0.17 |
| 6 | | | | 0.07 | 0.07 | | |
| 7 | | | | 0.12 | 0.12 | | |
| 8 | | | | 0.02 | 0.02 | | |
| 9 | | | | 0.18 | 0.18 | | |
| 10 | | | | 0.11 | 0.11 | | |
| 11 | | | | 0.07 | 0.07 | | |
| 12 | | | | 0.12 | 0.12 | | |
| 13 | | | | 0.02 | 0.02 | | |
| 14 | | | | 0.18 | 0.18 | | |
| 15 | | | | 0.11 | 0.11 | | |
| 16 | | | | 0.07 | 0.07 | | |
| 17 | | | | 0.12 | 0.12 | | |
| 18 | | | | 0.02 | 0.02 | | |
| 19 | | | | 0.18 | 0.18 | | |
| 20 | | | | 0.11 | 0.11 | | |
| 21 | | | | | 0.07 | | |
| 22 | | | | | 0.12 | | |
| 23 | | | | | 0.02 | | |
| 24 | | | | | 0.18 | | |
| 25 | | | | | 0.11 | | |
| 26 | | | | | 0.07 | | |
| 27 | | | | | 0.12 | | |
| 28 | | | | | 0.02 | | |
| 29 | | | | | 0.18 | | |
| 30 | | | | | 0.11 | | |
| 31 | | | | | 0.07 | | |
| 32 | | | | | 0.12 | | |
| 33 | | | | | 0.02 | | |
| 34 | | | | | 0.18 | | |
| 35 | | | | | 0.11 | | |
| 36 | | | | | 0.07 | | |
| 37 | | | | | 0.12 | | |
| 38 | | | | | 0.02 | | |
| 39 | | | | | 0.18 | | |
| 40 | | | | | 0.11 | | |

# AND SO... WHAT ACTION DO WE TAKE?

by

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## 1. Regulatory action

Most of the regulatory action is issued by the Norwegian Pollution Control Authority (SFT). Examples of recent action is given in Table 1. Even though action has been taken it has not always been effective enough. This has been revealed through monitoring. For example DDT has been prohibited since 1970 but still high concentrations are found in the Sørfjord region (Fig. 1). DDT containing insecticide was commonly used in the fruit orchard farms of the fjord area. Are the farmers still using the prohibited chemical? Is there still large quantities in the soil that gradually are being leached into the sea?

Restriction on the use of TBT has been applied since 1989, however, a team of Scottish scientists using imposex tests in dog whelk (Nucella lapillus) found signs of elevated levels (Fig. 2). TBT has been prohibited since 1989 with the exception of boats over 25 m, aluminium boats, or light metal structures below the water line. Is the legislation strict enough? Is it too early to see the effects? Is there any question as to the validity of the tests?

The concentrations of certain contaminants in nine fjord areas are so high that The Norwegian Food Control Authority (SNT) have issued restrictions on the sale and warnings on the consumption of certain seafood in these areas (per.com., K. Færder, SNT). For example, the warnings for Sørfjord and adjacent Hardangerfjord are: not to eat mussels or flounder liver from the Sørfjord, eat only "moderate" quantities of mussels from the Hardangerfjord, do not eat more than two meals of "bottom dwelling" fish per week and not more than one meal per week of cod liver from Sørfjord.

## 2 Remedial action

Skei (1992) argues that as the discharge of contaminants is reduced (through legislative control, increased public awareness, etc.) there will be a greater problem of secondary pollution, i.e. the flux of contaminants from contaminated sediment/waste-deposits into the water column.

