MONITORING WATER QUALITY IN THE FUTURE

VOLUME 3: BIOMONITORING

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"The paradox between attempting to analyze "too much" information and still not having enough - although frustrating - should not be discouraging, for this will lead to eventual acknowledgement by our administrators that complex problems do not have simple solutions. This is progress.
Biology without pollution is intricate, exacting and dynamic, while biology compounded by a single source of pollution may at times be overwhelming. Thus, biology with multiple-variable pollutants demands extraordinary insight as well as foresight into placing the problems into perceptive."

Salo, 1977 [1]

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PREFACE

Monitoring can be performed in many ways. It is known that the Member States of the European Union (EU) use different approaches in monitoring water quality. The project "Monitoring water quality in the future" was initiated in order to make recommendations concerning standardization, optimization, and organization of monitoring activities in the European Union. In the framework of this project five reports have been produced on methods and strategies for monitoring of water quality, with emphasis on mixture toxicity parameters, and on organizational aspects of monitoring on a European scale.

The project was co-funded by the European Commission, Directorate-General for Environment, Nuclear Safety and Civil Protection, Directorate for Nuclear Safety, Civil Protection and Industry, Environmental Control of Industrial Installations and Emission Division (CEC, DG XI, C5), the Netherlands Ministry of Housing, Spatial Planning and the Environment, Directorate-General for Environmental Protection, Directorate for Chemicals, External Safety and Radiation Protection (VROM/DGM-SVS) and the Netherlands Ministry of Transport, Public Works and Water Management, the Institute for Inland Water Management and Waste Water Treatment (RIZA). The project is carried out by representatives of VROM/DGM-SVS, RIZA, the International Centre of Water Studies (ICWS), the Research Institute of Toxicology (RITOX) of the University of Utrecht, the National Institute of Public Health and Environmental Protection (RIVM), AquaSense Consultants and DELFT HYDRAULICS. The overall project was supervised by a steering committee with the following members:

- Prof. Dr. C.J. van Leeuwen, Chairman (VROM/DGM-SVS);
- Ir. M. Hof (VROM/DGM-SVS);
- Ir. A. Roos (VROM/DGM-Directorate for Water Supply, Water and Agriculture (DWL));
- Mr. Ir. J. Vennekens (CEC, DG XI, C5);
- Dr. E. McDonnell/Ing. R. Goud (CEC, DG XI, C5);
- Ir. P.B.M. Stortelder (RIZA);
- Drs. D. de Zwart/Dr. W. Slooff (RIVM);
- Dr. P. Stoks (Water Transport Company Rhine-Kennemerland (WRK)).

This report, Volume 3: Biomonitoring, has been prepared by Drs. D. de Zwart (RIVM) and Dr. W. Slooff/Dr. J. Notenboom (RIVM; project leaders).

In order to broaden the basis of the overall project the several reports were peer reviewed by international experts on the concerning subject. This report was peer reviewed by:

- Dr. P. Logan, National Rivers Authority, Reading, UK
- Prof. Dr. G. Persoone, State University of Ghent, Laboratory for biological research in aquatic pollution, Ghent, Belgium

Their constructive criticism is greatly acknowledged.

This report, volume 3 deals with the options for application of biomonitoring techniques. Volume 3 has been produced under the supervision of a special project group consisting of delegates from VROM/DGM-SVS, RIZA. The delegates are:

- Drs. J. Botterweg (RWS/RIZA/Emissions)
- Drs. C. van de Guchte (RWS/RIZA/Ecotoxicology)
- Ir. M. Hof (VROM/DGM-SVS)
- Drs. I. Akkerman (RWS/National Institute for Coastal and Marine Management (RIKZ))

SUMMARY

INTRODUCTION

Monitoring of the water quality can be performed in many ways depending on the reasons and the objectives of a particular monitoring programme. In this project the following (routine) water quality monitoring objectives are used as a starting point with a focus on fresh surface water and effluent:

- identification of state (concentration) and trends;
- identification of mass flow (loads);
- testing of compliance with standards and classifications;
- early warning and detection.

Due to the enormous number of potentially polluting substances, a chemical-specific approach is insufficient to provide the information to protect surface waters from pollution effects. Therefore it is essential to develop chemical and biological tools to signal changes in and control the water quality.

In general terms the problems with the existing approach concern effective and efficient monitoring strategies. In 1993 the project "Monitoring water quality in the future" started in order to address these problems which will only increase in the future. In the framework of this project five reports have been produced, focusing on:

- Chemical Monitoring (Volume 1);
- Mixture toxicity parameters (Volume 2);
- Biomonitoring (Volume 3);
- Monitoring strategies for complex mixtures (Volume 4);
- Organizational aspects (Volume 5).

The specific objectives were to produce concise reviews of methods to signal changes in and control water quality (Volumes 1-3), to give a review of testing strategies for complex mixtures of chemical substances which can give more complete information at less costs (Volume 4) and to review existing practices and make recommendations concerning standardization, optimization and organization of monitoring activities in the European Union, with a focus on effectiveness and efficiency (Volume 5). In an executive summary overall recommendations are also made by drawing these together from the individual studies.

The present report (Volume 3) includes a short description of existing biomonitoring methodologies and measurement strategies, as well as a discussion on possibilities, developments, limitations and financial consequences.

BIOMONITORING

The introduction of biological variables in environmental monitoring activities added the terms biomonitoring or biological monitoring to our vocabulary. Different interpretations of what is considered to be a biological variable or biological observation caused a lot of confusion on which activities belong to biomonitoring. In this report the following names and definitions will be adopted for the different aspects of biomonitoring:

- **Bioaccumulation monitoring** for measurements on chemical concentrations in biological material.
- **Toxicity monitoring** for measurements on the direct biomolecular and physiological responses of individual organisms towards toxicants in an experimental setup, including bioassays and biological early warning systems.
- **Ecosystem monitoring** for measurements on the integrity of ecosystems which is in many cases related to all kinds of environmental perturbations. This type of biomonitoring will include inventories on species composition, density, diversity, availability of indicator species, rates of basic ecological processes, etc.

The word **integrated monitoring** will be reserved for coordinated monitoring activities comprising chemical and biological measurements in a variety of environmental media.

The present report will only deal with topics concerning toxicity monitoring and ecosystem monitoring. Bioaccumulation monitoring will be discussed in Volume 1 "Chemical Monitoring" of the related series of reports, while the topic of putting together an integrated monitoring system is reserved for Volume 4.

Using biomonitoring techniques, there are distinct differences in objective and operational strategy between:

- toxicity monitoring of effluents
- toxicity monitoring in receiving water bodies
- ecosystem response related monitoring in ambient waters

The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring. Firstly these methods measure effects in which the bioavailability of the compound(s) of interest is integrated with the concentration of the compounds and their intrinsic toxicity. Secondly, most biological measurements form the only way of integrating the effects on a large number of individual and interactive processes. Often biomonitoring methods are cheaper, more precise and more sensitive than chemical analysis in detecting adverse conditions in the environment. This is due to the fact that the biological response is very integrative and accumulative in nature, especially at the higher levels of biological organization. This may lead to a reduction of the number of measurements both in space and time.

A disadvantage of biological effect measurements is that sometimes it is very difficult to relate the observed effects to specific aspects of pollution. In view of the present chemical oriented pollution abatement policies and to reveal chemical specific problems, it is clear that biological effect analysis will never totally replace chemical analysis. However, in some situations the number of standard chemical analyses can be reduced, by allowing bioeffects to trigger chemical analysis (integrated monitoring), thus buying time for more elaborate analytical procedures.

Once it has been established that biomonitoring techniques provide valuable information to the solution of an environmental problem, suitable biological variables should be selected. The context in which these variables will be measured should be clearly indicated. Not all biological variables are equally fit for serving in a monitoring programme. Their suitability can be evaluated by checking against a number of requirements. Some of these are related to scientific and fundamental aspects, while others relate to efficiency, costs, logistic and policy aspects.
Some of the requirements for monitoring variables are mutually exclusive. It is generally accepted that ecological relevance is inversely related to criteria like sensitivity and specificity. Effects on a higher level of biological organisation (population, community, etc.) are highly biologically relevant, but may be insensitive (due to the availability of alternative pathways in an ecosystem, and complex regulating mechanisms) and are normally a-specific in their response to many perturbations. For biomolecular and physiological effects, the order of their compliance to the criteria mentioned above will be reversed.

Variables with a response that is restricted to only one type or group of pollutants or a specific type of perturbation are generally associated with processes having a low rank in the chain of causality. These types of monitoring variables have a high problem/solution directed bio-indicative capacity. Due to their distinct relation to specific aspects of pollution, they can be fruitfully used for control. The indicative value of ecological endpoints on a higher level of integration is to be found in signalling trends in combined ecosystem performance. However, this type of evaluation, in general, lacks the possibility to direct counter-active measures. In many cases it will only reveal the need for process studies on the underlying causes.

The types of biomonitoring variables available for distinguished biomonitoring objectives are presented [after the Organisation for Economic Cooperation and Development (OECD)]. Many of these tests and observations are procedurally well documented in internationally accepted guidance documents and standards. However, the degrees of freedom in the design of ecotoxicity tests with respect to the selection of test organisms, test criteria and test circumstances are manyfold. Therefore, many research groups continuously produce an endless stream of new procedures, which may all be capable of revealing specific aspects of ecotoxicity for specific situations. As an indication for the design variety of toxicity tests and field observations for the freshwater environment alone, about 120 different laboratory toxicity tests are presented in international literature, whereas about 100 different variables are given to describe community effects occurring in the field. Given the variety in monitoring objectives and biological variables, it will be evident that it is entirely impossible, within the scope of this report, to review all possible biomonitoring variables up to the level of species, processes and particular procedures. Pragmatically, only examples are given of variables and test for specific types of biomonitoring techniques in different environmental compartments.

Whatever data are produced, they are likely to be used for enforcement purposes and/or policy development. Both aspects may have legislative and economical implications. It is therefore vital that the data and the conclusions based on them are as free as possible of error. The production of reliable data for chemical safety assessment, requires the use of scientifically sound testing and monitoring procedures and the application of quality assurance in conducting tests and studies. Quality Assurance (QA) is a managerial concept intended to promote the reliability of data for use in risk assessment. Some requirements of quality assurance are briefly discussed.

Effluent toxicity monitoring can be applied for the following purposes:

- Testing and steering the progress of technology based improvement of effluent quality, to complement chemical specific assessment
- Permit compliance testing, provided that toxicological criteria are part of the permit formulation
- The prevention/reduction of effects occurring in receiving water bodies
- Early warning of calamities and accidental spills, provided that measures can be taken to contain the released toxicity
- The prediction of effects occurring in receiving water bodies

The first three of these objectives are strongly related to the **control function** of biomonitoring, while the following two objectives are mainly related to the **alarm** and the **prediction** function, respectively. In evaluating the quality of effluents for control and prediction purposes, it is generally accepted that a maximum of certainty should be attained within a minimum budget and time. For alarm purposes, however, timeliness is of more concern, while less certainty is required. The implications these deliberations have on the applied types of sampling, testing and evaluation strategies is discussed in detail from a conceptual point of view.

Ambient toxicity tests (i.e. toxicity tests on receiving waters and sediments) may be used in conjunction with effluent toxicity tests to provide additional valuable information. In particular, ambient tests may reveal or confirm the existence of toxic conditions in the receiving water, and may demonstrate the location of unknown toxic point-source or diffuse discharges. They may also be used to evaluate persistence, to evaluate the combined effects of multiple discharges, and to evaluate additivity, antagonism and synergism of effluents. Ambient toxicity testing mainly fulfils a **signalling function** for pollution control. Again the implications for the strategy design with respect to sampling site selection, sampling frequency and the selection of tests is discussed in detail.

An alternative to using toxicity tests with simple endpoints such as mortality, growth and reproduction to assess the environmental impact of an effluent is to conduct field surveys and analysis of the endogenous biota in the receiving water and to try and link the observed effects with the input of toxicity. However, it should always be realised that many more types of man induced or natural interferences than only the input and action of toxic compounds may be responsible for an observed degradation of the biological integrity of a given ecosystem. Ecosystem response monitoring can obviously also be performed with the sole objective of revealing the impacts of other than toxic stress. However, these applications fall beyond the scope of the present review.

As has been stated in the introduction of this report, as well as in the chapter introducing the concept of biomonitoring, the major objective of water pollution control is the safeguarding of the ecological integrity of a water system. To attain ecological integrity the combination of physical, chemical and biological characteristics should be favourable. Ecosystem monitoring should therefore be composed of the following types of measurements:

- Measurements on the **physical status** of the water body in terms of depth, shore development, substrate composition, flow, turbidity, temperature, canalization, mechanical disturbance, etc.
- Measurements on the **chemical status** of the water body in terms of concentrations of nutrients and salts, oxygen levels, pH and degradable organics, etc.
- Measurements on the **biological status** of a water body may involve quantitative and qualitative inventories of the incidence of biochemical or morphological deviations and diseases in individuals of particular species (eco-epidemiology), inventories of biological structure, and assessments of biological functioning. The majority of applied biological status evaluations are surveys on species composition.

It is discussed that both the physical and chemical status of a water body as part of the habitat for biological communities form the boundary conditions for biological status. This, so called, habitat evaluation identifies the possibilities for specific types of biota and ecological pathways to develop. As such, the availability of physico-chemical data and fundamental ecological insight are indispensable for setting standards and targets with respect to biological status (ecological objectives).

The discussions on the conceptual framework for measurement strategies in biomonitoring are followed by examples of biomonitoring schemes applied in a variety of countries throughout the world.

The different types of biomonitoring techniques are subsequently comparatively evaluated against the set of preformulated criteria for selection of appropriate (bio)monitoring variables. An attempt has been made to make an estimate of the capital and running costs per test or observation. However, it should be kept in mind, that the design of a monitoring network in terms of numbers and combination of tests is very much dependent on the local situation and the ultimate monitoring objective.

From the immense variety of biomonitoring variables being designed and applied for toxics control in the aquatic environment over the past few decades, it can be concluded that biomonitoring is generally considered to be a valuable source of pollution information. Since monitoring information requirements and monitoring objectives are very situation specific and are strongly dependent on national water management policies, it is very unlikely that the near future will show a global trend towards unification of standard biomonitoring protocols. For the coming decades, the diversity in scarcely applied monitoring variables and strategies will probably only increase. However, specifically with reference to the draft Directive on the Ecological Quality of Surface Water, a drive is felt within the European Community to unify the concepts of biological water quality evaluation.

Regarding the development of environmental toxicity tests for effluents and ambient water bodies, the driving force behind the continuous involvement of new test species needing adapted test protocols, is the wide-spread opinion of ecotoxicologists that the biotesting results only model real world effects when local species are used. Provided that a set of sufficiently diverse (reflecting the principle components of the aquatic food chain) and globally standardized tests are available and used, the scientific community would more efficiently spend time and money in trying to design universally applicable extrapolation methodologies based on sound statistical evaluations [see for instance 97]. At the moment only the acute ecotoxicity tests on Daphnia, fish and luminescent bacteria are (in the process of being) internationally standardized. For more chronic exposure international standardization relates to fish, algae and Daphnia only. The set of internationally standardized ecotoxicity tests should preferably encompass additional species from different trophic levels and functionality, e.g. waterplants, bacteria, molluscs, insect larvae, etc. Toxicity testing is restricted to a few highly specialized laboratories, and is not routinely practiced because of the high costs involved. Consequently there is an increasing demand for alternative tests which are rapid, user-friendly and more cost-effective, without neglecting ecological realism and possibilities for extrapolation.

At the moment, automated ecotoxicity early warning systems are mainly used for checking the quality of surface water before the water is used. Due to slow changes in water quality and considerable dilution, only real catastophes are liable to be detected. More effectively these monitoring techniques can be applied for the prevention of accidental industrial pollution. In this context, continuous automated toxicity monitoring devices should be installed and operated by high-risk industries at the end of the pipe in conjunction with effluent storage and clean-up facilities. At these locations, the water quality gradients in time are expected to be steep enough to allow for timely and reliable detection.

The evaluation of ecosystem effects measurements is generally done by comparing the results of inventories along established pollution gradients. The monitoring efforts could be evaluated a lot more effectively if it were possible to quantify the observed effects in an more absolute way. Setting of ecological objectives is now being considered in several countries in relation to "reference states". These "reference states" take into account the physico-chemical status of the watercourse and predict a "natural" biological community against which the "observed" community can be compared.

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1 INTRODUCTION

1.1 GENERAL

Water is one of the most important and basic natural resources. Water is not only one of the most essential commodities for our day-to-day life, but the development of this natural resource also plays a crucial role in our economic and social development process. While the total amount of water in the world is constant and is said to be adequate to meet all the demands of mankind, its quality and distribution over different regions of the world is uneven and contributes to the problems of availability and suitability. It is therefore imperative that man develops, uses and manages this scarce commodity as rationally and efficiently as possible. In order to execute this task, accurate and adequate information must be available about the behaviour of the environment under constantly changing human pressures and natural forces.

Water quality management generally involves the authorization of discharges of dangerous substances for which monitoring of discharges, effluent and influenced ambient water is essential. On a national and regional level, countries have issued several laws and directives related to water management and pollution control, including the prescription of monitoring activities. Examination of the different approaches applied in the European countries show great similarity, although the emphasis may differ because of geographical or institutional reasons. Moreover, directives are issued by the European Commission and have to be incorporated by the Members States in their national legislation. As early as 1975, the European Commission presented a directive for the quality of surface water to be used for the preparation of drinking water (Directive 75/440/EEC). More recently several directives related to the quality of ambient water and effluent were established. The directives for ambient water include standards for specific uses of the water system, while for each function a number of water quality variables have been chosen to describe the desired situation (i.e. directives concerning the quality of bathing water (76/160/EEC) or fresh waters needing protection or improvement in order to support fish life (78/659/EEC)). In contrast, the directives for effluent from specific types of industries generally specify the maximum allowable concentration for only one variable. For effluent, the general framework is laid down in Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community and is worked out in several daughter directives. In addition, regulations concerning new chemicals (Directive 93/67/EEC) and existing chemicals (Regulation 793/93/EEC) as well as biocides (proposed Directive) and plant protection products (Directive 94/43/EC) may require effluent and ambient water monitoring as well. In general, both European and national directives prescribe the monitoring effort in terms of sampling frequency, analytical methods and reporting.

Water quality monitoring is a complex subject, and the scope of it is both deep and wide. Its proper study has a direct relation and interface with chemistry, biology, physics, statistics, economics. Its scope is also related to the types of water-uses which are manifold and the nature of the sources of water such as ambient water (rivers and lakes), marine water and groundwater.

1.2 WATER QUALITY MONITORING

What is monitoring ?

Webster's dictionary defines monitoring as **(1)** to check and sometimes to adjust for quality or fidelity, **(2)** to watch, observe or check, especially for a special purpose, **(3)** to keep track of, regulate, or control (as a process for the operation of a machine). Note that both (1) and (3) involve adjustment, regulation, or control, which fit well with the various types of monitoring information. The following distinctions can be made between different monitoring activities [2]:

Survey: A finite duration, intensive programme to measure, evaluate and report the quality of the environment for a specific purpose;

Surveillance: Continuous, specific measurement, observation and reporting for the purpose of environmental quality management and operational activities;

Monitoring: Long-term, standardised measurement, observation, evaluation and reporting of the environment in order to define status and trends.

In the present project the word monitoring is defined in a less strict way to encompass all three types of activities.

Why monitoring ?

Clearly environmental monitoring must have a purpose and a function in the process of risk management and pollution control. In general a number of purposes for monitoring can be discerned:

- The **signal or alarm function** for the detection of suddenly occurring (adverse) changes in the environment. Preferably the monitoring system should be designed to immediately enable the tracing of causes;
- The **control function** for a verification on the effectivity of pollution control strategies and a check on compliance;
- The **trend (recognition) function** based on time series analysis of concentrations and loads to enable the prediction of future developments;
- The **instrument function** to help in the recognition and clarification of underlying processes by operational investigations (surveys).

The risk management process begins with activities that define the nature of the problem, followed by an integration of exposure assessment and effects assessment in order to estimate the probability and level of effects possibly occurring in the (aquatic) environment. The results of this risk assessment are considered along with economic, technological, social and political considerations to arrive at a control strategy. In this risk management process, (water quality) monitoring is essential in the following stages:

- **During problem formulation**; chemical and biological monitoring of ambient waters may indicate deviations from the normal (alarm and trend function), triggering problem recognition;
- **During the stage of analysis**; chemical monitoring of receiving waters as well as selected effluent can help in exposure characterization, while biological monitoring of the same can enlighten on the ecological effects to be expected (instrument function);
- **During the stage of risk management**; monitoring will help in the verification of control strategy results, and in checking compliance (control function).

It is stressed that in environmental control, monitoring should be applied as an instrument and not as an objective itself. The main reason for monitoring is to detect changes in the state and functioning of ecosystems at a stage such that timely counteractive measures can be initiated, developed, and evaluated. Sampling is only the first step in the monitoring process, that should be followed by the interpretation and evaluation of the monitoring results, to be concluded with a timely reporting of the achieved results. The period between sampling and reporting is often considerable, thereby devaluating the monitoring results for their intended use.

Monitoring objectives

Water quality monitoring is carried out for various reasons and the objectives of a particular monitoring programme have a direct bearing on the costs of carrying out the programme. In this project the following (routine) monitoring objectives of ambient water and effluent quality sampling programmes are used as a starting point:

- identification of state (concentration) and trends in water quality;
- identification of the mass flow (loads) in surface water and effluent;
- testing of compliance with standards and classifications for surface water and effluent;
- early warning and detection of pollution.

In practise, data from routine monitoring programmes are generally used for a variety of purposes in addition to those for which the programmes were designed. Identification of the state and trends in water quality is mainly important for policy and management, while the identification of the mass flow in rivers and waste water discharges is of particular importance at the boundaries between countries, districts or water systems. Mass flows are subject of international negotiations and are an input for mass balances for specific substances. Testing of compliance with standards (control) is related to the water quality objectives for surface water as prescribed in both national and international standards. The early warning monitoring programme to signal pollution due to (accidental) spills by industry and ships is especially important if ambient water of that particular river or water system is used for public water supply. Finally, data can also be used for various projects including research.

1.3 BACKGROUND OF THIS PROJECT

Monitoring is an important risk management tool to detect, control or to evaluate the human health or ecological effects of single chemicals or mixtures of chemicals. Traditionally, pollution control agencies all

over the world relied on chemical-specific approaches to regulate discharges of toxic pollutants. This approach involved specification of standards and limits to loads and concentrations of a number of priority pollutants in ambient water and waste water, among others based on their potential toxicity.

In the European Inventory of Existing Commercial Chemical Substances (EINECS) about 100.000 chemicals have been identified. From these compounds the concentrations of approximately 30-40 chemicals are regularly monitored in important European aquatic ecosystems. The major proportion of chemicals can not reliably be quantified in ambient water and effluent due to lack of analytical methods, or due to the prohibitive costs of sampling and laboratory analysis. Properly evaluated data on chemicals with respect to their long-term (eco)toxicity and environmental fate are also relatively scarce. Furthermore, data on the projected effects of individual compounds do not account for the interactions among pollutants or the combined effects of pollutants that may occur in the complex mixture of chemicals that comprise many industrial and municipal effluents as well as diffuse inputs to ambient waters. This implies that the likelihood of NOT managing the environmental impact of important chemicals is high. It is therefore understandable that water control authorities are taking a keen interest in developing both physical-chemical monitoring techniques including the development of mixture toxicity variables, and biological monitoring methods (toxicity studies and biomonitoring techniques) for the prediction and detection of ecological effects of waste loads to receiving water bodies.

Water quality monitoring is an important issue in various environmental programmes; i.e. the "Convention on protection and use of transboundary water courses and international lakes" was adopted in 1992 in Helsinki under the scope of the Economic Commission for Europe (ECE). In 1994 the European Environmental Agency started its work programme, to provide the European Commission and the Member States with the information on the state and trends of the environment in Europe, and to provide the European Commission with the information required to carry out tasks of identifying, preparing and evaluating measures and legislation in the field of environmental quality. For this purpose the Agency will develop and coordinate together with Member States an European information and observation network.

In line with the proposed directive on integrated pollution prevention and control (IPPC) future monitoring activities will have to be integrated; in the Fifth Environment Action Programme of the European Commission [3] integration is seen as an important part of the move towards a more sustainable development. With respect to water quality monitoring, future monitoring strategies will not only be influenced by this proposed directive on integrated pollution prevention and control but also by i.e. the proposed directive on the ecological quality of water and the proposed modification of the directive on pollution caused by certain dangerous substances discharged into the aquatic environment (76/464/EEC) taking into account the aims of the proposed directive on integrated pollution prevention and control. These proposed directives require the present water quality monitoring strategies used within the European Union to be re-evaluated, both at Commission and Member State level. In the framework of the development of future water quality monitoring strategies, one can already see a move from the single substance monitoring approach to an approach where complex mixtures and biological monitoring become important.

1.4 OBJECTIVES OF THIS PROJECT

In 1993 the project "Monitoring water quality in the future" started in order to address the problems with the existing approach which will only increase in the future; in general terms these problems concern effective and efficient monitoring strategies. Therefore, the general objective was to survey methods by which the enormous number of pollutants in effluent and surface water can be monitored in an effective and efficient way (i.e. better information at less costs). In addition, suggestions to harmonize and optimize water quality programmes within the European Union are made. More specific objectives of this project were:

1 To produce concise reviews of **methods to signal and control water quality** focusing on:

- Volume 1: Chemical Monitoring [4];
- Volume 2: Mixture toxicity parameters [5];
- Volume 3: Biomonitoring [6];

2 To give a review of **testing strategies for complex mixtures of chemical substances** which can give more complete information at less cost:

- Volume 4: Monitoring strategies for complex mixtures [7];

3 To review **existing practices and make recommendations concerning standardization, optimization and organization of monitoring activities** in the European Union, with a focus on complete information (effectiveness) and low cost (efficiency):

- Volume 5: Organizational aspects [8].

