CHAPTER 4

ECOTOXICOLOGY – HOW TO ASSESS THE IMPACT OF TOXICANTS IN A MULTI-FACTORIAL ENVIRONMENT?

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Abstract: Ecotoxicology assesses the fate of contaminants in the environment and contaminant effects on constituents of the biosphere. With respect to effects assessment, current ecotoxicology uses mainly reductionistic approaches. For concluding from the reductionistic approach to the effects of toxicant exposure in a multifactorial world, ecotoxicology relies on extrapolations: (i) from suborganism and organism effect levels, as determined in laboratory tests, to ecological levels, (ii) from few laboratory test species to the broad range of species and their interactions in the ecosystem and (iii) from the analysis of the effects of single toxicants under standardized laboratory settings to the toxicant response under real world conditions, where biota are exposed to combinations of chemical, biological and physical stressors. The challenge to ecotoxicology is to identify strategies and approaches for reducing uncertainty and ignorance being inherent to such extrapolations. This chapter discusses possibilities to improve ecotoxicological risk assessment by integrating mechanistic and ecological information, and it highlights the urgent need to develop concepts and models for predicting interactions between multiple stressors.

Keywords: ecotoxicology; risk assessment; effect propagation; interspecies extrapolation; multiple stressors

Introduction

Ecotoxicology is the science of contaminants in the biosphere and their effects on constituents of the biosphere (Newman, 1998). This scientific field draws from many disciplines, for instance, from chemistry for analysing and predicting fate and transport of chemicals in the environment, from toxicology for studying mechanisms of adverse effects of chemicals in organisms, or from ecology for assessing ecological consequences of chemical pollution.

C. Mothersill et al. (eds.), Multiple Stressors: A Challenge for the Future, 39–56.

Whereas the scope of ecotoxicology is well defined on the side of environmental chemistry, some inconsistency exists on the toxicological side. Contrary to toxicology which is concerned with effects of chemicals at the level of the individual organism and its constituent parts, ecotoxicology in principal aims to assess toxic impact at the ecological rather than the individual level. In practice, however, ecological assessment is often lacking (Forbes and Forbes, 1994), instead classical toxicological studies predominate so that ecotoxicology seems to differ from human toxicology mainly by using a broader array of target species. The bias of ecotoxicology towards toxicological rather than ecological studies is not an intended one, but reflects practical, methodological as well as conceptual limitations.

Ecotoxicology is a relatively new scientific discipline (Jorgensen, 1998). During the 1950s and 1960s, public awareness was increasing that anthropogenic substances released into the environment may not just dilute and virtually disappear, but that they could accumulate in biota and man, and may lead to adverse effects. Epidemics such as, e.g. the Minamata disease in Japan – caused by food web-enrichment of organic mercury – pinpointed to the possible problems arising from environmental pollution. In 1962, Rachel Carson published the book "Silent Spring" drawing attention to the consequences of pesticide accumulation in wildlife. Ecotoxicology as a term was coined, according to Truhaut (1977), in June 1969 during a meeting of a committee of the International Council of Scientific Unions. The first textbook on ecotoxicology was then published in 1977 (Ramade, 1977), still paying much attention to human health, but subsequent textbooks increasingly focused on genuine ecotoxicological issues such as, e.g. species differences in sensitivity, ecological determinants of residues, community toxicity, or ecotoxicology as a "hierarchical science" (e.g. Moriarty, 1983; Forbes and Forbes, 1994; Walker et al., 1996; Newman, 1998). From the beginning, ecotoxicology was strongly driven by managerial and legislative needs. Thus, much emphasis was given to technological goals such as the development of standardized toxicity tests. How well regulatory testing programmes fulfilling the legislative needs do protect ecosystems from long-term, insidious decline has always been debated. It is difficult to judge upon how many pollutant-related environmental problems have been avoided due to the application of regulatory testing programmes; however, there exist a number of examples where conventional ecotoxicological approaches failed to prevent or predict the environmental problems. One such example is endocrine disruption (Sumpter and Johnson, 2006). To improve the ability of retrospective as well as predictive assessment of pollutant impact on the biosphere, ecotoxicology is confronted with a number of challenges, some of which will be addressed shortly in the present communication.

