UNIVERSITY OF INSUBRIA, Como

Department of Science and High Technology

XVI PhD Course in Environmental Sciences

ECOTOXICOLOGICAL ASSESSMENT OF FRESHWATER ECOSYSTEMS

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A Rossella: mio più grande
esempio di coraggio, costanza
e determinazione.

Riassunto

L'ecotossicologia è una scienza recente nata come filiazione dalla tossicologia dalla quale ha derivato principi, concetti e metodi, coniugati però con l'ecologia. L'ecotossicologia è dunque la scienza dei veleni per l'ambiente e il suo scopo finale risulta essere la protezione degli ecosistemi.

La conservazione dell'ambiente ha un'importanza sia per quanto riguarda la salvaguardia dell'integrità degli ecosistemi per il loro valore intrinseco, sia per quanto riguarda i servizi che essi rendono all'uomo. Tra le risorse utili, l'acqua è indubbiamente è una delle più importanti, particolarmente vulnerabile, essenziale per la vita e lo sviluppo di tutti gli esseri viventi.

La tutela e il suo uso razionale è un obiettivo molto impegnativo che non può essere raggiunto con un approccio settoriale e di emergenza, ma richiede una politica preventiva che parta dalla cognizione della situazione al tempo presente ed incida poi sulle cause del degrado con una correttagestione ambientale.

Da questo punto di vista, a livello normativo un'importante novità è senza dubbio rappresentata della Direttiva Quadro sulle Acque (WFD 2000/60/EC), che impone ai Paesi membri la tutela e il recupero degli ecosistemi acquatici fissando obiettivi di qualità non più basati esclusivamente su indicatori chimici e fisici ma, soprattutto di tipo biologico ed ecosistemico.

L'approccio ecotossicologico risulta essere un valido strumento per la valutazione della qualità degli ecosistemi acquatici (sia comparto acqua che comparto sedimenti) sia attraverso il suo approccio classico con l'utilizzo dei test di ecotossicità in laboratorio su organismi target con lo scopo di identificare l'effetto che le sostanze pure possono avere sull'ambiente. Sia attraverso un approccio più oolistico che prevede l'utilizzo dei test di ecotossicità come strumenti di monitoraggio per identificare eventuali effetti negativi derivanti dalla presenza di miscele in ambiente (strumenti di monitoraggio *effect-based*). Quest'ultimo approccio risulta essere perfettamente in linea con quanto previsto dalle WFD portando i test di ecotossicità ad ricoprire un ruolo fondamentale all'interno di quelli che sono i classici metodi di monitoraggio basati esclusivamente sulla ricerca analitica delle singole sostanze in grado di provocare effetti avversi sull'ambiente (strumenti di monitoraggio *stressors-based*).

ECOTOXICOLOGICAL ASSESSMENT OF FRESHWATER ECOSYSTEMS

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1. INTRODUCTION

1.1 The Water Framework Directive

The European Water Framework Directive (EU WFD, 2000/60/EC), which was published in the Official Journal of the European Community on 22 December 2000, is probably the most significant legislative instrument in the water field to be introduced on an international basis for many years. It took over 10 years to develop and, to the end, engendered intense scientific and political debate within the Community.

The 1990s saw an emergence worldwide of holistic environmental management, integrated pollution control and countries embracing the ecosystem approach which combines natural and social sciences in tackling environmental problems (Apitz et al. 2006). This was most embodied in the Earth Summits in 1992 (Rio de Janeiro), 1995 (New York) and 2002 (Johannesburg) and the 1992 Convention of Biological Diversity. In these meetings, countries worldwide agreed to achieve environmental sustainability. Within Europe, this led to the proposal for an EU Directive on the Ecological Quality of Surface Waters which followed on from many countries adopting monitoring schemes and environmental quality objectives and standards.

The European Directive proposal for the Ecological Quality of Surface Waters was never adopted, possibly because of its high ecological bias and inadequate consideration of socio-economic impacts. But this embryo of an idea eventually resulted in the drafting of the EU Water Framework Directive which was finally adopted in 2000.

The Directive takes a broad view of water management and has its key objectives the prevention of any further deterioration of water bodies, and the protection and enhancement of the status of aquatic ecosystems and associated wetlands. It aims to promote sustainable water consumption and will contribute to mitigating the effect of flood and droughts. The Directive is seen by the Commission as a providing a framework for each country to develop a common basis for the protection and sustainable use of water. Its overall aim is to maintain and improve the aquatic environment through attention to quality issues, but incorporating the control of quality as an essential ingredient, recognising the impact that inadequate quality would have on the maintenance of good ecological quality.

In addition to establishing a new, common management system for the delivery of water policy, the EU Water Framework Directive contains a set of overall objectives. There are:

- Expand the scope of action to protect water to all forms of naturally occurring water in the environment, including surface and groundwater;
- Prevent further deterioration, and protect and enhance the status of aquatic ecosystems, and with regards to their water needs, terrestrial ecosystems and wetlands;
- Promote sustainable water use based on long-term protection of available water resources;
- Take specific pollution control measures, by reducing or eliminating discharges and emission and losses of priority toxic substances, to enhance the protection and improvement of the aquatic environment;
- Reduce pollution of groundwater;
- Contribute to mitigating the effect of flood and droughts;
- Undertake measure which will result in the achievement of "good water status" for all waters within a predetermined timescale.

Also the four most important and innovative features of the Directive are that it aims:

- To manage water as a whole on a river basin reflecting the situation in the natural environment;
- To use a combined approach for the control of pollution, setting emission limit values and water quality objectives;
- To ensure that the user bears the costs of providing and using water reflection its true cost;
- To involve the public in making decisions on water management.

The WFD has impacted various levels of environmental management of aquatic resources and has triggered the re-organization of water management by hydrological catchments, rather than by administrative borders, with the ultimate goal to improve the quality of surface water bodies. It has also been an important incentive towards harmonisation of classification and monitoring methods across Europe. The biotic communities of European surface waters are now the primary focus, used to assess the status of lakes, rivers and marine ecosystems and the success of management. The WFD has precipitated a fundamental change in management objectives from merely pollution control to ensuring ecosystem integrity as a whole. Deterioration and improvement of "ecological quality" is defined by the response of the biota, rather than by changes in physical or chemical variables (Hering et al. 2010).

From a scientific perspective, the implementation of the WFD is greatly increasing knowledge on the ecology of European surface waters, particularly in regions which have rarely been investigated: approximately 1900 papers have resulted from research projects associated with the implementation of the directive (query "Water Framework Directive" in SCOPUS at 4/12/2009). Many methods to sample and investigate aquatic ecosystems have been developed and large amounts of data are being generated.

The WFD sets common approaches and goals for the management of water in 28 countries (15 Member State (MS) countries and the 13 pre-accession countries which should conform in the long term with Community law). In this respect the recent changes in EU water policy marked with the WFD will have important overall implications in shaping developments in water policy and management at an international level.

Water has been a cornerstone of EU environmental policy. Water directives characterise the different phases of environmental policy evolution, from an emphasis on public health protection to environmental protection per se, and from ''end-of-pipe'' solutions to preventative and integrated management approaches. The WFD marks the beginning of a new era in EU environmental policy and also sets a precedent for the long debated balance between MS ''subsidiarity'' and uniform standards at European level (Kallis and Butler, 2001).

1.2 The Water Framework Directive Goals

The objectives of the WFD are to improve, protect and prevent further deterioration of water quality across Europe. The term "water" within the WFD encompasses most types of water body, and therefore the legislation applies not only to groundwater but also to all coastal and surface waters. The Directive aims to achieve and ensure "good quality" status of all water bodies throughout Europe by 2015, and this is to be achieved by implementing management plans at the river basin level.

The means is organisation and planning at a hydrologic (river basin) level and implementation of a number of pollution-control measures, if necessary on top of those demanded by existing legislation that regulates water quality and pollution.

For surface waters the objective is that of a ''good'' ecological and chemical quality status. Surface water is defined as of good ecological quality if there is only slight departure from the biological community that would be expected in conditions of minimal anthropogenic impact; a standard process is provided in the WFD for defining local standards accordingly. Quality elements for assessment are divided into biological elements (e.g. composition and abundance of flora and fauna), hydromorphological elements (e.g. quantity and dynamics of flow, river depth and width variation) and supporting physico-chemical elements (e.g. thermal/oxygenation conditions, salinity, nutrients, etc.) for rivers, lakes, transitional and ‘‘artificial/modified’’ waters (those created or resulting from a human physical modification and serving economic activities).

For each element a descriptive definition of a high, good, moderate, poor and bad status is given. Each authority should set standards for the elements most relevant to the pressures faced by the water body under its responsibility and classify waters accordingly.
Critical has been the provision for a process to define an additional list of chemical substances to be regulated (a ‘‘deadlocked’’ process due to Member States disputes on agreeing to standards for ‘‘daughter’’ directives). Chemical monitoring is expected to intensify and will follow a list of 45 priority chemicals (inorganic and organic pollutants and substances) that will be reviewed every 4 years (Directive 2013/39/CE).

