Aquatic Ecotoxicology

Advancing Tools for Dealing with Emerging Risks

Edited by

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CHAPTER 1

Introduction

Claude Amiard-Triquet

Abstract

The aquatic environment appears as the final destination for most of anthropogenic contaminants released from industry, agriculture, urbanization, transport, tourism, and everyday life. On the other hand, inland, coastal, and marine waters provide many services important for human well-being. The conservation of ecosystems and human health is based on a sound assessment of the risks associated with the presence of contaminants in the aquatic environment. The aim of this book is to use cross-analyses of procedures, biological models, and contaminants to design ecotoxicological tools suitable for better environmental assessments, particularly in the case of emerging contaminants and emerging concern with legacy pollutants.

Keywords: Aquatic environment; Bioaccumulation; Bioassays; Bioindicators; Biomarkers; Ecotoxicological tools; Emerging contaminants; Emerging risks; Exposure; Risk assessment.

Chapter Outline

Many different classes of contaminants enter the environment as a consequence of human activities, including industry, agriculture, urbanization, transport, tourism, and everyday life. Initially, air pollutants are atmospheric contaminants and solid wastes are terrestrial contaminants, whereas liquid effluents are aquatic contaminants. Processes involved in the fate of contaminants in each compartment—air, soil, water—lead to many intercompartment exchanges, governed by advection (e.g., deposition, run-off, erosion), diffusion (e.g., gas absorption, volatilization), and degradation, both biotic and abiotic (Figures 2.2 and 2.3, this book), and by large-scale transport from atmospheric and marine currents. The aquatic environment appears as the final destination for most of anthropogenic contaminants, and aquatic sediments, either deposited or in suspension, as the major sink for their storage with only few exceptions (e.g., perfluorooctanoic acid [PFOA], Post et al., 2012; water-soluble pesticides such as alachlor, atrazine, and diuron).

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It is generally admitted that about 100,000 molecules are introduced in aquatic media intentionally (e.g., pesticides, antifouling paints) and more often unintentionally as incompletely treated sewages or because of accidents. In most cases, several classes of contaminants are present concomitantly, thus being able to act in addition, synergy, or antagonism (Chapter 18).

The Millennium Ecosystem Assessment (MEA, 2005) highlights how ecosystem services are important as determinants and constituents of human well-being. Inland, coastal, and marine waters are important contributors of core services (nutrient cycling, primary production). They are also part contributors in providing services (water, food, biochemicals, genetic resources) and cultural services (such as recreation and ecotourism, aesthetic and educational benefits). As emphasized by Maltby (2013), applying approaches based on the ecosystem service concept to the protection, restoration, and management of ecosystems requires the development of new understanding, tools, and frameworks.

Legislation has been adopted on a worldwide scale to improve the status of aquatic ecosystems (e.g., United States' Clean Water Act, 1972; European Community Water Framework Directive, ECWFD, 2000). Environmental management aiming at the improvement of chemical and ecological quality in aquatic media must be based on robust risk assessments. Retrospective risk assessments are performed when sites have potentially been impacted in the past. When they show a degradation of environmental quality, the restoration of degraded habitats and ecosystems must be addressed. Prospective, or predictive, risk assessments aim at assessing the future risks of anthropogenic pressure such as climate change or releases of new chemicals into the environment. Strategies to limit the risks of both new and existing chemicals include the federal Toxic Substances Control Act (1976) in the United States, and a new chemical policy, Registration, Evaluation, and Authorization of CHemicals in Europe (2006).

1.1 Ecotoxicological Tools Currently Used for Risk Assessment in Aquatic Media

Conventional risk assessment (Chapter 2) in different environments aims at establishing a comparison between the degree of exposure expected or measured in the field and the effects induced by a contaminant or a class of contaminants. It is mainly based on the determination of predicted environmental concentrations (PECs) and predicted no effect concentrations (PNECs). The procedure has been described in the Technical Guidance Document on Risk Assessment in support of European Commission regulations (TGD, 2003). PECs and PNECS are then used in a risk quotient approach: very simplistically, if the PEC/PNEC ratio is lower than 1, the substance is not considered to be of concern; if the PEC/PNEC ratio is higher than 1, further testing must be carried out to improve the determination of PEC or PNEC with subsequent revision of PEC/PNEC ratio, or risk reduction measures must be envisaged (TGD, 2003).

Environmental quality standards ([EQS] concentration in water, sediment, or biota that must not be exceeded) are a major tool to protect the aquatic environment and human health

(Chapter 3). An overshoot of EQS at a given site triggers management actions (e.g., research for contamination sources, reduction of contaminant discharges). EQS for sediment and biota are needed to ensure protection against indirect effects and secondary poisoning. To date, no EQSs are available for sediments under the ECWFD (2000), partly because the total dose of a pollutant in sediment has a low ecotoxicological significance and the bioavailable fraction must be determined using specific methods (Chapter 3). In addition, different sediment quality guidelines are commonly used by official organisms in the US (National Oceanic and Atmospheric Administration) (Long et al., 1995; MacDonald et al., 1996), Canada (http://ceqg-rcqe.ccme.ca/), Australia (McCready et al., 2006), etc. An overshoot of these guidelines at a given site triggers additional investigations on the impacts and their extent.

Environmental monitoring is then indispensable to assess if environmental concentrations meet standards/guidelines (Chapter 3). An excellent example is provided by the Coordinated Environmental Monitoring Program undertaken under the OSPAR Commission that aims at protecting and conserving the Northeast Atlantic and its resources. Guidelines for monitoring of hazardous substances in sediment and biota are available at http://www.ospar.org/content/content.asp?menu=00900301400135_000000_000000. OSPAR monitoring guidance is regularly reviewed in collaboration with the International Council for the Exploration of the Sea and, where necessary, updated to take account of new developments such as the inclusion of new monitoring parameters.

However, chemical measurements of contaminants in environmental matrices pose a number of problems in many monitoring programs:

1. Analytical efforts focus on chemicals that are perceived to be relatively easy to analyze (heavy metals, DDT and its metabolites, γHCH, αHCH, some congeners of polychlorobiphenyls [PCBs], some individual polycyclic aromatic hydrocarbons [PAHs], etc.);
2. Complex mixtures present in multipolluted environments include many classes of compounds that are not yet accessible to analysis or are extremely expensive to analyze, particularly emerging contaminants (nanomaterials) or known contaminants of emerging concern (pharmaceuticals, personal care products) and their metabolites;
3. As previously mentioned for sediments, the total dose of a pollutant in any compartment of the environment (water, sediment, biota) has a low ecotoxicological significance since their physicochemical forms govern their bioaccessibility and biological effects.

1.2 How Can We Improve Risk Assessment?

To improve exposure assessment, it is indispensable to take into account the physicochemical characteristics of different classes of contaminants (Chapter 4). In the case of metals, a number of chemical speciation models allows a good characterization of the metal chemical species in a solution containing inorganic ligands and well-characterized organic ligands, particularly natural organic matter that is one of the most dominating processes in freshwater

and salinity (chlorinity) in seawater (Paquin et al., 2002; VanBriesen et al., 2010). Different procedures have been described to take into account bioavailability concepts in the risk assessment process or environmental quality criteria setting. The Free Ion Activity Model (FIAM) has been designed to take into account the central role of the activity of the free metal ion as a regulator of interactions (both uptake and toxicity) between metals and aquatic organisms (Campbell, 1995). As the FIAM, the Biological Ligand Model is a chemical equilibrium-based model but at the center of this model is the site of action of toxicity in the organism that corresponds to the biotic ligand. The Biological Ligand Model can be used to predict the degree of metal binding at this site of action, and this level of accumulation is in turn related to a toxicological response (Paquin et al., 2002).

Passive samplers are devices that rely on diffusion and sorption to accumulate analytes in the sampler (Mills et al., 2010). Among these techniques, diffusive equilibration in thin films and diffusive gradients in thin films allow a better understanding of the speciation of metals in the environment, differentiating between free-, inorganic-, and organic-bound metal species and organometallic compounds. Other passive samplers can be used for different classes of organic chemicals, also providing a partial determination of the physicochemical characteristics that govern fate and effects of contaminants. For instance, semipermeable membrane devices are relevant for nonpolar contaminants such as PAHs, whereas polar organic chemical integrative samplers are relevant for polar compounds such as detergents including alkylphenols, pharmaceuticals, and pesticides (Mills et al., 2010).

Chemical monitoring in different environmental matrices (seawater, freshwater, underground water, and effluents; sediment and leachates; organismal tissue and fluids) may be carried out by using either a priori or "global" approach. In the first case, the analyses focus on main classes of known contaminants, particularly the priority hazardous substances listed in European legislation (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:EN:PDF) and USEPA (http://water.epa.gov/scitech/methods/cwa/pollutants.cfm). For instance, the Joint Assessment and Monitoring Program guidelines for monitoring contaminants in biota (https://www.google.fr/#q=JAMP+Guidelines+for+Monitoring+Contaminants+in+Biota) and sediments (https://www.google.fr/#q=jamp+guidelines+for+monitoring+contaminants+in+sediments) provide procedures for metals (including organotin compounds), parent and alkylated PAHs, hexabromocyclododecane, perfluorinated compounds (PFCs), polybromodiphenyl ethers, PCBs, dioxins, furans, and dioxin-like PCBs. However, this a priori approach is not adapted for all the unknown contaminants present in mixtures in most of the aquatic media. The global approach combining biotesting, fractionation, and chemical analysis, helps to identify hazardous compounds in complex environmental mixtures (Burgess et al., 2013). Toxicity identification evaluation (TIE), which was mainly developed in North America in support to the US Clean Water Act and effects-directed analysis (EDA) that originates from both Europe and North America, differed primarily by the biological endpoints used to reveal toxicity (whole organism toxicity tests vs cellular toxicity tests able to reveal mutagenicity, genotoxicity, and endocrine disruption) (Figure 15.2, this book).

In the case of emerging contaminants such as nanomaterials (NMs), the situation is even more challenging because efficient methods and techniques for their detection and quantification in the complex environmental media are not yet available—not to mention difficulties currently insurmountable for investigating the transport and fate of NMs in water systems (Wong et al., 2013). However, advanced nuclear analytical and related techniques recently reviewed by Chen et al. (2013) are powerful tools that can be applied (1) to study their transformation in vitro; (2) to analyze the bio–nano interactions at the molecular level; and (3) for the study of in vivo biodistribution and quantification of nanoparticles in animals. But to date, many of these analytical resources located in large-scale facilities are not available for routine applications in nanotoxicology (Chen et al., 2013).

Processes leading to bioaccumulation of environmental contaminants in aquatic organisms are reviewed in Chapter 5. They include direct uptake of compounds from water (bioconcentration) as well as dietary uptake and incorporation of sediment-bound contaminants. Even when considering only waterborne exposure, bioconcentration factors (concentration in biota/concentration in water) indicate that nearly all the contaminants are incorporated at a level higher than encountered in water. As mentioned previously, the chemical characteristics of contaminants in water and other sources (preys, sediment) are a major driver of bioaccumulation but biological factors also influence bioaccumulation (Eggleton and Thomas, 2004; Abarnou, in Amiard-Triquet and Rainbow, 2009; Rainbow et al., 2011). The concepts of bioaccessibility and trophic bioavailability are often used concurrently. Release of a chemical from ingested food is a prerequisite for uptake and assimilation. Bioaccessibility of a food-bound contaminant can be measured by its extractability from food (or sediment frequently ingested with food by deposit-feeding invertebrates and flatfish). Trophic bioavailability should be used in the strict sense to describe the proportion of a chemical ingested with food which enters the systemic circulation (Versantvoort et al., 2005). Similarly, only a fraction of contaminants present in water is readily available for organisms (FIAM, Chapter 4).