Current Approaches in Ecotoxicological Risk Assessment

A comprehensive review of ecotoxicological risk assessment is beyond the scope of this chapter, instead only a short introduction into certain principles and technologies as they are currently used will be given. Ecotoxicological risk assessment aims to estimate levels of contaminants in environmental compartments, to evaluate effects of pollutants at various levels of biological complexity, and to relate environmental exposure to environmental effects (Newman, 1998; Ahlers and Diderich, 1998; Calow and Forbes, 2003; Bradbury et al., 2004). It can be either retrospective ("from effect to pollutant") or prospective ("from pollutant to effect"; Eggen and Suter, 2007).

The retrospective approach builds on monitoring of existing chemical exposure, bioaccumulation and adverse effects in wildlife. Monitoring can start with observing exposure or bioaccumulation, and then trying to relate this to biological or ecological change, or it can start from the observation of adverse changes in wildlife, and then trying to trace this back to chemicals as cause. A number of factors obscure exposure effect-relationships in field studies, for instance, bioavailability on the exposure side, or the impact of multiple stressors on the effect side. Thus, unequivocal demonstration of cause effect-relations is difficult in field studies. Instead, usually a weight-of-evidence approach has to be taken (Rolland, 2000; Burkhardt-Holm and Scheurer, 2007). The demonstration of causative relationships is supported by the existence of temporal or spatial parallelism between exposure and effects (Downes et al., 2002) as well as by a sound epidemiological design of monitoring studies, although the latter aspect is often neglected in ecotoxicology. A number of technologies help to reveal exposure effect-relationships in retrospective studies, such as bioassay-directed fractionation and biomarkers (Brack, 2003; Segner, 2003). Bioassay-directed fractionation is a procedure combining chemical fractionation and analysis with bioassays in order to identify those chemicals within a complex environmental sample which are responsible for a measured biological activity of the sample (Brack, 2003). An example of this methodology is provided by study of Desbrow et al. (1998) on identification of the chemical nature of the estrogen-active substances in effluents of wastewater treatment plants in UK. Biomarkers are sub-organismic parameters being responsive to chemicals and thus can be used either as indicators of exposure to or effects of chemical substances (Peakall, 1994; Van der Ost, 2003). Well-known examples of biomarkers include cytochrome P4501A, which is induced by chemicals activating the arylhydrocarbon receptor, for instance dioxins, or vitellogenin, which responds to chemicals activating estrogen receptors. The concept of biomarkers has attracted much attention in ecotoxicology, and indeed biomarkers are valuable as indicators

of exposure, as early warning signals of long-term or delayed toxicity, or as "signposts" for toxic modes of action, however, they are usually not predictive of adverse effects at higher levels of biological organization (Forbes et al., 2006; Hutchinson et al., 2006).