The ‘‘good’’ and non-deteriorating status is the minimum goal for all waters; in addition where more stringent requirements are needed for particular uses, ‘‘protected zones’’ should be established and higher objectives set within them. These should include at least areas already protected by Community legislation, i.e. drinking waters, bathing waters, nutrient (nitrate and urban w/w) sensitive designated areas and areas designated for the protection of habitats or species (including Natura 2000 sites). In addition other zones may be designated for the protection of economically significant aquatic species and for recreational activities. Finally the last but not least goal is that of ‘‘sustainable use’’ of water.

1.3 The Water Framework Directive Tools

The Directive aims to achieve and ensure “good quality” status of all water bodies throughout Europe by 2015, and this is to be achieved by implementing management plans at the river basin level. Therefore water resources should be managed across national boundaries, choosing a co-ordinated approach within each river basin and monitoring is a valid tool required by WFD to follow a risk assessment approach. The monitoring data should provide information on initial water quality, assess long-term changes from both natural and human activities, reveal short-term changes where waters are found to be at risk, and lead to measures to rectify the situation when problems may hinder compliance with WFD environmental objectives.

Monitoring is required to cover a number of ‘water quality elements’ including, physico-chemical, hydro-morphological, biological and chemical parameters.
Three basic types of monitoring of surface water are referred to in the WFD:

- *Surveillance* monitoring aims at assessing long term changes in natural conditions resulting from human activity;

- *Operational* monitoring is to be carried out as an additional measure for water bodies that are at risk of failing to meet the Directive's environmental objective;
- *Investigative* monitoring is to be only carried out in individual cases, e.g. where the environmental standards are not met due to unknown reasons with the aim of determining the causes of such failure.

Aquatic systems are complex and there are many problems associated with monitoring their quality. If good quality status is achieved only surveillance monitoring is required to ensure this is maintained. However, for water bodies which are determined to be at risk, or of moderate or poor quality, further information will be needed so that adequate remediation strategies can be implemented and subsequently monitored.

In Figure 1 is possible to see how each stage of monitoring requires the use of a suitable set of 'tools' to obtain meaningful and reliable data and indicates the extent and complexity of the information required for the successful management of water bodies. While most of the tools may be used for all types of monitoring (i.e. investigative, operational or surveillance), some may be more suited or specifically adapted to certain situations or sites. This choice will depend on their deployment characteristics, cost, robustness, sensitivity and the type of measure and information required.

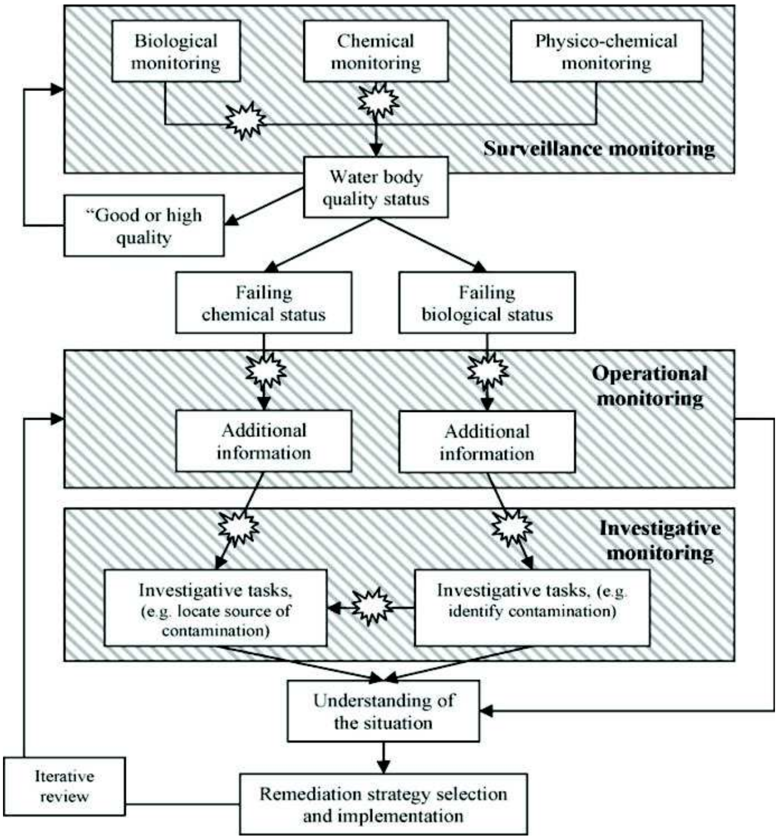


Figure 1 Simplified scheme for the three types of monitoring embedded in the Water Framework Directive, namely surveillance, operational and investigative monitoring. The use of emerging tools and technologies is represented by the star ( ) symbols (Figure from Allan et al. 2006).

The WFD does not mandate the use of a particular set of monitoring methods, but aims to ensure the establishment of an adequate monitoring programme based on the quality elements mentioned above. The additional cost of the monitoring necessary to underpin the Directive will be an important factor in determining the selection of particular tools. The successful implementation of the

WFD will rely on the availability of low-cost tools and technologies able to deliver appropriate and reliable data. In addition, as many large river basins encompass a number of countries, it is important to ensure that the data collected by different EU member states are of comparable and appropriate quality. To achieve this, new analytical methods, the production of relevant certified reference materials and the organisation of inter-laboratory trials and proficiency testing schemes will be required (Dworak et al. 2006).

Until few years ago, monitoring of water quality had generally relied on the collection, at prescribed periods of time, of spot water samples followed by extraction and laboratory-based instrumental analysis for both inorganic and organic pollutants. In most cases the collected water sample is analysed directly to measure the 'total' concentration of a particular analyte. This methodology is well established and validated and therefore has been accepted for regulatory and law enforcement purposes.

However, this approach is valid only if it provides a truly representative picture/status of the chemical quality of water at a particular sampling site. This is generally assumed. Research during the last two decades has shown that considerable limitations are associated with spot sampling to determine total pollutant concentrations (Gueguen et al. 2004).

Chemical monitoring programmes are used to asses both chemical and ecological status. Thus, chemical analysis is a fundamental step in the assessment of surface water status according to WFD. However, chemical analysis generally requires a priori knowledge about the type of substances to be monitored whereas, for technical and economical reasons, it is not possible to analyse, detect and quantify all substances that are present in the aquatic environment, in all water bodies. There is therefore a tendency to focus chemical monitoring on already regulated substances that are known to pose a threat to or via the aquatic environment. There were e.g. 7336 unique REACH registered substances in the year 2010 and it is not possible to plan a chemical monitoring programme on all substances that could pose a risk to the aquatic environment. Furthermore, to estimate the risk of effects related to the large number of substances that are present in the environment (including emerging pollutants, metabolites and transformation products), it would be necessary to develop a very large number of assessment criteria. Such assessment criteria for chemicals are generally developed substance by substance, based on laboratory studies, and usually do not consider the consequences of the co-exposure to multiple chemicals that occur in the environment, possibly giving rise to cumulative effects (Silva et al. 2002).

Similarly, for hydrophobic organic pollutants, sorption to DOC or colloidal matter and sediments may significantly alter their bioavailability. All these factors need to be accounted for during both sampling and subsequent sample extraction steps. Therefore, sample acidification for metals and extraction of whole-water (i.e. both suspended solids and water) samples for organic chemicals aiming to obtain ‘total’ concentrations do not necessarily provide a representative picture of the level of pollution.

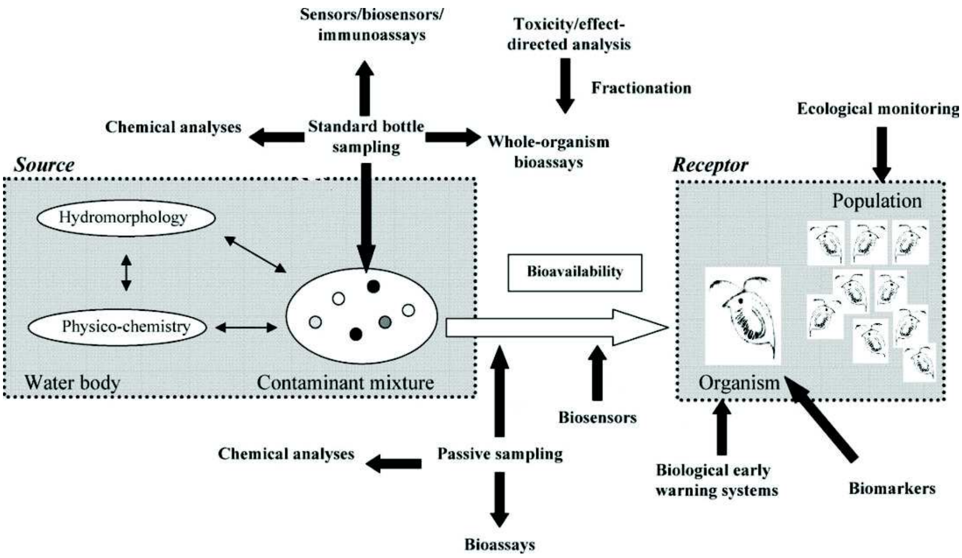


Figure 2 Suitability of existing and emerging techniques and methods for water quality monitoring under Water Framework Directive. Thin arrows represent the interaction of the hydromorphology, physico-chemical properties of a water body with contaminants present in the water. Thick arrows represent emerging monitoring strategies that may be employed to assess ecosystem health and water quality (Modified by Allan et al. 2006).