There are five primary ways in dealing with this (cf., Skei, 1992):

- **dredging** and disposal of the dredged material in a confined area;
- **incineration** (in cases of small amount and high toxicity);
- various **capping** techniques (sand, fly ash, cement, membranes, etc.);
- **fixation** by adding of chemicals (chemical immobilization/ solidification);

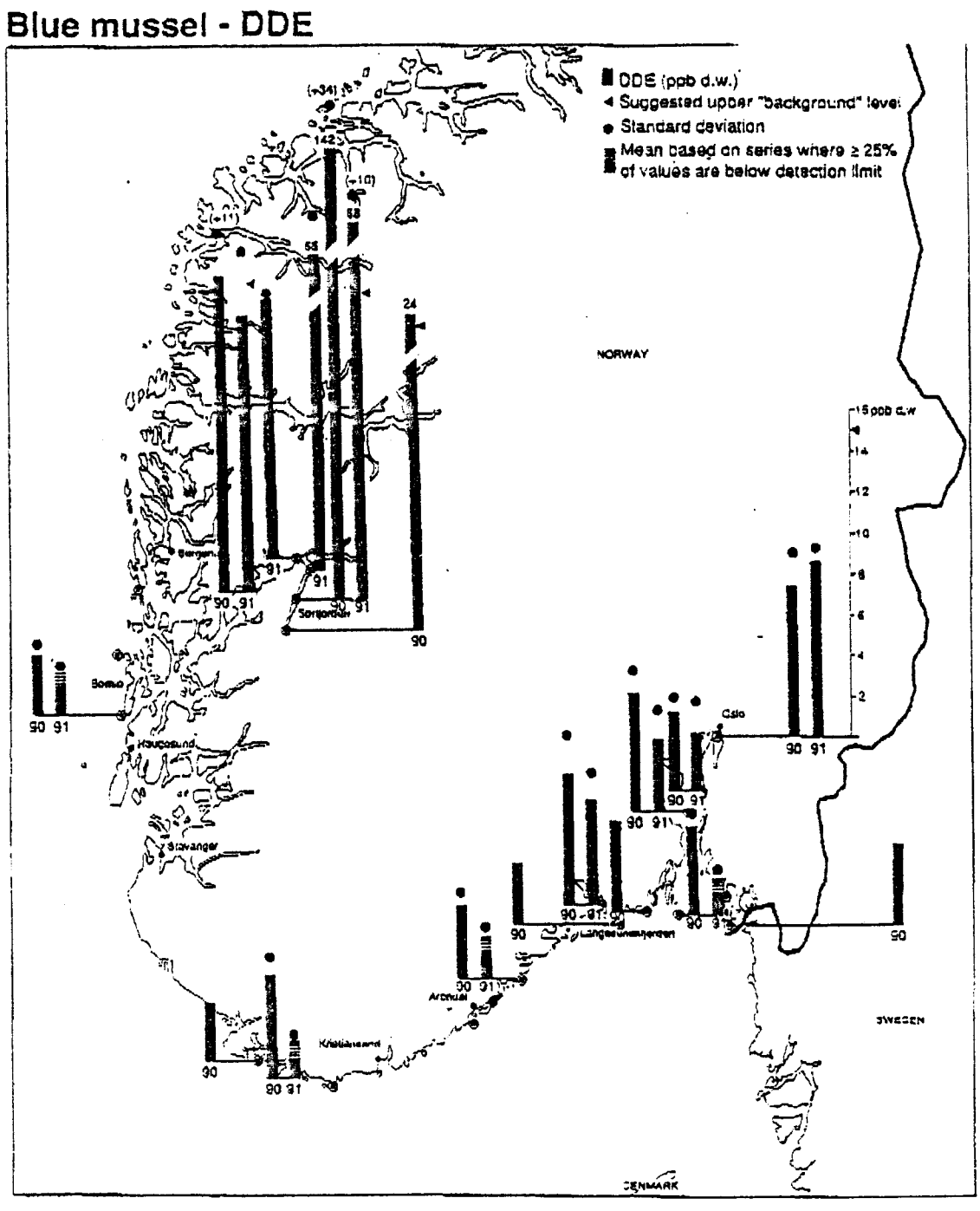


Fig. 1 Concentration of DDE in blue mussels from Sørfjord region (from Green, 1993)

- **reduction of mobility** of critical compounds by changes of pH/redox conditions.