Predictive ecotoxicological risk assessment aims to estimate environmental concentrations of chemicals, to evaluate the toxic hazard and ecological effects arising from these substances, and the likelihood of adverse effects to occur in exposed biota (Newman, 1998; Calow and Forbes, 2003; Bradbury et al., 2004). Key elements in the predictive approach are the "predicted environmental concentration" (PEC) and the "predicted no effect concentration" (PNEC). A PEC can be estimated from actually measured concentrations in the environment or from mathematical modelling; a PNEC can be derived, for instance, from concentration-response determinations in single species toxicity tests in the laboratory (Ahlers and Diderich, 1998). For risk characterization, the PEC is compared to the PNEC in order to estimate the probability of adverse effects to occur. Relevant effects may range from the suborganism level over population and communities to the landscape scale, and they may vary with the broad range of potential target species in an ecosystem. The advantage of the described approach is that it is straightforward and manageable; its disadvantage is that it appears to be at least sometimes too simplistic thereby missing environmentally relevant aspects of exposure and effect, which arise from the complexity of biological and ecological systems. Shortcomings exist particularly on the effects side. PNEC values are largely based on testing of single substances in acute or (sub)chronic laboratory tests, using a few selected "model" species, and using either suborganism or organism-level endpoints. It is easy to demonstrate toxic effects on suborganism and organism-level endpoints of individuals of single species in the laboratory, but we have insufficient understanding of how to extrapolate from the analysed effect level in the laboratory test to ecological levels (effect propagation), how to extrapolate from few laboratory test species to the broad range of other species being present in the ecosystem (interspecies effect extrapolation), and how to estimate from the analysis of the effects of single toxicants under standardized laboratory settings to the toxicant response of organisms under multifactorial real world conditions, where biota are exposed to combinations of chemicals and non-chemical stressors (multiple stressor extrapolation). It is evident that such extrapolations in ecotoxicological risk assessment bear important uncertainties; however, an additional problem arises from ignorance, i.e. our inability to take unknown processes and variables into account (Hoffman-Riem and Wynne, 2002). For instance, egg shell thinning as a consequence of DDT accumulation was overlooked as long as it was unknown that this is a target of DDT action. For practical as well as for principal reasons, ecotoxicological testing will never be able and does not aim

for fully reflecting environmental complexity but will always have to rely on reductionistic approaches. The problem is not the reductionism but that we need to learn more on how stressors and effects are interrelated – across and between species, across levels of biological organization, across time scales – and which processes and parameters are of key importance in determining the effects of toxicant exposure in a multifactorial environment.

Challenges in Ecotoxicological Effects Assessment

Given the limitations as discussed earlier, ecotoxicological effects assessment is confronted with a number of challenges:

- While during the early days of ecotoxicology, environmental pollution was often characterized by high levels of contaminants, acute spills, or dominance of high volume industrial chemicals, the situation has changed nowadays (Eggen et al., 2004). Enhanced regulatory practices and technical measures such as improved water treatment technologies or replacing persistent by more degradable substances successfully reduced overall environmental contamination in industrialized countries. In this situation, risks arising from low dose, chronic exposures are coming into focus. This includes questions on the importance of combined effects of chemicals at low concentrations or on combined effects of chemicals and other stressors such as altered habitat morphology or climate change. Further, although concentrations of "classical" toxicants are decreasing, at the same time, new contaminants are emerging such as pharmaceuticals and personal care products, which often show specific modes of toxic action not reliably detectable by the existing testing concepts and methodologies. The challenge is to clarify whether existing ecotoxicological concepts and tools are sufficient or how they have to be enhanced to be able to assess hazard and risks arising from the actual situation of environmental contamination.
- For many, if not the majority of existing chemicals, the available ecotoxicological information is rather limited. Even for high production volume chemicals, often not more than acute lethality data are available. At the same time, new regulations such as REACH in Europe are demanding more information on ecotoxicological properties of existing substances. It is a challenge to ecotoxicology to generate the required data but not by simply increasing the number of tests as this is confronted with many technical, economical and ethical problems, but by developing integrated testing strategies (Bradbury et al., 2004), which are taking advantage of mechanistic and ecological knowledge. Such a knowledge-based testing scheme would enable targeted testing for better chemical prioritization and hazard identification.

- Existing methodologies and concepts of ecotoxicology, despite the prefix "eco-", still reflect more toxicology than ecology. Although the emphasis on toxicology may be understandable from the historical development and pressing regulatory needs (see earlier), doubt remains that this approach might be too simplistic to inform ecological risk assessment (Calow and Forbes, 2003). The challenge is to improve this situation and to find concepts and tools that on the one hand are manageable and practical but on the other hand move ecotoxicological risk assessment closer to ecology.