A further factor is that continuously varying hydromorphological, and hydrological conditions and intermittent chemical releases associated with industrial/urban wastewater effluents, bed-sediment re-suspension and diffuse pollution (e.g. run-off from the periodic application of pesticides to agricultural land) lead to spacio-temporal variations in a water body’s physico-chemical characteristics (Warren et al. 2003). Spot water sampling therefore provides only a ‘snapshot’ of the situation at the set time of sampling and fails to provide information on the bioavailability of pollutants in water. Furthermore the use of repeated spot sampling alone is very expensive because of transport and analytical costs. The WFD wants rely on the effective use of a combination of monitoring methods according to their suitability for the questions being asked and characteristics of the given site of sampling. Deployment of time-integrated sampling systems e.g. passive samplers based on the uptake of truly dissolved contaminants or the establishment of continuous monitoring stations with both biological and chemical testing capabilities may provide, at lower cost, more useful data on the variability of contaminant concentrations or temporal changes in toxicity (Allan et al. 2006).

Furthermore a connection between these monitoring systems and a range of emerging techniques as ecological monitoring, biomarkers or bioassays may be useful in providing a more realistic assessment of impacts and exposure of aquatic organisms to specific contaminants or a mixture of contaminants present in the water and may give additional information in order to obtain a clearer picture of the biological and chemical quality of a water body.

In the Figure 2 it is possible to see where standard spot sampling/chemical analysis stands in relation to an inter-related scheme of emerging tools and how these different approaches to monitor the water quality complement one another, and when used together, provide a more representative picture of the system under study.

1.4 *Environmental Quality Standard*

The Water Framework Directive requires European Union Member States to ensure that all inland and coastal waters achieve "good" water quality status by 2015. This goal will be realised through a range of measures, including the use of environmental quality standards. An Environmental Quality Standard (EQS) is defined under the WFD as "the concentration of a particular pollutant or group of pollutants in water, sediment, or biota which should not be exceeded in order to protect human health and the environment". The term EQS is generally used to refer to a standard or limit value that is protective against the potential adverse effects of long-term chemical exposure.

The Commission establishes environmental quality standards so as to limit the quantity of certain chemical substances that pose a significant risk to the environment and to health in surface water in the European Union (EU). These standards are coupled with an inventory of discharges, emissions and losses of these substances in order to ascertain whether the goals of reducing or eliminating such pollution have been achieved.

Good ecological status (or potential) also requires that the concentrations of the specific pollutants (also called River Basin Specific Pollutants) are not in excess of the EQSs set at Member State level. When the quality standards are not met, this could be a reason for failing to achieve good ecological status.

Directive 2008/105/EC (Environmental Quality Standards Directive), on environmental quality standards in the field of water policy sets out environmental quality standards concerning the presence in surface water of certain pollutants and substances or groups of substances identified as priority on account of the substantial risk they pose to or via the aquatic environment.

The priority substances have been defined by the Water Framework Directive. The COMMPS (combined monitoring-based and modeling-based priority setting) procedure was used to identify substances of greatest concern in the European Community (Klein et al. 1999). The resulting list identified 33 priority substances including cadmium, lead, mercury, nickel and its compounds, benzene, polyaromatic hydrocarbons (PAH) and DDT total. Twenty priority substances are classed as hazardous.

The Directive sets also EQS in biota for 3 substances (mercury and compounds, hexachlorobenzene and hexachlorobutadiene). The Directive allows Member States to establish EQSs for sediment and/or biota at the national level and to apply those EQSs instead of the EQSs for water, established at the European Community (EC) level. The EQSs are concentrations that should be achieved, with the aim to protect human health and the environment. The trends of accumulating priority substances should also be monitored in sediment and/or biota and should not increase (no deterioration objective) in the water bodies. The European Commission has recently pubblicate the Directive 2013/39/EC, amending Directive 2008/105/EC, where the list of priority substances were reviewed through a prioritization procedure based mainly on monitoring and modelling data in compliance with article 16 of WFD.

The main reviews are:

- 15 additional priority substances, 6 of them designated as priority hazardous substances;
- stricter EQS for four existing priority substances and slightly revised EQS for three others;
- the designation of two existing priority substances as priority hazardous substances;
- the introduction of biota standards for several substances;
- provisions to improve the efficiency of monitoring and the clarity of reporting with regard to certain substances behaving as ubiquitous persistent, bioaccumulative and toxic (PBT) substances;

Specific pollutants are not “listed” in the same way as the priority substances although there is an indicative, not exhaustive, list in Annex VIII of the WFD, that includes a wide range of substances or group of substances that can be detected in surface water bodies. In order to assess the ecological status based on concentrations of specific pollutants, these need to be identified on water body and/or district level. For each such substance, it is necessary to develop EQS values on national level.

Analysis of priority substances and specific pollutants are restricted to stringent validation requirements, mentioned in the directive on technical specifications for chemical analysis and monitoring of water status (2009/90/EC). All methods of analysis applied by Member States for the purposes of chemical monitoring programs of water status have to meet certain minimum performance criteria, including rules on the uncertainty of measurements and on the limit of quantification of the methods. Article 4 of that directive describes the minimum performance criteria for all methods of analysis. However, even for some priority substances, current or proposed EQS values are lower than the Levels Of Quantification (LOQ) possible to achieve, as also described in Common Implementation Strategy (CIS) guidance no 19 and a recent report (Loos, 2012).

The approach used to derive EQS for the priority substances was developed by the Fraunhofer Institute (FHI) under contract to the EC using guidelines now published as Lepper, 2005. This approach was based largely on current European Technical Guidance Document (TGD) methods for derivation of predicted no-effect concentrations (PNECs) in chemical risk assessment (EC 2003). Using TGD methods, in which the lowest reliable toxicity test no-observed-effect concentration, or the lower 5th percentile from a species sensitivity distribution, is selected and an assessment factor applied to derive a PNEC that is assumed to be protective of all potentially exposed species. Two types of water column EQS were originally proposed for each priority substance:

- the annual average (AA) value or concentration of the substance concerned calculated over a one-year period. The purpose of this standard is to ensure the long-term quality of the aquatic environment;
- the maximum allowable concentration (MAC) of the substance measured specifically. The purpose of this second standard is to limit short-term pollution peaks.

For naturally occurring substances, such as metals, FHI recommended use of the ‘‘added risk approach’’ in order to take background concentrations into account. This approach assumes that indigenous populations of aquatic organisms are adapted to background concentrations of naturally occurring substances such as metals. Therefore, safe concentrations for these substances are calculated by adding a ‘‘maximum permissible addition’’ (equivalent to a PNEC) to the relevant background concentration (Crommentuijn et al. 2000). EQS were also proposed for sediments and biota, using the approaches recommended by the TGD.

New Technical Guidance for Deriving Environmental Quality Standards (EU EQS TGD; European Commission 2011) has now been developed under the WFD Common

Implementation Strategy. This guidance is based on the European Technical Guidance Document (ECB (European Chemicals Bureau) 2003) that was developed to support the risk assessments for new notified substances, existing substances and the placing of biocidal products on the market in the EU. This guidance proposes the use of assessment or uncertainty factors for the derivation of long-term EQS when there are less than nine chronic data points for different species. The lowest chronic value (EC10 or no observed effect concentration) is divided by the assessment factor in order to account for the possibility that the most sensitive species in the ecosystem is more sensitive than the most sensitive species tested.

1.5 Environmental risk assessment

Risk assessment is a general approach established for independent, neutral, science-based evaluation of the probable likelihood of harm (response) from exposure (dose of stressor) to deleterious elements in the environment. Risk assessment is borne out of the need to manage risks of any such negative occurrence in order to protect public health and our ecosystems.

Environmental risk assessment ERA is defined as procedures by which the likely or actual adverse effects of pollutants and other anthropogenic activities on ecosystems and their components are estimated with a known degree of certainty using scientific methodologies (Depledge and Fossi, 1994).

Recently the growing public awareness and concern about the consequences of major environmental events of our times at both local and global levels, for example, acid precipitation, global warming, biodiversity loss, ozone depletion, etc., has created renewed interest and urgency for appropriate approach for predicting these human-induced stressors in both terrestrial and aquatic ecosystems. Consequently, risk-assessment methodologies, particularly for ecological systems, are constantly evolving and improving upon methods used in the past

The process of ecological risk assessment (ERA) addresses ecological complexity and incorporates uncertainty in characterizing the impacts of natural and man-made disturbances on ecological resources. ERA integrates ecology, environmental chemistry, environmental toxicology, geochemistry, hydrology, and other fundamental sciences in estimating the probabilities of undesired ecological impacts. In theory, ERA can be viewed as a subset of basic disturbance ecology. In practice, ERAs derive from specific needs to assess human-induced impacts on the environment (Bartell, 2008).