Thus the Tissue Residue Approach has been developed to link toxicity to incorporated doses of contaminants rather than external doses (Chapter 5). However, the relationship between global concentrations in organisms and noxious effects is not simple. The limitation of uptake—responsible for the gap between bioaccessibility and bioavailability—has been described as contributing to the ability of organisms to cope with the presence of contaminants in their medium as well as increased elimination, or storage in nontoxic forms (Amiard-Triquet and Rainbow, in Amiard-Triquet et al., 2011). For instance, when an organism has high metal concentrations in its tissues, it does not necessarily exhibit toxicity effects (Luoma and Rainbow, 2008).

However, to date the results of bioassays generally remained expressed as an external concentration–effect relationship (Figure 1.1). In addition, these classical bioassays exhibit a number of weaknesses. Considering the conditions of exposure (X axis), acute concentrations are most often tested, whereas in the real world, low concentrations are present except

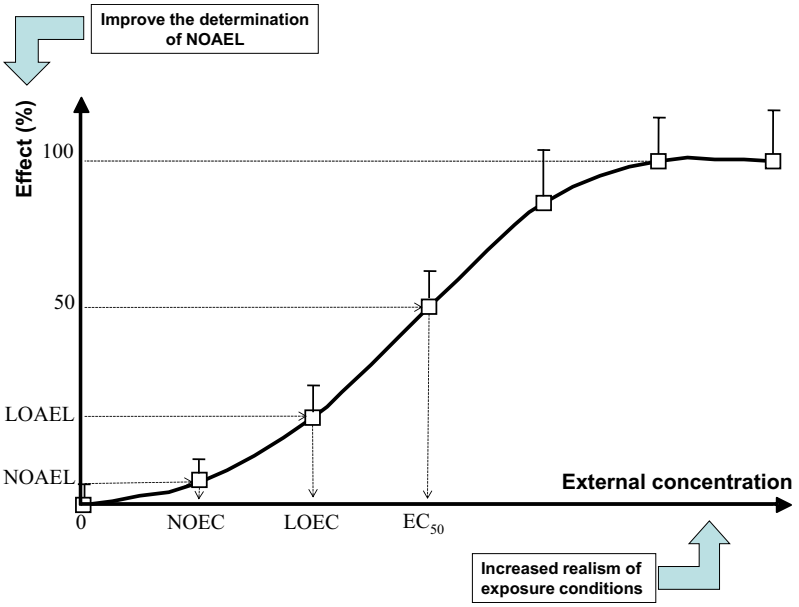

Figure 1.1
How to improve the assessment of the concentration–effect relationship in classical bioassays.

in the case of accidents. The contaminant under examination is generally added in water, whereas dietary and sediment exposures are neglected. Interactions between different classes of contaminants are neglected. Considering the observed effects (*Y* axis), test organisms are most often the equivalent for aquatic media of laboratory rats. Mortality tests and short-term tests are predominantly used. Proposals for improving bioassays (Chapter 6) include three pillars:

1. Improving the realism of exposure (low concentrations, chronic exposures, mesocosms, experiments in the field including transplantations);
2. Improving the determination of the no observed adverse effect level (for individual effects, prefer growth, behavior; develop subindividual effects such as biochemical markers; focus on those impacting reproduction since the success of reproduction is key for population fate);
3. Improve the statistical determination of toxicological parameters for instance by using the benchmark dose method as advocated by the European Food Safety Authority (EFSA, 2009) and the US Environmental Protection Agency (USEPA, 2012a).

Moreover, improving the extrapolation of experimental toxicity data to field situations needs a relevant choice of reference species (Chapter 9) by using organisms from the wild, representative of their environment as well as sensitive organisms or life stages (Galloway et al., 2004; Berthet et al., in Amiard-Triquet et al., 2011; Berthet, in Amiard-Triquet et al., 2013).

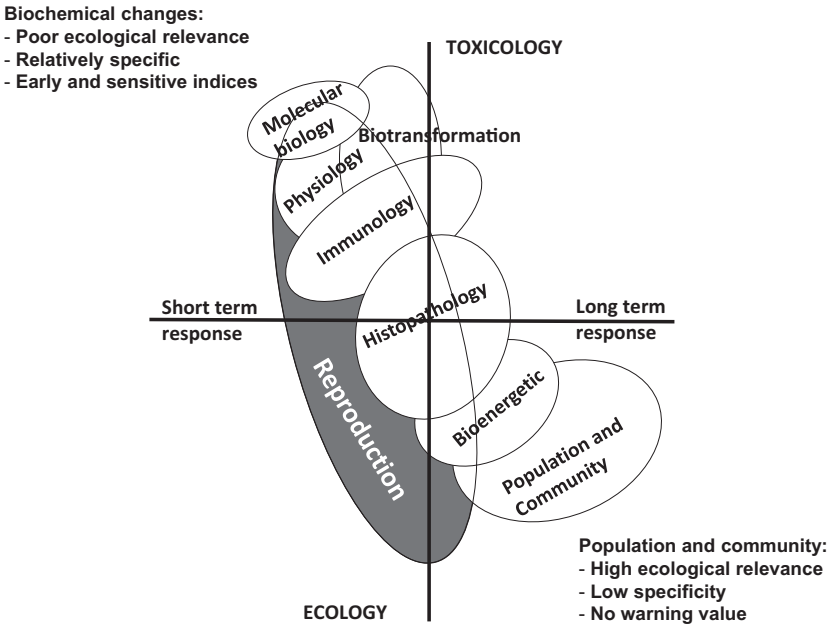

Figure 1.2
Pros and cons of biological responses at different levels of organization as biomarkers/bioindicators of the presence and/or effects of environmental stress including chemical contaminants. *Modified after Adams et al. (1989); Amiard-Triquet and Amiard, in Amiard-Triquet et al. (2013).*

In addition to chemistry and bioassays, the triad of analyses classically used for environmental assessment also includes the analysis of assemblages and communities (Chapman, 1990; ECWFD, 2000). Other tools are recommended including the use of biological responses at different levels of biological organization (Chapters 7 and 8) as biomarkers of the presence/effects of contaminants in the environment (Allan et al., 2006; Chapman and Hollert, 2006; Amiard-Triquet et al., 2013).

Because biomarkers were defined by Depledge in 1994 ("A biochemical, cellular, physiological or behavioral variation that can be measured in tissue or body fluid samples or at the level of whole organisms that provides evidence of exposure to and/or effects of, one or more chemical pollutants (and/or radiations)"), they are more and more frequently used despite recurrent criticisms about their responsiveness to confounding factors, their insufficient specificity of response toward a given class of chemicals, and their lack of ecological relevance. These weaknesses are analyzed in Chapter 7 and strategies to overcome these limits and take advantage of the potential of biomarker tools are recommended. The main achievement expected from the methodology of biomarkers is to provide an early signal of environmental degradation, well before effects at the community level become significant (Figure 1.2), a sign that severe environmental degradation has already occurred, thus leading to expensive remediation processes.

A comprehensive methodology initially proposed to assess the health status of estuarine ecosystems (Amiard-Triquet and Rainbow, 2009) may possibly be generalized to other aquatic ecosystems. The first step is based on the detection of abnormalities revealed by high level biomarkers, linking alterations at molecular, biochemical, and individual levels of organization to adverse outcomes in populations and communities (Mouneyrac and Amiard-Triquet, 2013), in keystone species or functional groups important for the ecosystem. When such impairments are revealed, the end-users need to know the nature of pollutant exposure, indispensable for any risk reduction measure or remediation decision. Core biomarkers validated in international intercalibration exercises and more specific of the main classes of contaminants are to be used for this second step such as those recommended in the Joint Assessment and Monitoring Program Guidelines for Contaminant-Specific Biological Effects (OSPAR Agreement, 2008-09). They include specific biological effects for monitoring metals, PAHs, tributyltin, and estrogenic chemicals. Used in battery, they are able to reveal the presence/effects of environmental mixtures (e.g., metals, PAHs, PCBs, pesticides, endocrine disruptors). And finally, analytical chemistry will be used to validate the hypotheses provided by biomarkers.

During the past decade, "omics" technologies (Chapter 8) covering genomics, proteomics, and metabolomics have emerged and their potential for the risk assessment of chemicals has been addressed (Garcia-Reyero and Perkins, 2011; Connon et al., 2012; Van Straalen and Feder, 2012; SCHER, SCENIHR, SCCS, 2013). Transcriptomics corresponds to a global analysis of gene expression; proteomics focuses on the functional responses of gene expression (proteins and peptides); and metabolomics measures the concentrations of endogenous metabolites (end products of cellular processes) or xenometabolites that represent enzymatic activity upon foreign substances such as environmental contaminants. Molecular approaches are clearly suitable as early warning systems and provide a powerful tool for high-throughput screening of substances/mixtures. Gene expression is also expected to be specific to the type of stress, and to respond quickly (hours to days), compared with tests based upon growth and reproduction that can last several weeks. Thus several regulatory authorities are considering how genomics tools could contribute to environmental pollution assessment (SCHER, SCENIHR, SCCS, 2013). According to Connon et al. (2012), these technologies have proven to be useful in elucidating modes of action of toxicants (a key point for the risk assessment of chemical mixtures, see Chapter 18). Omics have certainly an interest for ecotoxicology of mixtures because "the few ecotoxicogenomics studies that have considered mixtures suggest they may induce other genes than either of the constituent chemicals. On the gene expression level, a mixture appears like a new chemical" (SCHER, SCENIHR, SCCS, 2013). From 2002 to 2011, 41 studies were published with a focus on mixture toxicity assessment (for a review, see Altenburger et al., 2012).

Today, the relationship between molecular effects and responses at higher hierarchical levels (population, community) is largely unknown, despite several examples that suggest

the existence of mechanistic links between omics responses and effects at other levels of biological organization such as behavior, growth, predation risk, fitness, and mortality (Vandenbrouck et al., 2009; Connon et al., 2012; SCHER, SCENIHR, SCCS, 2013).

As underlined in a recent book devoted to ecotoxicology modeling (Devillers, 2009a), "the fate and effects of chemicals in the environment are governed by complex phenomena and modeling approaches have proved to be particularly suited not only to better understand these phenomena but also to simulate them in the frame of predictive hazard and risk assessment schemes." Modeling may be used in each field of the ecotoxicology triad: exposure, bioaccumulation, and effects (Chapters 11 and 12). For exposure, preference should be given to adequately measured, representative exposure data where these are available. For existing substances, monitoring programs often include only spatiotemporal spot check of environmental concentrations that have limited interest, whereas no measured environmental concentrations will normally be available for new substances as already mentioned for nanomaterials. Therefore, PECs must often be calculated (TGD, 2003). Measured data can then be used to revise the calculated concentrations. This exercise increased the confidence in the modeling of contaminant release into the environment as exemplified for radionuclides emitted by a nuclear reprocessing plant in northwest France since the model was modified using the long series of measurements that were available for some radionuclides (Nord-Cotentin Radioecology Group, 2000).