Each method has its pros and cons. The side effects of remedial or regulatory action may also have positive side effects like reducing the particle load, reducing sedimentation and increasing light conditions.

In the Minimata Bay where 150 tonnes of mercury were discharged during the 1950's, the dredging alternative was chosen at a cost of 92 million US dollars (1978) where the polluter was obliged to pay 65% of the cost (Ishikawa and Ikegaki, 1980).

Table 1

Examples of action taken by the Norwegian State Pollution Control Authority (SFT) to certain contaminants (SFT, 1992).

| Contaminant | Source/product | Action taken |
|---|---|---|
| Hg | Amalgam, thermometers, batteries | Obligatory amalgam-cleaning treatment at dental offices by 1992. Sale of mercury thermometers prohibited |
| Cd | Sea structure anodes, batteries, plastic stabilizers and dyes, artificial fertilizers | Controlled deposit of wasted rechargeable cadmium-batteries. Reduce the use of cadmium in plastic products |
| Cu | Antifouling paints and treatment of fishing nets | Regulation og use and discharge |
| Zn | Sea structure anodes | Prohibited use of zinc-containing anodes by 1995 |
| Pb | Traffic, batteries, gun-shot | Considering increase tax on lead-containing gasoline. Improve control of deposit of wasted batteries. Restrictions on use of lead-shot |
| Tin-organic compounds | Antifouling paint and fishing nets. Additive for plastics and wood treatment products | Reduce discharge of tin containing compounds |
| DDT | insecticides | Prohibited since 1970 |

The actual process of dredging may only aggrevate the problem by mobilizing contaminants at least on a short term scale (Förstner, 1987). Furthermore, there is a risk of seepage of contaminants into the water table or sea at the new relocation dump site. In the Sørfjorden, West Norway, the capping alternative was chosen (Skei, 1992). The inner fjord seabed had concentrations exceeding 10% Zn and 0.9% Pb (Figs 3 and 4). The capping incorporated three techniques: a permeable membrane, a 30 cm layer of shell sand and a piled wall enclosing the most contaminated area. The permeable membrane would theoretically allow some diffusion of gases and, hence prevent