Risk is defined as the probability that an undesired event will occur. Correspondingly, ecological risk refers to the probability of the occurrence of an undesired ecological event. Alternative definitions of risk include an evaluation of the consequences of the undesired event along with estimation of its occurrence.

ERA focused on the undesired ecological effects of different stressors defined as a substance or condition that causes stress on an entity in an ecosystem.

Different types of environmental stressors exist in nature. They include biological, chemical, or physical stressors that can be broadly categorized as biotic (living) or abiotic (nonliving) stressors. Unlike abiotic stressors, biotic stressors have only biological origin, that is, living organisms exerting stress on another biological organism.

Abiotic stressors consist of two major types: physical stressors and chemical stressors. Abiotic stressors originate from the ambient environment and may or may not have any biotic input. Abiotic stressors may include light intensity, temperature range, pH level (acidity or alkalinity), water availability, dissolved gases, nutrient availability, radiation level, heavy metal contamination, etc. A major abiotic stressor that has attracted the interest of environmental scientists is the amount and nature of chemical contaminants in environmental media – soil, water, and air.

Chemical stressors include various pesticides such as dichlorodiphenyltrichloroethane (DDT), or could be a chemical such as polychlorinated biphenyls (PCBs) discharged into water systems that could induce mutation in some freshwater species or heavy metal contamination.

The adverse ecological effects suffered as a result of exposure to stress is termed a ‘response’ (toxic reaction in toxicology). Reponses from an entity receiving a dose of a specific stressor could lead to changes in function or health of that entity receiving that stressor. Responses could be a reaction from either/both biotic and abiotic stressors. Stressor dose–response relationship is one of the major steps in ecological risk-assessment processes (Ishaque and Aighewi, 2008). The systematic steps for performing Ecological Risk Assessment, as applied to an identified stressor, are outlined in Figure 3 with particular reference to the stressor dose–response relationship as an integral part of the process; the others being exposure assessment, response assessment, and risk characterization.

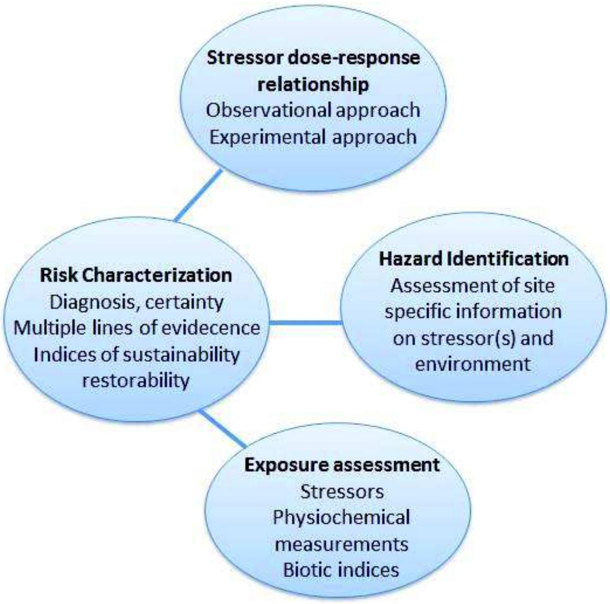


Figure 3 Risk-assessment process showing stressor–response relationship as a major component.

The ecotoxicity bioassay that permite to quantificate the toxicant impacts on different organisms would be a critical component of ERA. Toxicity bioassays are an important line of evidence in ERA because these tests provide relevant and direct measures of toxicity of contaminated media to biota (Fernández et al. 2005).

The chemical analysis infect does not allow an integration of the combined effects produced by the chemical mixture present at a polluted site. In addition, total concentrations can overestimate the real risk, as aging processes can strongly reduce the bioavailability and, subsequently, the toxicity of pollutants. Bioassays try to integrate these effects and help in evaluating the risk associated with exposure to bioavailable substances present in an ecosystem. As a result, there is an increasing need to incorporate toxicity tests in risk assessments and hazard identification. Ecotoxicological analyses are recommended for estimating the risk to ecological receptors associated with contaminants in the environment.

1.6 Biological monitoring as "emerging tool" for an Environmental Assessment

''Environmental hazard'' is a generic term for any situation or state of events that poses a threat to the surrounding environment. This term incorporates pollution and natural hazards (e.g., storms and earthquakes). Contamination of the environment by toxic substances has been associated with society since the beginning of industrialization. These substances enter ecosystems by many pathways, including industrial discharges and leakage, municipal waste, run-off from agricultural and forestry applications, and accidents. Air and water flow can disperse these contaminants over great distances. Environmental anthropogenic contamination originates from numerous sources of production, application and waste involving a multitude of chemicals that affect the biosphere. Traditionally, analytical chemistry was the tool used to target compounds of interest and to record their occurrence in the environment (Blasco and Picó, 2009). With this approach, evaluation of environmental contamination has been carried out by:

- periodical sampling of the compartments involved (e.g., water, air, soil and sediments);
- chemical analysis (including contaminant isolation and concentration from the sample and further determination, commonly by liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-MS (GC-MS) of the selected compounds);
- comparison of the measured levels of pollutants with the environmental quality standards (Brack et al. 2007).

This approach is based on monitoring of stressors: in the context of a water pollution event, stressors are typically specific chemicals that may cause undesirable effects on specific receptors (i.e., organisms that are exposed to these stressors). Stressor-based monitoring typically predicts the risk of effects, based on comparing the observed concentration of the stressor with the concentration known or considered to produce those effects (Beyer et al. 2013).

Stressor-based assessments may be unrealistic because a relatively small number of chemicals, recognized as hazardous, have been included in monitoring programs using this tactic. However, toxic effects in the environment can be caused by complex mixtures of known and unknown pollutants. The chemical method does not sufficiently cover unintentionally-produced chemicals (by-products) or metabolites that may cause effects even at low concentrations. Furthermore, the chemical approach is unfeasible, when regular or widespread sampling is needed (e.g., in ecosystems subject to spatial or temporal fluctuations in the levels of pollutants caused by the distribution of point or diffuse sources). In these circumstances, the cost of chemical analysis will be too high because of the labor and the transport involved in sampling and the need for a large number of analyses (Butt et al. 2008).

Beside a chemicals-driven strategy for assessing the ecological risk from pollutants, it is necessary to explore and to apply new strategies, combining both biological responses and chemical analysis, to identify toxic hotspots, to characterize chemicals likely to cause adverse biological effects, and, finally, to assess the ecological risk of the identified chemicals at relevant spatial scales (Brack et al. 2007). Environmental hazard assessments require rapid, inexpensive screening tests to characterize the extent of contamination (Farré et al. 2009). To complement chemical analysis with biological data, the development of biosensors and other biological approaches has grown steadily in recent years(González-Martínez et al. 2007). For all these reasons a monitoring based on effects is an essential tool for the complete implementation of Water Framework Directive (WFD) and related environmental strategies (Barcelo and Petrovic, 2006).

Currently effect-based monitoring tools are mentioned in some CIS (Common Implementation Strategy Working Group) guidance documents: CIS 19 (EC, 2009) (chapter 7 on Complementary Methods), CIS 25 (EC, 2010) (chapter 7 on Complementary Methods) and also shortly in CIS 27 (EC, 2011) related to sediment compliance checking.

For all these reasons environmental assessment should be based on both biological monitoring of ecosystems at the community level and chemical monitoring of priority and specific substances (Rice et al. 2008).

The key advantage with monitoring biological effects is that the overall response from exposure to multiple, bioavailable chemicals can be taken into account, on different levels of biological organisation, such as community, population, individual and/or suborganism levels. In this way a more holistic approach is possible.

For example biomarkers are a recent tool used as indicator of polluted environment and are defined as a change in a biological response of an organism (ranging from molecular through cellular and physiological responses to behavioural changes) that can be related to exposure to or toxic effect from, environmental contaminants. At the cellular and intracellular levels specific biomarkers, sensitive to the early detection of degradation of water quality, can be measured (Vasseur and Cossu-Leguille, 2003).

The use of whole-organism assays and the measurement of various biological responses provide an approach for the assessment of the quality of a water body (Allan et al. 2006b). This approach has taken on renewed importance as the aquatic fauna are the primary recipients of water pollutants. Biological monitoring may be performed at a number of levels. Whole- organisms can also be used in standardised toxicity tests, or by their integration into devices specifically designed to detect physiological and behavioural changes when the test species are subjected to a pollution event. At the highest level, the measurement of flora and fauna populations and communities forms an integral part of ecological status monitoring.

Bioassays are biological tools for the determination of the effect (positive or adverse) of a substance or a mixture by quantifying that effect on living organisms or their component parts.