Bioaccumulation results from various interacting mechanisms that depend on the characteristics of the compounds and on biological factors. Various attempts have been made to model bioaccumulation in order to describe and possibly to predict the fate of organic contaminants in food webs (Abarnou, in Amiard-Triquet and Rainbow, 2009). From a practical standpoint, the Canadian Center for Environmental Modeling and Chemistry has launched a Bioaccumulation Fish Model software (http://www.trentu.ca/academic/aminss/envmodel/models/Fish.html) that requires input data concerning chemical properties of the organic contaminant under assessment and properties of the fish and its environment. The correlation between the effects of molecules and their physicochemical properties is at the basis of the Quantitative Structure–Activity Relationship (QSAR) discipline. The QSAR models represent key tools in the development of drugs as well as in the hazard assessment of chemicals. They are an alternative to in vivo animal testing and are recommended in a number of legislations/regulations (e.g., Registration, Evaluation, and Authorization of Chemicals). Their potential is well-documented for endocrine disruption modeling (Devillers, 2009b) or for grouping of mixture components based on structural similarities (SCCS, SCHER, SCENIHR, 2011).

We have already mentioned that the responsiveness of biomarkers to confounding factors, and their lack of ecological relevance have partly hampered their use. Modeling the influence of confounding factors (for details, see Chapter 11) and the use of population dynamics models provide a significant improvement for a sound interpretation of biomarker data (Chapters 11 and 12).

1.3 The Choice of Biological Models for Bioassays, Biomarkers, and Chemical Monitoring

The pros and cons of using different species to support the ecotoxicological methodologies in biota are reviewed in Chapter 9. Standardized biological test methods validated by official bodies (International Organization for Standardization, Organization for Economic Co-operation and Development, ASTM International, Environnement Canada, etc.) are often based on laboratory reared organisms such as microalgae, zooplankton (e.g., daphnids) or fish (e.g., *Danio rerio*). Standard bioassay organisms can be relevant considering issues of comparability and consistency when the relative toxicity of different compounds is to be determined. However, the loss of genetic variation resulting from maintaining populations in the laboratory must be taken into consideration (Athrey et al., 2007). It is also needed to be clearly aware of this potential change of genetic pattern when extrapolating from laboratory to natural populations. Using organisms from the wild is certainly attractive to improve the environmental realism of bioassays in the framework of prospective risk assessment. In this case, test organisms must be obtained from relatively uncontaminated field sites to avoid the risk of undervaluation because of the tolerance acquired by organisms chronically exposed to contaminants in their environment (Amiard-Triquet et al., 2011). The species used in bioassays should be determined using an appropriate taxonomic key. All organisms should be as uniform as possible in age and size class (ASTM, 2012) to avoid any influence of these potential confounding factors (Chapter 7).

Confounding factors must also be avoided in the case of biomarkers and chemical monitoring in biota in support of retrospective assessment. Wild organisms collected in the field are used for the so-called "passive" biomonitoring whereas "active" biomonitoring is based on caged organisms that may be obtained from aquaculture or natural populations from clean areas. A clear advantage of active biomonitoring is the possibility of selecting organisms with the same history, age, and size.

For each of the major ecotoxicological tools (bioassays, biomarkers, and chemical monitoring in biota), a multispecies approach is recommended. The calculation of PNECs using statistical extrapolation techniques are based on the species sensitivity distribution (Dowse et al., 2013) and may be used only when many NOECs, determined in different taxa (e.g., algae, invertebrates, fish, amphibians) are available (Chapter 2). In the ECOMAN project (Galloway et al., 2004, 2006), various biomarkers were determined in common coastal organisms showing different feeding types (filter-feeding, grazing, and predation) and habitat requirements (estuary and rocky shore). The authors highlighted how this holistic integrated approach is essential to identify the full impact of chemical contamination for ecosystem management. The variability of biological responses (either in terms of bioaccumulation or effects) between different taxa and different feeding habits is well documented but even

within more restricted groups, dramatic differences may be expressed as exemplified in the case of silver in filter-feeding bivalves (Berthet et al., 1992).

Chapters 10 to 13 focus on some reference species that have allowed important achievements in ecotoxicological studies of the aquatic environment such as endobenthic invertebrates in the sediment compartment, gammarid crustaceans in freshwater, copepod crustaceans in estuarine and marine waters, and fish in different water masses. Because they belong to vertebrates, ecotoxicology of fish can take advantage of the more advanced research of mammal toxicology. For one decade, the fathead minnow *Pimephales promelas* and the mummichog *Fundulus heteroclitus* have been recognized as relevant models (Ankley and Villeneuve, 2006; Burnett et al., 2007).

Primary producers have a crucial role in the functioning of aquatic ecosystems particularly for their role in nutrient biogeochemical cycles and as the first step in food webs. Eutrophication (Hudon, in Férard and Blaise, 2013) including "green tides" of macroalgae as well as algal blooms involving harmful phytoplankton (Watson and Molot, in Férard and Blaise, 2013) is a clear sign of trophic disequilibrium. Changes in communities of both macrophytes and microalgae may be used to assess the ecological status of aquatic environment. Microalgae and macrophytes may also be used in toxicity tests including a number of standardized bioassays (Hanson; Debenest et al., both in Férard and Blaise, 2013) and as matrices for the determination of biomarkers of photosynthesis (Eullaffroy, in Férard and Blaise, 2013). The range of applications for microalgae in ecotoxicology include their potential for toxicogenomic studies, their use in flow cytometry (Stauber and Adams, in Férard and Blaise, 2013) and the determination of various biomarkers (metal chelators, stress proteins, defenses against oxidative stress, xenobiotic detoxification systems, reviewed by Torres et al., 2008) or the use of diatoms as indicators of metal pollution (Morin et al., 2012). Recent studies on phytotoxicity of engineered nanomaterials have revealed the toxic potential of these emerging contaminants toward both higher plants and algae (Petit, in Férard and Blaise, 2013; Chapter 17, this book). Ecotoxicological research with respect to phytoremediation has also been reviewed recently (Dosnon-Olette and Eullafroy, in Férard and Blaise, 2013). Thus a specific chapter in the present book would be largely redundant with these recent papers.

Rotifers are also a group that is not reviewed in this book. The use of rotifers in ecotoxicology has been documented in 1995 by Snell and Janssen. This review has been quoted 185 times until now, an eloquent testimony of the interest of the scientific community. It has been updated recently by Dahms et al. (2011) and Rico-Martínez (in Férard and Blaise, 2013).

There is no distinct chapter on bivalves living in the water column despite—or because—mussels and oysters are the among the most commonly used species in fundamental and applied ecotoxicology. Their role in biomonitoring programs will be evoked in Chapter 5 and their contribution to the study of emerging contaminants in Chapter 16 dedicated to

pharmaceuticals and care products. Canesi et al. (2012) claim that bivalve mollusks, in particular *Mytilus* spp., represent a unique target group for nanoparticle toxicity. Matranga and Corsi (2012) underscore the existence of "Mytibase," an interactive catalog of 7112 transcripts of the mussel *M. galloprovincialis* that can help using the "omic" tools for marine organisms. Very recently, Binelli et al. (2015) have reviewed the ecotoxicological studies carried out with the zebra mussel *Dreissena polymorpha* to suggest this bivalve species as possible reference organism for inland waters.

In many contaminated environments where living beings have been historically exposed to high anthropogenic pressures, ecotoxicologists are not in the role of providing tools to prevent further aggravation of the already existing problems. In this case, it is needed to qualify the ecological status of water masses in agreement with the different legislation aiming at the conservation/improvement of environmental quality, including the conservation of biodiversity with reference to a nearly undisturbed situation (Chapter 14). The ecological status can be determined by using biological indicators (bioindicators) as surrogates to indicate the quality of the environment in which they are present. They have been designed either at the level of species or communities. Sentinel species may be considered as any species providing a warning of a dysfunction or an imbalance of the environment, or, more restrictively, a warning of the dangers of substances to human and environmental health. In addition to bioaccumulative species (Chapter 5) and those used for the determination of infraindividual and individual biomarkers (Chapter 7), the sentinel species can be bioindicator species, providing information by their absence (or presence) and/or the abundance of individuals in the environment under study (Berthet, in Amiard-Triquet et al., 2013). Among bioindicators at the community/assemblage level, there are five biological compartments retained in the ECWFD (2000): phytoplankton, macroalgae, angiosperms, macrozoobenthos, and fish. Additional groups may be recommended such as meiobenthic groups (e.g., foraminifera, copepods, nematodes) and zooplankton (Dauvin et al., 2010).

The assessment methods used to classify the ecological status of rivers, lakes, coastal, and transitional waters in Member States of the European Community according to the ECWFD are available at http://www.wiser.eu/results/method-database/. Tools and methodologies used in assessing ecological integrity in estuarine and coastal systems have been reviewed by Borja et al. (2008) considering the situation in North America, Africa, Asia, Australia, and Europe. Many of the biotic indices in current use may not be specific enough in terms of the different kinds of stress. However, the AZTI's Marine Biotic Index (software freely available at http://www.azti.es) despite being designed to assess the response of soft-bottom macrobenthic communities to the introduction of organic matter in ecosystems, has been validated in relation to other environmental impact sources (e.g., drilling cuts with ester-based mud, submarine outfalls, industrial and mining wastes, jetties, sewerage works) (Borja et al., 2003). Positive correlations were particularly indicated between AZTI's Marine Biotic Index and metals or PCBs (Borja et al., 2000). In certain cases, more specific indices may be used such

as the nematode/copepod ratio (Carman et al., 2000) and the polychaete/amphipod ratio to identify petroleum hydrocarbon exposure (Gómez Gesteira and Dauvin, 2000).

The "static" look at structural ecosystem properties must be complemented using an approach toward the ecosystem function and dynamics (Borja et al., 2008). Monitoring and assessment tools for the management of water resources are generally more effective if they are based on a clear understanding of the mechanisms that lead to the presence or absence of species groups in the environment (Usseglio-Polatera et al., 2000). The theory of traits (life history, ecological and biological traits) states that a species' characteristics might enable its persistence and development in given environmental conditions (Logez et al., 2013). Biological Traits Analysis (BTA) could reveal which environmental factors may be responsible for a given observed impairment, thus providing causal insight into the interaction between species and stressors (Culp et al., 2010; Van den Brink et al., 2011). This is well illustrated by a trait-based indicator system that was developed to identify species at risk of being affected by pesticides, with reference to life history and physiological traits (Liess et al., 2008). In an experiment with outdoor stream mesocosms, long-term community effects of the insecticide thiacloprid were detected at concentrations 1000 times below those detected by the principal response curve approach (Liess and Beketov, 2011). There is now a widespread conviction that biological traits should be used for environmental risk assessment (Artigas et al., 2012). However, the BTA is not always more powerful than the traditional taxonomic approach as observed by Alvesa et al. (2014), studying the subtidal nematode assemblages from a temperate estuary (Mondego estuary, Portugal), anyway providing useful knowledge of the functional structure and characterization of nematode communities in the estuary. Thus it is necessary to analyze carefully the strengths, weaknesses, opportunities and threats of using BTA (Van den Brink et al., 2011). Improved data analysis and the development of relevant traits are key for a sound ecological risk assessment (Bremner et al., 2006; for details, see Chapter 14).