The most important conclusions of all the individual studies are summarized in an executive summary [9]. In this executive summary overall recommendations are also made by drawing these together from the individual studies. The conclusions and recommendations of the several reports are based on the experience of project participants and do not represent a consensus of all monitoring experts or managers and policy makers. Although some of the conclusions and recommendations in these reports may also be valid for groundwater, estuaries and seas, they have not been included within the realm of this project mainly for the sake of concentrating the scope of this project on fresh surface water and domestic and industrial effluent.

1.5 TARGET AUDIENCE OF THE SEVERAL SUB-PROJECTS

Given the content of the separate volumes, they are necessarily targeted for different audiences. Volumes 1-3 are geared for specialists involved in the technical aspects of monitoring. Volume 4 and 5 are more directed to managers of water quality programmes and policy makers (i.e. in environmental ministries). The executive summary is written for mainly managers and policymakers, though technical experts may be interested in how certain aspects fit into the larger picture of monitoring.

The present report deals with sub-project 3, and in this capacity presents a review of methodologies and measurement strategies for biological monitoring.

1.6 OUTLINE OF THE NEXT CHAPTERS

Based on the uses, selection criteria, requirements and available testing procedures, presented in chapter 2, a guided choice can be made to include certain biomonitoring variables in different measurement strategies for water pollution control. As the biomonitoring results may be used for regulatory purposes, it is essential that the tests and measurements are producing reliable results. The concept of quality assurance in biological monitoring is treated concisely in chapter 3. Considerations with respect to potential measurement strategies are presented in chapter 4. Several documents are available in international literature where the choice for including specific biomonitoring variables is already explicitly made. In the chapters 5-8 these documents are scanned for examples of biomonitoring schemes for effects measurement in effluents and ambient waters, where possible with emphasis on the detection of toxicity. By no means is the scan meant to produce a review of all monitoring activities possibly fulfilling the above objective. The review is limited to encompass well documented systematic developments having the prospect of being useful for pollution assessment and control, and for which the references were readily available.

There are distinct differences in objective (input/exposure restriction, compliance testing and priorization of remediation versus problem detection and strategy/policy verification) and operational strategy between:

- toxicity monitoring of effluents
- toxicity monitoring in receiving water bodies
- ecotoxicity alarm recognition in both effluents and ecosystems
- ecosystem response related monitoring in ambient waters

Therefore, the chapters 5-8 are divided accordingly.

In chapter 9 the groups of potential biomonitoring variables specified in paragraph 2.5 are subjectively evaluated against the full set of selection criteria given in paragraph 2.3.

Chapter 10 gives a short account of the authors view on omissions in the present status of the application of biomonitoring techniques and on developments considered desirable in the near future.

2 BIOMONITORING

2.1 DEFINITION AND TYPES OF BIOMONITORING

The introduction of biological variables in environmental monitoring activities added the terms biomonitoring or biological monitoring to our vocabulary. Different interpretations of what is considered to be a biological variable or biological observation caused a lot of confusion about which activities belong to biomonitoring. In the medical world, biomonitoring is solely defined as the concentration measurement of pollutants inside the human body. Naturalists generally also include measurements of the direct effects of disturbances on physiological processes in organisms. Measurements on the responses on a higher level of biological integration (populations, communities and ecosystems) naturalists classify as inventories. Finally, according to environmentalists, all varieties of biologically oriented measurements, as long as they are performed with the objective of protecting, preserving and correcting the biological integrity of natural systems, fall under the reign of biomonitoring. In this respect, biological integrity may be defined as "the maintenance of community structure and function characteristic of a particular locale" [10].

In this report the following names and definitions will be adopted for the different aspects of biomonitoring:

- **Bioaccumulation monitoring** for measurements on chemical concentrations in biological material.
- **Toxicity monitoring** for measurements on the direct biomolecular and physiological responses of individual organisms towards toxicants in an experimental setup, including bioassays and biological early warning systems.
- **Ecosystem monitoring** for measurements on the integrity of ecosystems which is in many cases diffusely related to all kinds of environmental perturbations. This type of biomonitoring will include inventories on species composition, density, diversity, availability of indicator species, rates of basic ecological processes, etc.

The word **integrated monitoring** will be reserved for coordinated monitoring activities comprising chemical and biological measurements in a variety of environmental media or compartments.

The present report will only deal with topics concerning toxicity monitoring and ecosystem monitoring. Bioaccumulation monitoring will be discussed in Volume 1 "Chemical Monitoring" of the related series of reports, while the topic of putting together an integrated monitoring system is reserved for Volume 4.

2.2 POSSIBILITIES OF BIOMONITORING

Both the occurrence of bioaccumulation and the occurrence of biological effects often have been demonstrated to provide useful and reliable information on the state of the environment. However, it is essential to realize that a biological response will only be fully expressed if the amplitude and exposure duration of the disturbing factor is matched with the sensitivity and response rate of the disrupted biological process. In Figure 1 the response rates of important biological processes to mild pollution are globally indicated. The slower response rates of processes on higher levels of biological organization are quite evident.

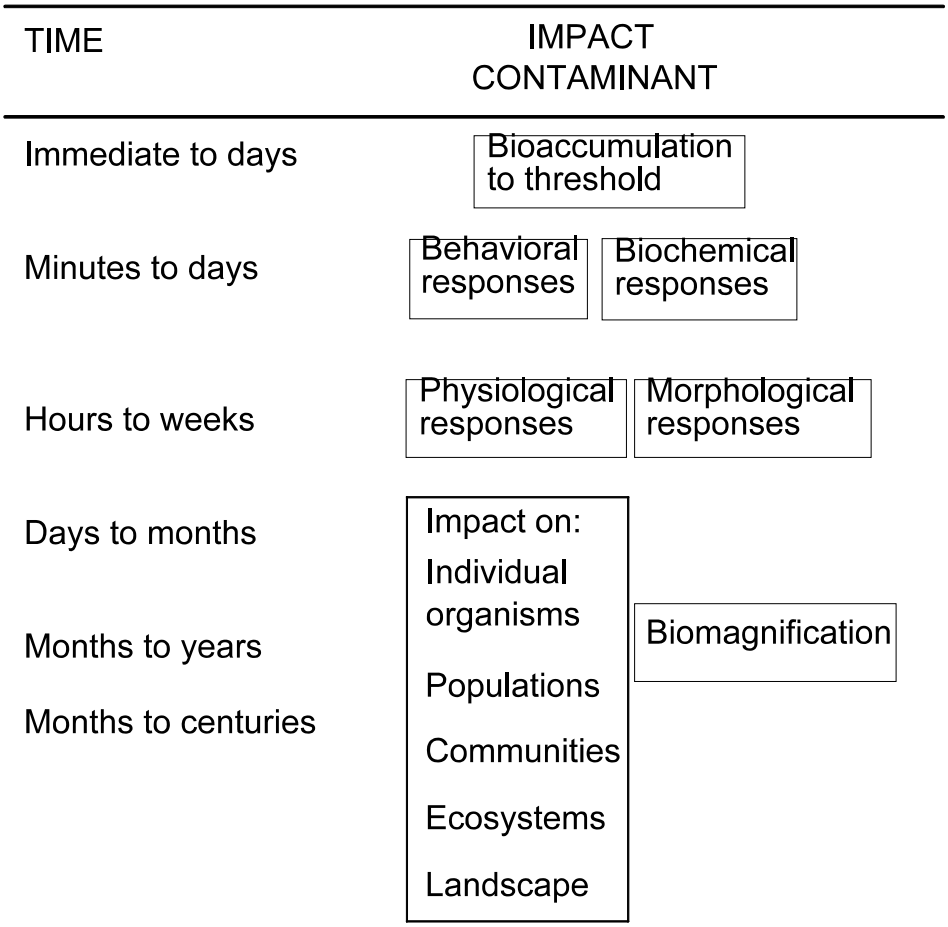

FIGURE 1 Rough estimates on the response rates of gross biological processes as a consequence of mild contamination [from 14]

Spatial gradients in physico-chemical variables and biological interactions are the cause for differences in populations of species and community structure. Depending on the tolerance, size, mobility and the radius of action of exposed species, these gradients can have a size varying between a single millimetre and several thousands of kilometres. As a consequence, specific types of environmental problems are related to their specific scales. As an example: the problems arising from the increased production of CO_2 are exerted on a global scale, while the effects of soil pollution caused by chemical dumping ("valleys of drums") are only expressed locally.

The different hierarchical scaling levels to be observed in both environmental pressure and the related effects negatively influence the possibilities for extrapolation of:

- Short-term to long-term effects
- local effects to effects on a larger scale
- effects on lower levels of organisation to higher level, integrated ecological effects level of organisation

It will be obvious that the differences in time, space and organizational scaling have important implications for the applicability of biomonitoring techniques. Especially with the design of monitoring networks (frequency, grid density and variable selection) these aspects are essential and to be considered with great care.

The use of biomonitoring methods in the control strategies for chemical pollution may have several advantages over chemical monitoring. Firstly these methods measure effects in which the bioavailability of the compound(s) of interest is integrated with the concentration of the compounds and their intrinsic toxicity.

Secondly, most biological measurements form the only way of integrating the effects on a large number of individual and interactive processes.

Often biomonitoring methods are cheaper, more precise and more sensitive than chemical analyses to detect adverse conditions in the environment. This is due to the fact that the biological response is very integrative and accumulative in nature, especially at the higher levels of biological organization. This may lead to a reduction of the number of measurements both in space and time.

A disadvantage of biological effect measurements is that sometimes it is very difficult to relate the observed effects to specific aspects of pollution. In view of the present chemical oriented pollution abatement policies and to reveal chemical specific problems, it is clear that biological effect analysis will never totally replace chemical analysis. However, in some situations the number of standard chemical analysis can be reduced, by allowing bioeffects to trigger chemical analysis (integrated monitoring), thus buying time for more elaborate analytical procedures.

2.3 CRITERIA FOR VARIABLE SELECTION

Once it has been established that biomonitoring techniques may provide welcome information to the solution of an environmental problem, suitable biological variables should be selected. The context in which these variables will be measured should be clearly indicated.

Not all biological variables are equally fit for serving in a monitoring programme. Their suitability can be evaluated by checking against a number of requirements [after 11]. Some of these are related to scientific and fundamental aspects, while others relate to efficiency, costs, logistic and policy aspects. In prioritizing monitoring variables, the following list should closely be checked. It is not possible to indicate a weighting to the different aspects.

SCIENTIFIC REQUIREMENTS:

- **Information contents with respect to environmental problems**: An observed effect in the considered biological variable preferably contributes to our understanding of the identified environmental problem (diagnostic value). The matching of temporal and spatial scales and dynamics of the observed biological variable and the expected disturbance or pollution are important aspects to consider.
- **Ecological information contents**: Observed effects in the considered variable are preferred not only to relate to mortality, growth and reproduction of individuals of the studied species, but also to the protection of populations, communities and eventually the ecosystem (diagnostic value).
- **Species specificity**: A response in the studied species is preferably representative for responses to be expected in other species.
- **Specificity to causes**: An observed effect in the variable under consideration should be indicative for the causes of the environmental problem identified.
- **Reversibility**: Especially for monitoring ecosystem responses and continuous in-situ exposure experiments (biological early warning systems), an important aspect to consider is the ability of the variable to return to its original state once the perturbation is removed.

EFFICIENCY REQUIREMENTS:

- **Quantitative aspects**: It is considered an advantage when the intensity of an observed effect is predictably related to the causing stress intensity (concentration-effect relationship).
- **Sensitivity**: The minimum stress intensity that will invoke an observable effect should preferably be low or in any case be matched with local conditions.
- **Response range:** The range of stress intensity resulting in a quantifiable effect is preferred to be large.
- **Response rate**: The response rate of the effect variable should be matched with the rate of change in the stress.
- **Natural variability**: In order to be able to discern stress caused effects from random fluctuation, the natural variability should be relatively low (signal/noise ratio).
- **Precision**: The variable under consideration should be measurable with a precision that enables the recognition of effects from variability.
- **Standardization**: It should be possible to standardize the method of measurement, also requiring interlaboratory tests on reproducibility.
- **Applicability**: For comparison among sites with similar environmental problems it is essential that the measurements are broadly applicable (not on species or processes only existing locally)
- **Cost effectiveness**: The results in terms of increased understanding of the problem should balance with the costs involved in monitoring the specific variable.

ADMINISTRATIVE CRITERIA:

- **Costs**: Funds and manpower to monitor the considered variable with the (minimum) required intensity (frequency, grid, duration) should be available. Cost breakdown should show capital investments, costs of infra structure and logistics, exploitation costs, cost of training, and labour costs.
- **Retrospection**: The selection of a proper monitoring variable is considerably helped by earlier successful use of it in a comparable monitoring situation.

POLICY ASPECT:

Biological variables for monitoring purposes used to be selected by individual scientists involved in the formulation of a monitoring programme. Naturally, this selection tended to be founded on the interests and limited specialization of the people involved. Especially in the US, policy-makers recently started to realise the crucial importance of proper variable selection not only for the efficiency and effectivity of monitoring programmes, but also for **biological relevance and social acceptance** [12, 13, 14]. These reports strongly recommend to base variable selection not only on the criteria mentioned above, but mainly on the ultimate objectives of the monitoring effort in terms of the protection of a specified asset of a water body to a specified level. This approach recognizes two different types of endpoints:

- **The assessment endpoint** is a formal expression of the actual environmental value that is to be protected. The most important property of assessment endpoints is societal relevance. In other words; it should be an environmental characteristic that is understood and valued by the public and by decision makers. In local risk assessments the most appropriate endpoints are generally the reduction of effects on valued indigenous populations such as game fish or harbour seals.
- **The measurement endpoint** is an expression of an observed or measured response to the hazard. It is a readily measurable environmental characteristic that corresponds to or is predictive of the valued characteristic chosen as the assessment endpoint.

The environmental science literature is replete with examples of effects on variables that were measured in the laboratory or in the field, but that can not be explicitly translated into a societally or biologically important environmental value. These monitoring efforts generally only result in the question "So What?" without any action taken. If monitoring variable selection is guided by first specifying assessment endpoints according to ecological objectives, the translation or extrapolation possibilities are built-in. The links between ecological objectives, assessment endpoints and measurement endpoints are not always translatable in terms of cause and effect but may simply be correlated. It is important to attempt to make these causal links if the measurements are to be relied upon to achieve the objectives. The process of defining measurement endpoints is easily understandable with the examples given in table 1.

Table 1: Examples of corresponding assessment and measurement endpoints

REGION	ECOLOGICAL OBJECTIVE	ASSESSMENT ENDPOINT	CAUSES	MEASUREMENT ENDPOINT
Wadden sea	Retain function as breeding ground for marine species	Presence of a balanced population of healthy harbour seals	PCB	Hepato-enzymatic reactions in fish
			Heavy metals	Metallothioneine masking reactions in mollusca
Rhine river	Ecological rehabilitation	Presence of an endogenous population of salmonids	Eutrophication	Biomass algae
			Heavy metals	Bioaccumulation in mollusca
			Toxicity	Sediment bioassays
Local industrial effluent discharge in river	- No impairment of local biota, water supply function, and fisheries downstream - Fish edible without health risk	1) No increased human health risk allowed after treatment to drinking water 2) No reduction allowed of fisheries volume and public demand	Toxicants in general	Effluent toxicity tests
				bioaccumulation in fish
				Tests for persistence of toxicity
				Mutagenicity tests
				Food chain inventories in receiving water

Some of the requirements for monitoring variables are mutually exclusive. It is generally accepted that ecological relevance is inversely related to criteria like sensitivity and specificity. Effects on a higher level of biological organisation (population, community, etc.) are highly biologically relevant, but may be insensitive (due to the availability of alternative pathways in an ecosystem, and complex regulating mechanisms) and are normally a-specific in their response to many perturbations. For biomolecular and physiological effects, the order of their compliance to the criteria mentioned above will be reversed.

Variables with a response that is restricted to only one type or group of pollutants or a specific type of perturbation are generally associated with processes having a low rank in the chain of causality. This type of monitoring variables (measurement endpoints) have a high problem/solution directed bio-indicative capacity. Due to their distinct relation to specific aspects of pollution, they can be fruitfully used for control.

The indicative value of ecological (assessment) endpoints on a higher level of integration is to be found in signalling trends in combined ecosystem performance. However, this type of evaluation, in general, lacks the possibility to direct counter-active measures. In many cases it will only reveal the need for process studies on the underlying causes.

2.4 POTENTIAL USERS OF BIOMONITORING DATA

Three groups of parties can be identified, who will be interested in the application of biomonitoring [15]:

- Effluent dischargers
- Regional and national water quality control agencies
- Water users

Effluent dischargers can apply biomonitoring techniques for testing the toxicity of their effluents. For this application it is essential that discharge permits contain criteria for ecotoxicity. Furthermore, discharges can use biotesting for evaluating the effectivity of technology based pollution control measures, and as an alarm notification for process failure.

Water quality control agencies can use biomonitoring for the formulation and validation of ecological water quality objectives, as well as checking their targets. In addition they can make use of biomonitoring data for tracing hidden sources of pollution, for setting permit criteria for the discharge of effluents, for checking the compliance of effluent dischargers, and for determining the effectivity of pollution control measures.

Watersupply agencies and other users of surface waters (e.g. fish farmers) can use biomonitoring techniques for indicating the presence of hazardous concentrations of unspecified pollutants in their intake.

Furthermore, biomonitoring data can also be used by the public and the government to monitor the performance of water regulators, to ensure that they are using their powers to the advantage of water users and the water environment.

2.5 POTENTIAL BIOMONITORING VARIABLES

Table 2 gives an indication of the types of biomonitoring variables available [after the Organisation for Economic Cooperation and Development (OECD), 94]. Many of these tests and observations are procedurally well documented in internationally accepted guidance documents and standards. However, the degrees of freedom in the design of ecotoxicity tests with respect to the selection of test organisms, test criteria and test circumstances are manyfold. Therefore, many research groups continuously produce an endless stream of new procedures, which may all be capable of revealing specific aspects of ecotoxicity for specific situations. As an indication for the design variety of toxicity tests and field observations it can be stated that for the freshwater environment alone, about 120 different laboratory toxicity tests are presented in international literature [105], whereas about 100 different variables are given to describe community effects occurring in the field [75, 76]. It will be evident that it is entirely impossible, within the scope of this report, to review all possible biomonitoring variables up to the level of species, processes and particular procedures. Table 2 pragmatically only gives examples of variables and test for specific types of biomonitoring techniques in different environmental compartments.

Table 2: Examples of biomonitoring variables

TEST or OBSERVATION TYPE	COMPARTMENT	ORGANISM or TEST METHOD	TEST or OBSERVATION CRITERIUM	REFERENCE
Laboratory toxicity test single species acute	Freshwater or effluents with or without concentration procedure	fish	lethality	[16]
		Daphnia	lethality immobilisation	[17, 18, 19]
		bacterial luminescence	light emission	[20, 21, 22]
		Daphnia IQ test	enzyme inhibition	[23, 24]
		Rotoxkit F	lethality	[25, 26, 27, 28, 29]
		Thamnotoxkit F	lethality	[30, 26]
		Toxichromotest	enzyme inhibition	[31]
		Ames-test SOS-chromotest Mutatox test	bacterial mutagenicity	[32, 33, 34]
	Saline water or effluents with or without concentration procedure	bacterial luminescence	light emission	[20, 21, 22]
		Rotoxkit M	lethality	[35, 36]
		Artoxkit M (brine shrimp)	lethality	[37, 26]
	Freshwater and saline Sediments	bacterial luminescence	light emission	[38]
	Freshwater sediments	Sediment chromotest	enzyme inhibition	[39]
Laboratory toxicity tests single species (sub)chronic	Freshwater or effluents	protozoa/bacteria	population growth	[40, 41]
		algae	population growth	[42, 106]
		Daphnia	reproduction	[43, 44, 45, 19]
		fish	ELS (early life stage), growth	[46, 47]
		Lemna test	colony growth	[48]
		fish	chromosome abberation	[49]
	Saline water or effluents	fish	ELS growth	[see 50]
	Freshwater sediments	*Daphnia* porewater test	reproduction	[51, 52]
		Chironomus sediment test	larvae development	[19]
	Saline sediments	oyster larvae sediment test	larvae development	[53, 54]

TEST or OBSERVATION TYPE	COMPARTMENT	ORGANISM or TEST METHOD	TEST or OBSERVATION CRITERIUM	REFERENCE
Laboratory toxicity tests suborganismal	Freshwater or effluents	in-vitro tissue tests	growth, lethality, histopathology	[see 75, p. 349-351]
Field toxicity tests (semi)continuous Early warning	Freshwater or effluents	fish	-ventilation -rheotaxis -swimming behaviour	[117]
		algae	productivity	
		bacteria	-luminescence -respiration	
		Daphnia	swimming activity	
		mussels	valve movement	
	Saline water or effluents	mussels	valve movement	
Field toxicity tests active monitoring	Freshwater and Saline water	caged organisms	lethality, growth, reproduction, bioconcentration, scope for growth, survival in air Biomarkers: -metallothioneine formation -lysosome stability -MFO-induction	[e.g. 55] [e.g. 56] [e.g. 57] [58] [e.g. 59] [see 60] [61]
Observations on effects in the field passive monitoring	Examples available for freshwater, saline water and sediments	eco-epidemiology in selected species -fish -*Chironomus*	incidence of diseases and morphological deviations	[e.g. 128, 129, 130, 131, 62, 63]
		indicator species	presence absence	[see 75]
		colonisation of artificial substrates	species composition, diversity, abundancy	[see 75]
		community structure -benthic macrofauna -diatoms	species composition, diversity, abundancy	[see 64 chapter 10, and 75]
		ecological functioning	primary productivity, respiration, biomass, turnover, degradation, material cycling	[e.g. 64, p. 10-33,]

3 THE CONCEPT OF QUALITY ASSURANCE

3.1 INTRODUCTION

Chemical safety is a world priority. Considerable effort is being devoted by governments and industries to ensure that the manufacture and use of chemicals will not have an adverse effect on human health or the environment. Many governments have introduced laws, regulations and guidelines designed to prevent human health risks and environmental degradation.

The production of reliable data for chemical safety assessment, requires the use of scientifically sound testing and monitoring procedures and the application of quality assurance in conducting tests and studies. Quality Assurance (QA) is a managerial concept intended to promote the reliability of data for use in risk assessment. QA is essential for toxicological and exposure studies to predict human health effects, for ecotoxicological laboratory or field studies to assess potential or actual environmental effects for ecosystems, and for studies to determine the fate of chemicals released into the environment.

QA is focused on organisational process and the conditions under which studies are planned, performed, monitored, recorded and archived. QA systems do explicitly not intend to interfere with the scientific design of the studies and their purposes. QA includes independent study monitoring assuring laboratory management and users of the data produced that facilities, personnel, methods, practices, records, and controls conform to accepted principles (often called Good Laboratory Practices: GLP). An effective QA system provides confidence that a study report meets pre-established quality criteria with respect to accuracy, integrity, completeness and clarity.

QA approaches have been laid down in national legislation [e.g. US-EPA, 65] and in guidance documents from international organisations. Major examples are the Principles of Good Laboratory Practice of the OECD [66, 67, 68], the series 9000 guides of the International Standardisation Organisation (ISO) [e.g. 69], and the QA principles and guidelines produced by the World Health Organisation (WHO) and the United Nation Environment Programme (UNEP) [70].

3.2 QUALITY ASSURANCE REQUIREMENTS

Study plan

A clearly written, comprehensive study plan is an essential element of quality assured chemical safety studies. The study plan should state the objectives, schedules and all methods for the conduct of a study, including an identification of critical passages in the progress of the study. Where possible, the study plan should refer to Standard Operating Procedures (SOP's). As the design specification for a study, the plan has an important QA function: it serves as the reference for measuring study performance. A properly specified study plan helps in the long-term planning of activities in terms of workload, manpower, facility and instrument allocation.

Standard operating procedures

Well documented, verified and traceable SOP's should be available in writing for the following aspects of a study:

- **Implementation of the QA programme**; describing organisational structure and procedures, as well as qualifications, facilities, authorities, and responsibilities.
- **Technical routines**; SOP's describing in detail how specific routine operations are to be carried out, to ensure that all personnel involved will be familiar with, and use the same procedures. This type of documents will prevent the introduction of indeterminant error in the generation, collection, handling and reporting of data.

Documentation and record keeping

Any study report, before it can be fully relied upon for accuracy and completeness of findings, and before any scientific conclusions can be derived from it, must be capable of being validated. This means that the information and conclusions stated in the report must be fully supported by "raw data" (all original observations, including laboratory worksheets, records, notes, memoranda, calculations, etc.) documented in the records of the laboratory. A complete data trail is required from the initiation of the study to the time when

the last data are recorded. The data trail should be detailed enough to allow an independent party to trace every aspect of the study.

The final report is the end product of a carefully planned and conducted study. The report must be well-organised and the evaluation, discussion and conclusions should accurately reflect all experimental data, including "outliers". It must contain a detailed account of the study, including statements on the why, when and how of deviations in applied methodologies and the original study plan.

GLP inspection and study audits

Inspecting facilities, critical activities and auditing final reports are very important tasks in a QA programme. The purpose of inspection is to verify that the study is being performed in accordance with the study plan, the SOP's, and applicable GLP. The goal is to detect and correct systematic or unintentional flaws in the study, before the quality of the study is violated. Auditing has two purposes. The first is to confirm that the results presented in the final report actually reflect the data that were collected. The second is to certify that any adverse circumstances that may have impacted the study are reported.

Standardisation and round-robin evaluation

For the comparability of data produced by different working parties, both on a national and international scale, it is preferred to use generally accepted and standardised methods. Since it is impossible to produce errorless analytical data, it is important to estimate the limits of uncertainty of the routinely produced data. In other words, it is important to establish the reproducibility of the routine analytical procedures used, both within and among different laboratories. Acceptable limits of variation should be set primarily by considering the data quality requirement rather than the characteristics of the analytical procedure. Statistical approaches to evaluate the quality of analytical results have recently been reviewed by Taylor [71, 72]. A well established procedure for comparing the analytical performance of different laboratories is round-robin testing, where each collaborating laboratory receives similar unknown samples for analysis. Round-robin testing generally is a last stage procedure in the process of standardisation.