A whole-organism bioassay relies on the measurement (as acute or chronic toxicity) of the biological response of a test organism to a mixture of contaminants present in a water (e.g. drinking, ground, surface or wastewater effluent) sample in a standardised test usually conducted in the laboratory (Persoone et al. 2003). The observed toxic impact is generally the result of the bioavailability of the complex mixture of pollutants that may be present in the sample but is also dependent on physico-chemical parameters (e.g. DOC content, pH) of the water. The use of multiple test species and trophic levels may be crucial for obtaining meaningful results or for fingerprinting, since many inter-comparisons of biological assays have shown differences in sensitivity to different chemicals or classes of compounds.

Observed toxicity may result from the presence and bioavailability of a mixture of contaminants whose toxicity is different from the sum of the toxicities of the individual components. Whole sample toxicity assessment can help identify, diagnose and control impacts in the environment arising from the release of hazardous chemicals in complex mixtures (Wharfe, 2004).

In many cases, toxicity of natural waters is too low to observe measurable effects, so preconcentration techniques may be used. Pre-concentration of river water followed by screening with a battery of bioassays is a technique developed in The Netherlands that may be used as part of WFD monitoring (Maas and van den Heuvel-Greve, 2005).

However it is important to assess the suitability of different effect based tools to realize how these emerging tools can help Member States to implement in a more pragmatic way the objectives of the water framework directive.

The suitability of any particular approach must be evaluated with respect to the cost and practicality of the method, and the ability of the method to provide information that can be translated into useful management information and to help achieve the monitoring programme objectives. A recent review was published on the application of the effect based tools for monitoring and predicting the ecotoxicological effects of chemicals in aquatic environment (Connon et al. 2012).

It is possible to identify several objectives for the use of effect-based tools in a WFD context, and a few of them are summarized below.

1. As screening tools, as part of the pressure and impact assessment to aid in the prioritisation of water bodies to study further.
2. To establish early warning systems, to prioritise further studies in areas that are not concluded to be at risk because they are located far from known local sources.
3. To take the effects from mixtures of pollutants into account
4. To support compliance checking.

1.7 Position of ecotoxicity test in the WFD

Within the WFD monitoring programmes, bioassays may be used with the aim of controlling the toxicity of wastewater treatment effluents, changes in toxicity after accidental spills or to determine the source of a pollutant. Consideration of the WFDs surface-water monitoring requirements for the classification of ecological status of waters necessitates the use of bioassays.

Within the purpose of identified the emerging techniques and methods for eater monitoring the European Commission (EC) was founded the project ‘‘Screening method for Water data InFormaTion in support of the Water Framework Directive (SWIFT-WFD)’’ from January 2004 until March 2007.

The directory aims to list the commercially available and prototype techniques or tools that may be considered for use in the water quality monitoring programmes necessary for the implementation of the WFD. This monitoring includes assessment of biological/ecological quality elements, chemical monitoring of both inorganic and organic priority pollutants and measurement of physico-chemical parameters.

The first major step of the SWIFT-WFD project was the production of an operational manual (Greenwood et al. 2004) which lists comprehensively several chemical and biological monitoring methods in use. This manual aims to provide easy access to the wide range of candidate chemical and biological assessment technologies for monitoring currently available or under development for supporting the WFD.

The principal aims of the project were:

- identify and consolidate information for the validation and demonstration of equivalence of a set of existing or emerging methods and tools for biological and chemical monitoring;
- decide whether, in appropriate circumstances, these technologies may be used to complement or replace traditional monitoring techniques;
- create a network with the capacity to provide advice and training on water monitoring using these emerging tools and methods; and,
- facilitate the link between water policy and research.

As part of this project, a “toolbox”, based on tools and techniques either available commercially or as prototypes, was elaborated in an attempt to answer the challenges of biological and chemical monitoring by providing improved information on a water body.

A set of tools is proposed in response to the challenges and environmental objectives faced by those in charge of monitoring that cannot be achieved by using traditional monitoring techniques. Well-defined roles or functions for types of tools have been identified to facilitate their introduction into and optimise their impact on monitoring programmes (Dworak et al. 2005).

In the context of the WFD, Guidance Document No 7 (EC, 2003) on monitoring, as part of the common implementation strategy (CIS), introduces three important terms: the notion of risk, precision and confidence. The WFD is based on a risk assessment exercise, where precision and confidence are described respectively as how close the measured value(s) or indicator is to the true value and the likelihood that this measured value is within the defined precision. Adequate precision and confidence in values obtained through monitoring are essential to allow an acceptable level of risk in the decision-making process.

In the Guidance Document No 7 in respect to the WFD provides a context within which Member States can either use or modify their existing methods and apply appropriate monitoring and assessment systems that will incorporate all the requirements of the WFD.
The Guidance advises that, when confidence in one particular method is low, several indicators ought to be used. This means the use of not only more than one method to measure a single parameter, or indicator, but also an adequate number of methods or parameters to obtain a more representative assessment of each quality element, with a useful level of precision and confidence.

The tool box concept was developed early in the SWIFT-WFD project following the completion of a directory of tools that may be used for WFD monitoring. The varied needs and requirements of monitoring imply that no one tool is capable of providing all the answers; rather, a suite of tools may be used to obtain a more representative picture of water quality (Allan et al. 2006a). The use of whole-organism assays and the measurement of various biological responses provide an approach for the assessment of the quality of a water body. This approach has taken on renewed importance as the aquatic fauna are the primary recipients of water pollutants. Whole-organisms can also be used in standardised toxicity tests, or by their integration into devices specifically designed to detect physiological and behavioural changes when the test species are subjected to a pollution event.

In order to enable the implementation partly to replace standard expensive chemical analysis (Maas et al. 2005) and adoption by all member states of whole-organism bioassays in regulatory monitoring, the tests need to be simple to undertake, follow standardised protocols, be economical and predictive, and applicable to species, population and communities. In addition, they need to exhibit a wide range of sensitivities to multiple chemicals with minimal matrix effects.

Even if currently not exist at regulatory level the obligation to use ecotoxicity test to evaluate the quality of water systems various studies were performed to this aim. Huschek end

Hansen (2005) used an endocrine effects assay test to realize an ecotoxicological classification of Berlin river system (water and sediments).

Their study have shown how "early warning signals" in ecosystems using sensing systems of biochemical responses (biomarkers) would not only tell us the initial levels of damage, but these signals will also provide us with answers by the development of control strategies and precautionary measures in respect to the WFD.

The current guideline (Directive 2000/60/EC) have been based in the detection of specific pollutants included in a list of priority organic pollutants for monitoring the wastewater treatment plants. For their monitoring, several analytical methods such as gas or liquid chromatography coupled to mass spectrometry have been developed to assess and maintain the quality of surface waters. Since chemical analysis alone are not real measurements of the toxicity effects on the aquatic ecosystem because toxicity is a biological response the use of biological assays can provide also a direct and appropriate measure of toxicity to complement the physicochemical measures of quality of wastewaters. Hernando et al. 2005 assessed the utility and validity of toxicity tests and to apply the toxicity tests for monitoring of wastewater treatment and proved that toxicity and chemical measures are complementary analytical tools for monitoring of wastewaters quality. And the use of single toxicity test or battery of tests is the better approach to evaluate the risk because they are reliable indices of the toxic impact of effluents in the aquatic environment. At the national level (Italy) this approach was considered already from Legislative Decree 152/99 presently revised by Legislative Decree 152/2006 that defines required toxicity testing for discharges of wastewater treatment plants, imposing specific limits.

1.8 Ecotoxicity testing for environmental samples

With the implementation of the Water Framework Directive, the use of bioassays will significantly increase across Europe. Consideration of the WFD's surface-water monitoring requirements for the classification of ecological status of waters necessitates the use of bioassays (Whadia and Thompson, 2007). This because the traditional approach to environmental assessment based on chemical analysis fails to provide an adequate interpretation of toxicity to biota in the ecosystem in the context of bioavailability.

An environmental toxicant can be defined as a substance that, in a given concentration and chemical form, challenges the organisms of the ecosystem and causes adverse or toxic effects (Lidman et al. 2005). This definition includes an element of chemical characterization, toxicity testing and ecoassessment. This triad of techniques, which can be used alone or in combination, forms a particular approach that is often employed in the environmental management of pollutants. How seen before environmental samples can be ecotoxicologically tested using any level of biological organisation from molecular to whole organisms and populations, communities or assemblages of organisms.

In order to obtain a reliable assessment of toxicity, specific aspects of testing need to be given particular consideration and attention:

- environmental samples taken for testing with bioassays need to be representative, and collection, storage and preparation procedures must not result in change in toxicity of the sample;
- test protocols need to be standardised;
- a measure of test variability needs to be ascertained;
- the effect of confounding variables (e.g., pH, DO, dissolved solids, and Eh) also needs to be ascertained.

For these assumption a single whole organisms assays result the good solution for obtain a compromise between ecological realisms and simplicity of testing (and sustainable costs, reproducibility, standardization).

Ecotoxicity tests have been developed to characterise the toxicity of individual chemicals in a specific biological system under investigation. There are international standards (or guidelines) for various tests. The most widely applicable standards have been produced by the Organisation for Economic Cooperation and Development (OECD) and the International Organisation for Standardisation (ISO).