1.4 Emerging Concern with Legacy Pollutants and Emerging Contaminants

Environmental contaminants may be assigned to two categories: legacy pollutants that have been present in the environment for decades and emerging chemicals that have only recently been detected and appreciated as possible environmental threats. Because effective analytical procedures have existed since the 1970s, the ecotoxicology of metals has been particularly well-developed (Luoma and Rainbow, 2008). Other legacy pollutants (PAHs, PCBs, dioxins and furans, and chlorinated pesticides such as DDT) became accessible to analysis more recently and because of the variety of environmental levels and biological effects among their different compounds/congeners/metabolites, analytical developments are still needed to improve their ecotoxicological assessment (Chapter 4). The ecotoxicological knowledge about PCBs, fire-retardants, cadmium, etc., was already important (Eisler, 2007) when more

recently their endocrine disrupting potential was discovered (Amiard et al., in Amiard-Triquet et al., 2013). To date, close to 800 chemicals are known or suspected to be endocrine disruptor compounds (EDCs), among which only a small fraction have been investigated with procedures that allows the identification of endocrine effects at the level of the whole organism (Bergman et al., 2013). Aquatic organisms are simultaneously exposed to many EDCs that can interact depending on their mode of action (estrogenic, antiestrogenic, androgenic, antiandrogenic and thyroid effects). It is impossible, for both technical and cost-effective reasons, to determine concentrations of all such compounds. Against this background, TIE or EDA-based strategies (Chapter 4) are particularly useful to characterize more accurately the environmental exposure to EDCs. Bioanalytical tools are developed using in vitro and in vivo models (OECD, 2012), including the generation of transgenic models such as tadpole fluorescent screens and fluorescent zebrafish transgenic embryos, for sexual and thyroid hormone disruptors (Brack et al., 2013). Low-dose effects, nonmonotonic dose responses, and the changes of biological susceptibility depending on life stage pose problems that cannot be solved by using classical strategies of risk assessment (Bergman et al., 2013). Chapter 15 will be dedicated to EDCs, a category of contaminants that are not defined as usually by their chemical characteristics (e.g., metals, PAHs, PCBs) but by the hazard associated to their presence in the aquatic environment. It will explore the more recent ecoepidemiological studies that try to explore the links between effects on the development, growth, and reproduction in individuals and the effects at the population and community levels.

Pharmaceuticals (Chapter 16) are submitted to precise regulations concerning their therapeutic value and potential secondary negative effects on human health but their environmental impact was not initially envisaged. Drugs are not totally assimilated in human organisms. Residues (urine) are released in the environment (with or without treatment in a waste water treatment plant) and numerous persistent molecules may be detected in natural waters. It should be noted that ecological footprints of active pharmaceuticals depend on risk factors that can differ substantially in low-, middle-, and high-income countries (Kookana et al., 2014).

Until recently, effects of pharmaceuticals on aquatic organisms were observed at very high doses "typically at least 1 order of magnitude higher than concentrations normally found in surface waters," suggesting that environmental doses were not deleterious (Corcoran et al., 2010) with the exception of antibiotic compounds in the environment and their potential for selection of resistant microbial strains. However, the lack of consideration given to the chronic nature of the exposures, the absence of knowledge on the significance of metabolites and transformation products resulting from the parent active pharmaceutical ingredients, or the potential for mixture effects were recognized (Corcoran et al., 2010; Kümmerer, 2010). More recent studies have demonstrated that at much lower doses and even realistic doses able to be found in the environment, deleterious effects may be observed. Pharmaceuticals can act as endocrine disruptors, the most potent being the synthetic estrogen 17-α-ethynylestradiol used in birth control pills (Kidd et al., 2007), but strong presumptions of effects in the field have

been recently published (Sanchez et al., 2011; Vieira Madureira et al., 2011). Also at low doses, behavioral effects were induced by antidepressant (fluoxetine), analgesic ibuprofen, and antiepileptic carbamazepine (De Lange et al., 2006; Gaworecki and Klaine, 2008; Painter et al., 2009; Di Poi et al., 2013). Recent research on the aquatic toxicity of human pharmaceuticals to aquatic organisms has been critically reviewed by Brausch et al. (2012) and the most critical questions to aid in development of future research programs on the topic has been extended to veterinary pharmaceuticals and personal care products (moisturizers, lipsticks, shampoos, hair colors, deodorants, and toothpastes) (Boxall et al., 2012). In 2010, the European Environment Agency held a specialized workshop that drew up proposals to reduce the environmental footprint of pharmaceuticals including (1) the eco-classification of all pharmaceuticals according to their environmental hazardousness and (2) the definition of environmental quality standards for pharmaceuticals; both of these approaches needing more data to describe the fate and long-term effects of pharmaceuticals in the aquatic environment (EEA, 2010).

Chapter 17 is dedicated to another category of emerging contaminants of environmental concern, the NMs. An NM is defined as any material that has unique or novel properties, because of the nanoscale (nanometer-scale) structuring. At this scale, physical and chemical properties of materials differ significantly from those at a larger scale. Nanomaterials can have one (e.g., nanosheet), two (e.g., nanotube), or three dimensions (e.g., nanoparticle) in the nanoscale. Engineered nanomaterials have multiple uses in nanometrology, electronics, optoelectronics, information and communication technology, bionanotechnology and nanomedicine (Royal Society, 2004). An inventory of nanotechnology-based consumer products introduced on the market (http://www.nanotechproject.org/cpi/) reveals that the number of products exploded from 54 in 2005 to 1628 in October 2013, the main product categories being in the field of health and fitness (personal care, clothing, cosmetics, sporting goods, filtration), home and garden, automotive, food, and beverages. The highest consumers were mainly in the United States (741 products), Europe (440), and East Asia (276). The major materials are silver, titanium, carbon, silicon/silica, zinc, and gold. The economic developments of nanotechnologies should not compromise the safety for human health and the environment. Products have already come to market, so first attention should be paid to postmarketing risks. Safety research should contribute to the sustainable development of nanotechnologies (Royal Society, 2004). The ecotoxicological history of nanomaterials parallels that of pharmaceuticals: nanotoxicity was first examined in tests carried out in the short term with very high doses, generally higher than mg/L^{-1} in water (Buffet, 2012; Gottschalk et al., 2013; Wong et al., 2013). In contrast, predicted concentrations of ENPs arising from use in consumer products are generally lower than the $\mu g/L^{-1}$ in water (Tiede et al., 2009; Sun et al., 2014) but with a severe degree of uncertainty (Gottschalk et al., 2011). However, recent studies carried out at much lower doses were able to reveal that all the nanoparticles tested were incorporated in the whole organisms but also enter the cells (Chapter 17) and even the nucleus (e.g., Joubert et al., 2013).

Procedures for improving the assessment of the risk of nanomaterials are reviewed/proposed in Chapter 17. They include a better characterization of exposure, not only by measuring environmental concentrations (Chapter 4) but also by considering the many physicochemical parameters that govern the fate and effects of NMs (Card and Magnuson, 2010; Chen et al., 2013). Bioaccumulation studies must evolve, taking into account not only the concentration in the whole organism/organ but the intracellular uptake and localization. Concerning both the evaluation of uptake and effects of NMs, different ways of incorporation must be explored (water, preys, sediment). Harmonization of test protocols can enable screening of different NMs and meaningful comparison between studies (Wong et al., 2013). However, innovating methods and techniques must be encouraged to improve the realism of test procedures.

PFCs are a large group of manufactured compounds that are widely used in everyday products (cookware, sofas and carpets, clothes and mattresses, food packaging, firefighting materials) and in a variety of industries (aerospace, automotive, building and construction, electronics) (NIEHS, 2012). Because of these widespread applications and their environmental persistence (OECD, 2002), PCFs are commonly detected in the environment and their presence in sediment and aquatic biota even in remote sites (Arctic biota) is well-documented. PFOA and perfluorooctane sulfonate (PFOS) are the PFCs that generally show the highest environmental concentrations (Wang et al., 2011 and literature cited therein). PFOS does not only bioconcentrate in fish tissues but it depurates slowly (50% clearance times of up to 116 days in the bluegill sunfish) (OECD, 2002). Using the limited information available, fish and fishery products seem to be one of the primary sources of human exposure to PFOS (USEPA, 2012b). PFOS and PFOA have a long half-life in humans (approximately 4 years), increasing the risk of adverse outcomes since different health effects are suspected in humans (USEPA, 2012b). Based on the assumption that consumption of fish by humans is the most critical route, Moermond et al. (2010) have proposed water quality standards in accordance with the ECWFD. The reader will not find a distinct chapter about PFCs in this book because it would be redundant with the recent reviews by Giesy et al. (2010) and Ding and Peijnenburg (2013).

Exposure of aquatic organisms to hazardous compounds is primarily through complex environmental mixtures, those that occur in water, sediment, and preys (Chapter 18). Interactions of chemical factors with physical and/or biological stressors in the environment are beyond the scope of this chapter (see the subsection on confounding factors in Chapter 7). Kortenkamp et al. (2009) have distinguished four categories of mixtures: (1) substances that are mixtures themselves (e.g., metallic alloys); (2) products that contain more than one chemical (e.g., cosmetics, biocidal products); (3) chemicals jointly emitted at any step of their lifecycle; and (4) mixtures of several chemicals emitted from various sources, via multiple pathways that might occur together in environmental media. Guidance for conducting cumulative risk assessments has been published by regulatory bodies in United States, United Kingdom, Norway, and Germany but except for the two first categories, risk assessments in the European Union deal mainly with individual substances (SCCS, SCHER, SCENIHR, 2011).

The main effort must be directed toward ecosystems where significant exposure is likely or at least plausible. Individual components in a mixture have specific and different physicochemical properties that govern their fate (and consequently effects) in the environment. Theoretically, it would be possible to identify each individual component of a mixture and then to determine a PEC for each of them but, in practice, this approach requires an unrealistic and extremely expensive analytical investment. Recently, strategies based upon the similarity of physicochemical properties (e.g., log K_{ow}, water solubility), and environmental-degradation potentials (e.g., photodegradation and hydrolysis rates), have been proposed for the identification of "blocks" of components that may be considered together with the help of QSARs (SCCS, SCHER, SCENIHR, 2011). However, to date, it seems much more difficult to take into account biological degradation that is a key process for the elimination of chemicals in the aquatic environment.

Mixture studies have been mainly conducted (1) to evaluate and quantify the overall toxicity of complex environmental samples (whole mixture approach) or (2) to reveal the joint action of individual molecules (component-based approach) (Kortenkamp et al., 2009). Recent studies try to fulfill the gap between these two approaches with promising results expected from the use of TIE and EDA (ECETOC, 2011; EC STAR, 2012).

Regulatory risk assessment of chemical mixtures needs at the minimum a sound knowledge of the different modes of action (MoA) of individual contaminants. It is generally admitted that the effects of a mixture composed of individual molecules with similar MoA can be estimated by summing the doses/concentrations, scaled for relative toxicity to take into account the different potency of each substance. This has been illustrated in the case of EDCs by Jin et al. (2012), who have examined the biological traits of the fish *Gobiocypris rarus* submitted to a coexposure to three estrogenic compounds (17β-estradiol, diethylstilbestrol, and nonylphenol) and Pottinger et al. (2013) in the case of a coexposure to four antiandrogenic compounds of the fish *Gasterosteus aculeatus*.