The following discussion cannot provide the solution to the open questions and problems, rather it tries to point out directions to be taken in the development of new concepts and tools for ecotoxicology.

GOING MECHANISTIC

To date, ecotoxicological testing has been rather phenomenological. However, ecotoxicology has to be more than describing that effects occur, but it needs explanatory principles (Newman, 1998). Understanding of how toxic effects occur is important in extrapolation, classification, and diagnosis of effects (Eggen et al., 2004; Miracle and Ankley, 2005; Segner, 2006) and it will reduce the risk to overlook or ignore possible adverse outcome of chemical exposure (Hoffmann-Riem and Wynne, 2002), as it has been the case, for instance, with endocrine disruption (Segner, 2006; Sumpter and Johnson, 2006). Standard ecotoxicological testing relies on apical endpoints which, due to their integrative nature, lend limited insight into causative processes. Thus, to achieve more knowledge on modes of toxic action or toxic mechanisms, additional endpoints have to be considered. Often, molecular and cellular parameters are used for this purpose. However, changing the level of biological analysis does not necessarily mean to move from the description of the effect to the understanding of the underlying mechanism. Actually, the so-called mechanistic research in ecotoxicology often has been descriptive again (Moore, 2002). The same comment applies for the use of specific technologies: it is not the use of a particular technique but it is the study design and interpretation what makes the difference. For instance, during recent years much emphasis has been given to the promises of genomic methodologies in predictive, mechanism-based ecotoxicology, but as put by Miracle et al. (2003) with respect to the use of these techniques: "If a well thought-out approach is neglected during experimental design and data interpretation, then we are simply left with standard toxicology in Technicolor".

There are a number of areas of ecotoxicological effects assessment where knowledge on modes of toxic action is helpful:

- Knowledge on modes of action helps in understanding time and concentration response-relationships (questions of thresholds, hormesis, U-shaped curves of endocrine disrupting compounds, relation between acute and chronic toxicity, concentration-dependent transitions in mode of action, etc.). For instance, the information that a chemical acts through the estrogen receptor pathway explains why this substance induces irreversible (organizational) effects in developing organisms while it induces transient (activational) effects in adults (Segner et al., 2006). Mechanistic knowledge identifies the processes being affected by the chemical and from this knowledge the risk for chronic effects may be inferred, e.g. the development of cancer as a consequence of mutagenic activity of a compound (Eggen et al., 2004).
- Knowing the mode of action by which a chemical substance induces adverse effects helps in extrapolation, both across species and across biological levels. Molecular or cellular processes are often more conserved than processes at higher levels of biological organisation, what facilitates interspecies extrapolation (Segner and Braunbeck, 1998; Miracle and Ankley, 2005; Hutchinson et al., 2006). For instance, an estrogen receptor ligand in man will be also an estrogen receptor ligand in a fish, however, the physiological consequences of the receptor activation differs between man and fish. Further, if we know that a toxicant impacts a specific biological process, we may understand why certain species are more sensitive than others, and which species are at particular risk.
- Knowing modes of action assists in the assessment and prediction of mixture effects (Escher and Hermens, 2002; Eggen et al., 2004). For instance, knowing that a set of chemicals act through the same receptor pathway is important to predict that a mixture of these compounds will behave in an additive way (Silva et al., 2002). This knowledge forms also the basis of the concept of toxic equivalency factors (Safe, 1990). Vice versa, knowing molecular targets of a toxicant helps to identify biological functions at risk and helps to understand why one and the same substance may lead to multiple effects. For instance, estrogen receptors do not only function in reproductive processes, but are involved in a variety of functions (see later).
- Information on modes of action assists in prioritizing, classification and testing of chemicals. Such knowledge provides input for computational methods and structure activity relationships (Schüürmann, 1998). Knowing which processes in the organisms are affected by a substance helps in designing targeted, knowledge-based testing strategies for the substance of concern (Eggen et al., 2004; Hutchinson et al., 2006; Segner,

2006), and this would reduce uncertainty in risk assessment and guard against surprises, as they happened, for instance, in the case of endocrine disruptors (Calow and Forbes, 2003; Segner, 2006).