The adoption of these tests for the toxicity evaluation of environmental samples led to contaminated media tests (Suter et al. 2000) or bioassays (Ferguson et al. 1998). In this case bioassays are used to toxicity evaluation in application to environmental samples with limited or complete lack of information on the level and the type of contamination is apt (Loibner et al. 2003). So bioassays can provide qualitative or quantitative evaluation. In the latter case, the assessment often involves an estimation of the concentration or the potency of a substance by measuring selected biological responses. Bioassays employ biological systems to detect toxicants in environmental samples (e.g., effluents, water, sediments, or soil) under investigation. A selected species provides a response considered to be representative of organisms indigenous to the environment potentially of concern. Furthermore the development of short-term bioassays to screen samples came about with the realisation that analysis of environmental samples for all suspected chemicals can prove very timely and expensive. With the use of the bioassays, samples or sampling areas can be prioritised after assessment, providing an indication of acute toxicity, genotoxicity or chronic effects has been attained (Whadia and Tomphson, 2007).

The primary advantage of using bioassays is that toxicity can be evaluated. Toxicity testing is not and never will be a substitute for chemical analysis because bioassays cannot identify the constituent toxicant(s) causing an effect; therefore the need for ecotoxicological testing to be complemented by chemical characterisation is considered critical. The use of bioassays provides a holistic approach that allows the toxicity evaluation of the total integrated effect of all constituent components, including toxicants and confounding variables, in a given complex sample matrix. The net assessment is the combined interactive evaluation of additive, antagonistic and synergistic effects of all sample components. As bioassays directly allow measurement of the potential environmental effects of complex sample matrices, their use for pollution monitoring and control in regulatory framework is becoming increasingly important (Scroggins, 1999). A major drawback in the use of ecotoxicity testing for routine purposes is in the complexity associated with culturing test organisms. This has profound implications for the cost of testing using conventional bioassays.

1.9 Ecotoxicology

The notion of toxicity has been in existence at least since the mid 16th Century. The Swiss physician Paracelsus wrote in 1567 that "…all substances are poisonous. There is nothing, which isn't poisonous. The right dose differentiates a poison from a remedy".

Ecotoxicology is a relatively new science concerned with contaminants in the biosphere and their effects on constituents of the biosphere, including humans (Newman and Zhao, 2008). Ecotoxicology is a combination of the terms ecology and toxicology. Ecology is the study of the relationships between plants and animals and their abiotic environment while toxicology is the study of poisons. The term ecotoxicology was coined by René Truhaut in 1969 who defined it as "the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context" (Truhaut, 1977).

The scope of ecotoxicology encompasses the interaction, transformation, fate, and effects of xenobiotics ('foreign compounds') on the organism, population, community, and ecosystem, from the regional to global level. To elucidate such broad scientific questions, the study of ecotoxicology incorporates concepts contributed from diverse fields including analytical and environmental chemistry, biochemistry, molecular biology, microbiology, immunology, physiology, behavioral ecology, soil science, limnology and oceanography, atmospheric science, environmental and chemical engineering, economics, public environmental policy, and other disciplines.

The ultimate goal of all toxicity testing is to provide data that can be used to establish biologically safe concentrations for toxicants. Ecotoxicological research was rapidly developing from the close of World War II and into 1960 due to the pollution of the environment induced by the rapid industrial development. Also, research was speeded up by several pollution events occurred with consequences universally acknowledged to be unacceptable. These watershed events included population crashes of raptor and piscivorous bird species due to DDT effects on reproduction, widespread water pollution, and epidemics of mercury (Minamata disease) and cadmium (Itai–Itai disease) poisoning. Expertise for dealing with such issues became essential to society and several practical sciences coalesced into the nascent science of ecotoxicology.

Policies were developed accordingly and ecotoxicology became an important part in environmental and ecological risk assessment. Contrarily to the approaches driven by analytical chemistry, ecotoxicological tests integrate all toxic signals and thus, it has been proposed to add toxicity-based criteria to the currently existing policies for the meaningful evaluation of the environmental hazard (Manusadzianas et al. 2003; Põllumaa et al. 2004; Kahru and Põllumaa, 2006).

Still others focus closely on resolving specific, practical problems such as assessing ecological risk due to a chemical exposure or the effectiveness of a proposed remediation action.

For example, the ecotoxicity tests applied today focus on effects to individual organisms, but predictions of consequences to populations and communities are a very high priority for ecotoxicologists. A major theme in ecotoxicology today is finding the best way of achieving scientific, technical, and practical goals while organizing a congruent body of knowledge around rigorously tested explanations.

The ecotoxicology developed mostly as aquatic toxicology and terrestrial ecotoxicological studies lag behind aquatic ones. Aquatic toxicology is the study of the effects of chemicals and other anthropogenic and natural materials and activities on aquatic organisms at various levels of organisation, from the subcellular to individual organisms, populations and ecosystems (Rand et al. 1995). It includes studies in the laboratory and on naturally occurring populations and communities.

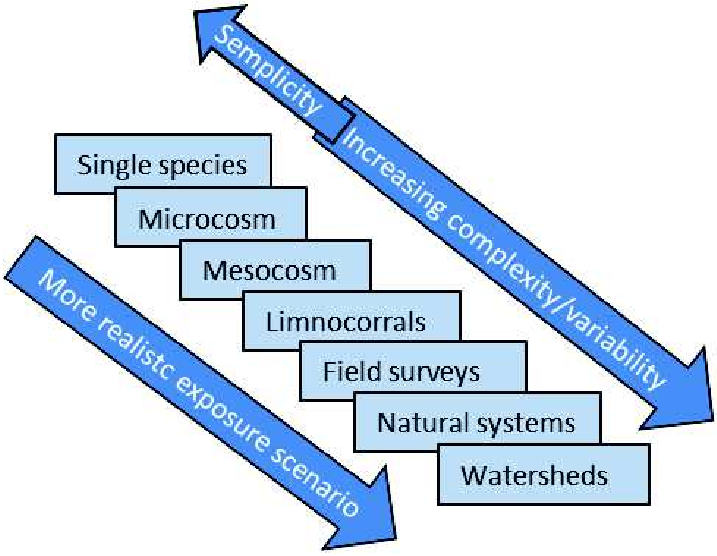


Figure 4 Schematic presentation of inverse relationship between simplicity and ecological realism in test systems of increasing complexity. These are also the aquatic toxicity tests used to establish biologically safe concentrations of potential toxicants.

Because of the long-standing interest in aquatic toxicity testing, a myriad of test species, types of tests and endpoints have been developed and utilised. Toxicity tests include a broad spectrum of tests, differing in the species and exposure media they use and the effects they measure. There are advantages and disadvantages associated with each of the test methodologies correlated to inverse relationship that existing between 'ecological realism' on one hand, and simplicity of testing on the other (Persone and Gillet, 1990).

As indicated in the Figure 4, as one moves from single species toxicity tests (which includes laboratory acute and chronic tests) to whole lake or natural system testing the complexity of the tests increases and therefore so do their expense. Generally, because of the characteristics of the tests, a tiered approach is used where testing begins with the simple tests and based on the data needs may progress across the tests.

1.9.1 Single species Toxicity Assays

Toxicologists are guided by principles and three of those are:

1. you only find what you are looking for . . . ;
2. the dose makes the poison . . . ;
3. only living material can measure toxicity . . . .

The third principle is, no instrument has been devised that can measure toxicity. Toxicity is the degree to which a compound or mixture is capable of causing damage or death. Chemical concentrations can be measured with an instrument but only living material can be used to measure toxicity.

The most commonly measured effects are lethal as mortality, or sublethal including development, growth, reproduction, behavior, physiology, and bioenergetics.

These effects are known as measurement endpoints; they are ecological attributes that may be adversely affected by exposure to site contaminants and that are readily measurable.

In addition, each measurement endpoint is closely related to an assessment endpoint. Because of this close relationship, a measurement endpoint can approximate or represent the assessment endpoint if the assessment endpoint is not amenable to direct measurement (U.S. EPA, 1992). Bioassay batteries used in environmental monitoring are often based on the concept of a simple food chain using at least three species from different trophic levels: typically a primary producer (e.g., green algae), a detritivore or filter feeder (e.g., waterflea), and a consumer (e.g., larval fish). Effects observed during single species toxicity tests could be measured under different exposure scenarios. They might be measured during acute (4 days or shorter) or chronic (longer than 10% of an individual's lifespan) exposures.