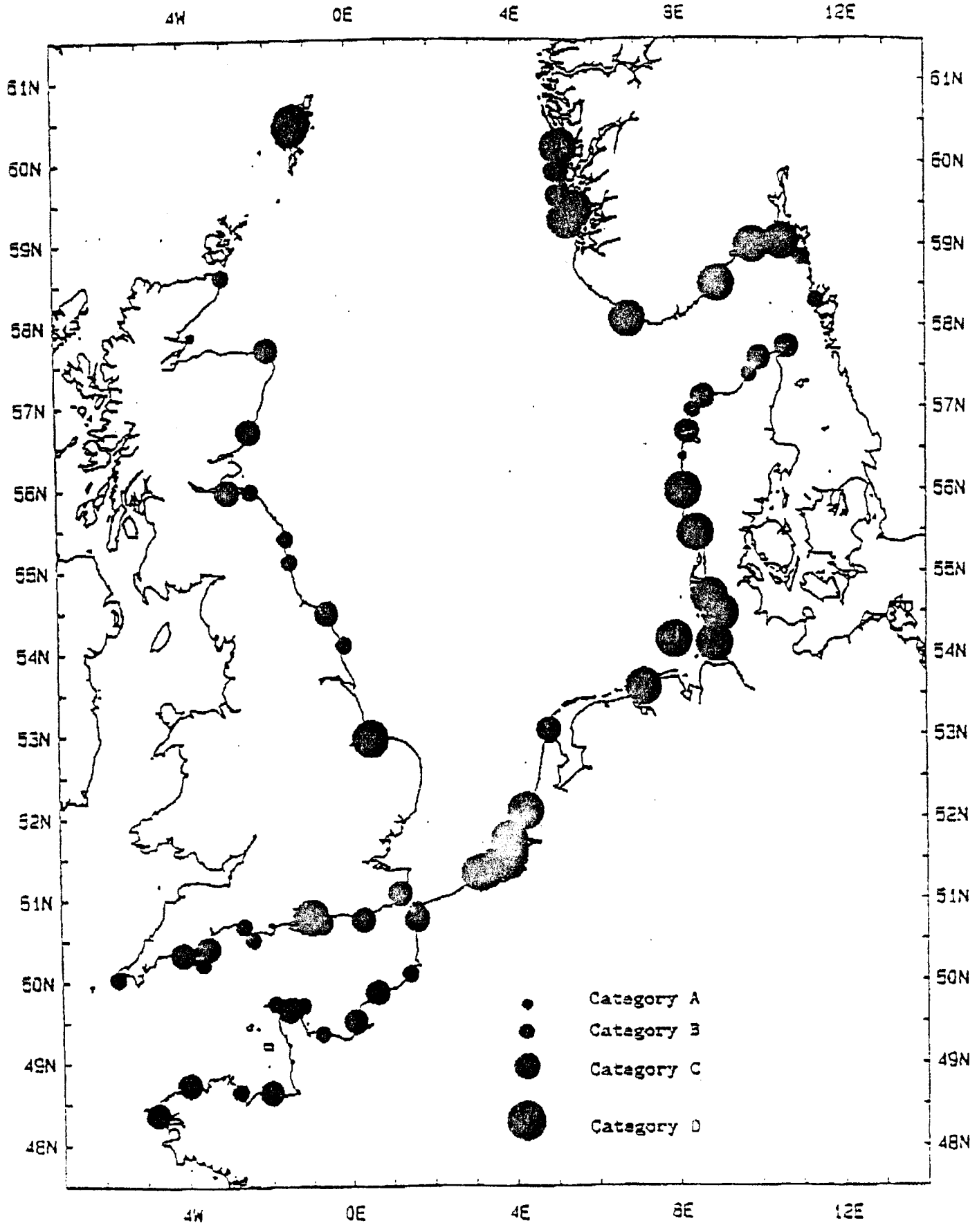


Fig. 2 Levels of TBT based on the imposex test (from Harding et al., 1992)

gas formation, but would not lead to a large flux of contaminants. The shell sand layer would allow for diffusion better than more clayey material, and it is thick enough to allow normal recolonization of the area, protect the membrane and serve as an absorption zone for any contaminants that might have leaked through the membrane. The wall protects the cap from erosion by storms of wave action.