In the case of a mixture composed of molecules with dissimilar MoA, it may be proposed to assess the effects using models of response addition (based on the probability of responses to the individual components) or effect addition (by summing of biological responses) (Chapter 18). Consequently, it is expected that mixtures composed of dissimilarly acting chemicals at levels below NOECs will not induce significant effects. However, at such low doses, the interpretation of data is tricky and controversial, as illustrated by the conclusions derived by different groups of experts (Kortenkamp et al., 2009; SCCS, SCHER, SCENIHR, 2011) from a study with fish (Hermens et al., 1985), two studies with algae (Walter et al., 2002; Faust et al., 2003), and one study using an in vitro cellular test (Payne et al., 2001). At higher doses, interactions either synergistic (supra-additive) or antagonistic (infra-additive) are more easy to identify. They include toxicokinetic, metabolic, and toxicodynamic interactions. Toxicokinetic interactions occur when a contaminant modifies the absorption of others (e.g., Tan et al., 2012; Su et al., 2013). Toxicodynamic

interactions occur when the different constituents of a mixture have a similar target, a situation encountered in the case of ligand–receptor interactions and well-documented for EDCs (Kortenkamp et al., 2009). Synergistic interactions have been documented for pesticides considering both biocidal products (Bjergager et al., 2011; Backhaus et al., 2013) and mixtures commonly detected in aquatic habitats (Laetz et al., 2009). The overall toxicity of a pharmaceutical mixture is in general substantially higher than the toxicity of each individual substance at its concentration present in the mixture (EEA, 2010). Antagonistic effects are reported in response to co-exposure to EDCs with known MoA, namely estrogens and antiestrogens (Sun et al., 2011; Wu et al., 2014). In such cases, the models are of no help and direct experimentation remains the only available tool (Chapter 18).

The aim of this book is to use cross-analyses of procedures, models, and contaminants to design ecotoxicological tools suitable for better environmental assessments, particularly in the case of emerging contaminants and emerging concern with legacy pollutants.

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CHAPTER 2

Conventional Risk Assessment of Environmental Contaminants

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Abstract

Conventional risk is assessed by using a four-tier approach including hazard identification, assessment of exposure (predicted environmental concentrations), hazard characterization (predicted no effect concentrations) and risk characterization based upon the risk quotient (predicted environmental concentration/predicted no effect concentration for water, sediment, and biota). This core procedure is used worldwide with some differences in the details and is described here using the European Union recommendations. Ecotoxicity databases have been compiled in many countries. The current procedures suffer many limitations, mainly because of the dominant use of standardized bioassays involving single species and unique substances, generally neglecting the dietary route of exposure and the effects of mixtures. The most important weaknesses include large uncertainties in extrapolating data across doses, species, and life stages, poor assessment of contaminants with nonmonotonic dose–response relationship, the lack of data (e.g., environmental degradation) for emerging contaminants, and the noninclusion of adaptation in polluted environments.

Keywords: Dose–effect relationship; Emission assessment; Environmental fate; Hazard identification; PECs; PNECs; Risk quotient approach; SSDs.

Chapter Outline

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Introduction

Environmental influences on health, particularly the role of water quality, have been recognized as anciently as in the treatise called "Airs, Waters, Places" by the Greek physician Hippocrates in the second half of the fifth century BC. However, environmental concern has developed more recently, particularly with the use of synthetic organic chemicals after the Second World War. "Silent Spring" by Rachel Carson (1962) has had a key role for environmental science and the society, documenting the detrimental effects on the environment—particularly on birds—of the unreasonable use of pesticides. 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" (published in Truhaut, 1977). The development of ecotoxicology has allowed the implementation of retrospective risk assessment, considering the effects of the dispersion of chemical compounds into the environment and possibly mitigating them, as also prospective risk assessment that aims at assessing the future risks from releases of new and existing chemicals into the environment.

Environmental risk assessment aims at the protection of ecosystems, considering their structure, functioning, and services. Predictive risk assessment aims at assessing the future risks from releases of chemicals into the environment. In the United States, the federal Toxic Substances Control Act gives the US Environmental Protection Agency (USEPA) the authority to regulate, and even ban, the manufacture, use, and distribution of both new and existing chemicals (Schierow, 2009). In Europe, a significant improvement occurred recently with a new chemical policy, REACH, for Registration, Evaluation, and Authorization of Chemicals (CEC, 2003). Procedures needed to reach this aim have been adopted in many countries (USEPA, 1998; ANZECC and ARMCANZ, 2000; CCME, 2007; NITE, 2010; Gormley et al., 2011) and supranational organizations such as the Organisation for Economic Co-operation and Development (OECD, 2012b) and European Environment Agency (EEA, 1998; TGD, 2003). These procedures are regularly updated using scientific enhancements. A systematic literature review conducted in the Elsevier database (ScienceDirect) using the terms "environmental risk assessment" AND "aquatic" have shown that about 200 papers were published from 2010 until May 13, 2014, including several reviews regrouped in the third edition of the *Encyclopedia of Toxicology*. The Society of Environmental Toxicity and Chemistry is also very active in this field, as shown by the workshop "Closing the gap between academic research and regulatory risk assessment of chemicals" held in 2013 in Glasgow, Scotland (http://www.setac.org/members/group_content_view.asp?group=90708&id=189652&hhSearchTerms=%22Environmental+and+risk+and+assessment%22, accessed 13.05.14). The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) is an independent association that cooperates in a scientific context with intergovernmental agencies, governments, health authorities, and other public and professional institutions with interests in

ecotoxicological and toxicological issues relating to chemicals. ECETOC's Targeted Risk Assessment tool calculates the risk of exposure from chemicals to workers, consumers, and the environment (http://www.ecetoc.org/research?q=Targeted%20Risk%20Assessment%20%28TRA%29%20tool).

Despite different guidelines having been launched in many national and supranational regulatory bodies, the procedures follow the same general scheme.

2.1 Principles for Environmental Risk Assessment

The meaning of the words *hazard* and *risk* is not totally clear for everybody, even in dictionaries. For example, one dictionary defines hazard as "a danger or risk," which helps explain why many people use the terms interchangeably. Among specialists of (eco)toxicology, hazard is defined as any source of potential damage, harm, or adverse health effects on something or someone under certain environmental conditions. However, it is clear that damage can occur only if organisms are exposed to hazard. Risk is the chance or probability that an organism will be harmed or experience an adverse health effect if exposed to a hazard.

The procedures currently in use for conventional risk assessment are depicted in Figure 2.1. The first step consists in the identification of hazard based on physicochemical properties, ecotoxicity, and intended use (EEA, 1998). Criteria for the selection of priority substances include their degree of persistency, toxicity, and bioaccumulation (for details, see

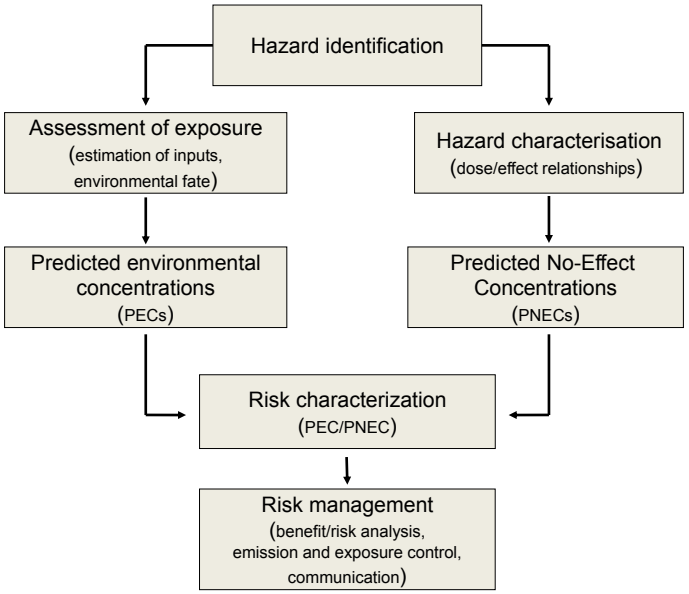

Figure 2.1
Principles for environmental risk assessment.

http://www.miljostatus.no/en/Topics/Hazardous-chemicals/Hazardous-chemical-lists/List-of-Priority-Substances/Criteria-for-the-selection-of-Priority-Substances/, accessed 13.05.14). In Europe, 45 substances or groups of substances are on the list of priority substances for which environmental quality standards were set in 2008 (amended in 2013), including selected existing chemicals, plant protection products, biocides, metals, and other groups such as polyaromatic hydrocarbons and polybrominated biphenyl ethers (PBDEs). The complete list is available at http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32013L0039 (accessed 4.12.14). Two lists have special significance to water quality regulatory programs in the US Clean Water Act: a list of 65 toxic pollutants and a list of 129 priority pollutants (http://water.epa.gov/scitech/methods/cwa/pollutants-background.cfm#pp, accessed 13.05.14).

When a hazard has been identified, there is the need to assess the fate and effects of pollutants. Studying the fate and the biogeochemical cycle of pollutants in ecosystems is crucial in determining the environmental risk assessment because knowledge is needed on the release of pollutants in water, air, and sediment/soil because many exchanges occur between them (Figure 2.2). It is also important to take into account the trophic transfer of pollutants from sediment (suspended in the water column or deposited) and microorganisms to invertebrates (filter-feeders or deposit feeders) then predatory fish (omnivorous, carnivorous, supercarnivorous). When these data are available, they can be used in models for predicted environmental concentrations (PECs). Modeling is particularly useful for certain emerging contaminants such as nanoparticles that cannot be directly measured in environmental matrices (Chapter 17).

When a substance has been recognized as hazardous, there is the need to carry out hazard characterization (Figure 2.1). This step mainly aims at determining the relationship between the concentration of a given pollutant in a medium and the noxious effects that this substance can induce in organisms. The main parameters that may be determined from experimental

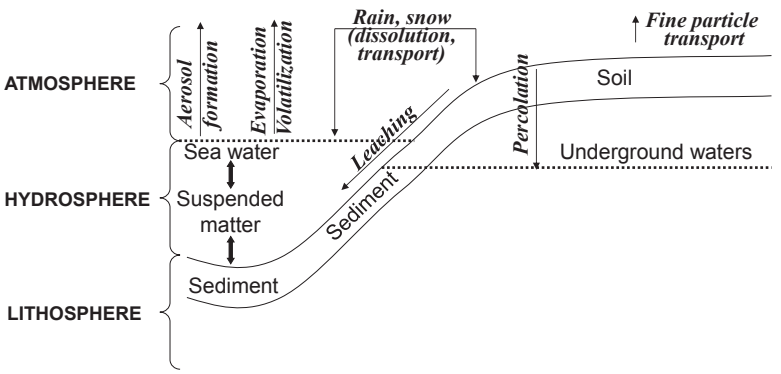

Figure 2.2
Contaminant dispersion in the physical environment.

tests include the no observed adverse effect level (NOAEL) corresponding to the no observed effect concentration (NOEC); the lowest observed adverse effect level corresponding to the lowest observed effect concentration (LOEC); and the median effect concentration (EC_{50}) producing a deleterious effect in 50% of the experimental population. When NOEC is available for a sufficient number of species belonging to different taxa/trophic level, modeling allows the determination of a predicted no effect concentration (PNEC) for the studied contaminant (Figure 2.1). Risk characterization is based on the risk quotient approach using the ratio PEC/PNEC. Very simplistically, if the PEC/PNEC ratio is greater than 1, the substance is considered to be of concern and risk reduction measures must be envisaged.

Environmental risk assessment has three main functions: (1) it allows the identification of environmental compartments and organisms at risk resulting from the use of a given substance; (2) it is the basis of risk management decisions (reduction of environmental inputs); and (3) it leads to restrictions or bans of certain substances including both new and existing chemicals.

2.2 Exposure: Determination of Predicted Environmental Concentrations

Chemical monitoring is labor-intensive and chemical analyses are expensive. In addition, until recently and until the use of passive samplers, traditional spot sampling procedures only reflected a short-lived situation. It is a serious problem because at a given site, water quality fluctuates greatly. Low concentrations of micropollutants are difficult to detect and the risk of secondary contamination when handling the samples is important. Thus, the available measured environmental concentrations have to be validated, taking into account the quality of the applied measuring techniques (OECD, 2000). Then representative data for the environmental compartment of concern will be selected. Another possibility is modeling. On the other hand, no measured environmental concentrations will normally be available for new substances (e.g., nanoparticles; see Chapter 17). Therefore, concentrations of these substances in the environment must be estimated.