4 POTENTIAL MEASUREMENT STRATEGIES

In order to fruitfully apply a set of specific testing procedures in a monitoring system it is essential to develop a balanced measurement strategy in terms of what to measure, where, how often, etcetera.

The most important step in setting the proper measurement strategy is clearly defining the objectives. Or in other words, we have to specify what we want to detect. The detectability of long-term trends in ecosystem pollution effects requires a thorough investigation of the natural variability in the observed variables, whereas the adequate recognition of suddenly occurring alarm conditions and effluent quality and compliance testing requires information on pollution load variability.

The following sub-chapters provide conceptual views on the development of measurement strategies for three distinct subjects of biomonitoring; respectively, (4.1) toxicity monitoring of effluents, (4.2) in receiving water bodies, and (4.3) biological impact monitoring. In these sub-chapters, the critical stages and options in the design of a monitoring system are mainly distilled from the US-EPA Technical Support Document for Water Quality-Based Toxics Control [96] and the OECD Monograph on the Use of Biological Tests for Water Pollution Assessment and Control [94].

4.1 EFFLUENT TOXICITY MONITORING

Objectives

Effluent toxicity monitoring can have five objectives:

- Testing and steering the progress of technology based improvement of effluent quality, to complement chemical specific assessment
- Permit compliance testing, provided that toxicological criteria are part of the permit formulation
- The prevention/reduction of effects occurring in receiving water bodies
- Early warning of calamities and accidental spills, provided that measures can be taken to contain the released toxicity
- The prediction of effects occurring in receiving water bodies

The first three of these objectives are strongly related to the **control function** of biomonitoring, while the following two objectives are mainly related to the **alarm** and the **prediction** function, respectively. In evaluating the quality of effluents for control and prediction purposes, it is generally accepted that a maximum of certainty should be attained within a minimum budget and time. For alarm purposes, however, timeliness is of more concern, while less certainty is required. As will be discussed in the following paragraphs, these deliberations do have major implications for the sampling, testing and evaluation strategies required.

Effluent sampling methods and frequency

In order to use effluent toxicity data for pollution control purposes, it is necessary to test effluent samples that are representative for the characteristics of the effluent. Since an effluent may vary significantly in quantity and toxicity either randomly or with regular intervals, the design of an appropriate sampling regime is difficult.

Effluent sampling must be designed to obtain samples which suit the desired objective of toxicity testing, whether that be to control long-term or short-term toxicity in a receiving water body or ring the alarm when sudden changes occur. Where possible, sampling regimes should be based upon a study of plant operation or pilot surveys to estimate the variation in the toxicity of an effluent. This will guide the establishment of the most efficient sampling programme based upon estimates of how best to allocate sampling frequency and whether grab samples or composite samples should be used, or whether on-site flow-through testing is the most advisable methodology.

- **On-site Continuous flow testing:**
  The test organisms may be exposed to fixed dilutions of a sample continuously collected from the effluent pipe. Where greater accuracy is required, the dilutions can be scaled to simulate the time-varying concentration of the effluent at the mixing zone boundary. It will be clear that this type of exposure is the only type applicable for early warning alarm monitoring.
- **Grab sampling:**

Grab sampling provides a discrete sample for static or renewal toxicity tests. Grab samples are recommended for short-term acute and sub-chronic toxicity tests of wastewaters that have a relatively constant composition with compounds that are not very dynamic in their behaviour. It should be noted that as grab samples reflect the toxicity of the effluent only at the time of sampling, toxicity results may vary with each sample. If the identification of toxicity peaks is of greatest concern, then grab samples must be taken regularly and randomly over a period of time that is dictated by a careful study of plant operation.

- **Composite sampling:**
  Composite samples are prepared by mixing together a number of grab samples. This mixing may be performed either time or volume proportional. Composite samples are usually used for chronic renewal testing. The process of averaging tends to dilute toxicity peaks.

Types of effluent toxicity tests

As long as adequate preservation (cool, dark) is provided for the effluent immediately after collecting the sample, the effluent can generally be expected to remain stable for a period of 24 hours. Therefore, unless other considerations, such as a requirement for continuous real-time monitoring (alarm recognition or extreme variability of the effluent) mandate that on-site testing be conducted, it is generally most cost-effective to have effluent toxicity testing done in an established laboratory. However, essentially a choice exists between the application of four types of testing strategies for toxicity determination of effluents. All four strategies leave the choice of test organism more or less open. In paragraph 2.5, a choice of organisms has been presented which were proven to be applicable for specific types of tests.

- **Laboratory static non-renewal testing:**
  In the static non-renewal test, a dilution series of an effluent sample is prepared at the beginning of the test. The organisms are exposed to this series for the entire duration of the test. This type of test is only appropriate for measurements of acute toxicity related to compounds that are not very dynamic in their behaviour.
- **Laboratory static renewal testing:**
  In a static renewal test the only difference with respect to the non-renewal test is that the test medium is replaced at regular intervals. The static renewal test is usually preferred over the non-renewal variety because less interference is to be expected from toxicant adsorption to the walls of the test vessels, from toxicant degradation and volatilization, from the uptake of toxicants by the organisms, and from the effects of build-up of waste products of the test organism or a depletion of oxygen.
- **Laboratory flow-through testing:**
  With this type of test, the concentration of toxicant can be maintained with a continuous flow of fresh dilution water by utilising a commercially available serial diluter.
  The advantages of laboratory flow-through testing as compared to static testing are:
  - A more representative evaluation of the effluent's toxicity
  - Metabolic wastes do not build up
  - Higher loading factors possible (more organisms in each test chamber)
  - Loss of volatile, adsorbing or degradable constituents from the effluents reduced

  The limitations are:
  - Large volumes of effluents and dilution water required
  - Complex and expensive
  - More resources, manpower, space and equipment required
- **On-site flow-through testing:**
  In this system, test organisms are exposed to an effluent which is diluted with receiving water pumped into test chambers from upstream of the discharge site. The effluent may be added with a serial dilution apparatus to maintain a specified concentration, or it may be added to the test system according to the plant's discharge schedule. The choice should be based upon the need to match the historical or anticipated discharge conditions.
  The toxicity response can either be evaluated by scoring the test criterium at regular intervals in time, or by (semi)continuous automated measurement with some kind of biological early warning system. The first option is normally associated with criteria like mortality, growth and reproduction, while the automated devices are more suitable to evaluate a physiological or behavioral response.

Tiered testing procedure and evaluation criteria

Many schemes for effluent monitoring for control purposes apply a tiered testing approach to foster reliable go or no-go decisions in a cost effective way. Tiered testing schemes start with gathering very course data on the involved risk for ecosystem damage, outside the mixing zone. The toxicity data are evaluated against predefined criteria, either showing that the risk for ecosystem damage is negligible or that the risk is unacceptable. Separate criteria are generally set for the risk for acute effects within the mixing zone and for effects of chronic exposure outside the mixing zone. When the first tier's data are not providing sufficient certainty for either of these cases, the complexity and reliability of the required tests is increased with each

tier until the required certainty is attained. The uncertainties related to the risk for ecosystem damage as a consequence of effluent discharge are the following:

- Variability in effluent composition
- Variability in effluent quantity
- Variability in flow and quality of the receiving water
- Uncertainties about the fate and behaviour of the toxicants in the effluent (degradation, evaporation, etc.)
- Uncertainties in the extrapolation to the sensitivity of local species
- Uncertainties in the extrapolation to chronic exposure effects

The first three of these topics can be handled by carefully adapting the sampling scheme, or by estimating worst case conditions. The uncertainties about the characteristics of the toxic compounds can be partially solved by applying appropriate testing schemes (flow-through, renewal and static testing). The uncertainties related to ecosystem sensitivity can be diminished by testing more species and using more realistic (chronic exposure) tests.

If certain aspects of uncertainty can not be resolved by applying these refinements in effluent testing protocols, monitoring will have to focus on the effects occurring in the receiving water body.

4.2 AMBIENT TOXICITY TESTING

Objectives

Ambient toxicity tests (i.e. toxicity tests on receiving waters and sediments) may be used in conjunction with effluent toxicity tests to provide additional valuable information. In particular, ambient tests may reveal or confirm the existence of toxic conditions in the receiving water, and may demonstrate the presence of unknown toxicants and the location of unknown toxic point-source or diffuse discharges. They may also be used to evaluate persistence, to evaluate the combined effects of multiple discharges, and to evaluate additivity, antagonism and synergism of effluents. A comparison of the toxicity contained in Rhine water concentrates with the supposedly additive toxicity of known constituents revealed that approximately 90% of the observed toxicity can not be explained by the chemical specific approach [73]. It can be stated that ambient toxicity testing mainly fulfills a **signalling function** for pollution control. This has specific implications for measurement strategy design with respect to sampling site selection, sampling frequency and the selection of tests.

Selection of sampling or exposure sites

The selection of stations for ambient toxicity evaluation is determined by site characteristics, where the following factors should be considered:

- For detecting toxic discharges, samples should be collected or organisms should be exposed at a number of sites upstream and, preferably beyond the mixing zone, downstream of a supposed discharge. Care should be taken to include a control station and recovery stations, as well as several intermediate stations with respect to the supposed pollution gradient.
- Knowledge of dilution isopleths allows placement of stations at locations where the expected concentrations correspond with the observed effect dilutions in effluent toxicity tests.
- Where data are available from earlier biological surveys, these data will help in guiding the choice of sampling locations.
- Presence of tributaries and other sources of pollution will influence positions and numbers of stations.
- Preferably sampling should be performed under low flow "worst case" conditions.

Sampling method and frequency

The problem of selection of sampling methods and frequency is of the same nature, but even more complex in ambient toxicity monitoring than in effluent sampling, because multiple independent effluent sources may be involved. Again a choice can be made of grab or composite sampling, as well as in-stream exposure. Since ambient testing is largely used as an investigative tool, the precision required for regulatory purposes is, however, not applicable.

Types of ambient toxicity tests

- **In-situ testing:**
  In this system, caged test organisms are exposed to the conditions in the receiving water body. This type of toxicity evaluation is also known as active biomonitoring. The toxicity response can either be evaluated by scoring the test criterium at regular intervals in time, or by (semi)continuous automated measurement with some kind of biological early warning system. The first option is normally associated with criteria like mortality, growth and reproduction, while the automated devices are more suitable to evaluate a physiological or behavioral response.

With this type of testing it is essential to continuously co-record variations in a number of physico-chemical variables (e.g. dissolved oxygen, temperature, pH, turbidity, etc.) which may cause effects not attributable to toxic agens or may affect the expression of toxicity.

- **Laboratory testing:**
  Depending on local circumstances, short-term (sub)chronic toxicity tests with sub-lethal endpoints may provide good results. Compared to acute tests, these tests are more sensitive to the dilution to be expected in ambient samples. Even short-term chronic exposure tests should at least be performed according to a static renewal scheme, where regular renewal with a freshly obtained sample will also account for some of the toxicant load variability.

In conducting both in-stream and laboratory ambient toxicity tests, the use of organisms compatible with the type of water is indispensable (e.g. freshwater or tropical organisms can not be exposed to sea water or polar conditions).

- **Laboratory testing with concentrated environmental samples:**
  Due to progressive dilution and sanitation, samples from many locations produce no or only marginal effects in short-term toxicity tests, thereby not revealing the fundamental relationship between concentration and effect which is crucial for toxicity evaluation. However, local conditions may very well attribute to certain aspects of long-term ecosystem instability. Solutions to detect the risk for subtle ecosystem perturbation with toxicity tests are either to considerably prolong exposure or to increase toxicant concentration. Increase of exposure duration will definitely increase the cost of testing. Toxicity testing with a dilution series of pollutant concentrates will nearly always provide information on the concentration/effect relationship, thereby allowing an estimate of the nearness of tolerance limits and a toxicity ranking. Many types of concentration procedures are available (e.g. liquid/liquid extraction, freeze drying, reverse osmosis, adsorption/elution techniques) of which, unfortunately, none is a-selective of toxicant characteristics. Since effective concentration factors varying between 50-100 are quite common in evaluating the toxicity of ambient water systems, the quantity of sample available for testing is considerably reduced. Therefore, the application of micro-volume tests (e.g. Microtox, Toxkits) is a must.

Ambient testing for persistence of toxicity

If the prime objective of the ambient toxicity study is to test whether the toxicity in a discharge is persistent or not, samples from several stations at appropriate intervals downstream of the discharge should be tested for remaining toxicity. If the decline in toxicity is abrupt, rather than the gradual decline accounted for by dilution, this may imply that the toxicants are degraded or otherwise excluded from exerting their action (e.g. adsorption, evaporation).

Ambient testing for multiple source situations

A multiple source situation is one in which more than one effluent discharges into a watercourse in such a way that their effects might overlap. It should be noted that multiple source situations are the ones most commonly encountered. Ambient toxicity evaluation is particularly useful in determining if effluent-related damage will occur in water which receive multiple effluents because the effect of these multiple discharges is measured in combination.

4.3 ECOSYSTEM RESPONSE MONITORING

Objectives

Toxicity testing of an effluent or ambient water may be used to demonstrate the probability of toxicity to the biota within a receiving water body. This is based on the assumption that an effluent that is toxic to one or more species in a test system is likely to be toxic to important components of the ecosystem, and therefore capable of causing adverse environmental impact. An alternative to using toxicity tests with simple endpoints such as mortality, growth and reproduction to assess the environmental impact of an effluent is to conduct field surveys and analysis of the endogenous biota in the receiving water and trying to link the observed effects with the input of toxicity. However, it should always be realised that many more types of man induced or natural interferences than only the input and action of toxic compounds may be responsible for an observed degradation of the biological appearance of a given ecosystem. Ecosystem response monitoring can obviously also be performed with the sole objective to reveal the impact of other than toxic stress. However, these applications falls beyond the scope of the present review.

As has been stated in the introduction of this report, as well as in the chapter introducing the concept of biomonitoring, the major objective of water pollution control is the safeguarding of the ecological integrity of a water system. To attain ecological integrity the combination of physical, chemical and biological characteristics should be favourable (Figure 2).

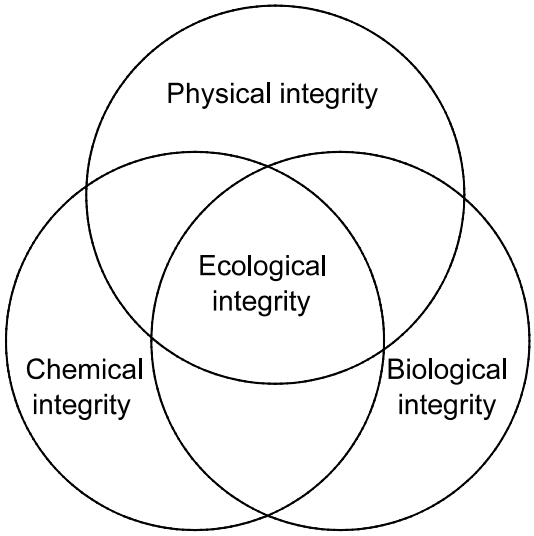

FIGURE 2 Elements of ecological integrity [95]

In order to evaluate the level of ecological integrity of water bodies, all of these aspects have to be addressed simultaneously. Therefore, ecosystem monitoring should be composed of the following types of measurements:

- Measurements on the **physical status** of the water body in terms of depth, shore development, substrate composition, flow, turbidity, temperature, canalization, mechanical disturbance, etc.
- Measurements on the **chemical status** of the water body in terms of concentrations of nutrients and salts, oxygen levels, pH and degradable organics.
- Measurements on the **biological status** of a water body may involve quantitative and qualitative inventories of the incidence of biochemical or morphological deviations and diseases in individuals of particular species (eco-epidemiology), inventories of biological structure (most commonly presented as species lists), and assessments of biological functioning.

Both physical and chemical status of a water body as part of the habitat for biological communities form the boundary conditions for the biological status (and also for the designated uses). This, so called, habitat evaluation identifies the possibilities for specific types of biota and ecological pathways to develop. As such, the availability of physico-chemical data and fundamental ecological insight are indispensable for setting standards and targets with respect to biological status (ecological objectives).

Advantages and disadvantages of ecosystem monitoring

Ecosystem surveillance can contribute uniquely to the monitoring of water quality in the following ways [78]:

- Biological communities act as continuous monitors of the water as opposed to intermittent samples taken for chemical analysis.
- Biological communities respond to a wide range of water quality determinants and pollutants, whereas chemical monitoring depends upon knowing what types of pollutants are likely to be present.
- Biological communities integrate the effects of mixed pollutants.

In the past, biological methods of assessing water quality have been criticized and from time to time have fallen into disrepute. This was probably due to their misuse and oversimplification in the hands of non-biologists. It is therefore necessary, in the defence of biological methods, to draw attention to their limitations:

- Although biological surveillance will detect ecological change indicative of a change in water quality, it will not identify the specific cause of this change. In case of toxic pollution, the cause has to be identified by chemical analysis as a complementary method.
- To monitor all water quality criteria and many different pollutants, ecosystem monitoring should ideally involve all components of the community. Such a comprehensive assessment will not be feasible. In practice a less comprehensive evaluation will suffice for most forms of pollution.

- The data generated by ecosystem monitoring are usually not comprehensible to non-biologists. This requires processing of the basic data in order to provide more acceptable grades or indices.
- Although ecosystem monitoring will detect ecological changes, a weakness in the system is in interpreting the observed changes in terms of water quality.
- Water indicated to be of poor ecological quality, is suspect for most uses. Water indicated to be of good ecological quality, may be good for most uses, but may not always be free from pathogens or harmful trace organics, and may not be of acceptable quality on human health grounds. To provide information on these aspects, bacteriological and chemical tests are required.

Eco-epidemiological monitoring

The incidence of diseases and morphological or biochemical deviations in local organisms can be regarded as an equivalent to the quantification of sub-lethal effects in toxicity testing. In order to be able to attribute the observed deviations to local circumstances, it is essential to limit the evaluation to organisms with a low mobility (e.g. sessile or territorial organisms).

Monitoring structural aspects of ecosystems

As has been stated earlier, biological structure is the integrated response of an ecosystem towards overall quality and appropriateness of its environment. Structure in a biocoenosis can be expressed to reflect different aspects:

- Species abundancy and distribution
- Community structure (species composition)
- Trophic structure (food web complexity, niche occupation)

In essence, all monitoring methods for ecosystem structure assessment boil down to sampling specified groups of biota, followed by counting and some kind of taxonomic or food requirement classification of the individual organisms sampled. Since the resulting type of data (species lists) are difficult to use for impact assessment, some 100 [74] numerical indices have been developed [see Hellawell 75, p. 427, 76 and 77] to reflect biological status:

A Indices for indicator species, based on the presence, absence or abundance of individual species
B Community richness indices, based on the number of taxa present
C Community abundancy indices, based on population size
D Community evenness indices, based on proportional community composition
E Community diversity indices, combining the information contents of B-D
F Biotic indices, combining the information contents of A-E

For reflecting the local impact of disturbance in aquatic ecosystems, it has often been demonstrated (e.g. De Pauw and Hawkes [78]) that relatively non-mobile organisms (e.g. benthic macro-invertebrate species and sessile diatoms) are the most reliable indicators. Benthic macro-invertebrates as a group have some assets making them particularly attractive for use as pollution bioindicators:

- They are ubiquitous and abundant throughout river systems
- They are relatively easy to collect
- They are relatively easy to identify
- They are generally confined to one locality of the river bed, and therefore indicative of local water quality
- They can act as continuous integrating monitors, due to constant exposure during their relatively long life spans
- They are a heterogeneous collection of evolutionary diverse taxa, which makes it likely that at least some will react to specific changes in water quality

Monitoring functional aspects of ecosystems

Energy flow and mineral cycling are the two driving forces behind ecosystem performance in terms of turnover, and fully determine the functionality of ecosystems for humanity in terms of crops and yield. In those macro ecosystem processes, the rates of many low level mechanisms can be determined. Examples of important quantifiable processes which can be used to indicate ecosystem performance in relation to environmental quality, are given below:

- Primary productivity
- Respiration
- Production over respiration
- Nitrification
- Degradation
- etc.

Assessment criteria

For both types of ecosystem biomonitoring, the assessment criteria are highly empirical due to a lack of understanding in fundamental ecology. Every ecologist, and in fact nearly every human being, has an idea what the balanced, healthy ecosystem in front of his door should look like, or what that system should be able to offer him. Therefore, the ecosystem quality criteria put to the structural indices and the rates of functioning reflect subjective judgements. For impact monitoring of environmental pollution, however, most of the monitoring results are only interpreted in a comparative way, which method is strongly relying on a proper selection of sampling sites. Using database information on best available sites within an ecoregion, targets for minimum acceptable ecological status can more objectively be estimated. The concept of specifying or predicting "reference states" or "yardsticks" with the aid of computer modelling, which is becoming widely used for comparison with observed states, can also be applied for making a quality judgement in more absolute terms. These methodologies which are being developed in several European countries and in the USA will surely be an important part of assessing water quality in the future as concluded from the European Conference on "River Water Quality - Ecological Assessment and Control" [79].

Sampling: site selection, methodology and timing

Since the evaluation of the monitoring results is generally based upon a comparison of results obtained at clean and polluted sites, it is very important that the sampling is performed at several stations along an established pollution gradient, and that a proper reference is included.

For monitoring ecosystem structure, the sampling can be performed either qualitatively or quantitatively. Care should be taken to make the sample sufficiently representative, by sampling a sizable stretch of a water body and a variety of appropriate habitats. A variety of sampling methods especially for the sampling of benthic macro-invertebrates is well documented and standardized by ISO [64, 80, 81, 82]. Especially for quantitative analysis, the use of artificial substrates is highly recommended [75, p. 407]. Sampling gear for fish and algae is also depicted by Hellawell [75].

There is a marked difference between the timing of sampling regarding the monitoring of structural and functional aspects of ecosystems. The slow and integrative character of subtle shifts in ecosystem structure and the seasonality of occurrence of species in a natural aquatic ecosystem allow for a less frequent but well timed sampling regime. Monitoring pollution induced changes in the rates of functional processes is far less time integrative and requires a more frequent sampling scheme, like with toxicity testing.

5 TOXICITY MONITORING OF EFFLUENTS

For the acceptability of the discharge of effluents and waste waters it is essential that they are environmentally safe, and that the designated uses of the receiving water are not likely to be affected. With respect to the release of chemicals, only a few aspects are of utmost importance:

Effluent properties:
- **Acute toxicity** is indicative for acute effects (e.g. fish kills) possibly occurring in the immediate vicinity of the discharge
- **Chronic toxicity** is reflecting the extend of possible sublethal ecological effects occurring in a larger proportion of the receiving water
- **Genotoxicity** reveals the risks for interference with the ecological gene pool leading to increased mutagenicity and/or carcinogenicity in biota and man. Unlike the normal toxicity, the incidence of genotoxic effects is thought to be only partially related to concentration (one-hit model)
- **Bioaccumulation and biomagnification capacity** is proportional to the risk of delayed effects and poisoning through the food chain

Modifying aspects:
- **Persistency and degradation of toxicity** determines the exposure duration and the affected area in the receiving water body
- **Bioavailability** influences the expression of toxicity, and may change during transport
- **Reactivity and combination toxicity** may positively or negatively alter the biological responses to the effluent
- **Dilution (effluent load per unit time vs retention time and flow in the receiving water)** is strongly influencing the expression of toxicity in the receiving water

With this in mind, it is not surprising that many schemes for effluent monitoring, control and priorization in international literature deal with tests and measurements revealing these types of effluent properties. The most characteristic schemes used in different countries are presented.

5.1 EUROPEAN COMMUNITY

France

In France, industrial effluents are regularly monitored for acute toxicity with daphnids. The toxicity data are only used as a base for discharge taxation [83]. It is proposed to add the Microtox test, chronic toxicity tests and a test on mutagenicity to the set of required bio-criteria [84].

Germany

German water authorities adopted a permit system for effluent emission where the requirements are based on fish toxicity [85]. They are now in the process of including criteria for daphnia, algae and luminescent bacteria as a screening requirement to trigger the requirement for fish tests on positive results. In this scheme the fish test (Goldorfe; *Leuciscus idus*) is still considered to be the only test producing definitive results.

The toxicity requirements are established per type of industry, in terms of the maximum number of times the effluents need to be diluted to produce a No Observed Effect Concentration (NOEC), defined as Gf for fish, Gd for daphnia, Ga for algae, and Gl for luminescent bacteria. Testing is limited to the exposure to only the appropriate Gx level, which should not produce any observed effect. The level of maximum allowable toxicity per industrial branch is based on the level which is considered to be attainable with state of the art process and/or treatment technology. Violating the toxicity requirements results in a levy which makes state of the art compliance a more economic option.

Ireland

In Ireland, compliance with toxicity limits for selected industries is ascertained by annual or bi-annual tests on representative samples of effluent. The test species most commonly used is the rainbow trout (*Salmo gairdneri*). Control authorities normally require results from 96-hour tests. The toxicity limits specified are developed on an industry specific basis with the categories given in table 3 [94]. The toxicity values in column 3 of this table are expressed as the minimum acceptable proportion of effluent (as a percentage) in a test resulting in 50% fish mortality after 96 hours of exposure. The Toxic Units (TU) in column 4 are defined as the maximum number of times an effluent may be diluted to produce the test criterium (TU = 100 / 96-hour LC_{50}, with the LC_{50} expressed as the percentage of effluent in the test).