- Finally, an important spin-off from mechanism-oriented work is the development of diagnostic tools (Eggen and Segner, 2003). Bioassays and biomarkers have been found to be most valuable in assessing environmental contamination. Currently, only a limited set of tools is available, being indicative for rather few stressors or modes of action, but novel technologies such as genomics and proteomics may generate a broader suite of diagnostic tools.

GOING ECOLOGICAL

Ecotoxicologists succeeded in demonstrating pollutant effects at all levels of biological organisation, from molecules to ecosystems; however, they are uncertain how effects at the different levels relate to each other (Newman, 1998). A similar problem is the question of interspecies extrapolation of toxicity data. The principal dilemma of ecotoxicological effects assessment is that we have to make simplifying assumptions and we have to use reductionistic approaches but we do not know if we use the right simplifications and we do not know the rules and conditions for translating the outcome of the simplified approaches to the systems to be protected. To say it with the words of Barnthouse et al. (1987): "There is an enormous disparity between the types of data available for assessment and the type of responses of ultimate interest. The toxicological data usually have been obtained from short-term toxicity tests performed using standard protocols and test species. In contrast, the effects of concern to ecologists performing assessments are those of long-term exposures on the persistence, abundance and/or production of populations". Although this statement has been formulated almost 20 years ago, substantial progress has not been achieved since then. Since for practical reasons, it will be not possible to abandon reductionistic approaches, the question is if these concepts and methods are indeed too simplistic to inform ecological risk assessment or how we could improve them to be more effective (Calow and Forbes, 2003).

Usually ecotoxicologists follow a bottom-up approach, i.e. investigating toxic effects at the suborganism and/or organism-level and then extrapolating to the levels of populations and communities. The reason behind this approach is that, although the changes at the population/community/ecosystem levels of biological organization are the ultimate concern, they are considered to be too complex and too far removed from the causative events to be useful in diagnosis and prediction of toxic effects of chemicals. Instead, the idea is that

toxic effects can be measured at the lower levels of biological organization and that these effects are prognostic for higher level consequences. In almost each review and textbook of ecotoxicology, a figure of the biological hierarchy is shown, with toxic effects propagating from the molecular through cellular and organism levels to populations or communities. What is neglected in this thinking is that there exists no linear effect propagation but at each level of the biological hierarchy, new properties emerge which are not predictable from the properties of the level below but which influence the outcome of the toxic impact (Fig. 1). For instance, a toxic cellular effect is not necessarily leading to a toxic response of the organism due to the existence of compensatory mechanisms at the supracellular level (Segner and Braunbeck, 1998). Exactly for this reason, the use of biomarkers to predict ecological effects is questionable (see earlier). Similarly, ecotoxicological test methodology puts emphasis on metrics such as survival, growth and reproduction since these changes in individual fitness are considered to be "ecologically relevant" and to influence directly the status of the population. However, translation of phenotypic variations in life history traits of individuals into demographic changes of the population depends much on the life history strategy of a particular species (Winemiller et al., 1992; Kooijman, 1998). A 50% loss of