2.2.1 Emission Assessment

Modeling PECs can use the measured values of a substance under examination in real releases. When such values are not available, the release rate of each given substance will be estimated based upon its use pattern. Emission scenario documents have been published under the auspices of the European Chemicals Bureau (TGD, 2003; part 4) for 12 categories of anthropogenic activities (chemical industry, metal extraction industry, refining and processing industry, biocides used in various applications, personal, domestic, and public domains, etc.) processing and producing chemicals able to enter the environment. The release estimation is based on the emission factors at different steps of the industrial process (production, formulation, processing, etc.), the production volume per time unit, the elimination in on-site treatment facilities (industrial activities), and the elimination in wastewater treatment

facilities (domestic and public domains). All the releases and the receiving environmental compartment(s)—air, soil/sediment, water—must be identified.

When detailed information on the use patterns, release into the environment, and elimination are missing, generic exposure scenarios are applied. The basic assumption is that substances are emitted into a model environment characterized by environmental parameters that can be average values or reasonable (excluding accidental situations) worst-case values. When more specific data on the emission of a substance may be obtained, the generic assessment may be improved for a more realistic result, including, for instance, topographical and climatological variability.

Local emissions ($Elocal_{i,j}$ in $kg \cdot d^{-1}$) can be calculated for each life-cycle stage i of the life-cycle and each compartment j according to the following formula:

$$Elocal_{i,j} = Fmainsource_i * 1000/Temission_i * RELEASE_{i,j}$$

with

$Fmainsource_i$ = fraction of release at the local main source at stage i,
$Temission_i$ = number of days per year for the emission in stage i ($d \cdot yr^{-1}$),
$RELEASE_{i,j}$ = release during stage i to compartment j ($t \cdot yr^{-1}$).

For the regional scale assessments, the emissions are assumed to be a constant and continuous flux during the year, thus the $Eregional_j$ is the total emission to compartment j (annual average) in $kg \cdot d^{-1}$. Regional emissions can be calculated by summing the release fractions for each stage of the life-cycle according to the following formula:

$$Eregional_j = 1000/365 * \sum_{i=1\ to\ n} * RELEASE_{i,j}$$

with $RELEASE_{i,j}$ = release during life-cycle stage i to compartment j ($t \cdot yr^{-1}$).

2.2.2 Behavior and Fate in the Environment

The fate of a given substance once released into the environment needs to be estimated by considering exchanges between physical compartments of the environment (Figure 2.2) and biotic and abiotic transformation processes. The quantification of distribution and degradation of the substance (as a function of time and space) leads to an estimate of PEClocal and PECregional. The PEC calculation is described for water (ground- and freshwaters, transitional and marine waters), soil and sediment, and air (TGD, 2003; part 2). Transport of the substance between the compartments must be taken into account to determine the full biogeochemical cycle of substances.

2.2.2.1 Abiotic and biotic degradation

In each physical compartment, a given substance is submitted to physicochemical and biological processes leading to its degradation. This includes:

- hydrolysis and oxidation in water;
- photolysis in surface water and in the atmosphere;

- microbial degradation in surface water, soil and sediment (as also in sewage treatment plants); and
- metabolization in macroorganisms.

The study of degradation does not need to be conducted if the substance is inorganic. For organic substances, test guidelines that may be used to conclude on ready biodegradability for organic substances have been recently reviewed (Kapanen et al., 2013) under the auspices of the European Chemicals Agency. Substance properties influence the applicability of specific test guidelines. Information on physicochemical properties enables the identification of the most appropriate test guideline. In addition, marine screening tests are available (OECD 306 "Biodegradability in Seawater").

According to the US Geological Survey (http://toxics.usgs.gov/definitions/biodegradation.html), biodegradation may be characterized for the purpose of hazard assessment as:

- primary, corresponding to the alteration of the chemical structure of a substance resulting in loss of a specific property of that substance;
- environmentally acceptable, meaning that biodegradation occurs to such an extent that undesirable properties of the compound are removed;
- ultimate, corresponding to the complete breakdown of a compound to either fully oxidized or reduced simple molecules (such as carbon dioxide/methane, nitrate/ammonium, and water). However, in some cases, the products of biodegradation can be more harmful than the substance degraded.

Degradation can occur under both aerobic and anaerobic conditions. A substance may be considered as easily degradable provided that the degradation rate is >60% over 28 days. In addition to the degradation rate, other useful parameters are the half-life (denoted DT_{50}, which is the time required for the disappearance of 50% of the applied substance) and the specific degradation rate constant k. By definition, the specific degradation rate constant is equal to the relative change in concentration per time:

$$k = (1/C) \cdot (dC/dt)$$

First-order kinetics implies that the rate of degradation ($mg \cdot L^{-1} \cdot day^{-1}$) is proportional to the concentration of substrate, which declines over time. With true first-order kinetics, the specific degradation rate constant, k, is independent of time and concentration. First-order kinetics are normally expected under the conditions prescribed for standardized tests (e.g., OECD 309 "Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test"). However, deviations from first-order kinetics may be observed, for instance, if the diffusion rate, rather than the biological reaction rate, limits the biotransformation rate.

2.2.2.2 Distribution

Generally, hydrophilic compounds are mainly present in water, whereas hydrophobic compounds are present in air, soil/sediment, and biota. Multimedia models have been developed

to examine the multimedia environmental fate of organic chemicals that are discharged to the environment. One of the models currently in use in the European regulatory framework (TGD, 2003; part 2) employs the fugacity concept and treats four bulk compartments: air, water, soil, and bottom sediment, which consist of subcompartments of varying proportions of air, water, and mineral and organic matter (Mackay and Peterson, 1991). These authors assume that equilibrium (equifugacity) applies within each compartment (i.e., between subcompartments), but not between compartments. Within the same compartment, partition coefficients may be useful to examine the distribution of chemicals.

The transfer of a substance from the water to the air subcompartment (e.g., volatilization from surface water, Figure 2.2) may be assessed by using its Henry's law constant. The air–water partitioning coefficient ($K_{air\text{-}water}$) can be estimated according to the following equation:

$$K_{air-water} = H/(R * TEMP)$$

with

H = Henry's law constant ($Pa \cdot m^3 \cdot mol^{-1}$),
R = gas constant ($Pa \cdot m^3 \cdot mol^{-1} \cdot k^{-1}$),
TEMP = temperature at the air–water interface (K).

Partition coefficients solid-water in suspended matter (Kp_{susp}), in sediment (Kp_{sed}), and soil (Kp_{soil}) expressed in ($L \cdot kg^{-1}$) are calculated according to the same approach (TGD, 2003; part 2).

$$Kp_{comp} = Foc_{comp} \cdot K_{oc} \text{ with comp} \in \{soil, sed, susp\}$$

with

K_{oc} = partition coefficient organic carbon-water ($L \cdot kg^{-1}$),
Foc_{comp} = weight fraction of organic carbon in compartment *comp* ($kg \cdot kg^{-1}$).

K_{oc} may be measured by adsorption studies (EC C18; OECD 106, 2000) or by the high-performance liquid chromatography method (EC C19; OECD 121, 2001). Kp may be expressed as the concentration of the substance sorbed to solids (in $mg_{chem} \cdot kg_{solid}^{-1}$) divided by the concentration dissolved in porewater ($mg_{chem} \cdot L_{water}^{-1}$). This formulation is similar to the distribution coefficient Kd used for inorganic substances (metals, metalloids, radionuclides) (OECD 106 "Adsorption - Desorption Using a Batch Equilibrium Method").

$$Kd = Cs(eq)/Caq(eq)$$

with

Cs(eq) = concentration of the inorganic substance adsorbed onto the solid phase ($mg \cdot kg^{-1}$) at equilibrium,
Caq(eq) = concentration of the inorganic substance dissolved in the aqueous phase ($mg \cdot L^{-1}$) at equilibrium.

2.2.2.3 Predicted Environmental Concentrations

Emissions can be assessed on a local scale (PEClocal) when only one source of emission is recognized; for instance, a sewage treatment plant (for a detailed example, see TGD, 2003; part 2). However, point source releases can also contribute to the environmental concentrations on a larger scale thus leading to the assessment of a PECregional. The concentrations of substances released from diffuse sources over a wider area are assessed on a regional scale (PECregional). Concerning the aquatic environment, procedures for the calculation of PEClocal and PECregional are described for surface waters, marine waters, and sediments. In addition, the PECoral is calculated to assess bioaccumulation and secondary poisoning of predators.

2.2.2.3.1 Calculation of PECs for the aquatic compartment

Figure 2.3 shows the most important fate processes in the aquatic compartment. For local PEC, the procedure recommended in the TGD (2003, part 2) is based on the assumption of complete mixing of the effluent in surface water whereas volatilization, degradation, and sedimentation are ignored because of the short distance between the point of effluent discharge and the exposure location. The calculation of the PEClocal for the aquatic compartment includes the calculation of the discharge concentration to a given water body, dilution effects, and removal from the aqueous medium by adsorption to suspended matter.

For PECregional, it is also important to take into account the general movement of the contaminated plume and the long-range transport of suspended particles by the river flow or drift and marine currents (Figure 2.3). In this case, volatilization, degradation, and sedimentation must be taken into account, should all the different processes of exchange exist between compartments (Figure 2.2). For regional computations, the TGD (2003, part two) recommend the use of multimedia fate models described by Mackay et al. (1992), Van de Meent (1993),

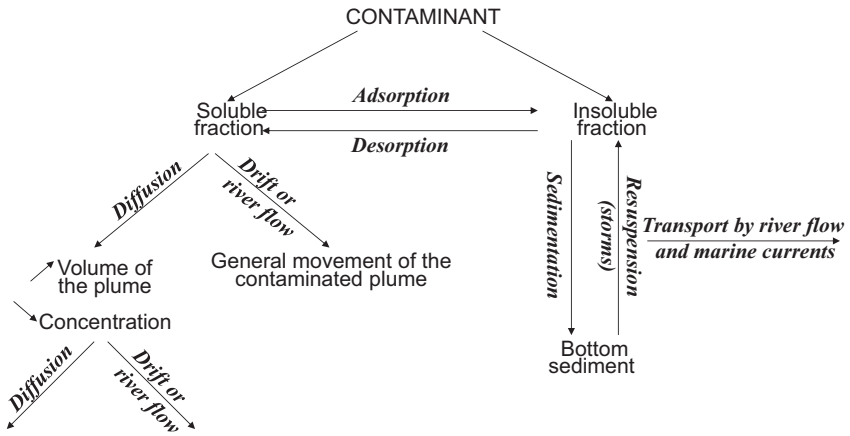

Figure 2.3
Circulation of contaminants in water masses.

and Brandes et al. (1996). In these models, each compartment (air, water, soil, and bottom sediment) is considered homogeneous and well mixed.

2.2.2.3.2 Calculation of PECs for sediment

The concentration in freshly deposited sediment is taken as the PEClocal for sediment and according to (Di Toro et al., 1991) may be determined by using the equation:

$$PEClocal_{sed} = K_{susp-water}/RHO_{susp} * PEClocal_{water} * 1000$$

with

$PEClocal_{water}$ concentration in surface water during emission episode ($mg \cdot L^{-1}$),
$K_{susp\text{-}water}$ suspended matter–water partitioning coefficient ($m^3 \cdot m^{-3}$),
RHO_{susp} bulk density of suspended matter ($kg \cdot m^{-3}$),
$PEClocal_{sed}$ predicted environmental concentration in sediment ($mg \cdot kg^{-1}$).