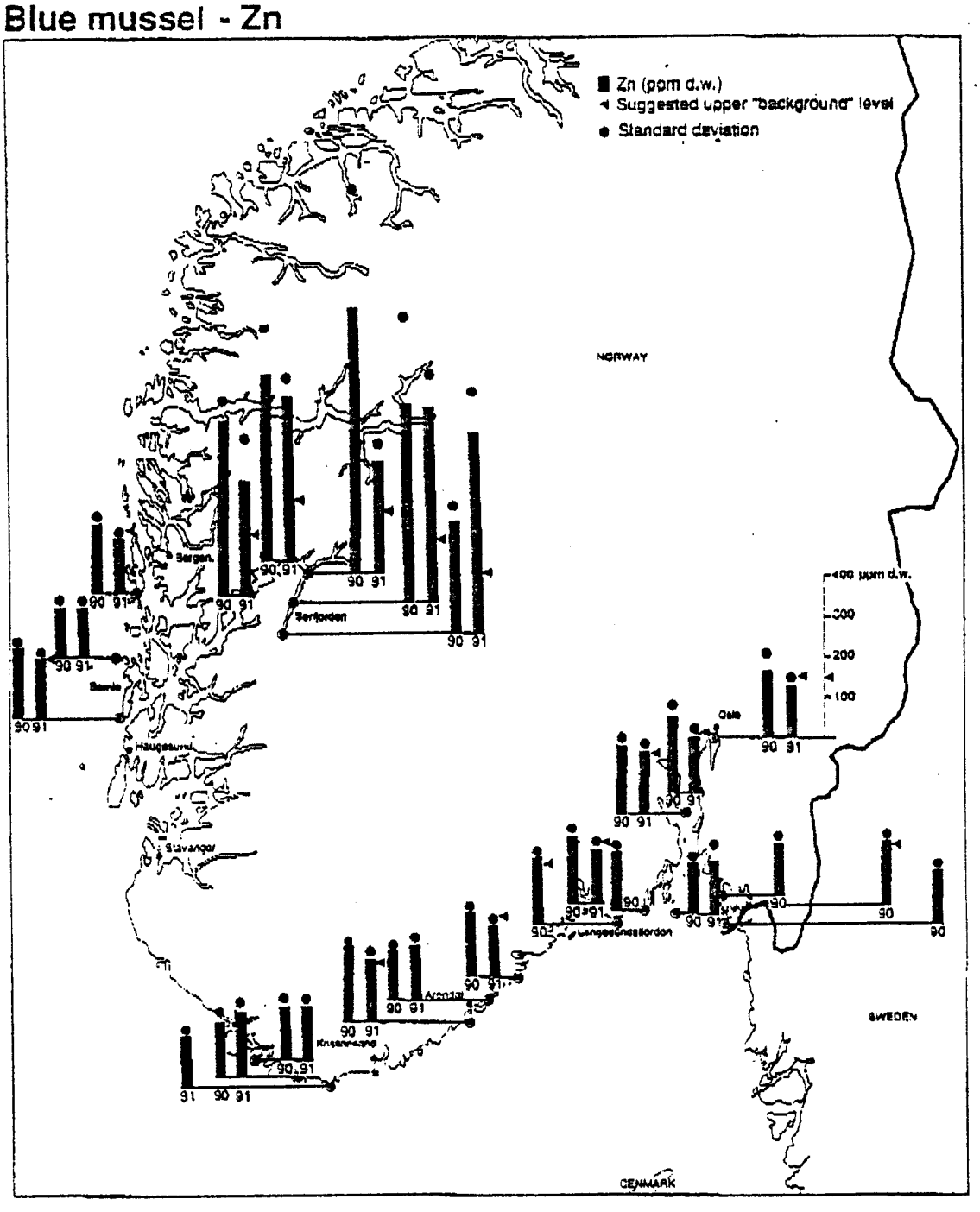


Fig. 3 Concentration of Zn in blue mussel from the Sorjord area (from Green, 1993)