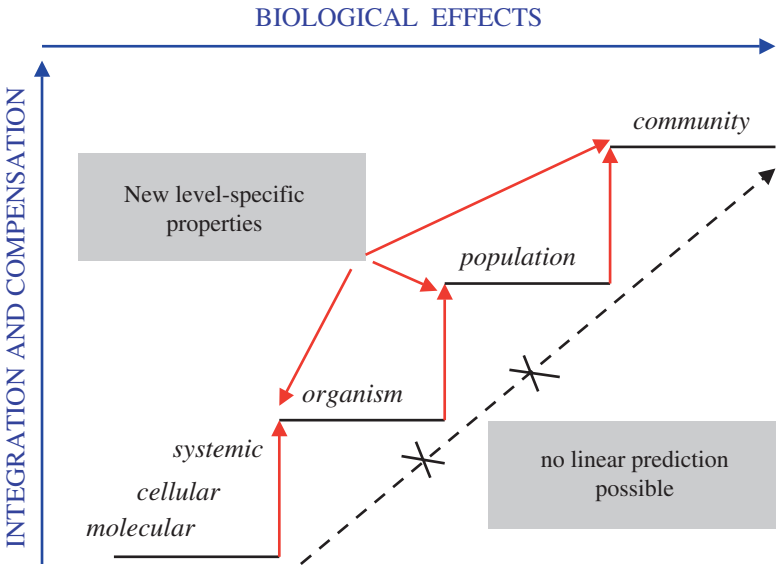

Fig. 1. The propagation of toxic effects along the levels of the biological hierarchy does not follow a linear, deterministic fashion, but the outcome of toxic exposure at a particular level of the hierarchy results on the one hand of integrating toxic and compensatory/protective mechanisms at the lower hierarchical levels and on the other hand it results on properties and processes newly emerging at the hierarchical level of concern.

fertility may have completely different demographic consequences for two species with contrasting life histories, e.g. an opportunistic and a periodic species (Gleason and Nacci, 2001). Laboratory tests often determine toxic effects for the most sensitive life stage, assuming that an adverse effect on this stage will be critical for population status. However, the most sensitive life stage might be not the most crucial factor for maintaining a viable population, since in many species, there is an overproduction of individuals at this stage what easily could compensate for the toxicant-induced losses (Newman, 2001).

This short discussion may already illustrate that inferring higher level effects from qualities of lower levels is problematic (Underwood and Peterson, 1988; Newman, 1998). What is needed to improve predictions across biological levels is to go beyond a linear thinking and to develop a better understanding of the processes and mechanisms how the different levels relate to each other. One possibility to overcome the limitations of current approaches is the use of appropriate modelling (Hutchinson et al., 2006). Models have been suggested for deriving adverse organism responses from cellular responses (Moore, 2002), but particularly also for predicting population responses from responses of individuals. For this it is important not to rely on "black box" modelling but to use physiological and ecological information. One possibility would be to combine toxicity data from laboratory tests with life history from natural populations to model population responses of the species of concern (Boxall et al., 2002). Kooijman (1998) pointed to the value of structured population modelling, in which individuals are not treated as identical copies, but accounts for the fact that individuals differ in many aspects from each other (age, size, sex, energy reserves, genetics, etc.). Modelling can be also valuable to identify which individual changes take a decisive influence on population viability (Grist et al., 2003; Gurney, 2006). Such knowledge could target toxicological testing towards the critical life history traits in order to quantify the sensitivity of population dynamics to changes in these vital traits (Calow and Forbes, 2003). While current testing in ecotoxicology relies on a fixed, pre-selected set of endpoints, a more ecological-oriented approach would select test endpoints on the basis of the analysis of the life history strategy of the species of concern. In this way, ecological knowledge could inform toxicological testing – an approach that to date has been surprisingly rarely used in ecotoxicology. Knowledge-based testing utilizing ecological information might be mutually complementary with knowledge-based testing utilizing mechanistic information (see earlier).

While the previous discussion addressed the problem of extrapolation across biological levels, another problem in informing on ecological risk from conventional single species laboratory tests is the extrapolation of toxic effects across species. When environmental contamination takes place, usually communities are exposed which comprise a large number