Organism	Exposure period	Test type	Toxicity Endpoint	Standard protocols
Green algae: ***Pseudokirchneriella subcapitata***	72-96 h	chronic	growth	ISO 8692, OECD 201
Waterflea: ***Daphnia magna***	48 h 21 days	Acute chronic	Mortality Fecundity	ISO 6341 OECD 202, 204 US EPA, 2002
Ceriodaphnia dubia	96 h 7-8 days	Acute chronic	Mortality fecundity	ISO 20665
Fish: ***Brachydanio rerio***	96 h	acute	Mortality	ISO 7346
Oncorhynchus ykiss	28 days	chronic	Growth	ISO 10229
Pimephales romelas	96 h 7 days	acute chronic	Mortality growth	US EPA, 2002

Table 1 Species, exposure periods, test types and endpoints used for some standardized in vivo bioassays in freshwater

Acute toxicity tests are short-term tests that measure the effects of exposure to relatively high concentrations of chemicals. The measurement endpoint generally reflects the extent of lethality.

A typical acute toxicity test exposes test organisms to a series of dilutions of a site's medium and records deaths occurring over a specified period of time, usually 24 to 96 hours. Results can be analyzed by comparing percent mortality of organisms exposed to site media to percent mortality of organisms exposed to uncontaminated media.

Usually in aquatic toxicity tests the results are expressed as an LC50 or an EC50. The LC50 is the median lethal concentration that caused 50% mortality to the test population in a given period of time. The time is generally 48 h for invertebrate test organisms and 96 h for fish. Since LC50s are point estimates, which are estimates of the effects from specific concentrations of contaminants, coefficients of variation can be calculated for them.

With some test organisms, toxicologists find death difficult to determine unequivocally. In tests using such organisms, toxicologists evaluate another effect, such as immobility, that correlates closely with death. In these situation data can be analyzed to estimate the EC50: the effective concentration at which 50 percent of the organisms displayed the effect in a given period of time. When an acute toxicity test reports an EC50, the test results will specify the effect, the test duration, the test species, and the life cycle stage of the test species. Like the LC50, the EC50 is a point estimate and a coefficient of variation can be calculated for it.

Chronic toxicity is toxicity that develops over longer periods of time that measure the effects of exposure to relatively lower, less toxic concentrations. For a chronic toxicity test, the measurement endpoint concerns a sublethal effect or both lethality and sub-lethal effect.

Sublethal effects may include growth reduction, reproductive impairment (number of offspring produced or eggs laid), nerve function impairment, lack of motility, behavioral changes, and the development of structural abnormalities.

Results from chronic toxicity tests can be analyzed in several ways. One is simply by a direct comparison between percent effect occurring in organisms exposed to site media and those exposed to uncontaminated media.

Results can also be expressed as the lowest observable effects concentration (LOEC), or the no observable effects concentration (NOEC). These are calculated statistically as the lowest concentration significantly different from the control and the highest concentration not statistically significantly different from the control group, respectively. For the majority of chemicals the concentrations causing chronic effects are less than the concentrations causing acute effects. However, there are cases where too little as well as too much can cause problems for organisms.

In general, acute and chronic toxicity tests differ in the amount of time required to perform them, their cost, and their resolution.

- Because chronic tests extend through either a life cycle or a critical developmental phase, they generally require more time to perform than acute tests with the same type of test organisms.
- Requiring more time to complete than acute tests, chronic tests also can require more funds. A chronic test also may require more resources and increased numbers of laboratory analyses, further increasing the cost of the test.
- Chronic tests have greater resolution than acute tests. For example, consider a chronic test that exposes invertebrates to site surface water and records the number of young they produce. In a highly toxic medium, the organisms will die. In a less toxic

medium, they may survive, but their reproductive capacity may be impaired when compared with controls maintained in an uncontaminated medium.

Numerous species have been used as test species in aquatic acute and chronic toxicity tests, including fish, invertebrates, macrophytes, algae, and bacteria.

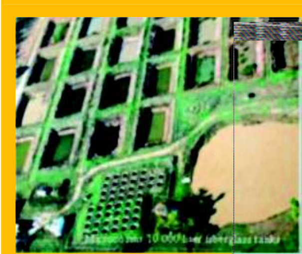
Invertebrates are composed of a large and very diverse group of animals, consisting of more than 30 different phyla, several of which include more than 1000 different species (Ruppert et al. 2004). For instance, the largest invertebrate phylum is the Arthropoda, consisting of more than 1 million species of which insects and crustaceans are the two largest groups. Since relatively few insects have aquatic larvae, crustaceans therefore are the most numerous and ecologically important group of invertebrates in fresh water ecosystems. Crustaceans also play an important role in regulatory toxicity testing as a part of the base set of organisms required for assessing risks to both aquatic and terrestrial environments (EU Commission 2003).

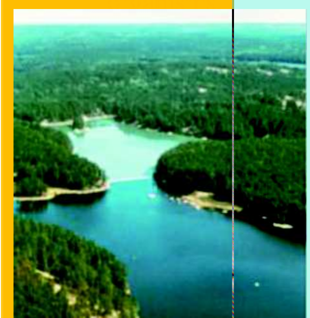
Hence, it is not surprising that most ecotoxicological tests on chemicals using invertebrates have been performed with crustaceans in particular with water fleas. These organisms belong to the order Cladocera (Latreille 1829) and family Daphniidae (Straus 1820). The family includes, among others, *Ceriodaphnia dubia* (Dana 1853), and *Daphnia magna* (Müller 1785). This species are the most commonly used invertebrate species in regulatory chemicals testing, and which are also included in several guidelines and international standards for acute and chronic tests (e.g. OECD, ISO).

i. Field Surveys

Field surveys have been used to evaluate whether or not an ecosystem has been impacted. While toxicity tests may infer potential population and community level effects, field surveys are the only means for demonstrating actual population and community level effects. Survey data identify the “problem” and the extent of the problem. Organisms are exposed in the “real world” and measured effects represent an integrated response to the temporal and spatial variations in exposure and contaminant concentrations in the field. However with survey data alone, the causes for observed effects are difficult to determine. Results from field surveys and measures of ecological status are often highly variable, reflecting the high degree of variability (both spatial and temporal) in natural communities and, in some cases the problems inherent in sampling the biological community. Furthermore procedures for quality control exist for field surveys but they are not nearly as well established or clear-cut as are protocols for other components of the ecological assessment.

A classic example of the use of field surveys is to measure the status of an ecosystem above and below an outfall. There are several problems with this approach: first, and one that is sometimes hard to avoid, is the statistical requirement of independence, that is, the downstream sites are not independent of the upstream sites. Second, measuring an ecosystem response is often much more difficult than measuring the toxicity of a sample. In text box below it is possible to find key information regarding the other intermediate test methodologies.





Microcosms and mesocosms

The factors that increase the 'realism' of microcosm tests over single species tests are that there are multiple species in a microcosm (<15m^3 water volume). Because there are multiple species, structural as well as functional endpoints can be evaluated. However, the structure of a microcosm is not such that the system can support all trophic levels found in larger 'cosms'.

Mesocosms (>15m^3 water volume) generally have a better developed community structure than microcosms but usually are still not large enough to maintain some top level predators and do not have all the complexities of natural systems such as larger ponds, lakes, or streams. The endpoints measured in mesocosms include changes in structure and function of the developed communities and through the use of multivariate techniques all the data collected in a study.

Limnocorrals

Limnocorrals are enclosures placed in natural lakes. Unlike mesocosms that require a period of time for the development of resident communities of organisms, limnocorrals take advantage of the already developed communities in the lake. Changes in the structure and/or function of the assemblages of organisms in the limnocorrals are measured, as are the overall effects through the use of multivariate statistics.

Experimental lake

While there are not a large number of experimental lakes research facilities in the world, the Canadian and Ontario governments have established one such area in Northwestern Ontario. The experimental lakes area (ELA) includes 58 small lakes and their drainage basins, plus some additional stream segments. Studies on nutrient enrichment in two basins of a lake divided by a plastic curtain, showed that in this lake limiting levels of phosphorus could control eutrophication.

1.10 The position of sediment in the WFD

Regulatory issues for the aquatic environment in the past have predominantly focused on the contamination of the water matrix. But, in recent years, both on the research front and in the regulatory framework, substantial interest has been generated on sediment contamination and its effects on aquatic organisms. In contrast to aquatic toxicity testing where test protocols have been standardized, testing protocols for sediments still lack harmonization.

Sediments are an essential component in the management of the aquatic environment. In fact, contaminated sediments may represent a serious risk for aquatic organisms and ecosystems because: (1) they accumulate contaminants and serve as a sink and source of pollution to the ecosystem they are connected with (Delistraty and Yokel 2007); (2) they integrate pollutant concentration over time (Roig et al. 2011); (3) they are extremely important to the food chain and serve as a reservoir of contaminants for bioaccumulation and trophic transfer (Kwok et al. 2010); and (4) their contaminants' concentration may be several orders of magnitude higher than in the water column (Tuikka et al. 2011). For all these reasons, it is extremely important to perform an integrative analysis of the chemical characteristics and the ecological responses of sediments in the process of environmental risk assessment concerning the freshwater reservoirs.

Several authors recommend that the ecological risk assessment of sediments include not only the analysis of the contaminants bioavailability, but also its toxicological profile (Chapman et al. 2002; Tuikka et al. 2011). Considering the toxicological assessment of sediments, a number of authors reported the importance of carrying through a battery of toxicity tests, with species from different trophic levels, both on the pore water, which contain dissolved pollutants, and on the solid-phase fraction (whole sediment) to which the biota are exposed through direct contact and/or by ingestion (Chial and Persoone 2002; Davoren et al. 2005; Roman et al. 2007).