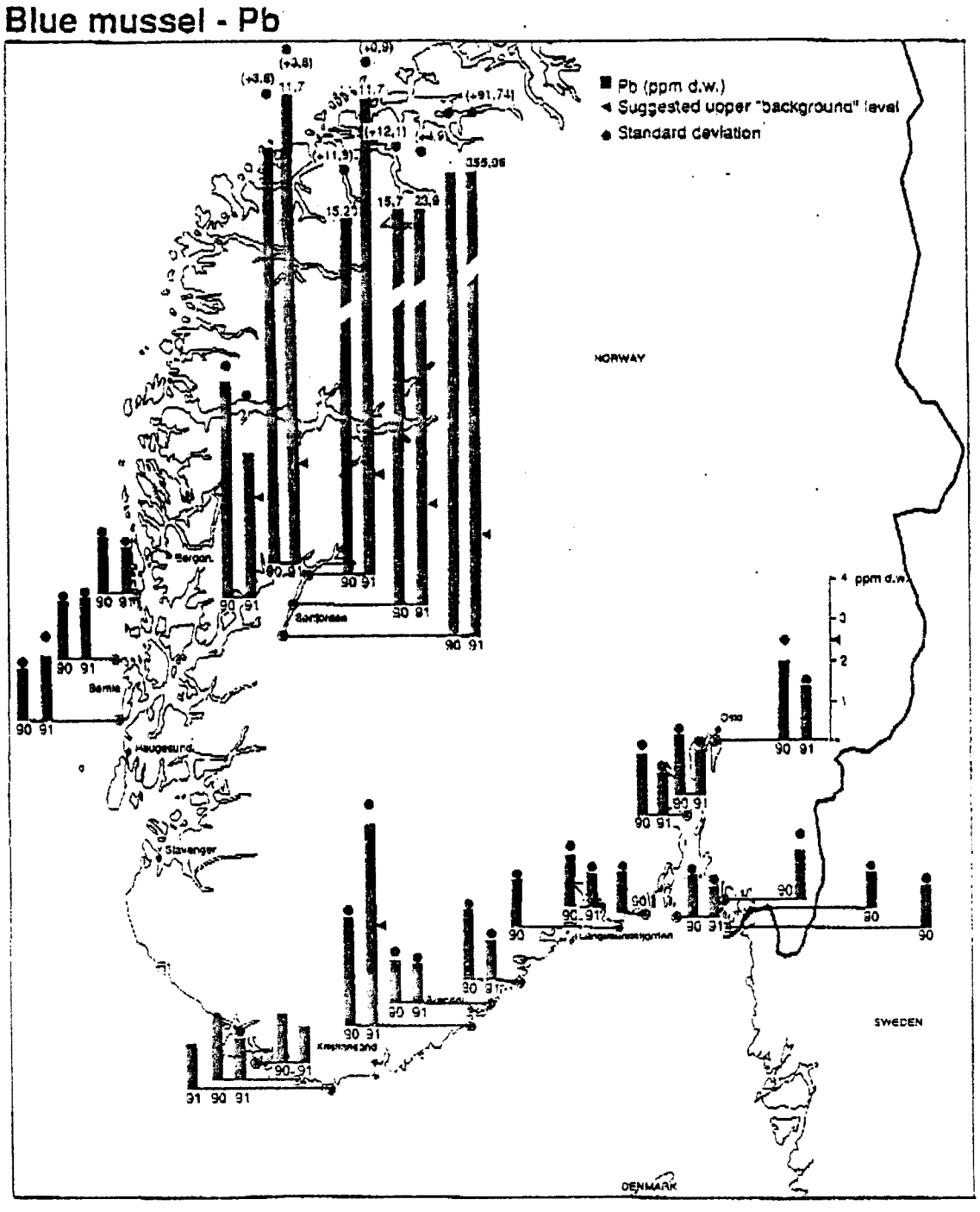


Fig. 4 Concentration of Pb in blue mussel from the Sorjord area (from Green, 1993)

## 3 Precautionary principle

The current debate around the precautionary principle strikes at the heart of the enigma for improved local and global environmental quality. The traditional approach to pollution control has been not to impose regulation without sufficient scientific evidence that the emissions are harmful. The

precautionary approach is to impose regulation without sufficient evidence of harmful effects. An important example of the latter was the agreement for 50% reduction in nutrient inputs to the North Sea agreed at the Ministerial Conference for the North Sea in 1987 and reconfirmed in 1990. This political decision has little or no scientific foundation but obviously infers enormous consequences especially to industries, the agronomical sector and municipalities.

Peterman and M'Gonigle (1992) liken these approaches to the judicial equivalents that a man is innocent until proven guilty (the traditional approach) or he is guilty until proven innocent (the precautionary principle). In the former case we attempt to reduce the number of innocent imprisoned but face the problem that more guilty people go free. The statistical parallel of this, is minimizing the chance of committing a Type I error (rejecting null hypothesis when it is true) but consequently we increase the chance of committing a Type II error (accepting the null hypothesis when it is false). We cannot have one without the other. Hence, in the extreme application of the precautionary principle we maximize a Type I error and minimize a Type II error which would have the consequence of putting considerable burden on industry to show proof of innocence. Whereas in the traditional approach, "environmentalists" have often enormous problems to show proof of guilt even though the effects are apparent. The traditional approach has lead to what Peterman and M'Gonigle have termed a **permissive regulatory system**. By enhancing the chances of committing a Type II error we may experience detrimental and perhaps irreversible effects far more serious and more encompassing than the original problem (reduction in fish stocks, acid rain, human health risk).