In this equation, the $PEClocal_{sed}$ is derived from the corresponding water body concentration, assuming a thermodynamic partitioning equilibrium. This model has certain limits underlined in the TGD (2003, part 2), such as the frequent lack of equilibrium distribution between water and suspended matter is also an underestimation of sediment concentration when release to the surface water predominately occurs as particles.

The multimedia fate model will be used again to calculate PECregional for sediment. The scenario used for freshwater PEC calculations must be modified to allow dispersive exchange between the coastal zone to the continental seawater (Figure 2.3). The results from regional models should be interpreted with caution because PECs (water, sediment) are averaged for all the regional compartments and are considered homogeneous and well mixed. However, there is a considerable uncertainty in the determination of input parameters (e.g., degradation rates, partitioning coefficients) and much higher concentrations can be encountered locally (TGD, 2003; part 2).

2.2.2.3.3 Calculation of PECs for biota

Contaminant concentrations in fish and their predators is a result of uptake from the aqueous phase and intake of contaminated food (prey species, sediment). Direct uptake from the water phase is predominant for hydrophilic substances (octanol–water partition coefficient $\log K_{ow} < 4.5$), whereas intake from food becomes increasingly important for lipophilic substances ($\log K_{ow} \geq 4.5$). The calculation of PECoral uses the bioconcentration factor (BCF) and the biomagnification factor (BMF). The TGD (2003, part 2) provides equations allowing the calculation of a given substance's BCF from the value of the K_{ow} for this substance. However, experimentally determined BCF values are often preferable and standardized procedures have been recently updated (OECD 305 "Bioaccumulation in Fish: Aqueous and Dietary Exposure"). The BMF is defined (TGD, 2003; part 2) as the relative concentration in

a predatory animal compared to the concentration in its prey (BMF = Cpredator/Cprey). It is recommended to use lipid normalized Cpredator and Cprey.

$$PECoral_{predator} = PEC_{water} * BCF_{fish} * BMF$$

with

$PECoral_{predator}$ = PEC in food ($mg \cdot kg^{-1}_{wet\ fish}$),
PEC_{water} = PEC in water ($mg \cdot L^{-1}$),
BCF_{fish} = bioconcentration factor for fish on wet weight basis ($L \cdot kg^{-1}_{wet\ fish}$),
BMF = biomagnification factor in fish (–).

When carrying risk assessment, it is important to validate the models used for the calculation of PECs considering measured data from monitoring programs and from high-quality literature.

2.3 Ecotoxicity: Determination of Predicted No Effect Concentrations

Once the hazardous effects of concern are identified, it is then needed to assess the relationship between dose (concentration) and response (effect) in different species. When results are available for different trophic levels and taxa, it is then possible to determine PNECs (Figure 2.1).

2.3.1 Hazard Characterization

Hazard characterization is mainly based on toxicity data obtained by using experimental toxicity tests. In both the European REACH and US Toxic Substances Control Act regulations, the level of ecotoxicity assessment is linked to the tonnage of new or existing substances produced by chemical industries. Standard information requirements for aquatic toxicity data under REACH includes short-term toxicity testing on invertebrates (preferred species *Daphnia*) and growth inhibition of aquatic plants (algae preferred) for a tonnage of 1–10 tons $year^{-1}$. Short-term toxicity testing on fish is needed for a tonnage of 10–100 tons $year^{-1}$. In addition, long-term toxicity testing on invertebrates (preferred species *Daphnia*) and fish are needed for tonnages >100 tons $year^{-1}$ (Tarazona et al., 2014).

Many standardized bioassays have been published by many regulatory bodies (OECD, International Organization for Standardization, ASTM, USEPA) for sediment and water toxicity testing (listed in Cesnaitis et al., 2014; Tarazona et al., 2014). The most commonly used are single-test species, carried out in dramatically simplified laboratory "ecosystems," most often without food and substratum. Tests can be carried out under static, semistatic, or flow-through conditions (EC, 2005). Static describes aquatic toxicity tests in which test solutions are not renewed during the test. Flow-through described tests in which solutions in test vessels are renewed continuously by the constant inflow of a fresh solution or by a frequent intermittent inflow. Semistatic describes aquatic tests in which test solutions are

replaced periodically during the test. Depending on the strategy adopted, the level of exposure integrated over the whole duration of the test may be highly variable. However, many reports provide only the nominal concentration at the beginning of the test because monitoring the real concentrations in experimental units is costly and labor-intensive.

In the oldest acute tests, the observed endpoint was most often lethality, whereas more recently, growth, reproduction parameters, and even behavior are chosen as endpoints in long-term toxicity tests (for examples, see Cesnaitis et al., 2014; Tarazona et al., 2014). Short-term toxicity tests allow the determination of EC_{50}s when the endpoint is a sublethal effect or median lethal concentrations (LC_{50}) when the endpoint is lethality. Long-term toxicity tests are relevant for the determination of NOECs and LOECs. The determination of these parameters is shown in Figure 2.4. In this example, six doses have been tested. No difference was observed between the response of controls and the response of specimens exposed to D1, whereas a significant change was observed at D2. Thus D1 corresponds to the observed NOEC and D2 to the observed LOEC. In fact the true values of NOEC and LOEC termed "biological" are between D1 and D2. The major disadvantage of this procedure is that it assimilates the NOEC and LOEC to the corresponding experimental doses. Thus the results are deeply influenced by the dose spacing and also by the number of tested organisms at each dose (influencing the statistical significance). Less "rustic" derivations of LC_{50}/EC_{50} and NOEC values from raw values (probit analysis, analysis of variance, and post hoc statistical tests) are given in the TGD (2003, part 2).

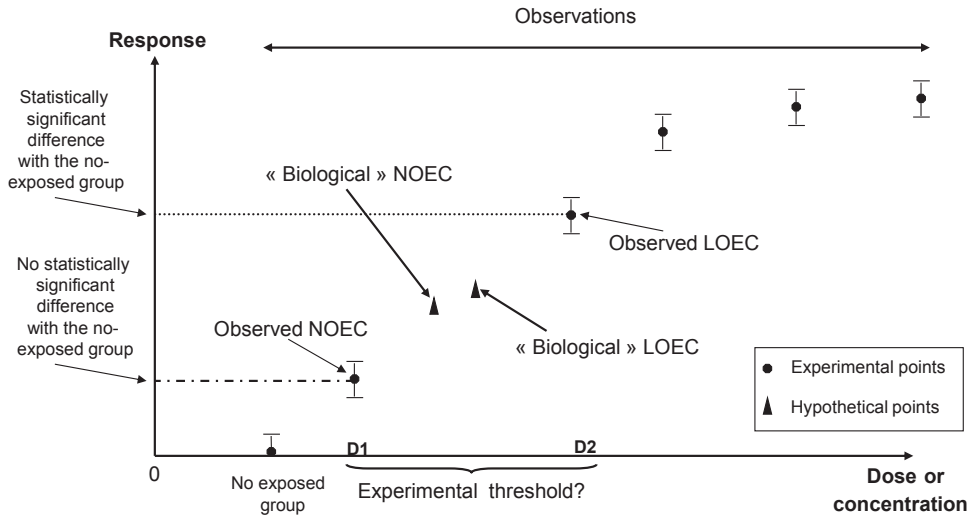

Figure 2.4
Experimental determination of the dose–response relationship: biological versus observed NOEC and LOEC.

However, it remains necessary to improve the determination of the NOECs that will be used at the next step (Figure 2.1) for the calculation of PNECs. Thus, another approach has been proposed by both European and US agencies (EFSA, 2009; USEPA, 2009) (Figure 2.5). First, a software freely available online allows the modeling of the dose–response curve (USEPA software) by choosing the model that fits well with the experimental points. Then, it is needed to choose a level of effects (benchmark response)—e.g., BMR_{10} corresponding to the benchmark dose (BMD_{10}) that induces a response in 10% of experimental specimens. Last, it is possible to choose the limit of the confidence interval (90 or 95%). The lower benchmark dose (BMDL) is the lower limit of the confidence interval at 90 or 95% of the BMD.

2.3.2 Calculation of PNECs

It is very important to evaluate available data with regard to their completeness and their reliability and relevance for environmental hazard and risk assessment (adequacy). The TGD (2003, part 2) puts forward general guidelines on the evaluation of ecotoxicity data. Two main points must be examined: the procedures used to carry out the study and the way the results have been interpreted. Greater weight should normally be attached to studies carried out according to standardized bioassays, conducted following good laboratory practices (GLP; European Directive 2004/10/EC; OECD, 1998; CFR, 2011a,b). The adequacy of a bioassay also lies in the way that the performance and results are described (critical pieces of information are missing; the design of the test is insufficiently detailed, etc.).

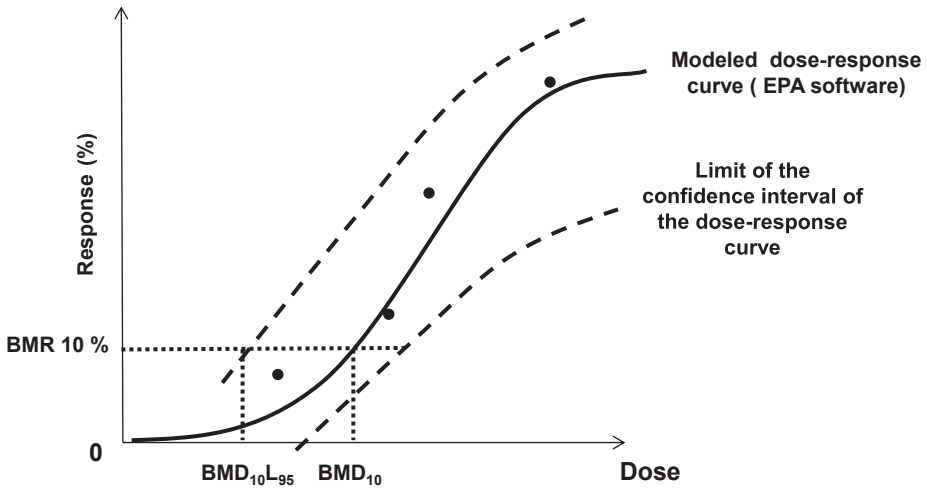

Figure 2.5
Assessment of the different parameters of the benchmark dose (BMD). BMR_{10}, 10% of experimental specimens are affected; $BMD_{10}L_{95}$, lower limit of the confidence interval at 95% of the BMD.

Determination of the PNECs takes into account the number of bioassay results available for different species representative of different trophic levels and the availability of long-term toxicity data. PNECs in current use for risk assessment in the aquatic environment are $PNEC_{aquatic}$ (fresh- and seawater), $PNEC_{sediments}$, and PNECoral (because of secondary poisoning).

2.3.2.1 Calculation of $PNEC_{aquatic}$

PNEC calculations are carried out using either assessment factors or statistical extrapolation techniques.

The use of assessment factors is needed to take into account the uncertainties resulting of (1) intra- and interlaboratory variation of toxicity data, (2) intra- and interspecies variations (biological variance), (3) short-term to long-term toxicity extrapolation, and (4) laboratory data to impact field extrapolation. The uncertainty decreases with larger and more relevant datasets, thus allowing the use of lower assessment factors (AFs). The AFs applicable in the framework of the European regulations are shown in Table 2.1. Seawater AFs are consistently higher than freshwater AF because fewer marine data are available. It also remains questionable if marine species are more sensitive than freshwater species. A recent literature review (Klok et al., 2012) based on 3627 references concludes that there is no systematic difference in sensitivity to pesticides between fresh- and saltwater species.