of species differing in physiology, ecology and toxicant sensitivity. In principal, to protect an ecosystem against adverse effects of toxic substances, it would be necessary to test all the different species occurring in the ecosystem for their sensitivity towards the substance of concern. For practical reasons, however, toxicity testing is usually done only with a limited number of species, and the results from these species are then extrapolated to predict the response of other species and of the whole community. The limitations in this approach are evident. Regulatory risk assessment tries to overcome this limitations by the use of numerical "safety factors", i.e. data from standard toxicity tests are divided by fixed extrapolation factors in order to account for the uncertainties in the extrapolation from simplified laboratory tests to environmental reality and to derive a threshold value (PNEC) below which adverse ecological effects are defined to be unlikely (Ahlers and Diderich, 1998; Calow and Forbes, 2003). However, it is clear that safety factors suffer from serious limitations and represent rather a formal than a scientifically based approach. An alternative approach to extrapolate from single species toxicity tests to communities is the concept of species sensitivity distribution (SSD) (Posthuma et al., 2002). The basic assumption in the SSD concept is that the sensitivities of a set of species can be described by some kind of statistical distribution. The available ecotoxicological data on different species are seen as a subsample from this distribution and are used to calculate a concentration which is considered to be safe for most species. Thus, this concept supports prediction of toxic effects for multiple species assemblages, and it has the potential to incorporate spatial information as well as information on mixture effects (Posthuma and de Zwart, 2005).

Finally, methodologies have been developed to directly test species interactions instead of extrapolating from single species data. One such method attempting to assess ecological complexity is toxicity testing in laboratory microcosms or outdoor mesocosms (Cairns and Cherry, 1993; Newman, 1998). These systems which harbour multiple species assemblage have more ecological realism than do single species laboratory tests and still possess more tractability than do field studies. Micro- and mesocosms harbour multiple species assemblages and therefore are able to perform community impact assessments in relation to toxic exposure. The extent to which such systems represent natural systems remains a subject of debate, however (Williams et al., 2002). Another methodology for using communities in toxicity assessment is pollution-induced community tolerance (PICT) which was introduced by Blanck et al. (1988) as a tool in predictive and retrospective risk assessment. It is based on the assumption that sensitive species within a community will be replaced by more tolerant species after exposure to a toxicant, increasing the tolerance

of the whole community. Experimentally, PICT can be measured by structural and functional parameters such as species number or photosynthetic activity (Schmitt-Janssen and Altenburger, 2005).

GOING MULTIPLE

In their environment, organisms are exposed to multiple chemicals and multiple stressors (a stressor is defined here as any factor that extends homeostatic or protective processes beyond the limits of the normal physiological or ecological range leading to reduced fitness: Sibly and Calow, 1989; Moore, 2002). The questions to ecotoxicologists are how toxic impact is modulated in the presence of mixtures or in combination with other stressors, and how the risk resulting from the interaction between chemical, physical and biological stressors can be assessed and predicted. Exposure to one stressor may change the response and sensitivity of the biological system to a second stressor. Such modulations could be of particular relevance under conditions of chronic, low-dose exposure, when chemical impact alone may provoke only subtle changes but it may be enhanced by interaction with other stressors. However, it has to be kept in mind that biological systems have evolved under conditions of fluctuating environments and multiple impacts. Thus, species have developed adaptive systems which enable ecological success in the presence of stressors, but these adaptive systems may differ in their capacities towards specific stressors, what further complicates assessment of combined effects. Overall, to better account for the multifactorial scenario to which organisms, populations and communities are exposed to in their environment, it is necessary to consider the various stressors in an integrated way instead of considering each stressor in isolation. Consequently, van Straalen (2003) suggested that ecotoxicology should become part of a broader scaled stress ecology.

Empirically, stressor interactions have been shown in many studies. For instance, season and temperature can overlay chemical induction of exposure biomarkers (Mackay and Lazier, 1993; Behrens and Segner, 2005), the nutritional status of exposed organisms can modify their sensitivity towards toxicants (Lanno et al., 1989; Braunbeck and Segner, 1992), or toxicant exposure can increase the susceptibility to degenerative and/or infectious diseases (Bayne et al., 1985; Odum, 1985; Heugens et al., 2001; Newman, 2001; Kiesecker, 2002). The infectious disease triad (Odum, 1985; Newman, 2001) summarizes such interactions in that it presents the likelihood of disease as a function of the balance between host, disease agent, and environmental factors. Pollutants, as part of the environmental milieu in which the host and the pathogen interact, can change the balance between host and pathogen, for instance, by altering the immunological competence of the