Table 3: Irish industry specific criteria for whole effluent toxicity

PRIORITY GROUP	CATEGORY	96-HOUR LC_{50}	TU's
A	Chemical or pharmaceutical manufacturing	4%	25
B	Metal extractions, plating, or finishing	10%	10
C	Textiles, tanning, paper and glass making	20%	5
D	Agricultural and food industries, untreated municipal sewage	70%	1.4
E	Treated municipal sewage (secondary)	100%	1

In order to encourage the optimum selection of sites for new industries, it is recommended that receiving waters at all times must provide a minimum of 20 dilutions in the immediate vicinity of the discharge for each Toxic Unit discharged. Flow measurements, mixing and dispersion studies are therefore a necessary addition to monitoring toxicity limits of effluents.

The Netherlands

For the control of water quality, the Netherlands government identified two pathways in a tiered procedure as depicted in Figure 3:

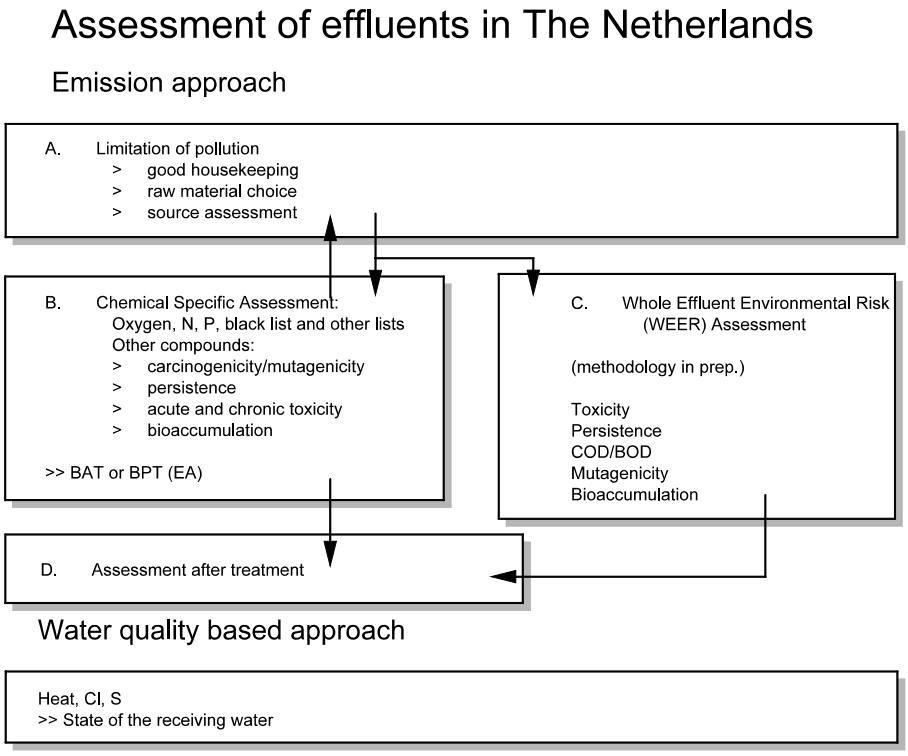

FIGURE 3 The Netherlands system for water quality control [after 86]

- The first path to follow, **the emission approach**, requires dischargers to apply best available and/or best affordable technologies for the reduction of the environmental risk of their effluents with respect to good housekeeping, process control, choice of (raw) materials, and effluent pretreatment. At the moment this process is only iteratively guided by chemical-specific evaluation of effluent quality. In a combined effort, the Ministry of Housing, Spatial Planning and the Environment, together with the Ministry of Transport, Public Works and Water Management, are in the process of developing a whole effluent evaluation system that will complement the chemical-specific approach. The whole effluent evaluation method will only be applied to selected effluents (large quantities, high risk) to assist in formulating additional pollution reduction strategies. The method will be comprised of effluent tests on mutagenicity, persistence, chemical and biological oxygen demand (COD and BOD), acute and chronic toxicity, and bioaccumulation [86] as intrinsic properties of the effluent.
- After effluent quality is considered to be acceptable, the **water quality based approach** will be followed, in which the remaining risks for effects in the receiving water are evaluated. In this framework, ambient water quality, inside and outside the mixing zone, will be verified against compound specific water quality objectives, designated use requirements, the presence of actual toxicity (TRIAD; see chapter on ambient

water quality monitoring) and biological integrity (biological water quality objectives). The results of the remaining risk evaluation may lead to the requirement of further risk reducing measures in the effluent.

- Additionally, the possibilities for setting **permit limit requirements** in the sense of whole effluent toxicity are also being evaluated.

United Kingdom

At the moment in the UK, no effluent permit requirements are based on whole effluent toxicity. Proposals are made to include Direct Toxicity Assessments (DTA) as a complement to the chemical-specific permit deduction [87] method. The developed strategy design [88] will be verified in 1994-1995. Biological testing is only started after desk studies have identified the need for them. The biological testing involved, will initially only consist of acute toxicity screening with luminescent bacteria (Microtox) and a 24 hr *Daphnia* lethality test for freshwater or a 24 hr Oyster larvae test for estuarine or marine waters to reveal the need for further testing. The results of these tests classify the permit requirements for the effluent in four categories. The most stringent class requires the effluent to be monitored with 3 or 4 acute toxicity tests (Freshwater: 72/96 hr algal growth inhibition test with *Selenastrum*, 48 hr *Daphnia* lethality test and a 96 hr fish lethality test with *Salmo trutta*, *Oncorhynchus mykiss* or *Cyprinus carpio*. Marine/estuarine water: 72/96 hr algal growth inhibition test with *Pheodactylum* or *Skeletonema*, 48 hr oyster embryo/larvae development test and a 96 hr fish lethality test with *Pleuronectes paltessa* or *Scopthalmus maximus*). The second stringent class prescribes effluent monitoring with one of the screening tests after verification with the above mentioned 3 or 4 acute tests. A third lower level of toxicity leaves the obligation for toxicity monitoring to one of the screening tests only, and at the fourth level no toxicity monitoring will be required. Measurements of chronic toxicity are not considered, neither are evaluations of accumulation, persistency, degradability and genotoxicity.

Sweden

In Sweden, industrial effluents are to be characterized by chemical composition, toxicity, bioaccumulative capacity and degradability [89]. The evaluation is performed according to the following tiered procedure:

step 1
- **Degradability** is measured as BOD_7/COD
- **Acute toxicity** is evaluated for fish, crustaceans, algae, and higher plants (model organisms)
- **Bioaccumulation capacity** is estimated by extraction with an organic solvent, followed by the separation of the lipophilic compounds with Thin Layer Chromatography (TLC). The migration distances give information on possible Bio-Concentration Factors (BCF). The compounds of interest can be isolated from the TLC-plate and analyzed by GC/MS
- **Chemical analysis**, including group variables like Absorbable Organic Halogenids (AOX) or Total Organic Chlorine (TOCl)

step 2
- **Degradability**; added test with possibly a characterization (toxicity or bioaccumulation) or identification of the non-degradable fraction
- **Biological effects measurements**; chronic toxicity and mutagenicity tests
- **Bioaccumulation and Chemical evaluation**; involve more, and more elaborate analysis

step 3

Step III is only prescribed in general terms, but should be tailored for the specific effluent on the basis of the results from tier I and II.

5.2 OTHER EUROPEAN COUNTRIES

Norway

Norway has a standardized test programme for permit derivation, comprising the Ames Mutagenicity test, acute and chronic toxicity tests and a biodegradation test. For monitoring purposes, it is advised to start screening the toxicity of an effluent with a comparatively large diversity of tests. The determination of precise concentration-effect relationships can then be restricted to the most sensitive types of organisms [90].

5.3 THE NORTH AMERICAN CONTINENT

Canada

Environment Canada recently developed an evaluation system, based on effluent toxicity testing, capable of ranking the environmental hazards of industrial effluents [91]. This so called Potential Ecotoxic Effects Probe (PEEP) incorporates the results of a variety of small-scale toxicity tests into one relative toxicity index to prioritize effluents for sanitation. In the index no allowance has been made for in-stream dilution, therefore the actual risk for environmental effects is NOT modelled. The tests performed on each effluent are the following:

A) **Bacterial assay** (*Vibrio fischeri* (new name of *Photobacterium phosphoreum*), Microtox, 15 min. exposure, acute sub-lethal criterion: light inhibition)
B) **Microalgal assay** (*Selenastrum capricornutum*, 96 hr exposure, chronic sublethal criterion: inhibition of multiplication)
C) **Crustacean assay** (*Ceriodaphnia dubia*, 7 days exposure, chronic criteria: lethality and inhibition of reproduction)
D) **Bacterial genotoxicity test** (*Escherichia coli*, SOS-test, 2 hr exposure, criteria: DNA repair for direct acting genotoxicants [without S9 activation] and DNA repair for genotoxicants requiring metabolic activation [with S9 activation])

The above tests are all performed on the effluent samples as they are. The A), B) and D) tests are also performed on effluent samples that are stored in the dark at room temperature for a period of 5 days after addition of an inorganic nutrient solution and a diverse microbial seed, to simulate biological treatment.

All test results are expressed as threshold values (LOEC's), and subsequently transformed to represent toxic units (TU's). The entire scheme results in a total number of 10 TU's per effluent. The results are put through the following calculation to produce the PEEP-index.

$$PEEP = \log_{10} \left[1 + n \left(\frac{\sum_{i=1}^{N} TU_i}{N} \right) Q \right]$$

Where: N is the total number of bioassays performed
n is the number of bioassays indicating toxicity
Q is the flow rate of the effluent in m^3/hr

Based on the correlation matrix of all bioassay data obtained with 37 effluents, it can be concluded that none of the bioassays produces data that are redundant. In other words, all bioassay procedures add to the information content of the PEEP-index.

In the 37-effluent study, the effluents of pulp and paper industry proved to be consistently far more toxic than those of other types of industries (PEEP $>$ 5). The same study revealed that approximately 90% of the total toxic discharge is caused by the added toxicity of only three effluents in 37. These effluent pipes are clearly considered the most rewarding for counteractive measures.

USA

In 1984, the US Environmental Protection Agency (EPA) [92] recommended the use of "biological techniques as a complement to chemical-specific analysis to assess effluent discharges and express permit limitations". Already in 1985 [93] a guidance document was produced on the use of effluent toxicity test results in the process of granting permits for discharge. The Organisation for Economic Cooperation and Development (OECD) [94, 95] in 1987 and 1991 fully adhered to the guidelines provided by the US-EPA. The discharging industries are required to provide quality assured data on toxicity according to a tiered approach, where the in-stream dilution is the first screening level, and increasing toxicity requires more complicated and definitive testing with increasing numbers of species from different trophic levels, at increasing frequencies. The permit requirements are set to the level where there is a minimal risk for ecosystem damage outside the in-stream mixing zone. Inside the mixing zone some non-lethal effects are allowed to occur, depending on the types of organisms and their duration of residence in the dilution plume. The 1985 scheme was rather complicated with respect to determining the balance between the projected in-stream toxicity and the uncertainty/reliability. Since new policies and regulations have been promulgated and a vast amount of knowledge and experience has been gained in controlling toxic pollutants, the testing and evaluation scheme was greatly simplified, while retaining its integrity, in 1991 [96]. The general outline of the EPA method is presented in Figure 4. Genotoxicity is addressed in a chemical-specific way with respect to human health only, based on the Average

Daily Intake (ADI) with drinking water and the ADI with fish consumption. The aspect of bioaccumulative capacity is also dealt with in a chemical-specific way.

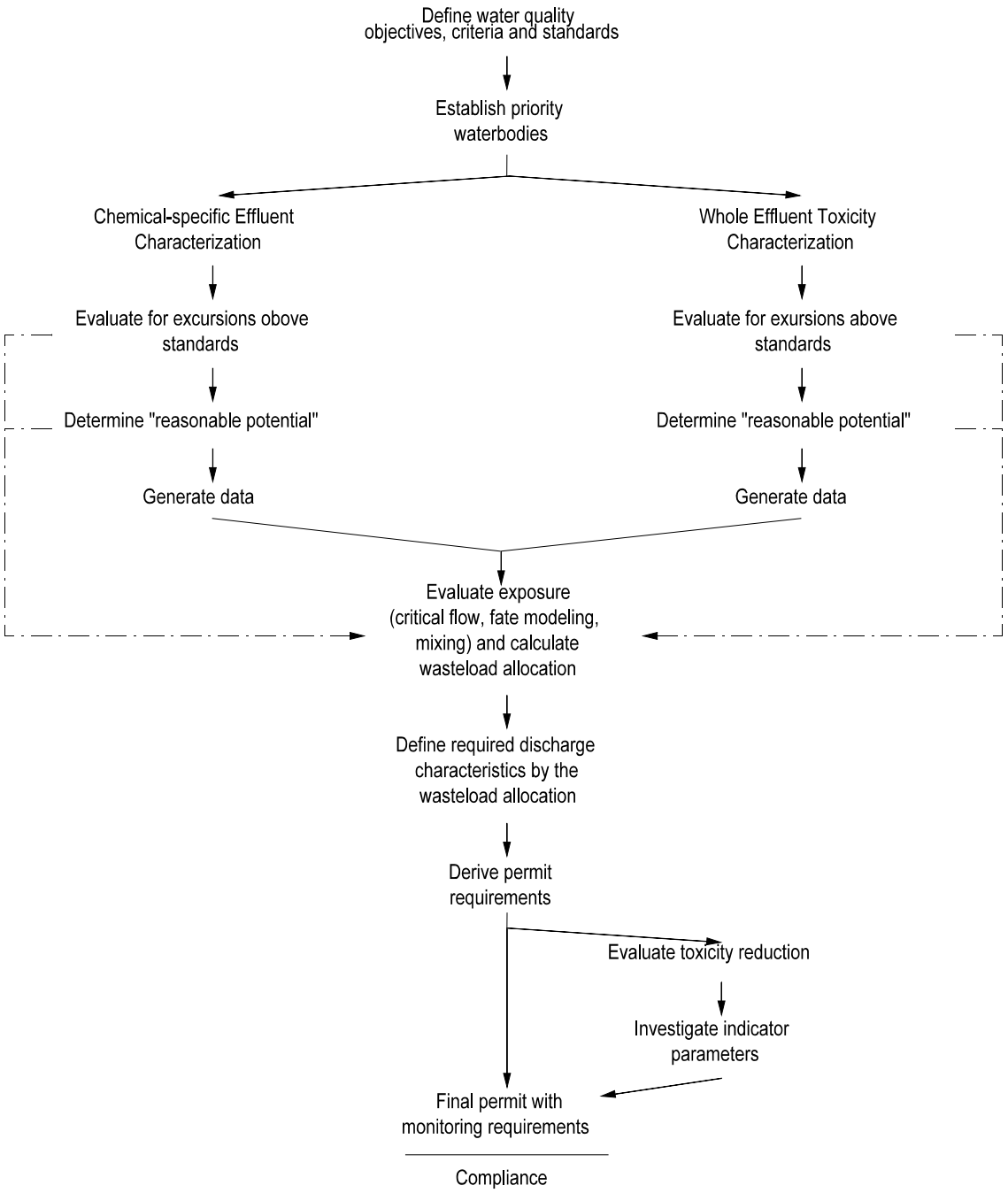

FIGURE 4 Outline of the EPA effluent toxicity control method [96]

The biological approach (whole effluent) to toxics control for the protection of aquatic life involves the use of acute and chronic toxicity tests to measure the toxicity of wastewaters. Whole effluent tests employ the use of standardized, surrogate freshwater or marine (depending on the mixture of effluent and receiving water) plants (algae), invertebrates, and vertebrates. An acute toxicity test is defined as a test of 96-hours or less in duration, in which lethality is the measured endpoint. A chronic test is defined as a long-term test in which sub-lethal effects, such as fertilization, growth, and reproduction are usually measured in addition to lethality. Traditionally, chronic tests are full life-cycle tests or a shortened test of about 30 days known as an "early life-stage test". However, the duration of most EPA tests have been shortened to 7 days by focusing on the most sensitive early life-cycle stages. For this reason the EPA chronic tests are called short-term chronic tests. The 1991 guidance document [96] extensively references the available test methods, including tests for persistency of toxicity and combination effects.

In the tests, conducted in the laboratory, an effluent sample is collected, diluted, and placed in test chambers with the chosen test species. An example of a dilution series used in both chronic and acute tests is 100, 50, 25, 12.5, and 6.25 percent, along with a control. In acute tests the number of live organisms remaining in each test concentration is recorded after 24, 48, 72, and 96 hours. The short-term chronic tests are evaluated on the basis of recordings of the incidence of all kinds of abnormalities at regular intervals. At termination of the test, the result for an acute test is calculated as an LC_{50} concentration (median lethal concentration). The chronic tests produce the highest concentration percentage tested that causes no significant adverse impact on the most sensitive of the criteria for that test (No Observed Effect Concentration; NOEC) as the result. Alternative results are the lowest concentration tested that causes a significant effect (Lowest Observed Effect Concentration; LOEC), or the effluent concentration that would produce an observed effect in a certain percentage of test organisms (e.g. EC_{10} or EC_{50}). The advantage of using the LC or EC over the NOEC and LOEC values, is that the Coefficient of Variation (CV) variation can be calculated. Since toxicity involves an inverse relationship to the effect concentration (test result; the lower the EC, the higher the toxicity), all test results are converted into toxic units (TU). The number of toxic units in an effluent is defined as 100 divided by the EC measured (expressed as a dilution percentage). Two distinct types of TU's are recognized by the EPA, depending types of tests involved (acute: $TU_a = 100 / LC_{50}$, and chronic $TU_c = 100 / NOEC$). Acute and chronic TU's make it easy to quantify the toxicity of an effluent, and to specify toxicity based effluent quality criteria.

Dilution water is an important part of effluent toxicity testing. Dilution water may either be standard laboratory water and/or the receiving water. Sometimes the receiving water is used to dilute the effluent because it more closely simulates effluent/receiving water interactions (bioavailability, combination toxicity, and salinity).

Due to degradation and partitioning (a.o., bioconcentration), the composition and concentration of the effluent may change during the test exposure. The effluent, however, will be discharged continuously into the receiving water body. Furthermore, the effluent may not be consistent in pollution load, volume, and/or chemical composition. The guidance document provides all kinds of considerations on test and sampling strategies to cope with variability in the effluent and reality in prolonged continuous exposure.

The evaluation strategy applied to the combined data on in-stream dilution and multiple data on effluent toxicity, involves a comparison of the calculated concentration of the effluent in the receiving water under worst case conditions (RWC = Receiving Water Concentration) with statistically derived "safe" concentrations of that specific effluent (the Critical Continuous Concentration (CCC), based on chronic testing, and the Critical Maximum Concentration (CMC), based on acute testing). RWC as well as CCC and CMC are expressed as TU's. Action is taken when RWC > CCC or RWC > CMC. The statistics to generate the environmentally safe levels closely resemble those used in The Netherlands [97]. As a minimum input from toxicity testing it is required to perform acute toxicity tests on 3 different species quarterly for a period of at least one year. Additionally some extrapolation to chronic toxicity has to be provided, or chronic toxicity has to be tested, depending on the rate of in-stream dilution. If the dilution is less than 1:100, chronic testing is required. If none of the CCC or CMC are violated and the dilution is less than 1%, then it has to be demonstrated that combination effects will not occur in the receiving water (use up-stream dilution water in toxicity tests), and that the toxicity is non-persistent (repeatedly test effluent/up-stream water samples after progressive storage under realistic conditions).

The EPA realized that setting water quality criteria with respect to toxic load, though playing an important role in assuring a healthy aquatic environment, have not been sufficient to ensure appropriate levels of environmental protection. Sediment contamination, which can involve deposition of toxicants over long periods of time, is responsible for impacts in receiving waters in quite some areas. At the moment the EPA tackles sediment related problems by means of the chemical-specific approach, based on equilibrium partitioning sediment quality criteria which are relating aquatic toxicity to equilibrium concentrations to be expected in sediments. However, action is taken to include ambient sediment toxicity testing in the whole effluent characterization approach. Appropriate testing tools still have to be developed, standardized and/or validated.

The primary objective of the United States Clean Water Act (1987) is "…. the restoration and maintenance of the chemical, physical, and biological integrity of the Nation's waters.". To meet this objective, EPA rightfully states that water quality criteria should address biological integrity. Therefore, the Agency recommends that the water quality authorities begin to develop and implement biological criteria in their water quality standards. In order to verify the compliance of water bodies to their assigned standards, ecosystem monitoring is considered a necessity. In the guidance document on water quality based toxics control [96], it is explicitly stated that the chemical-specific and the whole effluent approaches for controlling water quality should eventually be integrated with ecological bioassessment approaches. In the chapter on ambient biomonitoring a detailed discussion will be held on the views and methodologies presented by the EPA on this subject.

5.4 THE REST OF THE WORLD

From other countries there is no or only scarce information on the use of effluent toxicity monitoring techniques.

6 AMBIENT TOXICITY MONITORING

For ambient toxicity monitoring, the same general remarks can be made as for toxicity monitoring of effluents.
A variety of bioassays can be used in concert to generate biological effect information for deriving ecological effects-based environmental quality criteria, evaluate hazards to organisms on a site-specific basis, and evaluate the effectiveness of cleanup operations.

6.1 EUROPEAN COMMUNITY

The Netherlands

Since 1990, the Netherlands Ministry of Transport and Public Works, added a number of biomonitoring variables to the National Water Quality Monitoring Programme (MWTL), which was previously oriented purely on chemical variables. Within the MWTL, separate biomonitoring programmes are formulated for fresh and tidal waters. Within the freshwater monitoring programme [98, 99], a number of biological variables are directly related to the toxicological condition of water and sediments, while others reflect the overall biological integrity (see chapter on ecosystem biomonitoring). This monitoring programme is explicitly meant to reveal general trends in water quality and not to detect the effects of local discharges or other human activities. The ecotoxicological programme is comprised of the following measurements, which are performed once every 4 years at a total number of 14 stations:

- Accumulation of heavy metals (Cd, As, Zn, Cu, Pb, Hg-total, and Hg-methyl) and organic micro pollutants (PCB, OCB, PAH) in eel (*Anguilla anguilla)*
- Accumulation of the same compounds in zebra mussels (*Dreissena polymorpha*)
- Acute bioassays on surface water concentrates with daphnids and luminescent bacteria (due to the dynamic nature of waterborne toxicity, these determinations are performed monthly). The concentration step is essential, since surface water quality is not acutely toxic anymore, due to progressive sanitation.
- Semi-chronic bioassays on sediment and pore water toxicity with chironomids and daphnids

The results of the sediment and pore water toxicity tests are evaluated with the sediment quality TRIAD-methodology [100, 101], to produce an integrated quality judgement, together with a verification based on chemical and ecological quality criteria.

The results of the toxicity tests on water concentrates can be combined to produce a pT-value [102] as an indication for the risk of adversely affecting the biota in the ecosystem. At the moment, the XAD-concentrate tests are only providing information on the presence of a selection of organic micro pollutants. In the near future, the pT-methodology will be expanded to include tests on more species and biological processes, and to include concentrates with different specificity (e.g. heavy metals). This expansion may greatly enhance the level of realism in the risk estimation.

The applied ecotoxicological monitoring techniques are in general not (yet) used for the detection of local problems, but only for determining water quality on a national scale. Application for local pollution control purposes would require a larger number of measurements in time and space.

The tidal waters biomonitoring programme [103] has been in operation since 1989. The ecotoxicological variables in this programme can be summarized as follows:

- Mixed Function Oxidase (MFO) activity in fish liver (*Limanda limanda)*
- Bioaccumulation in mussels (*Mytilus edulis*) and the fish species flounder (*Pleuronectes platessa*) and common dab (*Limanda limanda*)
- Marine sediment toxicity tests with oyster larvae (*Crassostrea gigas*)

United Kingdom

In the UK a large proportion of the sewage sludge produced is disposed off in the sea at a number of licensed sites. A responsibility for monitoring and surveillance of these sites with biological methods has been formulated to involve measurement of effects on species numbers and diversity (see chapter 8, Ecosystem biomonitoring), as well as active biomonitoring of physiological and biochemical responses in caged mussels (*Mytilus edulis*). The physiological response in the mussel is equivalent to a reduction in the Scope for Growth

(SFG), which is a quantification of the energy budget available for growth, based on measurements of feeding, assimilation, respiration and excretion rates under controlled conditions after exposure. The exposed mussels also show effects on sub-cellular biomarker levels, such as a decrease in the stability of lysosome membranes in the digestive gland, an increase in metallothioneine formation as detoxification complex for heavy metals, and an increase in the induction of mixed function oxidase enzymes capable of partial detoxification of organic xenobiotics [104].

6.2 OTHER EUROPEAN COUNTRIES

Norway

Norwegian water pollution control authorities on some occasions use an Algal Assay Procedure (AAP) to assess local water quality and to detect discharges of toxic as well as nutrient rich wastewater in polluted rivers, lakes and fjords. Based on yield and growth rate in laboratory toxicity experiments with natural water samples and test algae, with and without nutrient addition, the water can be classified into different quality categories revealing toxicity and nutrient conditions [94].

6.3 THE NORTH AMERICAN CONTINENT

Canada

The National Contaminated Sites Remediation Program (NCSRP) was initiated in 1991 for the remediation of high priority contaminated sites in Canada. For the selection of appropriate bioassays to provide ecotoxicological information on soil, freshwater sediment and freshwater contamination, the international literature was extensively reviewed and authorities on the application of bioassays for contamination assessment were contacted in order to compile state-of-the-art possibilities [105]. Potentially suitable bioassays identified were evaluated using a two step approach. The first step assessed methodology completeness in a two-tiered approach. In the first tier, the presence/absence of three essential criteria for a suitable bioassay (appropriate printed method, reference toxicant, and acceptability criteria) was determined. In the second tier, 12 criteria that are desirable in a bioassay were assessed. Based on these two sets of criteria, bioassays were categorized as immediately usable, requiring some additional work to become usable, and still requiring substantial work to become usable. The second step involved an assessment of aspects making a potentially usable test fit for application in the field (representativity of trophic level, sensitivity, field validation and ecological relevance). Based on methodological completeness (step 1) and applicability (step 2), two batteries of bioassays were recommended for each of the three media. The first set is for screening purposes and contains acute and short-term tests, while the second set is designed to produce definitive data on the basis of chronic tests and a broadened trophic spectrum of acute tests.