Consequently, sediment quality has emerged as an important and critical consideration for protection of benthic ecosystem health, fisheries conservation, and protection of surface water quality in both marine and freshwater environments (Babut et al. 2005; Wenning et al. 2005).

Despite the importance of the integrated sediment management in the implementation of the WFD, there is no dedicated legislation (guidelines) at the European level in what concerns to managing the quantity and quality of sediments in the reservoirs (Reis et al. 2010). Recently the Environmental Quality Standards Directive 2008/105/EC (EQS) marked an important step in the use of sediment and biota as matrices for chemical-status assessment under WFD. Analysis of contaminants in sediments and biota is widely recognized as a cost effective approach in water-quality monitoring to describe the general contamination level, to supply reference values for local and regional monitoring and to identify areas of concern where additional monitoring effort is needed. Because of the explicit reference in the EQSD to the use of sediments and biota as preferred matrices for the monitoring of substances with accumulation potential Member States asked the EC to publish a guidance document to enhance the degree of harmonization among EU countries in chemical monitoring of sediments and biota, taking into account best available techniques, standard procedures and common practices.

The purpose of analysing the levels of priority substances in sediments under the WFD might be: a) to monitor the progressive reduction in the contamination of priority substances and phasing out of priority hazardous substances and b) to demonstrate conditions of “no deterioration” in sediment quality. This is implicit in the need to ensure adequate provision of pollution prevention and control.

The presence of contaminated sediments might be one of the obstacles to achieving “good ecological status” for a waterbody. One widely accepted way of obtaining an initial indication of the likely causes of a waterbody’s poor ecological status is the sediment quality triad (Chapman, 1996): the simultaneous observations of sediment chemistry, sediment toxicity tests and, in the field, the benthic community. The observed concentrations of sediment-associated chemicals can be compared with sediment quality guidelines, if these are available. Over the years, research has demonstrated that contaminated sediments that exceed sediment quality guidelines do not always result in toxic effects in sediment toxicity tests or in the benthic community as a result of decreased bioavailability of the sediment-associated contaminants.

Sometimes the opposite has been observed, i.e. sediment that meet a suite of sediment quality guidelines has caused adverse effects to the benthic community in the field or in laboratory toxicity tests because of combination toxicity or the presence of unidentified compounds. This demonstrates need to better understand the relation between sediment contamination (a hazard) and its actual risk to the functioning of the ecosystem (ecological status) in order to be able to take effective measures to restore the ecological status of a given water body (Heugens et al., 2001).

Sediment toxicity tests play an important role in prospective risk assessment for contaminants (Diepens et al., 2013). Historically, toxicity testing mainly used aquatic animals. It has been recognised, however, that by testing animals in the aquatic phase, the role of sediment as an exposure route is neglected and these tests are not sufficient to assess environmental hazards to benthic invertebrates. Consequently, there is an urgent need to evaluate the role of toxicity tests with benthic species in sediment risk assessment procedures.

The ideal sediment toxicity test provides accurate and reproducible results. This requires standardised tests with well-defined endpoints that are linked to the related protection goals. Hence, test guidelines produced by international (e.g. OECD, ISO, US EPA, ASTM) bodies are highly appreciated. These test guidelines are preferably ring tested. In a ring test, the performance of a method is evaluated across different laboratories and countries. Such a ring test is required, e.g. by the OECD, to approve the test as a guideline. Another regulatory requirement is that the standard sediment species to be used should be easy to obtain or culture, should be ecologically and ecotoxicologically relevant and should represent specific trophic levels or taxonomic groups that allow extrapolation to the wider array of sediment organisms occurring in the field. Benthic invertebrates are used most often because they are often highly abundant in ecosystems and differ in morphological, physiological, behavioural and ecological characteristics (i.e. traits). These traits influence the uptake potential, metabolic capacity, exposure routes and bioaccumulation, and thus the sensitivity of invertebrate species to contaminants (Rubach, 2011). Moreover, benthic invertebrates provide an important ecosystem function, which underlines the importance of protecting the biodiversity and functionality of benthic communities.

Many retrospective tests are available in which contaminated field sediments are tested with single species in the laboratory or in situ. Tests mainly focus on single species and short-term effects, with exposures of 4-10 d (Burton and Scott, 1992), which seems insufficient to detect effects at the population level, and to reach a steady state in exposure. Tests regarding long-term effects, full life-cycles, multiple generations or their implications at population level are less well developed. Full life-cycle and multi-generation tests are more useful for risk assessment and setting quality standards for sediment-dwelling organisms, since they include all sensitive life stages of an organism (Tassou and Schulz 2011). However, these tests are timeconsuming and expensive. Various short- and long-term standard methods have been validated using ring tests, and are internationally accepted.(Sánchez et al., 2005).

Early sediment toxicity testing methods and regulatory instruments were developed in North America. Originally, aquatic species (e.g. Daphnia sp.) were tested in the aqueous phase. These species, which predominantly dwell in the water column, cannot be used to test the toxicity of the solid phase directly, which is why they have been used as a surrogate measure of the toxicity to benthic species by testing them in pore water and elutriate. Pore water contains the bioavailable fraction and therefore is important for exposure to infaunal species. Elutriate tests provide information on the leaching capacity of sediment-associated contaminants and were used to mimic the open water disposal of dredged material, thus representing the potential adverse effects to aquatic organisms due to sediment disturbance.

Nevertheless, simulation of in situ exposure of organisms to contaminated sediments is most realistic when wholesediment samples are used (Liss and Ahlf, 1997).

After the early phases of sediment toxicity testing, benthic organisms were introduced in pore water, elutriate or sediment tests with and without an overlying water phase. The first standard protocols for whole sediment tests with benthic invertebrates were developed in the 1990s by the American Society for Testing and Materials (ASTM) that approved three standard guides that provided general guidelines for conducting whole-sediment toxicity tests with the freshwater benthic invertebrates *Hyalella azteca* (an amphipod), *Chironomus tentans* and *C. riparius* (insect).

Species	Endpoint	Method
***Chironomus riparius* (insect)**	Emergence	OECD 218, 219, 233
***Hyalella azteca* (crustacean)**	Reproduction	ASTM, 2010
***Lumbriculus variegatus* (annelid)**	Reproduction, growth Bioaccumulation	OECD 225 US EPA, 2000; ASTM, 2000

Table 2 Selection of most commonly freshwater benthic invertebrate species and endpoints used in standardized methods.

Despite the importance of sediments for an ecological assessment of water ecosystems only a handful of new sediment toxicity bioassays have been developed in the past 20 yr. There are still surprisingly few standardized bioassays for benthic species compared with pelagic species. Part of this inactivity can be explained by a scarcity of research funding by governmental agencies for exploratory studies of contaminated sediments. An additional factor that may be slowing the development of new benthic bioassays is the fact that few benthic species can be cultured easily.

Presently, the freshwater benthic invertebrate species most commonly used in standardized sediment toxicity bioassays are summarized in Table 2.

The limited suite of sediment toxicity assays is problematic for the assessment and regulatory processes, such as developing species sensitivity distributions from which sediment guidelines are derived (e.g., predicted no effect concentrations (PNECs)), or extrapolating findings to widely varying benthic populations and communities. Sediment quality guidelines have also largely remained unchanged in the past 2 decades (Burton, 2013). The exception is that new PNECs are currently being developed as part of the European Commission's Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) and Water Framework Directive programs. As in water, no sediment guidelines exist for contaminants of emerging concern (CECs), such as pyrethroids, polybrominated diphenyl ethers, pharmaceuticals, and personal care products. Many of the CECs, like most chemicals, tend to sorb to solids and therefore will accumulate in sediments.

Another critical aspect of sediment assessments that is presently being studied is that of resuspension of contaminated sediments. It is somewhat perplexing that, although many of the contaminated sediment sites in the world are in harbors, where resuspension is a frequent daily event, little is known about the ecological consequences of these events.

All of this suggests that contaminated sediments will increase in importance. It will be increasingly difficult to remove and dispose of contaminated sediments due to the cost and sheer magnitude of the problem.

Judging by what we have seen so far, prospective compound-specific risk assessment regarding sediments remains a crucial challenge in the European risk assessment framework. At present, chemical risk assessment for sediment-related biota is performed under conditions associated to a number of uncertainties (Brils, 2008).

Ecotoxicological tests that involve the sediment compartment present a number of methodological challenges because sediments are complex and heterogeneous in terms of their physical, chemical, and biological characteristics (Chapman and Hollert 2006; Hollert et al. 2007; Höss et al. 2010). As a result of this heterogeneity, toxicants can be available in different forms in the various compartments. Therefore there is currently a need for developing an appropriate sediment toxicity testing framework for the prospective risk assessment of chemical compounds and recent works have been carried out with this purpose (Beketov et al. 2013; Diepens et al. 2013).