Peterman and M'Gonigle (1992) further argue that the problem encountered by these approaches underline the need for improved regulatory procedures. This is especially relevant, considering the increasing number and severity of surprise effects of human activities and the difficulty in demonstrating causal effects scientifically and hence, legally. One alternative, they point out, is the reduction in the use of potentially harmful substances. Examples of this are the Oslo Commission's 'Prior Justification Procedure' and the American "Massachusetts Toxics Use Reduction Act' which are remedies more oriented towards the production processes than end-of-pipe emissions. This new alternative they term **preventative design**.

But monitoring programs focus on end-of-pipe effects and hence, it is important that investigators (scientist and policy makers alike) have a clear concept idea of the statistical power of the methods they employ. They should know what is the minimum change that is detectable by the method chosen. Peterman and M'Gonigle (1992) point out that conclusion about safety or no effect are associated with an agreed level of statistical power.

## 4. Improvement of monitoring design

During the course of this workshop there has been considerable emphasis placed on the importance of selecting goals, agreeing on and following guidelines and rational approach to assessment of the data material.

It is evident that we do not have the resources to monitor just for the sake of monitoring. There is an ever increasing competition for limited funds compounded by the increasing costs of fieldwork and chemical analyses in order to obtain high quality data. It is paramount that we have a clear

grasp of the purposes of the work and realize the statistical power of our methods in order to know what changes are detectable. It is perhaps important at this point to review some of the positive aspects of monitoring (from J.A. Berge, pers.comm):

- Can be directly related to known/accepted limits such as for health risk or "reference"
- May be directly related to known changes in inputs (eg., local discharges)
- Can be related to other matrices (sediment, seawater)
- May be used to trace a source of contamination by establishing a gradient
- May create a basis for immediate action
- May give authorities important feedback on consequences of remedial action

Improved monitoring is needed and the guiding light should be **KIS** - Keep it Simple! but do it thoroughly. It is my impression that ambition level of many monitoring programmes generally far exceeds the ability to reach the goals. This goes for the North Sea area as well as the Mediterranean. A simple robust programme carried out by all participating countries not only provides a good data base (high quality, easier assessments) but while doing so necessitates an improved infrastructure to which all countries can actively participate.

One step to simplify spatial distribution of contaminants would be to reduce the monitoring species to a few that are widely distributed such as the Mediterranean mussel (Mytilus galloprovincialis) and red mullet (Mullus barbatus). It is self evident that the large number of species monitored as shown in Table 2 only compound the problem of assessment. However, with just a few selected species some areas may go unmonitored.

The North Sea Task Force - Quality Status Report (SNTF - QSR) has been suggested as a good way to assess a particular region. The principle of a concentrated short-term effort has an appealing tone to policy makers and it tends to key the participants for extra effort. The goals of the NSTF required the knowledge of many different sciences and hence, was bound to be more wholistic touching on processes that steer the ecosystem. This approach however, does require a fairly good infrastructure and positive international atmosphere. These problems may be partly circumvented by incorporating fewer laboratories/institutions covering larger regions and devoting more effort to ensure that these have been intercalibrated and use GLP.

## 5. The future of monitoring in biota

Even with stricter controls on the emissions of pollutants it is evident that some degree of monitoring will be necessary in order to control regulatory action and accommodate public pressure. However, it is unlikely that monitoring alone will be the only prerequisite for regulatory action.

There is definitely a need for better data on emissions; both on the amount and type and how these vary in time and space. Without this information it is nearly impossible to convince anyone of dose response relationships.