For freshwater sediments, 19 sediment-dependent species falling into 8 major groups of organisms were identified in connection with toxicity testing. The screening battery of tests is recommended to include:

- Algal growth inhibition test (*Selenastrum capricornutum*) [106]
- Amphipod survival test (*Hyalella azteca*) [107]
- Midge survival test (*Chironomus tentans*) [108]
- Mayfly larvae survival test (*Hexagenia spp.*) [109]. This test is recommended when the genus forms a significant element of the benthic community, but not as a general screening test.

The definitive set of tests consists of bioassays covering the first, third and fourth tests of the screening set and an extended version of the amphipod test including sexual maturity as an endpoint.

For freshwater 119 species falling into 22 major groups of organisms were screened for applicability in testing. Of these, bacteria (5 species), algae (26 species), invertebrates (30 species), amphibians (2 genera), fish (25 species) and vascular plants (1 species) had appropriately documented methodologies. Ten of the 25 methods considered satisfied the other two essential criteria, and only 7 had the maximum score for the desirable criteria. Based on this evaluation, the following screening tests were recommended for the usable battery of tests for freshwater quality evaluation:

- Algal growth inhibition test (*Selenastrun capricornutum)* [106]
- Crustacean survival test (*Daphnia spp.*) [110]
- Bacterial luminescence test (*Photobacterium phosphoreum*) [111]

The recommended definitive set of tests consists of the same algal and bacterial bioassays, replaces the acute *Daphnia* test with the sub-chronic *Ceriodaphnia dubia* test [112], and adds a fish bioassay (either the fathead minnow larvae (*Pimephales promelas*) or the rainbow trout test (*Oncorhynchus mykiss*)). It is

recommended to focus priority attention to further development of methods for testing with the freshwater bacterium *Pseudomonas putida*, the rotifer *Brachionus calyciflorus* and the vascular plant *Lemna gibba* (duckweed).

For further strengthening of ambient toxicity evaluation, a number of research priorities have been identified, amongst which:

- Development of a standard sediment for benthic invertebrate tests
- Development of a test for rooted aquatic plants
- Re-evaluation of bacterial species for testing
- Development of a test with *Lemna minor*
- Determination of a reference toxicant for the test with *Lemna gibba*
- Conduct comparative testing with *Chironomus* species
- Preparation of a handbook providing statistical guidance for battery tests

USA

In addition to effluent toxicity testing, the US-EPA also recommends that the regional offices and the States monitor surface water for toxicity. In ambient toxicity tests, researchers collect samples at stations along a receiving water body and test the toxicity of the samples using modified toxicity testing procedures [96], normally involving a 100% Daphnia or Microtox test (no dilution series). In this way, regulatory authorities can assemble a toxicity profile of the receiving water. The data from these tests can be used in several ways [113]:

- to establish priorities for water pollution control resources
- to uncover the existence of in-stream toxicity
- to reveal the presence of illegal point source discharges
- to reveal the presence of other sources of pollution (such as, leaking hazardous waste dumps, and non-point source runoff that contains toxicants)

At several of the trial sites investigated for formulating the strategy for Water Quality Based Toxics Control [96], ambient testing disclosed previously unknown leaking waste disposal sites [114, 115].

6.4 THE REST OF THE WORLD

From other countries there is no or only scarce information on the use of ambient toxicity monitoring techniques.

7 CONTINUOUS BIOLOGICAL MONITORING WITH EARLY WARNING SYSTEMS

Biomonitoring methodologies that can be applied to both effluents and ambient waters, are the hardware monitoring devices that automatically measure the physiological or behavioral state of a biological entity in a continuous or semi-continuous way [116]. Sudden changes in the environment are timely detected by a biological response in the observed variable. These responses are evaluated in real-time to produce an alarm when a threshold is exceeded. When these types of biological early warning systems (BEWS) are used to test the quality of ambient waters, they only can act to trigger a study on the underlying causes. No immediate action can be taken to correct the situation. Application at intake points for water supply and other uses (process water) may help to recognize poor water quality, in which case water intake can be temporarily suspended. The actual strong point of such devices can be found in situations in or close to an effluent discharge point, where the response directly relates to adverse effluent quality. This application provides the opportunity to take counteractive measures before effects are liable to occur in the receiving water system. Obviously, this type of biological monitoring only provides information additional to "classical" (discrete) biomonitoring, when the effluent is highly variable both in concentration or in composition (batch processing).

7.1 EUROPEAN COMMUNITY AND RELATED COUNTRIES

France; Germany; The Netherlands; United Kingdom

In France, England, Germany and The Netherlands quite a lot of effort has been put in the design and validation of automated biological early warning systems, which recently (May 1993) culminated in a demonstration conference jointly organized by the German Bundes Gesundheitsamt (BGA, Institut fur Wasser-, Boden- und Lufthygiene), the Bundesministerium fur Forschung und Technologie, and SETAC. During this conference the results obtained with a large number of BEWS-devices (of which many are commercially available at the moment) were presented [117, 118, 119]. The major German conclusions of a 4-year comparative study on the applicability of some 20 BEWS-devices was that a battery of tests, based on different species and physiological responses, should be operated simultaneously in order to detect environmental levels of a wide range of toxicants. Test based on the following principles are suggested to be installed at crucial monitoring stations by the German BGA:

- swimming behaviour of daphnids
- productivity of algae
- reduction of light in luminescent bacteria

Additionally, the installation of a BEWS based on the valve closing reaction of bivalves is recommended for installation.

The present monitoring systems responding to the swimming behaviour of fish are considered not be sensitive enough.

For a fully equipped monitoring station the price tag for BEWS-devices will be around 100 k$.

Laboratory tests on the sensitivity of these monitoring systems suggest that they are likely to respond at concentrations close to acute lethal levels [120]. The current average and peak concentrations of surface water pollutants that can be identified and of which information on toxicity is available are generally far below these levels. Nevertheless, during field tests of a fish (swimming behaviour of *Leuciscus idus*) and a *Daphnia* monitoring system in the rivers Rhine and Meuse in The Netherlands, a number of alerts were recognized [120]. These alerts may be attributed to the combined effect of known and unknown compounds under prevailing field conditions.

Within the framework of the EC Council Regulation 2242/87 on Community Actions for the Environment, a project has been carried out in The Netherlands to demonstrate the applicability of biological early warning systems for protection of the environment against toxic industrial waste water discharges [121]. The responses of a monitoring system based on the rheotaxis reaction of the fish species *Leuciscus idus* was tested for a prolonged period on 3 different industrial effluents. From the experiences gained in this demonstration project, the following main conclusions can be drawn:

- At all three industrial sites a number of biological responses related to chemically verified peak discharges occurred during the test period.

- 50 percent of the responses could not be explained by chemical analysis.
- The frequency and magnitude of discharge peaks seem to justify continuous control.
- The possibilities for adequate intervention following an alarm, and the willingness to interfere in production processes are limited.

The same conclusions can be drawn from a demonstration project conducted by the Netherlands Organisation of Applied Science (TNO) on effluent induced valve movement responses in the mussel *Mytilus edulis* [119].

Several water supply intake stations in The Netherlands are at the moment equipped with BEWS. However, the intake of surface water is not (yet) automatically suspended when poor water quality is detected.

The Dow Benelux petrochemical industries at Terneuzen, The Netherlands, is up to now the only industrial complex using a BEWS-device based on the shell closure response of oysters (*Ostrea edulis*) to automatically control the process of cooling water chlorination. In this application the BEWS is actually hooked up to their process controller to produce reliable information on the amount of chlorination required to prevent mussels from blocking heat exchange piping, while reducing the amount of free chlorine in the effluent discharge.

8 ECOSYSTEM BIOMONITORING

It is very important to realize that nearly all biological responses observed in a natural ecosystem may be generated by a variety of either man-induced or natural stress factors. The nature of underlying causes, like toxic conditions, can hardly ever directly be concluded without further investigations. In this respect, ecosystem biomonitoring only can act as an "environmental thermometer" with limited diagnostic capacity. However, the acquired information on the integrity of an ecosystem is very closely related to the ultimate objective of environmental protection, which is preventing effects to occur.

8.1 EUROPEAN COMMUNITY

The EC-countries in general

Nearly all countries within the European Community (except Greece) have some kind of biological quality index for running freshwaters, based on the indicative capacity of benthic macro-invertebrates. The methods applied are different for nearly all countries. As a demonstration of variety, the major properties are summarised in table 4 [78].

Table 4: Biological water quality indices used in the EC countries

COUNTRY	SAMPLING METHOD	ANALYSIS	LEVEL OF TAXONOMIC DETERMINATION	STANDARDISATION	RANGE OF QUALITY INDEX
Belgium	Qualitative	Qualitative	OFG	N	0-10
Denmark	Qualitative	Qualitative	FGS	N	1-4
France	Quantitative Qualitative	Quantitative	F	N	0-20
Germany	Qualitative	Quantitative	S	N	1-100/1-4
Greece	-	-	-	-	-
Ireland	Qualitative	Qualitative	FGS	N	0-5
Italy	Qualitative	Qualitative	OFG	R	0-14
Luxembourg	Qualitative	Qualitative	OF	N	0-10
Netherlands	Qualitative	Qualitative	FGS	R	100-500
Portugal (equals Belgium)	Qualitative	Qualitative	OFG	-	0-10
Spain (equals UK)	Qualitative	Qualitative	F	-	0->150
UK	Qualitative	Qualitative	F	N	0->150/0-10

O = Order G = Genus N = National
F = Family S = Species R = Regional

The methodologies applied in Belgium, The Netherlands and in the UK will be treated in detail under the appropriate heading.

Belgium

In 1978 the Belgium Ministry of Public Health took the decision to develop and adopt a generally applicable biological assessment method for running waters based on the analysis of benthic macro-invertebrate communities. It was decided to combine the advantages of the English Trent Biotic Index (TBI) [122] and the French Indice Biotique (IB). This resulted in the formulation of the Belgian Biotic Index (BBI) [123]. The modifications mainly deal with the standardisation of the sampling procedure (usually 5 minute kick sampling with a handnet) and the level of taxonomical identification standardised at the genus or family level. The BBI produces a quality score from 0 to 10, where a higher value stands for better quality (more sensitive species present). The index values are divided over five water quality classes, each represented by different colour codes in the nation wide water quality maps produced since 1979. To facilitate the application of the BBI, a detailed description of the method and the taxonomical keys needed for the identification of the macro-invertebrates was published both in French [124] and in Flemish [125]. In addition to the BBI method, other assessment methods are applied in particular situations and for particular purposes. For monitoring large rivers, Descy et al. [126] have used periphytic diatoms to evaluate short-term changes of water quality. For long-term evaluation of water and habitat quality, fish inventories also proved to provide useful information [127].

France

Next to evaluating macro fauna inventories with a biotic scoring system, French water quality control agencies use morphological characteristics of stationary individuals and colonies of freshwater oligochaetes to numerically quantify the degree of pollution in water and sediments [94].

Ireland

In Ireland, the efficacy of effluent toxicity limitation is at least every three years tested by means of conducting biological surveys in receiving waters [94].

The Netherlands

In the early 80's, Slooff et al. [128, 129, 130, 131] conducted an elaborate eco-epidemiological study on the health, growth, fecundity and morphological deviations in endogenous populations of the fish species *Abramis brama*. A grand total of 7000 specimen of this fish species were examined from a variety of sampling stations (River Rhine, River Meuse, Lake Braassem, Lake IJssel). A clear positive relation could be observed between the pollution status at the site where the fish originated and the incidence of skeletal deformities and liver enlargement. Fish from more polluted sites show an increase in the number of eggs produced, but a decrease in the size of the eggs and probably recruitment. The calculated body length of 5-year old fish of different spawning years (1966-1976) showed a marked increase which may be related to the observed general improvement of water quality. A clear relation between toxic stress and the incidence of neoplastic lesions (carcinoma) could not be demonstrated.

Under auspices of the National Water Quality Monitoring Programme (MWTL) of the Ministry of Transport, Public Works and Water Management, a number of ecosystem monitoring activities are taking place in larger freshwater bodies and tidal waters.

The freshwater ecosystem monitoring programme consists of the following measurements, which are performed at stations that coincide with the stations of the chemical and ecotoxicological monitoring programme:

- Phytoplankton abundance, biovolume and species composition
- Zooplankton abundancy, biomass and species composition
- Benthic macrofauna inventories
- Zebra mussel abundancy
- Macrofauna inventories (artificial substrate colonisation)
- Submerged and emergent water plant inventories
- Fish inventories
- Water fowl inventories
- Incidence of mandibular deviations in chironomids

The tidal water programme [103] has the following variables (the stations are also coincident with the chemical monitoring stations):

- phytoplankton abundance and species composition
- Vegetation development of macroalgae, seagrass and salt marsh vegetations
- Inventories of macrozoobenthos
- Shore dwelling bird inventories
- Sea bird inventories
- Sea mammal inventories

- Inventories on commercial fish stock (including the incidence of fish diseases)

For both the freshwater and the tidal water systems, the biological integrity is expressed as the deviation from biological water quality criteria, by means of a so called AMOEBA radar plot [132] (Dutch acronym for General Method of Ecosystem Description and Assessment), where the actual abundancy of keynote, indicator or assessment endpoint species is expressed as a percentage of a projected optimum (reference state or target state).

Figure 5 presents an example of an AMOEBA evaluation for Lake IJssel.

At the moment attemps are being made to use a Habitat Evaluation Procedure to formalize the definition of the reference state [133, 134].

The CUWVO (Commission for Execution of the Water Pollution Control Act) [135] identified detailed biological water quality objectives and criteria for different types of freshwater systems in The Netherlands. For running waters, the STOWA (Foundation for Applied Studies on Water Management) refined the CUWVO

FIGURE 5 Example of an AMOEBA evaluation of biological integrity

recommendations into a biological macrofauna based qualification system [136].

Regional water quality authorities all operate their own biomonitoring systems with different variables and different ways of storing the monitoring data. As a consequence, the monitoring results of individual

authorities can not be assembled in one fully comprehensive database, thereby degrading the value of individual efforts for pollution control on a nation-wide scale. Recently, efforts have been made to align the individual monitoring activities, to form a National Aquatic Monitoring Network [137].

United Kingdom

In the 1970 river water quality survey, a biological method was introduced for the first time to supplement the established chemical classification. The system was simple, recognizing four classes, each characterized by groups of animals indicative of different water qualities. The four classes were defined as:

A Rivers with a diverse invertebrate fauna, including an appreciable proportion of Plecoptera (stonefly nymphs) or Ephemeroptera (mayfly nymphs), Trichoptera (caddisfly larvae) and Amphipoda (freshwater shrimps). Salmon, trout and grayling fisheries should be present when purely ecological factors favour the presence of these fish. Otherwise good mixed course fisheries should indicate the presence of a variety of species.

B Rivers with a varied invertebrate fauna, in which Plecoptera and Ephemeroptera populations may be restricted. Trichoptera and Amphipoda are usually present in reasonable numbers. Good mixed-coarse fisheries will prevail, and trout may be present but will rarely be dominant.

C Rivers in which the variety of macro-invertebrate organisms is restricted and the community is dominated by the Isopod *Asselus aquaticus*. Some Amphipoda may be present, but Trichoptera and Ephemeroptera are relatively rare.

D Macrofauna is absent or severely restricted to pollution tolerant Oligochetes and Chironomids. Rivers known to be incapable of supporting fish life.

The application of this biological classification system to a national survey including all rivers in England and Wales was a severe test. The report admits to having some reservations about the adequacy of the biological classification for application to all types of rivers. The most obvious inconsistencies between the chemical and the biological classifications arose in the slow flowing rivers of East Anglia. This is due to the fact that the system used, which required an appreciable proportion of Plecoptera, Ephemeroptera, Trichoptera and Amphipoda, was designed for the riffles of fast flowing rivers. Such a community is not found in the sluggish muddy bottomed rivers of East Anglia, however good the water quality. This illustrates the need to take into account the type of river in the interpretation of benthic communities in relation to water quality.

Following the disappointing results of the 1970 biological classification of rivers, no biological classification was included in the 1975 survey, but a Biological Monitoring Working Party (BMWP) was set up by the Department of Environment (DoE) to recommend on a biological classification system for future use in national river pollution surveys. The working party failed to agree on a biological classification system for river water quality, but finally recommended a classification system for "the biological condition of rivers" based on a scoring system [138]. The score was intended to monitor changes at defined points on a river over a period of time, and was not to be used to compare different stations or different rivers. Economic constraints, in terms of available resources, dictated that the system should be simple, necessitating a compromise between ecological validity and logistic feasibility. Only qualitative sampling and identification to family level is required. The system is based on a score derived from points attributed to different invertebrate families according to their degree of intolerance to organic pollution. Again the BMWP score is liable to give higher marks for fast flowing rivers than for sluggish rivers. The system was applied to the 1980 national river pollution survey. The published results [139] indeed show that lowland rivers score lower than upland rivers because of the effects of current and substrate differences. Although comparison of scores in different rivers is discouraged, it is tempting to consider a river with a higher score in some way superior to a river with a lower score.

A further criticism of the BMWP score system is that being dependent on the numbers of taxa, the score is highly depending on sample size, sampling effort and sampling efficiency. For this reason the Average Score Per Taxon (ASPT), which is independent of the numbers of taxa and therefore less sensitive to sampling errors and seasonality, is preferred by some biologists [140]. However, since the ASTP does not take the number of taxa into account, it looses information and is less sensitive to toxic pollution.

New developments in biomonitoring and biological water quality classification in the UK are related to the comparison of the actual biological community with a computerised prediction of the community composition or BMWP-score for a given water body in its un-polluted state [141]. The computer model RIVPACS uses sets of environmental variables, like flow, depth, temperature, substrate composition, etc., to calculate the species composition at a site were the site to be un-polluted. The ratio of the actual over the predicted situation will produce an Ecological Quality Index (EQI). The 1990 national river water quality survey used the computer model RIVPACS to predict the BMWP-score for all 8796 sites and to produce EQI-values which in theory are independent of the type of river and only related to pollution [142].

UN-ECE

In 1979 arrangements were made in the "Convention on Long Range Transboundary Air Pollution" (CLRTRAP), on the initiative of the United Nations Environmental Programme (UNEP) and the United Nations Economic Commission for Europe (UN-ECE) to prevent and fight long range air pollution. The CLRTRAP appointed an executive body which initiated several expert, coordinating and working groups, including the "Working Group on Effects". This working group started several International Cooperative Programmes (ICP's) in specific areas such as forests, freshwaters, materials and crops. The ICP on Assessment and Monitoring of Acidification of Rivers and Lakes was established in 1985 with the objective to monitor the degree and geographical extent of acidification in surface water. The data collected were to provide information on the correlation between acid deposition and the physical, chemical and biological status of lakes and streams in terms of dose/response relationships on a regional basis. The first task of the ICP was to produce a manual to enable the collaborating countries to produce qualified and comparable monitoring data [143]. Since then, a growing number of countries (14 in 1993) with an increasing number of sites (200 in 1993) are taking part in this monitoring programme. The biological effects part of the monitoring programme is composed of inventories of invertebrate benthic fauna, diatoms and fish in terms of diversity, species composition and abundance. In monitoring acidification it is necessary to find an evaluation system where the biological effects of acidification are distinguished from the effects of other perturbations. This is achieved by using an index based on the presence/absence of acid sensitive species according to Raddum et al. [144]. The focus of this model is species specific tolerance towards acidic water, while other environmental factors are of secondary importance. The presence of a sensitive species will mainly be determined by acidity, while the abundance is determined by other factors.

Under the UN/ECE Convention on Protection and Use of Transboundary Watercourses and International Lakes (Helsinki, 1992), the Task Force on Monitoring and Assessment is in the process of providing guidelines for monitoring and assessment. The Task Force, which is composed of national designated experts from 20 countries, will have drafted guidelines available by mid 1995.

8.2 OTHER EUROPEAN COUNTRIES

Norway

The industry as well as densely populated areas along the Norwegian coastline are very often situated along fjords, representing well defined point sources of pollution. Fjords are deep marine intrusions of the main land that act as sediment traps. Since the majority of pollutants are associated with particulate material, these sediments act as sinks for pollution. The greater part of the bottom area in fjords consist of soft sediments which act as substrate for the so called "soft-bottom fauna". Normally this fauna is very abundant and diverse. The number of animals bigger than 1 mm typically reaches 1000-2000 per m^2, representing 60-90 different species. Studies in a number of fjords in Norway showed a marked negative correlation between the diversity of the soft-bottom fauna and pollutant discharge amount, the proximity to discharge sites and sediment pollutant load [145].

8.3 THE NORTH AMERICAN CONTINENT

USA

Discrepancies between results of a so called Habitat Evaluation Procedure (HEP) and an evaluation of biological integrity, as indicated by biosurveys and bioassessments [146], and many more in 96] are considered to indicate the combined action of toxic pollution, eutrophication, low oxygen levels, altered temperatures and pH and other disturbances.

The first step in this procedure is to determine a biological reference state for a given water body, with respect to the types and numbers of organisms that ideally should be present. Normally a selection will be made on the most valued species (biological water quality criteria/objectives, the assessment endpoints!!!). It should be noted that the ecological or societal value of a species may be very different from its value as an economic resource. In order to accomplish this, the suitability of a particular habitat for a particular species can be modelled. In this aspect, the habitat is defined as the set of physical, chemical and biological constraints, that a locality is imposing on the species. The inputs for these so called Habitat Suitability Index Models (HSI) are quantified relationships in the form of species optimum curves for specific aspects of their environment. The result of running a HSI model is a numeric score proportional to the most optimal conditions for the species, where optimal habitat quality is defined to allow for maximum density of the observed species. From these HS-indexes the local species abundancy can be calculated for each area. When biosurveys (inventories on the presence and abundancy of species), executed as the second step, indicate a deviation from

the projected population success of a species, it can be concluded that environmental factors are playing a role, that were not included in the HEP Procedure.

Tables 5 & 6 represent the ecological water quality indicators formulated by EMAP [13]

Table 5: Linkages between potential environmental values (assessment endpoints), measurements, metrics and response indicators for EMAP inland surface waters.

Environmental values (assessment endpoints)	Measurements	Metrics	Indicators (with scoring criteria)		
Number or % of fishable waters			Fishability index (0 or 1)		
Gamefish sp. present	Species identification	Relative abundance	Absent (0)		
Gamefish abundance	Number of individuals	Catch per unit effort (CPUE)	< 0.002 per 1/8 hr (0)		
Gamefish size	Individual length/weight	% keepers, % trophy	< 50% keepers (0)		
Fish appearance	External anomalies	% anomalies	> 1% with anomalies (0)		
Fish edibility	Toxic concentrations	Cunsumption criteria violations	Restricted > once/year (0)		
Fishery sustainability	Individual weight, length, scales, stocking records, catch restrictions	age/size structure,	Juveniles absent (0)		
			< 50% wildfish (0)		
		% wildfish, % keepers	< 50% keepers (0)		
Trophic condition					
Noxious algal blooms, surface scums	Pigment concentration, visual, sediment diatom sp. & abundance	% blue-green algae	> 10% (0)		
		% nuisance species	> 10% (0)		
Macrophytes	Macrophytes	% of lake macrophyte dominated	> 25% littoral zone (0)		
Low transparency	Secchi depth	Secchi depth	< 2 m (0)		
Noxious taste/odor	Threshold odor	Threshold Odor Number	> 10 (0)		
Fish kills	Hypolimnion oxygen concentration	% depth < 3 mg DO/l	> 50% (0)		
Trophic state	Pigment concentration, total phosphorus, Secchi depth, total nitrogen	Trophic state index	(1)	(3)	(5)
			<30	30-60	>60
Change of trophic state	Sediment diatom sp. & abundance	% oligo/meso/eu and dystrophic species	Rate of hisorical trophic state change		
		Dominant sp: % epiphytic, % planktonic			
Biological integrity			Biointegrity index[1]		
			(1)	(3)	(5)
"Dead" lakes/streams	No of individuals[2]	CPUE	<33%	33-67%	>67%[3]
Decline in species richness	Sp. identification and number[2]	No. of species	<33%	33-67%	>67%[3]
Decline in sensitive species	Sp. identification and number[2]	% sensitive	<33%	33-67%	>67%
Increase in tollerant species	Sp. identification and number[2]	% tolerant	>25%	10-25%	<10%
Increase in exotic species	Sp. identification and number[2]	% exotic	>10%	1-9%	<1%
Evidence of kills	Questionnaire	Kill frequency	1-5 yr	>5 yr	no kill
Increased anomalies	External anomalies in fish	% anomalies	<5%	1-5%	<1%
Decreased abundance	Number of individuals (fish)[2]	CPUE	<33%	33-67%	>67%[3]
Decreased maximum ind. size	Individual length and weight (fish)	% old/growth	<1%	1-10%	>10%
Historical dislocation	Diatom species and abundance	% similarity	<25%	25-75%	>75%

[1] The index of biotic integrity condition (IBC) will be used for stream fish and macroinvertebrate assemblages. Criteria for both are based largely on regional reference site values
[2] Includes sedimentary diatoms, fish and birds for lakes; macroinvertebrates, fish and birds for streams
[3] Determined from regional reference site values

Table 6: Linkages between potential environmental values (assessment endpoints), measurements, metrics and exposure indicators for EMAP inland surface waters.