host and thereby rendering the host more susceptible to the pathogen, or, alternatively, by reducing survival of the infective stage of the pathogen in the environment. Vice versa, pathogens can alter response of the host to toxicants in that the diseased organism may be more sensitive to toxic impact than the healthy organism (Carlson et al., 2002; Köllner et al., 2002). Again, however, one must not neglect the capability of the host for adaptive strategies. For instance, Burki et al. (2007) showed that when rainbow trout was exposed simultaneously to a parasite and an environmental estrogen, the response of the fish was dominated by the parasite, while the estrogenic response was largely suppressed.

The multiple stressor issue has different facets in retrospective and in predictive risk assessment. In retrospective studies, a major problem is to disentangle the impact of toxicants from the influence of other factors including natural environmental change. If an adverse change is observed, this may directly result from toxicant action, it may result from factors other than toxic chemicals, or it may result from the combined action of several factors. Epidemiological and weight-of-evidence methodologies are essential to sort out the role of toxicants in complex exposure scenarios (Rolland, 2000; Burkhardt-Holm et al., 2005; Burkhardt-Holm and Scheurer, 2007). For predictive studies, principal concepts or models are needed as it is not realistic to use empirical testing for the infinite number of possible combinations. In mixtures of chemicals, the interactions may lead to amplification (synergism), reduction (antagonism) or additivity of the stand-alone effects of the individual compounds. Several models have been found useful under laboratory settings to predict the combined effects of chemicals in a mixture (e.g. Könnemann, 1981; Hermens et al., 1984; McCarthy et al., 1992; Silva et al., 2002; Altenburger et al., 2003; Monosson, 2005). How complex the assessment of the combined effect of a chemical mixture containing substances with different modes of action can be has been illustrated by Altenburger et al. (2004). The extrapolation from single species laboratory tests on mixture toxicity to the in situ risk of chemical mixtures for an assemblage of species adds further complexity, and requires development of new methodologies (De Zwart and Posthuma, 2005). An additional challenge is to develop concepts and models for predicting interactions between chemical and physical or biological stressors. As indicated above, it is known empirically that physical and biological entities can modulate chemical toxicity and vice versa, however, few attempts have been made to date to quantitatively analyse and to predict such interactions (Folt et al., 1999; Koppe et al., 2006).

Finally, when talking on “going multiple”, we should not only focus on exposure of organisms to multiple stressors, but we need also to consider that one and the same toxicant may induce multiple biological responses. This includes not only the fact that toxicants show a transition in mode of action

with exposure duration and concentration (Slikker et al., 2004; Schäfers et al., 2007), but it points also to the fact that a chemical can interact with multiple targets in a biological system, what may result in unexpected toxic effects. For instance, studies on estrogen-active substances focused primarily on their effect on sexual and reproductive parameters; however, estrogens have a series of functions beyond the reproductive system. Studies during recent years have revealed that, for instance, estrogen-active environmental substances can interfere with the arylhydrocarbon receptor pathway (Navas and Segner, 2001; Cheshenko et al., 2007), the growth hormone/insulin-like growth factor system (Berishvili et al., 2006; Filby et al., 2006), with the immune system (Segner et al., 2006) or with the neurosensory system (Kallivretaki et al., 2007) and thereby are able to disrupt a broad array of target systems.

Acknowledgements

The author gratefully acknowledges the financial support by the EU projects EDEN (Endocrine Disrupters: Exploring Novel Endpoints, Exposure, Low Dose and Mixture Effects in Humans, Aquatic Wildlife and Laboratory Animals) and MODELKEY (Models for Assessing and Forecasting the Impact of Environmental Key Pollutants on Marine and Freshwater Ecosystems and Biodiversity).

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