## Table 2

Some Mediterranean organisms used in contaminant monitoring (from Jeftić et al., 1990). H=Hg (Tab.19), P=PCB, D=DDT or derivatives (Tab.24). Monitoring organisms used in MED POL Phase II are shown in bold print (Jeftić et al., 1990; UNEP, 1989). The different regions of the Mediterranean are indicated in roman numerals.

| Species | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Engraulis encrasicolus (European anchovy) | fish | | H | | H | | H | | | | | | |
| Mullus barbatus (red mullet) | fish | | HPD | H | HPD | HPD | H | H | HPD | HPD | PD | | H |
| Mullus surmuletus (mullet) | fish | | H | | PD | PD | | | | H | | H | |
| Mytilus galloprovincialis (Mediterranean mussel) | biv. | | HPD | | HPD | HPD | H | | HPD | H | | H | H |
| Nephrops norvegicus (Norwegian lobster) | crus. | | H | | HP | | H | | | | | | |
| Sarda sarda | | | H | | | | | | | | | | |
| Thunnus thynnus (tuna) | fish | | HD | | H | | | | HD | | | H | |
| Xiphias gladius (swordfish) | fish | | H | H | | | | | H | | | | |
| Perna perna | | | | | | | | | | | | | |
| Thunnus alalunga | fish | | | | | | H | | | | | | |
| Lithophaga lithophaga | biv. | | | | | | | H | | | | | |
| Trachurus mediterraneus (mackerel group) | fish | | | | | | | H | H | | H | | H |
| Carcinus mediterraneus | crus. | | PD | | | PD | | | HD | D | PD | | |
| Merluccius merluccus (European hake) | fish | | | | | | | | H | H | | | H |
| Mugil auratus (mullet) | fish | | | | | | | | H | H | | | |
| Mugil cephalus (mullet) | fish | | | | | | | | H | | | | |
| Penaeus kerathurus (swimming prawn) | crus. | | | | | | | | H | H | | | |
| Boops salpa (bogue?) | fish | | | | | | | | | H | | | |
| Upenaeus moluccensis | | | | | | | | | | H | H | | |
| Boops boops (bogue) | fish | | | | | | | | | H | | | |
| Dentex dentex (common dentex) | fish | | | | | | | | | H | | | |
| Dentex gibbosus (dentex) | fish | | | | | | | | | H | | | |
| Donax trunculus | biv. | | | | | | | | | H | | | |
| Epinephelus aenus | fish | | | | | | | | | H | | | |
| Pagellus acarne (pandora) | fish | | | | | | | | | H | | | |
| Pagellus erythrinus (common pandora) | fish | | | | | | | | | H | | | H |
| Surida undosquamis | | | | | | | | | | | H | | |
| Sphyraena sphyraena | fish | | | | | | | | | | H | | |
| Parapenaeus longirostris | crus. | | D | | | | | | PD | PD | PD | | H |
| Diplodus sargus (seabream) | fish | | | | | | | | | | H? | | |
| Lithognatus mormyrus (stripped seabream) | fish | | | | | | | | | | H? | | |
| Mactra corallina corallina | biv. | | | | | | | | | | H? | | |
| Mactra corallina stultorum | biv. | | | | | | | | | | H? | | |
| Arcularia gibbosula | gas. | | | | | | | | | | H? | | |
| Macropipus depurator | crus. | | | | | | | | | | H? | | |
| Sardina sp. (pilchard) | fish | | | | | | | | | | H? | | |

There is also a need to know what form these pollutants are when they come into contact with the target organism. The water phase of contaminants is important for uptake in seaweeds, whereas the particle phase is important for mussels and concentrations in prey species for fish is a determining factor for most fish species.

We need to know more about the biochemistry of contaminants in the organisms we monitor; accumulation rates, storage/release, mobility under different life-stages or conditions. There is also a pressing need to know what effects these changes have on the organism (behavioural studies, $LC_{50}$, MRL etc.).

On a community level it is important to know how the contaminant functions at different tropic levels in the food chain.

Though it is often difficult to observe effects on a community level the chemical/behavioural study of selected smaller "key" monitoring species may be a far better approach.

At the end of the day the quality of our environment is not the sole responsibility of politicians, scientists or activists. What standard are we, as a population, willing to accept?

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