Environmental values (assessment endpoints)	Measurements		Indications
Are fish safe to eat?	Concentrations of muscle toxics (metals, organics)		Violation of consumption criteria > 1/year
Are waters becoming more eutrophic?	P, N, Ca, Cl, TSS, Secchi, Temp., pigments		Trophic State Index (TSI)
	Lake sedimentary diatom spp. & abundance		% eutrophic, mesotrophic, oligotrophic
Are waters being more saline?	Cl, Tds		Total chlotride, total dissolved solids
	Lake sedimentary diatom spp. & abundance		% halophilic, halophobic
Is water acidity changing?	pH, DIC, ANC, Al, SO_4, NO_3, Cl, DOC, TN, Na, K, Mg, Ca, NH_4		ANC, anions, cations
	Lake sedimentary diatom spp. & abundance		
Are waters warming?	% canopy (streams)		% canopy (<25%, 25-50%, 51-75%, >75%)
			% sternothermal, metathermal, eurythermal
Are water becoming more turbid?	Secchi, turbidity, TSS		Secchi depth, turbidity, total suspended solids
Are sediments toxic to aquatic life?	*Hyalella*, *Ceriodaphnia* & *Pimephales*		Toxicity significantly greater that controls
	Mortality & reproduction or growth		
What is the critical level of habitat alteration for eliminating habitat-sensitive fish and wildlife?	LAKES	% littoral dominance, temp. & DO profiles, lake area, max. depth, level fluctuations, substrate cover, depth variation, shoreline development, land use and vegetation	Lake Habitat Quality Indices (LHQI)
To what degree is biological impairment due to natural habitat conditions?	STREAMS	Widths, depths, substrate, cover, embeddedness, flow, channel alteraion/complexity, pool/riffle/run/bend, bank stabilty, riparian vegetation, immediate land use	Stream Habitat Quality Index (SHQI) (% regional reference site value <25%, 26-50%, 51-75%, >75%)

A large number of biosurvey methods to assess biological integrity in different types of aquatic habitats are presented in literature reviews on biological criteria [77, 147], as well as in a compilation of symposium proceedings [148], and an implementation progress report [149].

Another, but closely related, approach for the verification of biological integrity, can be found in simple checking against pre-formulated ecological indicators and criteria for specific types of water systems [13]. Deviations from the nominal indicate adverse water quality. The US Environmental Monitoring and Assessment Program identified a large number of potentially valuable water quality indicators together with

scoring criteria. Again, the indicators are closely linked to assessment endpoints. As an example, a selection for inland freshwaters is given in the tables 5 and 6 [13].

8.4 THE REST OF THE WORLD

India

With the aid of the Netherlands International Cooperation Programme on the Environment, The Indian Central and State Pollution Control Boards designed a river water quality evaluation system comprised of chemical, biological and bacteriological variables [150]. Groups of variables related to specific aspects of pollution or effects are combined in a total of 9 indices that can be presented in a kind of AMOEBA figure, to disclose the deviations from target index values. The following figures give (Figure 6) the relation of the different indexes with specific aspects of pollution, (Figure 8) the measured variables, and (Figure 7) an example of the graphic presentation relevant to decision-taking and pollution control.

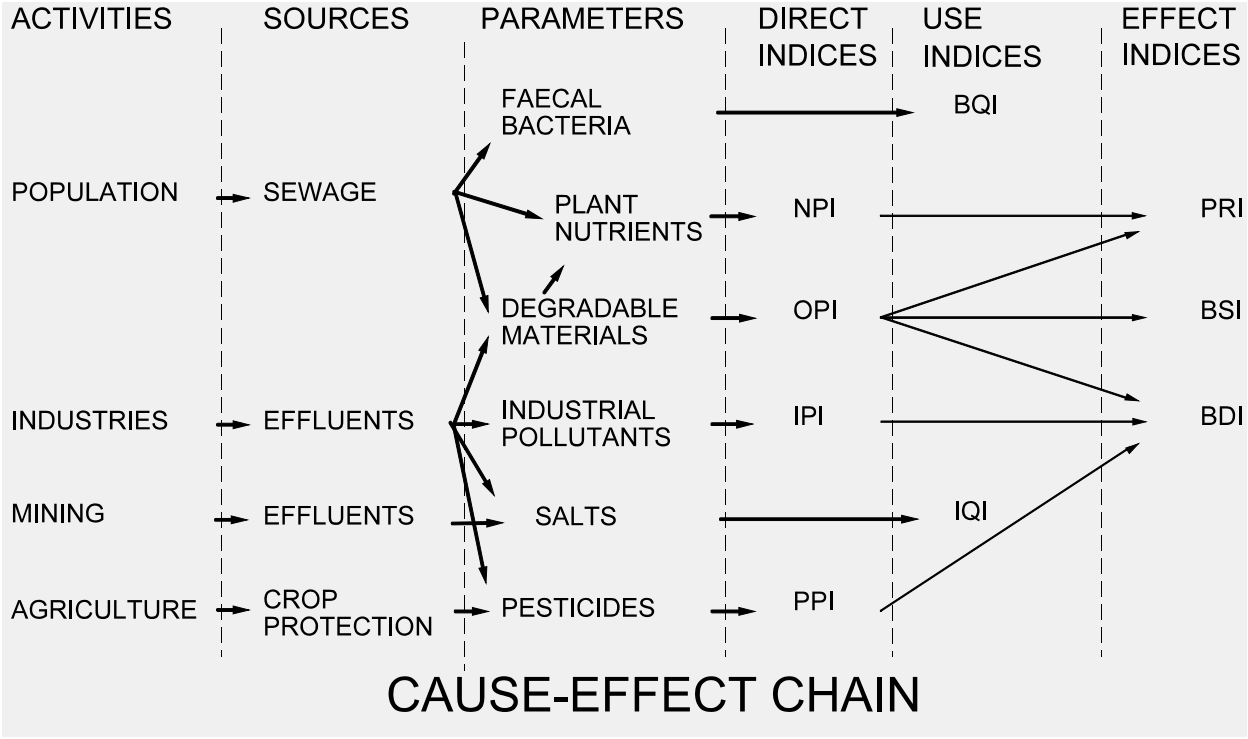

FIGURE 6 The relation of pollution indices and specific aspects of pollution

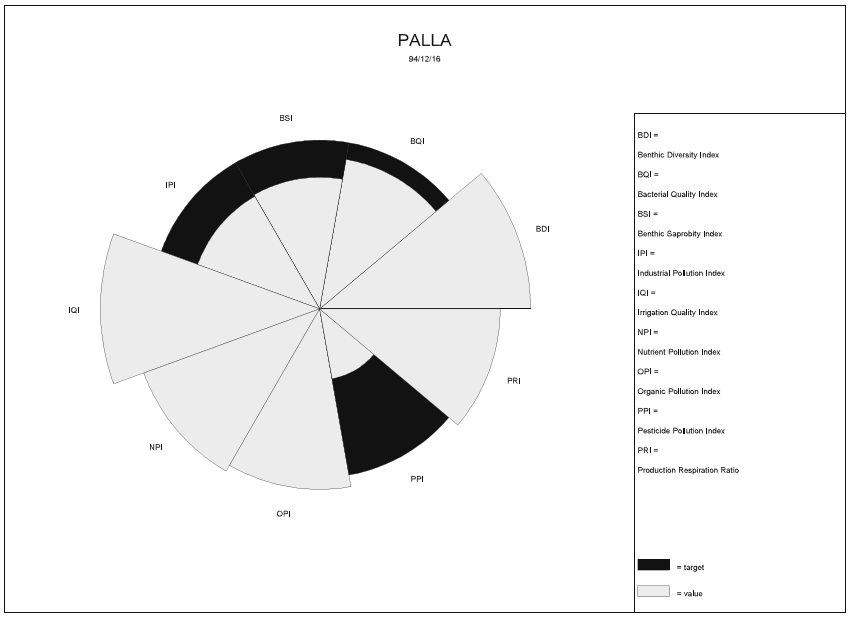

FIGURE 7 Example of a river water quality revealing AMOEBA figure

NON-TOXIC PHYSICO-CHEMICAL POLLUTION		TOXIC POLLUTION of INDUSTRIAL AND AGRICULTURAL ORIGIN		USAGE QUALITY		BIOLOGICAL EFFECTS		
OPI	NPI	PPI	IPI	BQI	IQI	BSI	BDI	PRI
avg. 24 hr temperature	ammonium-ion	chlorinated pesticides	heavy metals	*Escherichia coli* bacteria or thermotolerant bacteria	sodium adsorption ratio	Saprobity score benthic macrofauna (BMWP)	Diversity of benthic macrofauna (SCI)	24 hr P/R ratio
BOD	total Kjeldahl nitrogen	organo-phosphorus pesticides	oil	viral indicators	conductivity		Species deficit score (SDS)	
COD	nitrate	acute toxicity	PAH	etc.	pH			
minimum 24 hr DO saturation	total P	etc.	phenol		etc.			
ammonium-ion	ortho P		cyanide					
etc.	chlorophyll		PCB					
	maximum 24 hr DO saturation		free ammonia					
	turbidity		nitrite					
	etc.		acute toxicity					
			etc.					

FIGURE 8 The parameters measured (monthly)

9 BIOMONITORING VARIABLE EVALUATION

In paragraph 2.3 a range of criteria are given for the selection of usable groups of biomonitoring variables. These criteria are used in table 7 to **comparatively** evaluate the applicability of the groups of variables presented in paragraph 2.5. The pre-specified criteria, except estimates of capital and operational investments for single measurements and the standardization, are **subjectively** scored for each of the biomonitoring variables on a scale of 1 to 9 indicating minimum and maximum compliance or appropriateness. The minus sign (-) and the question mark (?) are used to indicate that a specific criterion is respectively not applicable or unquantifiable. The column for evaluation standardization shows a P if standardization is in the process of being formulated, while I and N are standing for standardization at an international or national level, respectively. As has been discussed earlier, it should be realized that there will be no variable scoring high for all criteria, and that there is no use in adding or averaging the criterion scores, since the ultimate choice of a biomonitoring variable will largely depend on the monitoring objective in a particular situation.

It proved to be impossible to make generally applicable estimates of budget requirements needed to produce valuable results in a systematic biomonitoring approach due to the fact that local situation would normally require an indeterminate number of different tests and measurements to be performed an indeterminate number of times at an indeterminate number of stations.

Table 7: Evaluation of groups of biomonitoring variables against criteria for variable selection.

TEST or OBSERVATION TYPE	COMPARTMENT	ORGANISM or TEST METHOD	TEST or OBSERVATION CRITERIUM	SCORE FOR SEL. CRITERIA: 1-9 = very bad to very good prospects, - = not applicable, ? = unknown															
				SCIENTIFIC					EFFICIENCY								ADMINISTRATIVE		
				ENV.-INFORMATION	ECO-INFORMATION	SPECIES-SPECIFICITY	PROBLEM-SPECIFICITY	REVERSIBILITY	CONC./EFFECT-REL.	SENSITIVITY	RESPONSE-RANGE	RESPONSE-RATE	SIGNAL/NOISE	PRECISION	STANDARDISATION	COST-EFFECTIVITY	RETROSPECTION	EQUIPMENT COSTS order of magnitude in K$ per facility or apparatus	RUNNING COSTS order of magnitude in K$ per test or observation
Laboratory toxicity test single species acute	Freshwater or effluents with or without concentration procedure	fish	lethality	5	5	7	5	-	9	5	8	6	6	7	I	6	9	10	0.4
		Daphnia	lethality immobilisation	5	5	7	5	-	9	5	8	6	6	7	I	7	9	5	0.3
		bacterial luminescence	light emission	5	5	5	5	-	9	5	8	9	7	8	N	9	8	20	0.1
		Daphnia IQ test	enzyme inhibition	5	5	7	5	-	9	4	8	8	6	7	P	7	5	5	0.3
		Rotoxkit F	lethality	5	5	7	5	-	9	5	8	7	6	7	N	9	7	1	0.1
		Thamnotoxkit	lethality	5	5	6	5	-	9	4	8	7	6	7	P	7	7	1	0.1
		Toxichromotest	enzyme inhibition	5	5	5	5	-	9	4	8	8	6	7	P	9	5	3	0.1
		Ames-test SOS-chromotest Mutatox test	bacterial mutagenicity	4 4 4	4 4 4	3 3 3	7 7 7	- - -	9 9 9	7 7 7	8 8 8	7 8 8	6 6 6	8 8 8	I P P	7 7 7	9 6 6	5 1 20	0.3 0.2 0.1
	Saline water or effluents with or without concentration procedure	bacterial luminescence	light emission	5	5	5	5	-	9	4	8	9	7	8	N	9	8	20	0.1
		Rotoxkit M	lethality	5	5	6	5	-	9	4	8	7	6	7	N	9	6	1	0.1
		Artoxkit (brine shrimp)	lethality	5	5	6	5	-	9	4	8	7	6	7	P	9	6	1	0.1
	Freshwater and saline Sediments	bacterial luminescence	light emission	5	5	5	5	-	9	4	8	9	7	8	N	9	7	20	0.1
	Freshwater sediments	Sediment chromotest	enzyme inhibition	5	5	5	5	-	9	4	8	8	6	8	P	7	6	3	0.1
Laboratory toxicity tests single species (sub)chronic	Freshwater or effluents	protozoa/bacteria	population growth	7	7	6	5	-	9	6	8	6	6	8	N	7	8	5	0.5
		algae	population growth	7	7	6	5	-	9	7	8	6	6	8	I	7	8	10	0.5
		Daphnia	reproduction	7	8	7	5	-	9	7	8	5	6	7	I	7	8	5	0.7

TEST or OBSERVATION TYPE	COMPARTMENT	ORGANISM or TEST METHOD	TEST or OBSERVATION CRITERIUM	SCORE FOR SEL. CRITERIA: 1-9 = very bad to very good prospects, - = not applicable, ? = unknown															
				SCIENTIFIC					EFFICIENCY								ADMINISTRATIVE		
				ENV.-INFORMATION	ECO-INFORMATION	SPECIES-SPECIFICITY	PROBLEM-SPECIFICITY	REVERSIBILITY	CONC./EFFECT-REL.	SENSITIVITY	RESPONSE-RANGE	RESPONSE-RATE	SIGNAL/NOISE	PRECISION	STANDARDISATION	COST-EFFECTIVITY	RETROSPECTION	EQUIPMENT COSTS order of magnitude in K$ per facility or apparatus	RUNNING COSTS order of magnitude in K$ per test or observation
		fish	ELS (early life stage), growth	7	8	7	5	-	9	7	8	5	6	7	I	7	8	20	1.5
		Lemna test	colony growth	7	7	6	5	-	9	7	8	5	6	7	N	7	7	5	0.7
		fish	chromosome abberation	6	6	6	5	-	9	?	8	5	6	7	P	7	4	10	1.0
	Saline water or effluents	fish	ELS growth	7	7	6	5	-	9	7	8	5	6	7	N	7	4	5	0.7
	Freshwater sediments	*Daphnia* porewater test	reproduction	7	7	6	5	-	9	7	8	5	6	7	N	7	8	5	0.7
		Chironomus sediment test	larvae development	7	7	6	5	-	9	6	8	5	6	7	N	7	8	5	0.7
	Saline sediments	oyster larvae sediment test	larvae development	7	7	6	5	-	9	7	8	5	6	7	N	7	8	5	0.7
Laboratory toxicity tests suborganismal	Freshwater or effluents	in-vitro tissue tests	growth, lethality, histopathology	4	4	-	5	-	9	?	8	7	6	7	?	7	?	?	?

TEST or OBSERVATION TYPE	COMPARTMENT	ORGANISM or TEST METHOD	TEST or OBSERVATION CRITERIUM	SCORE FOR SEL. CRITERIA: 1-9 = very bad to very good prospects, - = not applicable, ? = unknown															
				SCIENTIFIC					EFFICIENCY							ADMINISTRATIVE			
				ENV.-INFORMATION	ECO-INFORMATION	SPECIES-SPECIFICITY	PROBLEM-SPECIFICITY	REVERSIBILITY	CONC./EFFECT-REL.	SENSITIVITY	RESPONSE-RANGE	RESPONSE-RATE	SIGNAL/NOISE	PRECISION	STANDARDISATION	COST-EFFECTIVITY	RETROSPECTION	EQUIPMENT COSTS order of magnitude in K$ per facility or apparatus	RUNNING COSTS order of magnitude in K$ per test or observation
Field toxicity tests(semi)continuous Early warning	Freshwater or effluents	fish	-ventilation	7	4	5	5	6	6	4	4	9	6	4	N	6	7	50	1/wk
			-rheotaxis	7	4	5	5	6	6	4	4	9	6	4	N	6	7	25	1/wk
			-swimming behaviour	7	4	5	5	6	6	4	4	9	6	4	N	6	7	50	1/wk
		algae	productivity	7	4	5	5	7	6	4	4	9	7	7	N	7	6	10-25	1/wk
		bacteria	-luminescence	7	4	5	5	9	9	4	9	8	7	8	N	7	5	40	1/wk
			-respiration	6	4	5	5	7	6	4	4	8	7	8	N	7	6	20	1/wk
		Daphnia	swimming activity	7	4	5	5	6	6	5	4	9	6	4	N	6	7	15	1/wk
		mussels	valve movement	7	4	5	5	8	6	4	6	9	6	4	N	7	7	20	0.5/wk
	Saline water or effluents	mussels	valve movement	7	4	5	5	8	6	4	6	9	6	4	N	7	7	20	0.5/wk
Field toxicity tests active monitoring	Freshwater and Saline water	caged organisms	lethality, growth, reproduction, bioconcentration,																
			survival in air,	8	9	6	4	6	6	7	4	5	5	4	-	7	5	10	1/wk
			scope for growth	6	7	5	3	6	6	7	4	6	4	3	-	6	5	15	1
			Biomarkers: -																
			metallothioneine formation	5	6	5	8	8	8	6	6	6	5	6	-	7	5	20	0.5
			-lysosome stability	5	6	5	3	7	7	6	6	6	5	6	-	7	5	15	0.5
			-MFO-induction	5	6	5	8	8	8	6	6	6	5	6	-	7	5	15	0.5 /st
Observations on effects in the field passive monitoring	Erxamoles available for freshwater, saline water and sediments	eco-epidemiology in selected species -fish - Chironomus	Incidence of diseases and morphological deviations	8	9	5	4	7	5	7	6	4	3	7	-	5	6	10	1/st
		indicator species	presence absence	7	7	5	5	7	5	7	5	3	5	7	N	7	7	5	1/st

TEST or OBSERVATION TYPE	COMPARTMENT	ORGANISM or TEST METHOD	TEST or OBSERVATION CRITERIUM	SCORE FOR SEL. CRITERIA: 1-9 = very bad to very good prospects, - = not applicable, ? = unknown																
				SCIENTIFIC					EFFICIENCY									ADMINISTRATIVE		
				ENV.-INFORMATION	ECO-INFORMATION	SPECIES-SPECIFICITY	PROBLEM-SPECIFICITY	REVERSIBILITY	CONC./EFFECT-REL.	SENSITIVITY	RESPONSE-RANGE	RESPONSE-RATE	SIGNAL/NOISE	PRECISION	STANDARDISATION	COST-EFFECTIVITY	RETROSPECTION	EQUIPMENT COSTS order of magnitude in K$ per facility or apparatus	RUNNING COSTS order of magnitude in K$ per test or observation
		colonisation of artificial substrates	species composition, diversity, abundancy	8	9	7	4	7	5	8	5	3	3	7	N	6	7	5	1/st
		community structure -benthic macrofauna -diatoms	species composition, diversity, abundancy	8	9	7	4	7	5	8	5	3	3	7	N	5	7	5	1/st
		ecological functioning	primary productivity, respiration, biomass, turnover, degradation, material cycling	8	9	8	4	8	5	8	6	6	4	7	-	6	5	?	?

/wk = per week
/st = per station

10 CONCLUSIONS

From the immense variety of biomonitoring variables being designed and applied for toxics control in the aquatic environment over the past few decades, it can be concluded that biomonitoring is generally considered to be a valuable source of pollution information. Since monitoring information requirements and monitoring objectives are very situation specific and are strongly dependent on national water management policies, it is very unlikely that the near future will show a global trend towards unification of standard biomonitoring protocols. For the coming decades, the diversity in scarcely applied monitoring variables and strategies will probably only increase. However, specifically with reference to the draft Directive on the Ecological Quality of Surface Water, a drive is felt within the European Community to unify the concepts of biological water quality evaluation.

Regarding the development of environmental toxicity tests for effluents and ambient water bodies, the driving force behind the continuous involvement of new test species needing adapted test protocols, is the wide-spread opinion of ecotoxicologists that the biotesting results only model real world effects when local species are used. Provided that a set of sufficiently diverse (reflecting the principle components of the aquatic food chain) and globally standardized tests are available and used, the scientific community would more efficiently spend time and money in trying to design universally applicable extrapolation methodologies based on sound statistical evaluations [see for instance 97]. At the moment only the acute ecotoxicity tests on Daphnia, fish and luminescent bacteria are (in the process of being) internationally standardized. For more chronic exposure international standardization relates to fish, algae and Daphnia only. The set of internationally standardized ecotoxicity tests should preferably encompass additional species from different trophic levels and functionality, e.g. waterplants, bacteria, molluscs, insect larvae, etc. Toxicity testing is restricted to a few highly specialized laboratories, and is not routinely practiced because of the high costs involved. Consequently there is an increasing demand for alternative tests which are rapid, user-friendly and more cost-effective, without neglecting ecological realism and possibilities for extrapolation.

At the moment, automated ecotoxicity early warning systems are mainly used for checking the quality of surface water before the water is used. Due to slow changes in water quality and considerable dilution, only real catastophes are liable to be detected. More effectively these monitoring techniques can be applied for the prevention of accidental industrial pollution. In this context, continuous automated toxicity monitoring devices should be installed and operated by high-risk industries at the end of the pipe in conjunction with effluent storage and clean-up facilities. At these locations, the water quality gradients in time are expected to be steep enough to allow for timely and reliable detection.

The evaluation of ecosystem effects measurements is generally done by comparing the results of inventories along established pollution gradients. The monitoring efforts could be evaluated a lot more effectively if it were possible to quantify the observed effects in a more absolute way by applying modelling techniques to predict the "natural" biological community against which the "observed" community can be compared.

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GLOSSARY AND ABBREVIATIONS

Abiotic Not associated with living organisms.

Absorbed dose Amount of substance absorbed by an organism or by specific tissues.

Absorption Process of active or passive transport of a substance across biological membranes or other barriers into an organism. Absorption through the gills is an important transport route for many aquatic species.

Abundance The degree of plentifulness.

Accidental pollution An unexpected occurrence, failure or loss, either at a plant or along a transportation route, resulting in a release of hazardous materials.

Acclimatization (1) Processes, including selection and adaptation, by which a population of microorganisms develops the ability to degrade a substance or develops a tolerance to it. (2) In toxicity tests: allowing organisms to adjust to their environment prior to undertaking a study.

Accumulation Successive additions of a substance to a target organism, organ or to an environmental compartment, resulting in an increasing amount or concentration of the substance in the organism, organ or environment.

Accuracy (statistical) Indicates how close the measured magnitude of a quantity is to the true magnitude of the quantity in the population, whereas preci- sion indicates the reproducibility in the magnitude of the measured quantity or the variability of that magnitude of the quantity among samples in the population.

Acute Within a short period in relation to the life span of the organism, usually ≤4 days for fish. It can be used to define either the exposure (acute test) or the response to an exposure (acute effect).

Acute to Chronic Ratio (ACR) *See* Application factor.

Acute toxicity The concentration at which specified effects occur shortly after the start of an exposure. *See* chronic toxicity and subchronic toxicity.

Adaptation (1) Change in an organism, in response to changing conditions of the environment (specifically chemical), which takes place without any irreversible disruptions of the given biological system and without exceeding normal (homeostatic) capacities of its response. (2) Process by which an organism stabilizes its physiological condition after an environmental change.

Added risk Difference between the incidence of an adverse effect in a treated group of organisms and the spontaneous incidence of the effect in a control group of the same organisms.

Additive effect An effect which is the result of chemicals acting together and which is the simple sum of the effects of the chemicals acting independently. *See* synergism/potentiation.

Additive toxicity The toxicity of a mixture of chemicals which is approximately equivalent to that expected from a simple summation of the known toxicities of the individual chemicals present in the mixture (i.e. algebraic summation of effects).

Adsorption The adhesion of molecules to surfaces of solids.

Adverse effect Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of its functional capacity or impairment of its capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Aerobic Requiring molecular oxygen.

Age class A group of organisms of the same age within a population.

Age composition The distribution of organisms among the various age classes present in the population.

Age distribution The configuration of a population in terms of how its abundance is distributed into various age classes.

Aggregate variables In the report series also referred to as group variables, or group parameters. (Combinations of) analytical-chemical biochemical or biological procedures which can be used to determine a specific element or a chemically defined group of (toxic) compounds in water. Bioassays are sometimes referred to as "biological aggregate variables": Biological tests which determine the mixture toxicity of (complex) mixtures of organic and inorganic compounds.

Ambient concentration The concentration of a chemical in a medium resulting from the addition of an incremental concentration to a background concentration.

Ambient standard *See* environmental quality standard.

Ambient water quality monitoring
A monitoring programme for the assessment of water quality over an unlimited period of time at fixed locations.

Ambient waters Natural (receiving) water bodies.

Anaerobic Not requiring molecular oxygen.

Analysis (versus assessment)
A formal, usually quantitative, determination of the effects of an action (as in risk analysis and impact analysis).

Analysis of extrapolation error
A method of risk analysis in which the probability density of an assessment endpoint, with respect to the concentration of a chemical (or other measure of exposure), is estimated from the statistical extrapolations between toxicological data and the assessment endpoint.

ANalysis Of VAriance (ANOVA)
A method for testing the significance of differences between means by dividing the total variation into separate parts attributable to different treatments.

Antagonism The interaction of (two) chemicals having an opposing, or neutralizing effect on each other, or - given some specific biological effect - a functional interaction that appears to have an opposing or neutralizing effect, other than that which might otherwise be expected.

Anthropogenic Caused by or influenced by human activities.

Application factor (AF)
A unitless value, giving the factorial difference between acute and chronic toxicity of a chemical.

AquaSense A consulting and engineering company for water related problems, Amsterdam, The Netherlands.

Artefact Finding or product of an experimental or observational technique that is not properly associated with the system being studied.

Assessment (versus analysis)
(1) The combination of analysis with policy-related activities such as identification of issues and comparison of risks and benefits (as in risk assessment and impact assessment). (2) An evaluation of the physical, chemical and biological nature of water in relation to natural quality, human effects and intended uses, particularly uses which may affect human health and the health of the aquatic system itself.