The calculation of $PNEC_{aquatic}$ using statistical extrapolation techniques is based upon the species sensitivity distribution (SSD). SSDs are cumulative distributions of measures of species sensitivity to a stressor or toxicant. In the case of triclosan depicted in Figure 2.6, the normal model provided the best fit of the models tested among the possible range of distributions (European Commission, 2011; Health Canada, Environment Canada, 2012). SSDs are used to estimate concentrations that will protect a certain percentage of a community. It is frequently accepted that 5% of sensitive species may be neglected. For instance, in the case depicted in Figure 2.6, the provisional hazardous concentration 5% (HC_5) is 115 $ng \cdot L^{-1}$. Only a substantial amount of toxicity data from several taxonomic groups can result in a robust HC_5. In Europe, to meet the requirements of the REACH guidance, the database must contain preferably more than 15, but at least 10 NOECs/EC_{10}s (effect concentration producing a deleterious effect in 10% of the experimental population), from different species covering at least eight taxonomic groups. For estimating a quality standard for the freshwater community, fish and a second family in the phylum Chordata (e.g., fish, amphibian), a crustacean (e.g., cladoceran, copepod, ostracod, isopod, amphipod, crayfish), an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge), a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca), a family in any order of insect or any phylum not already represented, algae and higher plants would normally need to be represented (European Commission, 2011). Similar taxa requirements have also been adopted in the water quality guidelines of other countries (CCME, 2007).

Table 2.1: Assessment factors to derive a $PNEC_{aquatic}$ in fresh- or seawater

Available Data	Assessment Factor
Freshwater	
At least one short-term LC_{50}/EC_{50} from each of three trophic levels of the base set (fish, *Daphnia*, and algae)	1000
One long-term NOEC (either fish or *Daphnia*)	500
Two long-term NOECs from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50
Long-term NOECs from at least three species (normally fish, *Daphnia*, and algae) representing three trophic levels	10
Species sensitivity distribution method	1–5
Field data or model ecosystems	Reviewed on a case-by-case basis
Seawater	
Lowest short-term LC_{50}/EC_{50} from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans, and fish) of three trophic levels	10,000
Lowest short-term LC_{50}/EC_{50} from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans, and fish) of three trophic levels + two additional marine taxonomic groups (e.g., echinoderms, mollusks)	1000
One long-term NOEC (from freshwater or saltwater crustacean reproduction or fish growth studies)	1000
Two long-term NOECs from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish)	500
Lowest long-term NOECs from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels	100
Two long-term NOECs from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) + one long-term NOEC from an additional marine taxonomic group (e.g., echinoderms, mollusks)	50
Lowest long-term NOECs from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels + two long-term NOECs from additional marine taxonomic groups (e.g., echinoderms, mollusks)	10

Modified after TGD (2003), part 2.

The calculation of PNECs again includes an assessment factor, but much lower than normal as few toxicological data are available (Table 2.1):

$$PNEC = HC_5/AF \quad (\text{with} \quad 1 < AF < 5)$$

The exact value of the AF depends on the overall quality of the database concerning the mode of exposure (chronic vs acute studies, mesocosm/field studies), the biological models tested (representative of different taxonomic groups, feeding strategies and trophic levels, sensitive life stages), the goodness of fit of the SSD curve, or the size of confidence interval around the

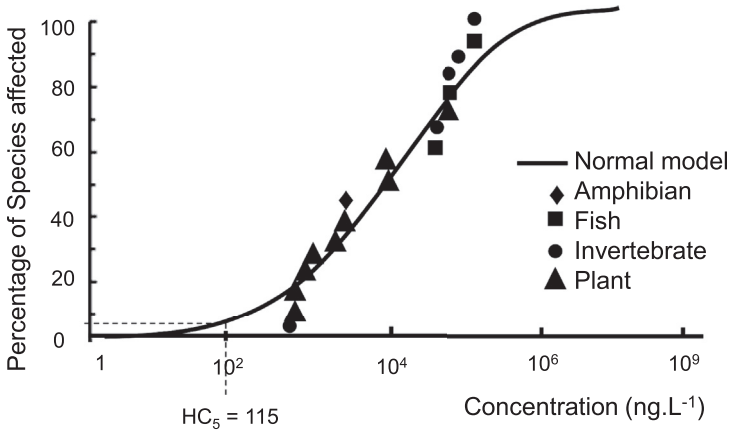

Figure 2.6

Species-sensitivity distribution for the preliminary assessment of triclosan using chronic toxicity data for freshwater organisms. HC_5, hazardous concentration 5%. *Modified after Health Canada, Environment Canada (2012).*

fifth percentile. Information on the mode of action of chemicals is also important to judge of the relevance of taxonomic groups tested, realizing that the mode of action may differ between short-and long-term effects and between taxonomic groups (e.g., special sensitivity of algae to copper; of insects and also crustaceans to insecticides).

Many procedures for environmental risk assessment are based upon single-species laboratory acute toxicity data (Raimondo et al., 2013). They seem relatively robust for estimating environmental quality standards because the geographical distribution of the species used to construct SSDs generally do not have a significant influence (Maltby et al., 2005; Feng et al., 2013; Wang et al., 2014). However, it remains questionable if SSDs derived from single-species laboratory acute toxicity data can be used to protect species assemblages in aquatic ecosystems. This question has been addressed for insecticides by collating single-species acute toxicity data and (micro)mesocosm data (Maltby et al., 2005). These authors concluded that "the corresponding median HC_5 (95% protection level with 50% confidence) was generally protective of single applications of insecticide but not of continuous or multiple applications. In the latter cases, a safety factor of at least five should be applied to the median HC_5".

In the case of an intermittent release (less than once per month and for no more than 24 h), the major risk is due to acute toxic effects. Thus, it is recommended (TGD, 2003; part 2) to calculate a $PNEC_{water}$, applying an assessment factor of 100 to the lowest LC_{50}/EC_{50} of at least three short-term tests from three trophic levels.

2.3.2.2 Calculation of $PNEC_{sediment}$

Sediments in both freshwater and marine environments are the final sink for most of the contaminants entering the aquatic environment as a consequence of the high sorption capacity

of particulate matter. Substances with a low partitioning coefficient (Kd for inorganic substances, K_{oc} or K_{ow} for organic substances) have a low capacity to bind to sediments. Thus to avoid extensive testing of chemicals a log K_{oc} or log K_{ow} of ≥3 can be used as a trigger value for sediment effects assessment (TGD, 2003; part 2).

The calculation of the $PNEC_{sed}$ according to the equilibrium partitioning method has been described for both fresh- and seawater. The following equation is applied:

$$PNEC_{sed} = (K_{susp-water}/RHO_{susp}) * PNEC_{water} * 1,000$$

with

$PNEC_{water}$ = PNEC in water ($mg \cdot L^{-1}$),
RHO_{susp} = bulk density of wet suspended matter ($kg \cdot m^{-3}$),
$K_{susp\text{-}water}$ = partition coefficient suspended matter–water ($m^3 \cdot m^{-3}$)
and $PNEC_{sed}$ = PNEC in sediment ($mg \cdot kg^{-1}$).

Sediment toxicity testing with endobenthic species is widely developed (Chapter 10), and these bioassays are extensively used in the framework of REACH (Cesnaitis et al., 2014) and in other countries, based on test guidelines published by OECD, USEPA, and ASTM. A $PNEC_{sed}$ can be derived from these tests using assessment factors (Table 2.2) according to a strategy similar to the one already described for the determination of $PNEC_{aquatic}$ (for detail, see TGD, 2003; part 2).

Table 2.2: Assessment factors to derive a $PNEC_{sediment}$ in freshwater or marine environment

Available Test Result	Assessment Factor
Freshwater	
One long-term test (NOEC or EC_{10})	100
Two long-term tests (NOEC or EC_{10}) with species representing different living and feeding conditions	50
Three long-term tests (NOEC or EC_{10}) with species representing different living and feeding conditions	10
Seawater	
Short-term freshwater or marine test	10,000
Two short-term tests including a minimum of one marine test with an organism of a sensitive taxa	1000
One long-term freshwater sediment test	1000
Two long-term freshwater sediment tests with species representing different living and feeding conditions	500
One long-term freshwater and one saltwater sediment test representing different living and feeding conditions	100
Three long-term sediment tests with species representing different living and feeding conditions	50
Three long-term tests with species representing different living and feeding conditions including a minimum of two tests with marine species	10

Modified after TGD (2003), part 2.

2.3.2.3 Calculation of PNECoral

Secondary poisoning can arise from the uptake of contaminants from prey and sediment (bioaccumulation, biomagnification) in addition to uptake from water (bioconcentration). Bioaccumulation is termed biomagnification when the transfer of chemicals via the food chain results in an increase of body concentrations at higher levels in the trophic chain. Most of the commonly studied compounds that possess K_{ow} in the 10^4–10^9 range exhibit a capacity to be bioconcentrated and thus, depending on their persistency, to be bioaccumulated and biomagnified in food webs. The maximum bioaccumulation potential has been observed for compounds with $\log K_{ow}$ between 5.5 and 7.5 (Abarnou, in Amiard-Triquet and Rainbow, 2009).

The risk to the freshwater fish-eating predators is calculated as the ratio between the concentration in their food ($PECoral_{predator}$) and the no-effect-concentration for oral intake ($PNECoral_{predator}$). The calculation must be preferably based on toxicity data provided by long-term studies, including NOECs established considering not only survival but also growth or reproduction. The following equations are applied respectively for fish-eating birds and mammals:

$$NOEC_{bird} = NOAEL_{bird} * CONV_{bird}$$

with

$NOEC_{bird}$ = NOEC for birds ($kg \cdot kg_{food}^{-1}$),
$NOAEL_{bird}$ = NOAEL for birds ($kg \cdot kg^{-1}\,bw \cdot d^{-1}$),
$CONV_{bird}$ = conversion factor from NOAEL to NOEC ($kg\,bw \cdot d^{-1} \cdot kg_{food}^{-1}$).

$$NOEC_{mammal,\,food\_chr} = NOAEL_{mammal,\,oral\_chr} * CONV_{mammal}$$

with

$NOEC_{mammal,food\_chr}$ = NOEC for mammals ($kg \cdot kg_{food}^{-1}$),
$NOAEL_{mammal,oral\_chr}$ = NOAEL for mammals ($kg \cdot kg^{-1}\,bw \cdot d^{-1}$),
$CONV_{mammal}$ = conversion factor from NOAEL to NOEC ($kg\,bw \cdot d^{-1} \cdot kg_{food}^{-1}$).

The PNECoral is ultimately derived from the toxicity data (food basis) applying an assessment factor. The following equation is applied:

$$PNECoral = TOX_{oral}/AF_{oral}$$

with

PNECoral = PNEC for secondary poisoning of birds and mammals (in $kg \cdot kg_{food}^{-1}$),
AF_{oral} = assessment factor applied in extrapolation of PNEC
and TOX_{oral} = $LC50_{bird}$, $NOEC_{bird}$, or $NOEC_{mammal,food,chronic}$ (in $kg \cdot kg_{food}^{-1}$).

The assessment factors for extrapolation differ from 30 to 3000 when the TOX_{oral} is a LC_{50} or an NOEC established by using either a short-term or a chronic test. In the marine