Assessment criteria Criteria set for primary ecological quality objectives.

Assessment endpoint Primary ecological objective.

AWWA American Water Works Association .

Background concentration
The concentration of a chemical in a medium prior to the action under consideration or the concentration that would have occurred in the absence of a prior action.

BAT Best Available Techniques.

Battery toxicity testing
The parallel application of a range of different toxicity tests.

Benefit A gain to a population. Expected benefit incorporates an estimate of the probability of achieving a gain.

Benthic Living on the bottom of aquatic systems.

Best Available Techniques (BAT)
BAT addresses the latest (state of the art) of technical and operational developments for preventing or minimizing emissions of polluting substances to the environment without prescribing specific methodologies.

Best Environmental Practice (BEP)
BEP means the most appropriate combination of measures to prevent diffuse pollution or to ensure the safe operation of pollution control facilities.

Bias The error caused by systematic deviation of an estimate from the true value.

Bioaccumulation The net accumulation of a chemical in an organism from combined exposure to its surrounding environment and its food.

Bioactivation Biotransformation of a compound to a more toxic product.

Bioalarming The application of biomonitoring methods for early warning purposes.

Bioassay *See* toxicity test.

Bioavailability The direct availability of a compound in a specific situation (environmental matrix, speciation) to be taken up by organisms and to exert an effect in biota.

Biochemical mechanism
A chemical reaction or series of reactions, usually enzyme catalyzed, which produces a given physiological effect in a living organism.

Biochemical Oxygen Demand (BOD)
The amount of dissolved oxygen consumed by microbiological action when a sample is incubated usually for 5 days at 20˚C and in the presence of a nitrification inhibitor.

Bioconcentration The net accumulation of a chemical from water by an organism.

Biodegradation Breakdown of a substance catalysed by enzymes.

Biological monitoring (1) Analysis of the amounts of potentially toxic substances or their metabolites present in body tissues and fluids as a means of assessing exposure to these substances and aiding timely action to prevent adverse effects. (2) The term is also used for an assessment of the biological status of populations and biocommunities at risk in order to protect them and to have an early warning of possible hazards to human or environmental health.

Biomagnification The tendency of a contaminant concentration to increase with trophic level in an ecosystems and to exceed the concentration expected from bioconcentration.

Biomagnification factor
Quantitative measure of a chemical's tendency to be taken up via the food. It is obtained in feeding experiments by dividing the concentration of a chemical substance in a living organism by the concentration of the chemical substance in its food at steady-state.

Biomarkers Molecular changes in exposed organisms that are taken as indicators of pollution/stress.

Biomonitoring *See* Biological monitoring.

Biotic indices Use of biota to indicate quality of surrounding environment.

Biotransformation Enzyme-catalyzed conversion of one xenobiotic compound to another.

Bioturbation Mixing of sediment/soil by biological action, e.g. burrowing.

Blank Is used interchangeably with the term control.

BOD Biochemical Oxygen Demand.

Body burden Total amount of a chemical present in an organism at a given time.

Broodstock Adult test organisms used for breeding purposes.

Carcinogenicity Process of induction of malignant neoplasms by chemical, physical or biological agents.

Carrying capacity (K) The maximum number of organisms that can be supported in a given unit of habitat. Often computed as the long-term average abundance.

Cell line A defined population of cells which has been maintained in a culture for an extended period and which has usually undergone a spontaneous process of transformation conferring an unlimited culture lifespan on the cells.

CEN European Standardization Committee.

Chemical-specific approach
The evaluation of environmental quality based on chemical concentrations and toxicity of individual compounds.

Chromosomal aberration
An abnormality of chromosome number or structure.

Chromosome The heredity-bearing gene carrier situated within the cell nucleus and composed of DNA and protein.

Chronic Extended or long-term (conventionally taken to include at least 1/10 of the life span of an organism). Long-term effects are related to changes in metabolism, growth, reproduction or the ability to survive.

Chronic toxicity The concentration of a chemical corresponding to the geometric mean of the NOEC and LOEC in tests that have a chronic exposure. *See also* acute toxicity.

Coefficient of Variation (CV)
The standard deviation of a sample relative to the mean. It can be expressed as a fraction or as a percentage.

Community Collection of potentially interacting species populations living together.

Complex mixture A mixture of many different substances in an effluent or in the environment.

Compliance (testing) Evaluation (of water quality) in accordance with governmental permits or regulatory requirements.

Composite sampling A sampling procedure where the ultimate analysis is performed on a mixture of subsamples.

Concentration (1) The quantifiable amount of a chemical per unit volume or unit weight of air, water, food, sediment, tissue, or any other medium. (2) The process of isolating/concentrating specific compounds in a smaller volume.

Concentration-response curve
A curve describing the relationship between exposure concentration and percentage response of the test population.

Conductivity A numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on temperature and the concentration of ions in solution, their valence and mobility.

Congeners (1) Substances which by structure, function or origin are similar to another and may be fit by the same structure-activity relationship. (2) Referring to species belonging to the same genus.

Contemporary background concentrations
Environmental concentrations of compounds upstream from a discharge.

Continuous effect A response that can be measured on a continuum from zero (possibly negative) to positive values such as growth and reproduction. *See* quantal effect.

Continuous-flow Tests in which solutions in test vessels are renewed continuously by the constant inflow of a fresh solution, or by a frequent intermittent inflow (same as flow-through).

Control A treatment in a toxicity test that duplicates all the conditions of the exposure treatments but contains no test material. The control is used to determine the absence of toxicity of basic test conditions (e.g. health of test organisms, quality of dilution water).

Control/dilution water
The water used for diluting the test substance and for the control test.

Cost-benefit analysis The procedure for determining whether the expected benefits from a proposed action outweigh the expected costs.

Criterion The level of exposure (concentration and duration) of a contaminant in a particular medium that is thought to result in an acceptably low level of effect on populations, communities, or uses of the medium (e.g. water quality criteria, air quality criteria).

Criterium An environmental criterium is an estimate of the concentration of a chemical or other constituent in water which if not exceeded, will protect an organism, an organism community, or a prescribed environmental use with an adequate degree of safety.

Culture (1) A stock of animals or plants raised under defined and controlled conditions to produce healthy test organisms. (2) As a verb, it means to carry out the procedure of raising organisms.

Cytotoxic Causing disturbance to cellular structure or function often leading to cell death.

Damage A loss of inherent quality suffered by an entity.

Degradability The property of a compound to transform into molecular fragments.

Delayed effects Effects that occur some time after exposure. Carcinogenic effects of chemicals typically have a long latent period; the occurrence of a tumor may take years after the initial exposure.

Delft Hydraulics A consulting and engineering company for water related problems, Delft, The Netherlands.

Demography the study of populations, especially their age structure and growth rates.

Detection limit The detection limit is typically defined as the analyte concentration yielding an analytical signal equal to two or three times the standard deviation of a blank measurement. Detection at or close to this level is unreliable.

Detergent (surfactant)
A cleaning or wetting compound which possesses both polar and non-polar terminals or surfaces allowing interaction with non-polar molecules which renders them miscible with a polar solvent. A detergent is a formulation containing surfactants.

Deterministic analysis An analysis in which all population and environmental variables are assumed to be constant and accurately specified.

Deterministic model A mathematical model which is completely specified and does not include a stochastic component.

Detoxification (1) A process which renders a toxic molecule less toxic by biotransformation, removal, or masking of active functional groups. (2) To treat patients suffering from poisoning in such a way as to reduce the probability and/or severity of harmful effects.

Detritus Organic debris from decomposing plants and animals.

Detrivorous Organisms feeding on detritus.

Diffuse pollution Non-point source pollution.

Dilution water (diluent)
Water used to dilute the test material in an aquatic toxicity test in order to prepare either different concentrations of a test chemical or different percentages of an effluent for the various test treatments. The water (negative) control in a test is prepared with dilution water only.

Distribution Dispersal of a xenobiotic and its derivatives throughout an organism or environmental compartments, including tissue binding and localization.

Diversity index Measure of richness of biota (number of taxa) and (usually) the evenness of their distribution in communities.

Dose A measure of integral exposure. Examples include (1) the amount of a chemical ingested or injected, (2) the amount of a chemical actually taken up and (3) the product of the ambient exposure concentration and the duration of exposure.

Dose-effect curve Demonstrates the relation between dose and the magnitude of a graded effect, either in an individual or in a population. Such curves may have a variety of forms. Within a given dose range they may be linear but more often they are not.

Dose-response assessment
The process of characterizing the relationship between the dose of an agent administered or received and the incidence of an adverse health effect in exposed populations.

Early warning (monitoring)
Describes monitoring activities undertaken to ensure the timely detection of accidental pollution in order to minimize adverse effects on downstream water uses. Data from early warning monitoring are further used to trace the causes of the pollution.

EC European Community. *See* EEC.

EC(D)n Concentration (dose) that affects designated criterion (e.g. behavioral trait) in n% of the population observed. The LC(D)50 is known as the median effect concentration/dose. *See also* LC(D) and IC(D).

ECE Economic Commission for Europe.

Eco-epidemiology The study on the general occurrence of diseases, deviations and deformities in populations of natural species.

Ecological risk analysis
Determination of the probability and magnitude of adverse effects of environmental hazards (chemical physical, or biological agents occurring in or mediated by the ambient environment) on nonhuman biota. *See also* ecological risk assessment and environmental risk analysis.

Ecological risk assessment
The process of defining and quantifying risks to nonhuman biota and determining the acceptability of those risks. *See also* ecological risk analysis.

Ecosystem Collection of populations (microorganisms, plants and animals) that occur in the same place at the same time and that can therefore potentially interact with each other as well as their physical and chemical environment and thus form a functional entity.

Ecosystem functioning
A full description of biological, chemical and biochemical processes taking place in an ecosystem.

Ecosystem monitoring
Monitoring the biological response of natural ecosystems, ecosystem structure and the composition of the biological community in an ecosystem.

Ecosystem structure The composition of the biological community in an ecosystem and the interrelationships between the individual populations of species (e.g. food web structure).

Ecotoxicity The property of a compound to produce adverse effects in an ecosystem.

Ecotoxicology The study of toxic effects of chemical and physical agents in living organisms, especially on populations and communities within defined ecosystems; it includes transfer pathways of these agents and their interaction with the environment.

EEC European Economic Community. Generally simply referred to as European Community (EC) or European Union (EU). The EC is also used as an abbreviation for the European Commission, an institution of the Community.

EEC *See* Estimated Environmental Concentration.

Effect A change in the state or dynamics of an organism or other ecological system resulting from exposure to a chemical or other stressor (equivalent to response but used when the emphasis is on the chemical).

Effects assessment The component of an environmental risk analysis that is concerned with quantifying the manner in which the frequency and intensity of effects increase with increasing exposure to a contaminant or other source of stress (also known as dose-response assessment or toxicity assessment).

Effluent Wastewater - treated or untreated - that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes discharged into surface water.

EINECS European Inventory of Existing Commercial Chemical Substances.

Elimination The combination of the process of metabolism and excretion which result in the removal of a compound from the organism.

ELS Early Life Stage.

Emission Release of a substance from a source into the environment.

Emission based approach Pollution control by regulating emissions.

Emission standard A quantitative limit on the emission or discharge of a potentially toxic substance from a particular source. The simplest example is a uniform emission standard where the same limit is placed on all emissions of a particular contaminant. *See* limit value.

End-of-pipe The latest possible moment for an effluent to be monitored, just before it is discharged (generally to surface water).

Endogenous Arising within or derived from the body.

Endpoint A response measure in a toxicity test, i.e. the measurement(s) or value(s) derived from a toxicity test which characterize the results of the test (e.g. NOEC or LC50).

Endpoint, Assessment A quantitative or quantifiable expression of the environmental value considered to be at risk in a risk analysis. Examples include a 25% or greater reduction in gamefish biomass or local extinction of an avian species.

Endpoint, Measurement A quantitative summary of the results of a biological monitoring study, a toxicity test, or other activity intended to reveal the effects of a hazard. Examples include catch per unit effort, standing crop, and LC50.

Endpoint, Test A type of measurement endpoint. The numeric summary of the results of a toxicity test. Examples include the LC50 and NOEC.

Environmental compartments Soil, water, sediment, biota.

Environmental fate Destiny of a chemical or biological pollutant after release into the natural environment.

Environmental Impact Assessment (EIA) A type of assessment that attempts to reveal the consequences of proposed governmental actions as an aid to governmental decision making.

Environmental Quality Objective (EQO) The quality to be aimed for in a particular aspect of the environment, for example, the quality of water in a river such that coarse fish can maintain healthy populations. Unlike an environmental quality standard, the EQO is not usually expressed in quantitative terms.

Environmental Quality Standard (EQS) The concentration of a potentially toxic substance which can be allowed in an environmental component, usually air (air quality standard), or water, over a defined period. Synonym: ambient standard. *See* limit value.

Environmental risk analysis Determination of the probability of adverse effects on humans and nonhuman biota resulting from an environmental hazard (a chemical, physical, or biological agent occurring in or mediated by the environment).

Environmental transport The movement of contaminants from their point of release through the various media to locations where exposure is assumed to occur.

Enzyme A protein which is a catalyst (i.e. a substance which in minute amounts promotes chemical change without itself being used up in the reaction), by virtue of its power of increasing the reactivity of a specific substance or specific substances (called the substrate).

Enzyme induction *de novo* synthesis of an enzyme or activation of an existing enzyme.

Enzyme inhibition A process leading to the reduced activity of an enzyme.

Enzymic (or enzymatic) process A chemical reaction or series of reactions catalysed by an enzyme or enzymes. An

enzyme is a protein which acts as a selective catalyst permitting reactions to take place rapidly in living cells under physiological conditions.

EP *See* Equilibrium Partitioning.

EPA (US) Environmental Protection Agency.

Epibenthic Living on the bottom of aquatic systems.

Epifauna Living on sediments of aquatic systems (see infauna).

Episodic Discontinuous effect, e.g. due to accidental spill or periodic stormwater discharges from sewers.

Equilibrium The state of a system in which no further change is occurring and in which the free energy is at a minimum. At equilibrium, the rate of the forward reaction is equal to the rate of the reverse reaction so that a small change in the opposite direction is balanced by a small change in the opposite direction. *See also* steady-state.

Equilibrium partitioning The distribution of a chemical over different environmental compartments at equilibrium.

EROD assay 7-Ethoxyresorufine-O-deethylation enzyme assay; determines cytochrome P_{450} 1A enzyme activity.

EROD 7-Ethoxy-Resorufin O-Deethylase.

Estimated (or Expected) Environmental Concentration (EEC) The concentration of a material estimated as being likely to occur in environmental waters to which aquatic organisms are exposed as a result of planned manufacture, use, and disposal.

EU European Union. *See* EEC (European Economic Community).

Eukaryote An organism (e.g. plant and animal) whose cells contain a membrane-bound nucleus and other membranous organelles. *See* prokaryote.

European Inventory of Existing Commercial Chemical Substances (EINECS) A list of all chemicals either single or as components in preparations supplied to a person in a Community Member State at any time between 1 January 1971 and 18 September 1981.

Eutrophic Nutrient rich (aquatic) system with a high or excessive rate of biological production. *See* oligotrophic.

Eutrophication A complex series of inter-related changes in the chemical and biological status of a water body most often manifested by a depletion of the oxygen content caused by decay of organic matter resulting from a high level of primary productivity and typically caused by enhanced nutrient input.

Excretion Removal of a substance or its metabolites from organism by elimination of a biological material including urine, faeces, expired air, mucus, milk, eggs, and perspiration.

Existing chemicals Chemicals listed in the EINECS (EEC legislation). *See also* EINECS.

Exogenous Resulting from causes or derived from materials external to an organism. *See also* endogenous.

Exponential growth The growth of cells, organisms or populations in which the number/mass increases exponentially and the growth at any time is proportional to the number/mass present.

Exposure (1) Concentration, amount or intensity of a particular physical or chemical agent or environmental agent that reaches the target population, organism, organ, tissue or cell, usually expressed in numerical terms of substance concentration, duration, and frequency (for chemical agents and microorganisms) or intensity (for physical agents such as radiation). (2) Process by which a substance becomes available for absorption by the target population, organism, organ, tissue or cell by any route.

Exposure assessment The component of an environmental or human risk analysis that estimates the exposure resulting from a release or occurrence in a medium of a chemical, physical, or biological agent. It includes estimation of transport, fate, and uptake.

Exposure, Internal The concentration of a contaminant in an organism or in a specific organ or tissue.

Extinction probability The probability that a population will become extinct within a specified interval of time.

Extrapolation An estimation of a numerical value of an empirical (measured) function at a point outside the range of data which were used to calibrate the function or the use of data derived from observations to estimate values for unobserved entities or conditions.

Extrapolation factor A quantity used in effects and exposure assessments to adjust estimated exposures or concentrations/doses for uncertainties, to make corrections in the data, or to increase safety.

Fate Disposition of a material in various environmental compartments (e.g. soil or sediment, water, air, biota) as a result of transport, partitioning, bioconcentration, transformation, and degradation.

First-order process/reaction A chemical process or reaction where the rate of reaction is proportional to the amounts of chemicals present.

Fitness When used in a Darwinian sense, refers to capacity to reproduce and survive.

Flow-through Tests in which solutions in test vessels are continuously renewed by the constant inflow of fresh solution, or by frequent intermittent inflow (same as continuous-flow).

Generation time The average time between the birth of parents and the birth of their offspring.

Genetic toxicology The study of chemicals which can produce harmful heritable changes in the genetic information carried by living organisms in the form of deoxyribonucleic acid (DNA).

Genotoxicity Ability to cause damage to genetic material or an adverse effect in the genome, e.g. mutation, chromosomal damage etc. that may lead to a cancer. *See* carcinogenicity and mutagenicity.

Good Laboratory Practice (GLP) Fundamental rules incorporated in national regulations concerned with the process of effective organization and the conditions under which laboratory studies are properly planned, performed, monitored, recorded and reported.

Grab sampling Procedure where the analysis is performed on a sample taken at a specific location and time.

Group parameters or variables *See* aggregate variables.

Growth The increase in size or weight as the result of proliferation of new tissues.

Habitat evaluation Evaluation of the appropriateness of environmental conditions for the occurrence of specific biota.

Hazard (toxic) Is the set of inherent properties of a chemical substance or mixture which makes it capable of causing adverse effects in man or the environment when a particular degree of exposure occurs. *See also* risk.

Hazard assessment Comparison of intrinsic ability to cause harm (*see* hazard) with expected environmental concentration, often a comparison of PEC with PNEC. Sometimes referred to as risk assessment.

Hazard identification Is the identification of the adverse effects which a substance has an inherent capacity to cause, or in certain cases, the assessment of a particular effect. It also includes the identification of the target populations and conditions of exposure.

Hazard quotient The PEC/PNEC ratio, i.e. the characterization of environmental and/or health risks by combining the results of the exposure assessment (PECs) with the result of the effect assessment (PNECs or NOAEL). Although there is a clear difference between hazard and risk, hazard and risk quotients are often used as synonyms.

HCp (HC5) Hazardous Concentration for p% (5%) of the species derived from a so-called statistical extrapolation procedure.

Homeostasis The tendency in an organism toward maintenance of physiological and psychological stability.

Hydrophilic Describing the character of a molecule or atomic group which has an affinity for water.

Hydrophobic Describing the character of a molecule or atomic group which has a tendency to escape from water.

Hydrosphere Water above, on or in the earth's crust, including oceans, seas, lakes, groundwater and atmospheric moisture.

IC(D)n Concentration that induces a n%-inhibition of a designated process in an exposed population. The IC(D)50 is known as the median inhibitory concentration/dose. *See also* EC(D) and LC(D).

ICWS (International Centre of Water Studies) A consulting and engineering company for water related problems, Amsterdam, The Netherlands.

Immune response Selective reaction of the body to substances that are foreign to it or that the immune system identifies as foreign, shown by the production of antibodies and antibody-bearing cells or by a cell-mediated hypersensitivity reaction.

Immunoassays Immunochemical detection methods based on a reaction between a target analyte and a specific antibody.

Immunotoxic Poisonous to the immune system.

Immunotoxicology The science that deals with the immunotoxic effects of chemicals.

Impermeable The extent to which the membrane, skin or exoskeleton prevents the passage of molecules (e.g. water, ions, proteins, fats, or toxicants).

in vitro In glass, referring to studies in the laboratory usually involving isolated organs, tissues, cells or biochemical systems.

in vivo Within the living organism.

Index (water quality) Aggregated environmental data conveying the (general) state of the aquatic environment as a grading on a scale (usually a scale of 0 - 100).

Indicator A characteristic of the environment, e.g. a species, that provides evidence of the occurrence or magnitude of exposure or effects. Formal expressions of the results of measuring an indicator are referred to as measurement endpoints. Abundance, yield, and age/weight ratios are indicators of population production. A low cholinesterase level is an indicator of exposure to cholinesterase-inhibiting pesticides.

Indicator species A species that is surveyed or sampled for analysis because it is believed to represent the biotic community, some functional or taxonomic group, or some population that cannot be readily sampled or surveyed.

Indigenous species Naturally occurring species (native, autochtonous).

Infauna Lives in sediments of aquatic systems (*see* epifauna).

Integrated monitoring
The method of monitoring both chemical and biological aspects in concert.

Inter-laboratory testing
Synonym of ring-test.

Interstitial water The water within sediment or soil that surrounds the solid particles. The amount of interstitial water is calculated and expressed as the percentage ratio of the weight of water in the sediment to the weight of the wet sediment.

Invertebrate (macro-) All lower organisms characterized by the absence of a vertex (insects, larvae, worms, crustacea, etc.).

IPPC Proposed directive on Integrated Pollution Prevention and Control.

IRC International Commission for the protection of the Rhine against Pollution.

ISO International Standards Organisation.

Isopleths Lines indication areas of equal magnitude (concentration, effects, properties).

ITn Time for a toxicant to Inhibit a specified process in n% of the population observed. The IT50 is known as the median inhibitory time. *See also* LTn.

Joint action Two or more chemicals exerting their effects simultaneously.

KIWA Service centre for quality control and research in the drinking water construction and environmental sectors, Nieuwegein, The Netherlands.

K_{ow} *See* octanol-water partition coefficient.

Larva A recently hatched fish or other organism that has physical characteristics other than those seen in the adult.

LC(D)n The concentration/dose of a substance in water that is estimated to be lethal to n% of the test organisms. The LC50 is known as the median lethal concentration. The LC-values and their 95% confidence limits are usually derived by statistical analysis of mortalities in several test concentrations, after a fixed period of exposure. The duration of exposure must be specified (e.g. 96-h LC50).

Lentic Non-flowing water; lakes, ponds.

Lethal Causing death. Death of fish is often defined as the cessation of all visible signs of movement or other activity.

Life cycle Series of stages, from a stage in one generation to the same stage in the next generation, e.g. egg-larva-adult-egg. *See also* life history.

Life history Sometimes seen as synonymous with life cycle, but may also be defined as a segment of a life cycle, e.g. egg to adult.

Life-cycle study A chronic (or full chronic) study in which all the significant life stages of an organism are exposed to a test material. Generally, a life-cycle test involves an entire reproductive cycle of the organism.

Limit value The limit at or below which Member States of the European Community must set their environmental quality standards and emission standards. These limits are set by Community Directives.

Lipophilic (1) Having an affinity for fat and high lipid solubility and (2) a physicochemical property which describes a partitioning equilibrium of solute molecules between water and an immiscible organic solvent, favouring the latter and which correlates with bioaccumulation.

Load The amount of waste received per unit time (waste load, critical waste load).

Loading Ratio of the animal biomass to the volume of test solution in an exposure chamber.

LOEC(L) Lowest Observed Effect Concentration (Level). The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls. When derived from a life-cycle or partial life-cycle test, it is numerically the same as the upper limit of the MATC. The LOEC is generally reserved for sublethal effects but can also be used for mortality, which might sometimes be the most sensitive effect observed. *See* N(O)EC.

Lognormal distribution A positively skewed probability distribution of a variable that, when subjected to a logarithmic transformation takes the shape of a normal distribution.

Lotic Flowing water such as rivers and streams.

LTn Time required to kill n% of the population observed. The LT50 is known as Median Lethal Time (MLT). *See also* ITn.

Margin of safety The ratio of the no observed adverse effect level (NOAEL) to the estimated exposure intake or dose.

Matrix (sample) The collection of all the constituents in a sample. The analytical matrix refers specifically to the matrix of the analytical sample (including the analyte) which may differ from that of the initial sample due to the substances added or removed in the various sample treatment stages.

Maximum Acceptable Toxicant Concentration (MATC) The hypothetical toxic threshold concentration lying in a range bounded at the lower end by the highest tested concentration having no observed effect (NOEC) and at the higher end by the lowest tested concentration having a significant toxic effect (LOEC) in a life- cycle (full chronic) or partial life-cycle (partial chronic) test. This may be represented as NOEC < MATC < LOEC. Calculation of a MATC requires quantitative life-cycle toxicity data on the effects of a material on survival, growth, and reproduction.

Measurement error Error that results from inaccuracy and imprecision in the measurement of parameter values.

Media The various compartments - water, suspended material, sediment, biota - that can be distinguished within the aquatic ecosystem and over which a compound introduced in the aquatic ecosystem will be distributed based on the characteristics of the compound in its interactions with these compartments.

Median effective concentration *See* ECn.

Median lethal concentration *See* LCn.

Meiofauna Animals living in interstices of soil or sediment of aquatic systems.

Metabolic activation The biotransformation of relatively inert chemicals to biologically reactive metabolites.

MFO *See* Mixed Function Oxidase.

Microtox™ A test involving the "luminous" marine bacterium *Vibrio fischeri* (*Photobacterium phosphoreum*). Reductions in light output are taken as a measure of chemical stress.

Migration (population) The movement of an individual or group into or out of a new population or geographical region.

Mineralization Complete conversion of organic substances to inorganic derivatives.

Mixed function oxidase or mono-genase Enzyme that catalyses reactions between an organic compound and molecular oxygen in which one atom of the oxygen molecule is incorporated into the organic compound and one atom of the oxygen molecule is reduced to water. Involved in the metabolism of many natural and xenobiotic compounds giving both unreactive products and products of different or increased toxicity from that of the parent compound.

Model A formal representation of some component of the world or a mathematical function with parameters which can be adjusted so that the function closely describes a set of empirical data. A mathematical or mechanistic model is usually based on biological, chemical or physical mechanisms, and has model parameters that have real world interpretation. In contrast, statistical or empirical models are curve-fitting to data where the math function used is selected for its numerical properties. Extrapolation from mechanistic models (e.g. pharmacokinetic equations) usually carries higher confidence than extrapolation using empirical models (e.g. the logistic extrapolation models). A model that is able to describe the temporal change of a system variable under the influence of an arbitrary "external force" is called a dynamic model. To turn a mass balance model into a dynamic model theories are needed relating the internal processes to the state of the system expressed by e.g. concentrations. Such elements to build dynamic models are called process models.

Model error The component of uncertainty associated with a lack of correspondence between the model and the real world.

Monitoring (water quality)
Long-term, standardised measurement, observation, evaluation and reporting of the aquatic environment in order to define status and trends.

Monitoring network A spatial net of sampling stations where samples are taken with predetermined time intervals in such a way that changes in environmental variables can be detected, both in space and time.

Mutagenicity Introduction of heritable changes (mutations) of the genotype in a cell as a consequence of alterations or loss of genes or chromosomes (or parts thereof). Any chemical that causes mutations is said to be mutagenic. Some mutagenic chemicals are also carcinogenic. *See* carcinogenesis.

Mutatox™ Test system that assays mutagenicity using *Vibrio fischeri* (*Photobacterium phosphoreum*).

N(O)EC No (Observed) Effect Concentration. The highest concentration of a test substance to which organisms are exposed, that does not cause any observed and statistically significant adverse effects on the organism as compared with the controls. For example, the NOEC might be the highest tested concentration at which an observed variable such as growth did not differ significantly from growth in the control. The NOEC customarily refers to sublethal effects, and to the most sensitive effect unless otherwise specified. NEL, NOAEL, NEC and NOEC are used as equivalent terms.

Neoplasm A heritably altered, relatively autonomous growth of tissue. A neoplasm is composed of abnormal cells, the growth of which is more rapid than that of other tissues and is not coordinated with the growth of other tissues.

Neurotoxic Any toxic effect on any aspect of the central or peripheral nervous system. Such changes can be expressed as functional changes (such as behavioral or neurological abnormalities) or as neurochemical, biochemical, physiological or morphological changes.

No observed effect concentration
See N(O)EC.

Non-target organisms Those organisms which are not the intended specific targets of a particular use of a pesticide.

Normal distribution The classical statistical bell-shaped distribution which is symmetric and parametrically simple in that it can be fully characterized by two parameters: its mean and variance. The normal distribution is observed in situations where many independent additive effects are influencing the values of the variates.

Octanol-water partition coefficient (K_{ow})
The ratio of a chemical's solubility in n-octanol and water at equilibrium.

Oligotrophic Nutrient poor (aquatic) system, see eutrophic.

Organelle A structure with a specialized function which forms part of a cell.

Parameter uncertainty
The component of uncertainty associated with estimating model parameters. It may arise from measurement or extrapolation.

Parthenogenesis Virgin birth; eggs develop without fertilization.

Partition coefficient The concentration ratio of a compound between two different liquid or solid phases.

Parts per billion (ppb)
µg/L or 1 µg/kg or ng/g.

Parts per million (ppm)
mg/kg or mg/L or μg/g.

Parts per thousand (ppt)
g/L or g/kg. This ratio is used to express the salinity of seawater.

Parts per trillion (pptr)
ng/L or ng/kg.

PCA Principal Component Analysis: a multivariate technique to derive a set of orthogonal parameters (principal components) from a large number of properties.

PEC Predicted Environmental Concentration. The calculated concentration of a chemical in a particular medium at a particular location at a particular time.

Pelagic organisms Free swimming aquatic organisms.

Percentiles Divides frequency distribution into 100 equal portions. Hence the 95 percentile is that value that 95% of the population do not exceed.

Persistence Attribute of a substance that describe the length of time that the substance remains in a particular environment before it is physically removed or chemically or biologically transformed.

Pesticide Those chemicals used in agriculture and non-agricultural areas to control the severity and incidence of pests and diseases which reduce e.g. agricultural yields or hinder other processes. Pesticides are used to control bacteria, fungi, algae, higher plants, nematodes, molluscs, mites and ticks, insects, rodents (e.g. mice and rats) or other organisms. This generic term is used to describe respectively: bactericides, fungicides, algicides, herbicides, nematocides, molluscicides, acaricides, insecticides and rodenticides. In addition, they have a number of non-agricultural uses.

pH The negative logarithm of the activity of hydrogen ions in gram equivalents per litre. The pH value expresses the degree or intensity of both acidic and alkaline reactions on a scale from 0 to 14, with 7 representing neutrality, numbers less than 7 signifying increasingly greater acidic reactions, and numbers greater than 7 indicating increasingly basic or alkaline reactions.

Photodegradation Any break-down reaction of a chemical that is initiated by (ultraviolet) sunlight, or more accurately, by the influence of a high-energy photon. This can either be direct photodegradation, in which the photon photolyses or ionises the molecule of interest itself, which then reacts with additional species in its neighbourhood, or indirect photodegradation, in which the molecule under consideration reacts with ions or radicals that were created by photolysis of other species.

Photoperiod The duration of illumination and darkness within a 24-h day.

PICT Pollution-Induced Community Tolerance. Index that uses extent of adaptation in a community to stress as indication of prior exposure.

PLS Partial Least Square analysis: a multivariate technique to relate Y-values for series of objects to a set of X-variables for the objects.

PMN Pre-Manufacture Notification. Regulation for new chemicals as required by TSCA in the USA.

PNEC(L) *See* Predicted No Effect Concentration (Level).

Pollutant A potentially harmful agent that occurs in the environment, products or at the workplace as a result of human actions.

Pollution Release to the environment of a chemical, physical, or biological agent that has the potential to damage the health of humans or nonhuman organisms.

Population A group of interacting and typically interbreeding organisms (sharing genes) of the same species.

Population biomass The total mass or weight of organisms in a population, given by the sum of the masses or weights of all of the members of the population.

Population growth rate
The rate of population growth per unit time.

Population size The total number of organisms in a population.

Pore water *See* interstitial water.

Potentiation The effect of a chemical which enhances the toxicity of another chemical. Also called synergism.

PPP (Polluter Pays Principle)
Principle that places the financial burden for the prevention and control of pollution on the party responsible for its generation, leading to precautionary actions.

Precautionary principle
The general principle to do all that can reasonably be expected to prevent unnecessary risks. *See also* ALARA.

Predicted Environmental Concentration (PEC)
The concentration in the environment of a chemical calculated from the available information on certain of its properties, its use and discharge patterns and the associated quantities.

Predicted No Effect Concentration/Level (PNEC/PNEL)
The maximum level (dose or concentration) which on the basis of current knowledge is likely to be tolerated by an organism without producing any adverse effect.

Predictive risk assessment
A risk assessment performed for a proposed future action such as use of a new chemical or release of a new effluent.

Preliminary test *See* screening test.

Probability A quantitative statement about the likelihood of occurrence of a specific outcome. Probability values can range from 0 to 1.0.

Probit/log transformation
A plot of the probability unit obtained from the standardized normal distribution versus the logarithm of the concentration or the dose of a substance when a quantal or graded response has been measured. A linear plot provides evidence that the distribution is lognormal. Estimates of the L(E)C50 and L(E)D50, as well as the standard deviation for the distribution, can than be made.

Probits A probit or probability unit is obtained by modification of the standard variate of the standardized normal distribution by addition of a constant value of 5 (to avoid negative numbers). Conversion of cumulative percent response to probits followed by plots against concentration or dose can give useful information about the distribution of the response and estimates of the L(E)D50 or L(E)C50 values.

Prokaryote Simple unicellular organisms, primarily the bacteria and cyanobacteria, that do not have nuclei to house their genetic material. They have a few subcellular structures (cf. eukaryote).

pT Toxic potency.

QA Quality Assurance.

QC Quality Control.

QSAR Quantitative Structure-Activity Relationship. A model for estimating the biological effect of a compound based on information concerning the known or calculated effect of structural elements of the compound and/or (general) physico-chemical characteristics of the compound.

Quality assurance (QA)
All those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality.

Quality control (QC) (1) Operational techniques and activities that are used to fulfil requirements for quality. (2) In toxicology: procedures incorporated in experimental protocols to reduce the possibility of error.

Quality criteria These are quality guidelines based on the evaluation of scientific data.

Quality guidelines These are numerical limits or narrative statements which are set to support and maintain designated uses of the environment or to protect human health.

Quality objectives These are numerical limits or narrative statements which have been established to protect and maintain human health or designated uses of the environment at a particular site.

Quality standards These are fixed upper limits of exposure for certain chemicals that are recognized in enforceable laws by one or more levels of government. Well-known examples of standards are the air, water and soil quality standards as well as threshold limit values for air pollutants at the workplace.

Quantal effect Discontinuous response such as death or survival or presence/absence of a behavioral response (*see* continuous effect).

Range-finding test *See* screening test.

RAP Rhine Action Plan.

Receiving water Surface water (e.g. in a stream, river, or lake) that receives a discharged waste.

Reconstituted water Deionized or glass-distilled water to which reagent-grade chemicals have been added. The resultant synthetic fresh water is free from contaminants and has the desired pH and hardness characteristics.

Reference compound A pure compound with known toxicological, ecotoxicological and/or physicochemical properties, that can be used to check the response of a toxicity test or an analytical-chemical procedure.

Reference environment
A generalized description of the environment into which contaminants will be

released and in which organisms will be exposed. Reference environments are used when there is no specific site at risk.

Reference site A relatively unpolluted site used for comparison to polluted sites in environmental monitoring studies, often incorrectly referred to as a control site.

Regression analysis A statistical procedure for determining the constants and coefficients in regression equations from an analysis of observed data for two or more variables. *See also* regression coefficient.

Regression coefficient A parameter that describes the rate of change of a dependent variable with respect to an independent variable; any coefficient in a regression equation, such as the parameters a and b in the linear regression equation $y = a + bx$. *See also* regression analysis.

Relevant margin The margin in the information that is still relevant for the policy and management of surface water. The relevant margin is determined by the aim and use of the information and is thus independent of measurement accuracy or limits of detection.

Remediation Concerned with correction and cleanup of chemically contaminated environmental sites.

Replicate A single test unit such as a container or aquarium, containing a prescribed number of organisms exposed to one concentration/dose of the test compound. In an aquatic toxicity test comprising five test concentrations and a control, and using three replicates, 18 aquaria would be used. For each concentration or control, there would be three aquaria or replicates. A replicate is an independent test unit, and therefore, any transfer of organisms or solutions from one replicate to another would invalidate the test.

Reproducibility Measure of the extent to which different laboratories get the same result with the same reference test compound.

Reproductive toxicology The study of the adverse effects of chemicals on the embryo, foetus, neonate and prepubertal animal and the adult reproductive and neuro-endocrine systems.

Resistance time The period of time for which an organism can live beyond the incipient lethal level.

Response Changes in the state or dynamics of an organism or other ecological system resulting from exposure to a chemical or other hazard (synonymous with effects but used when the emphasis is on the reaction of the organism to the chemical as in "dose-response relationship").

Retrospective risk assessment A risk assessment performed for hazards that began in the past and may have ongoing effects such as waste disposal sites and oil spills.

RIKZ National Institute for Coastal and Marine Management, The Hague/Haren, The Netherlands.

Ring-test (1) A conjoint test conducted under strictly standardized and uniformly applied conditions to assess the precision and accuracy with which different laboratories can determine the toxicity of a chemical or effluent. (2) A test designed to measure statistically the reproducibility of a test method, or to compare the results obtained from the use of different test methods.

Risk Is the probability of occurrence of an adverse effect on man or the environment resulting from a given exposure to a chemical or mixture. It is the likelihood of suffering a harmful effect or effects resulting from exposure to a risk factor (usually some chemical or physical or biological agent). Risk is usually expressed as the probability of occurrence of an adverse effect, i.e. expected ratio between the number of individuals that would experience an adverse effect in a given time and the total number of individuals exposed to the risk factor. The term absolute risk is sometimes expressed per unit dose (or exposure) or for a given dose (exposure).

Risk (Toxic) The predicted or actual frequency of occurrence of an adverse effect of a chemical substance or mixture from a given exposure to humans or the environment (cf-Hazard).

Risk assessment is a process which entails some or all of the following elements: hazard identification, effects assessment, exposure assessment and risk characterization. It is the identification and quantification of the risk resulting from a specific use or occurrence of a chemical compound including the establishment of dose-response relationships and target populations. When quantitative data on dose-response relationships for different types of population, including sensitive groups, are

unavailable, such considerations may have to be expressed in more qualitative terms.

Risk characterization This is the estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartments due to actual or predicted exposure to a substance. It may include "risk estimation", i.e. the quantification of that likelihood. It is also the summary and description of the results of a risk analysis for a risk manager or for the public and other stakeholders.

Risk classification This is the valuation of risks in order to decide if risk reduction is required. It is the complex process of determining the significance or value of the identified hazards and estimated risks to those concerned with or affected by the decision. It therefore includes the study of risk perception and the trade-off between perceived risks and perceived benefits.

Risk control The type and level of control required for a specified level of risk.

Risk estimation The quantification of dose-effect and dose-response for a substance and linking exposure to the probability and nature of an effect.

Risk evaluation The complex process of determining the significance or value of the identified hazards and estimated risks to those concerned with or affected by the decision. It therefore includes the study of risk perception and the trade-off between perceived risks and perceived benefits.

Risk identification The identification of the substance of concern, its adverse effects, target populations, and conditions of exposure.

Risk management This is a decision-making process that entails considerations of political, social, economic, and engineering information with risk-related information to develop, analyze and compare regulatory options and to select the appropriate regulatory response to a potential health or environmental hazard.

Risk perception This is an integral part of "risk evaluation". The subjective perception of the gravity or importance of the risk based on the subject's knowledge of different risks and the moral and political judgement of their importance.

Risk quotient A comparison of exposure with effects, i.e. the PEC/PNEC ratio. This risk quotient is often used to express a risk of a particular chemical. *See also* hazard quotient.

Risk reduction This is taking measures to protect man and/or the environment against the risks identified.

Risk-benefit analysis This is the next consideration after risk-classification. It is the process of drawing up a balance sheet of the respective risks and benefits of a proposed risk-reducing action. It is a multifactorial task in which the risk manager has to consider not only the risk assessment but also other important aspects such as technical feasibility, economic factors, social/cultural factors and legislative/political factors.

RITOX Research Institute of Toxicology, Utrecht, The Netherlands.

RIVM National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

RIZA Institute for Inland Water Management and Waste Water Treatment, Lelystad, The Netherlands.

Round-robin test Synonym of ring-test.

Run-off The portion of the precipitate on the land that ultimately reaches streams and hence the sea.

Safe concentration Concentration of material to which prolonged exposure will cause no adverse effect.

Safety factor A factor applied to an observed or estimated toxic concentration or dose to arrive at a criterion or standard that is considered safe. Safety factor and uncertainty factor are often used synonymously. *See* uncertainty factor.

Safety, Toxicological This is defined as the high probability that adverse effects will not result from exposure to a substance under specific conditions.

Salinity The total amount of salts, in grams, dissolved in 1 kg of water. It is determined after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized. Salinity can also be measured directly using a salinity/conductivity meter.

SCE *See* Sister Chromatid Exchange.

Screening The application of analytical techniques to obtain a broad impression of all compounds present in samples from the aquatic environment and encompassed by the analytical window of the technique under concern (i.e. the known as well as the previously unidentified compounds that can be detected).

Screening test (preliminary or range-finding test)
(1) A test conducted to estimate the concentrations to be used for a definitive test. (2) A short-term test used early in a test program to evaluate the potential of a chemical (or other material) to produce some selected adverse effect (e.g. mortality).

Seasonality A regular pattern - in phase with seasonal changes - in a time series of data, relating to seasonal characteristics like biological growth and multiplication or temperature.

Self-monitoring Monitoring of effluents by the dischargers on a voluntary or regulatory basis.

Self-purification The ability of (healthy) ecosystems to metabolize pollutants introduced in the aquatic environment therewith restoring the original ecological balance.

Semi-static Exposure system in which the test volume is renewed at intervals during the study.

SFG Scope For Growth.

Significance (statistical)
See statistically significant effects.

Sister chromatid exchange
A reciprocal exchange of DNA between the two DNA molecules of a replicating chromosome.

SOP (Standard Operating Procedure)
Formal written procedures of all methods to be followed during the course of a (monitoring) programme, or individual tasks within a (monitoring) programme. SOPs are also referred to as protocols. They should be up to date, safely archived, and correspond to genuine practices.

Spawning The release of eggs or sperm from mature adult fish, or refers to behaviour related to the readiness of mature adult fish to release gametes.

Speciation Determination of the exact chemical form or compound in which an element occurs in a sample, for instance the determination of whether arsenic occurs in the form of trivalent or pentavalent ions or as part of an organic molecule and the quantitative distribution of the different chemical forms that may coexist.

SQT Sediment Quality Triad.

Stable age distribution
The relative age class abundancies that are approached by a population if it is allowed to grow exponentially.

Standard An environmental quality standard is the limiting concentration of a chemical (or degree of intensity of some other adverse condition, e.g. pH) which is permitted in an environmental compartment (soil, effluent or waterway). Standards are established for regulatory purposes and are determined from a judgment of the criteria involved. The standard is dependent on the use (e.g. potable water or agricultural water for irrigation). Standards are derived from criteria, often by applying safety factors (e.g. quality standards for air, water and soil).

Standard (water quality)
The limiting concentration of a chemical (or degree of intensity of some other adverse condition, e.g, pH) which is permitted in an effluent or waterway. Standards are established for regulatory purposes and are determined from a judgement of the criteria involved. The standard is dependent on the use (e.g, potable, agricultural) of the water to be protected.

Static Exposure system in which the test volume is not renewed during the study.

Static renewal Describes a toxicity test in which test solutions are renewed (replaced) periodically, usually at the beginning of each 24-hour period. Synonymous terms are "batch replacement", "renewed static", "renewal", "static replacement" and "semi-static".

Statistical methods, (non)parametric
Nonparametric statistical methods only make use of the relative order present in a data series to draw conclusions, whereas parametrical methods make use of the actual numerical values of the data to draw conclusions.

Statistically significant effects
Effects (responses) in the exposed population that are different from those in the controls at a statistical probability level of $p < 0.05$. Biological endpoints that are important for the survival, growth, behaviour, and perpetuation of a species are selected as criteria for effect. The endpoints differ depending on the type of toxicity test conducted and the species used. The statistical approach also changes with the type of toxicity test conducted.

Steady-state The dynamic equilibrium state of a system in which matter flows in and out at equal rates so that all of the components remain at constant concentrations (dynamic equilibrium). *See also* equilibrium.

Stochastic Pertaining to or arising from chance according to the laws of probability.

Stock solution A concentrated aqueous solution of the substance to be tested. Measured volumes of a stock solution are added to dilution water to prepare the required strengths of test solutions.

Stoichiometry The quantitative relations between the elements in a compound or between the reactants and the products in a chemical reaction.

STP Sewage Treatment Plant.

Strategy The policy or plan according to which work is performed or done.

Stratification Separating into horizontal layers.

Stress The proximate cause of an adverse effect in an organism or a system.

Subacute *See* subchronic.

Subchronic Short-term tests that give an indication of long-term effects often by focusing on critical (sensitive) stages. Sometimes referred to as subacute, but, in the light of this definition, this would seem misleading. The period of exposure often does not exceed 10% of the life span.

Sublethal Any observable behavioral, functional or morphological response of an organism to a toxicant other than death.

Sum variables In this report series also referred to as sum parameters .The summed concentrations of a selection of target compounds.

Surfactant *See* detergent.

Surrogate A test organism, or population that is cultured under laboratory conditions to serve as substitutes in toxicity testing for indigenous organisms, communities or populations.

Surveillance Continuous, specific measurement, observation and reporting for the purpose of water quality management and operational activities.

Survey A finite duration, intensive programme to measure, evaluate and report the quality of the aquatic environment for a specific purpose.

Survival time The time interval between initial exposure of an organism to a harmful chemical and death.

Susceptibility The condition of organism or other ecological system lacking the power to resist a particular disease, infection or intoxication. It is inversely proportional to the magnitude of the exposure required to cause the response.

Suspended matter Small particles of inorganic and organic material, originating from natural and/or anthropogenic sources, that are suspended in water (effluent or surface water). Due to the very small particle size they do not settle in running waters.

Synergism A phenomenon in which the toxicity of a mixture of chemicals is greater than that which would be expected from a simple summation of the toxicities of the individual chemicals present in the mixture.

TBI Trent Biotic Index. *See* biotic indices.

Teratogen Agent that, when administered prenatally (to the mother), induces permanent structural malformations or defects in the offspring.

Teratogenesis Potential to cause defects in embryonic and foetal development caused by a substance.

Test material A chemical, formulation, effluent, sludge, or other agent or substance that is under investigation in a toxicity test.

Test solution or test treatment Medium containing the material to be tested to which the test organisms are exposed. Different test solutions contain different concentrations of the test material.

Threshold Dose or exposure concentration below which an effect is not expected.

Threshold Effect Concentration (TEC) The concentration calculated as the geometric mean of NOEC and LOEC. Chronic value or subchronic value are alternative terms that may be appropriate depending on the duration of exposure in the test. The TEC is equivalent to the MATC (maximum acceptable toxicant concentration) used in other countries.

TIE Toxicity Identification Evaluations.

Time Weighted Average concentration (TWA) The concentration of a substance to which a person is exposed in the ambient air, averaged over a period, usually 8 h. For example, if a person is exposed to 0.1 mg/m^3 for 6 h and 0.2 mg/m^3 for 2 h, the 8-h TWA will be $(0.1 \times 6 + 0.2 \times 2) + 8 = 0.125$ mg/m^3.

Tolerance The ability to experience exposure to potentially harmful amounts of a substance without showing an adverse effect.

Topical Pertaining to a particular area, as in a topical effect that involves only the area to which the causative substance has been applied.

Toxic Able to cause injury to living organisms as a result of physicochemical interaction.

Toxic unit The strength of a chemical (measured in some unit) expressed as a fraction or proportion of its lethal threshold concentration (measured in the same unit). The strength may be calculated as follows: toxic unit = actual concentration of chemical in solution / LC50. If this number is greater than 1.0, more than half of a group of aquatic organisms will be killed by the chemical. If it is less than 1.0, half the organisms will not be killed. 1.0 toxic unit = the incipient LC50.

Toxicant An agent or material capable of producing an adverse response (effect) in a biological system, seriously injuring structure or function or producing death.

Toxicity The inherent potential or capacity of a substance to cause adverse effects on an living organism, seriously injuring structure or function or producing death.

Toxicity curve The curve obtained by plotting the median survival times of a group of test organisms against the concentration on a logarithmic scale.

Toxicity identification evaluation (TIE)
Describes a systematic sample pre-treatment (e.g. pH adjustment, filtration, or aeration) followed by tests for toxicity. This evaluation is used to identify the agent(s) that are primarily responsible for lethal or sublethal toxicity in a complex mixture.

Toxicity test The determination of the effect of a substance on a group of selected organisms under defined conditions. A toxicity test usually measures either (a) the proportions of organisms affected (quantal) or (b) the degree of effect shown (graded or quantitative), after exposure to specific levels of a stimulus (concentration or dose, or mixture of chemicals).

Toxin Natural poison; a toxic organic substance produced by a living organism.

Toxkit™ Kits for running cyst-based toxicity tests.

Trend A systematic change in the amount of one or more constituents in the aquatic environment or the condition of the aquatic ecosystem as a whole.

Triad (of water quality monitoring approaches)
The integrated application of three types of monitoring approaches - chemical analysis, bio-assay testing and ecosystem monitoring - in assessing the state of the aquatic environment.

Tumor (neoplasm) Growth of tissue forming an abnormal mass. Cells of a benign tumor will not spread and cause cancer. Cells of a malignant tumor can spread through the body and cause cancer.

Turbidity The extent to which the clarity of water has been reduced by the presence of suspended or other matter that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample. It is generally expressed in terms of Nephelometric Turbidity Units.

Uncertainty Imperfect knowledge concerning the present or future state of the system under consideration; a component of risk resulting from imperfect knowledge of the degree of hazard or of its spatial and temporal pattern of expression.

Uncertainty factor A factor applied to an exposure or effect concentration or dose to correct for identified sources of uncertainty. *See also* safety factor.

Upstream water Surface water that is not influenced by a particular effluent due to the flow of water.

VROM-DGM Ministry of Housing, Spatial Planning and the Environment, Directorate General for the Environment, The Hague, The Netherlands.

Wastewater A general term that includes effluents, leachates, and elutriates.

Water quality based approach
Pollution control by specifying quality objectives for receiving water bodies.

Weight composition The distribution of organisms among the various weight classes present in the population. The sum of individual weights over all weight classes equals the population biomass.

WHO World Health Organization.

Whole-effluent assessment
Estimation of all the potential hazardous effects (mutagenicity, acute and chronic toxicity, bioaccumulation characteristics, persistence etc.) of complete effluent mixtures on the ecosystem and/or individual organisms.

WMO World Meteorological Organization.

WRK Water Transport Company Rhine-Kennemerland, Nieuwegein, The Netherlands.

WWTP	Waste Water Treatment Plant.
XAD	Amberlite XAD-4 and XAD-8 macroporous resins.
Xenobiotic	A man-made chemical or material not produced in nature and not normally considered a constitutive component of a specified biological system. This term is usually applied to manufactured chemicals.