Tolerance to Environmental Contaminants

Environmental and Ecological Risk Assessment

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Preface

Tolerance may be defined as the ability of organisms to cope with stress, particularly the chemical stress resulting from the anthropogenic input of one or more of many different toxic contaminants into the environment. Tolerance has been described in many organisms from bacteria to fungi, from phytoplankton to terrestrial flowering plants, and from invertebrates like worms to vertebrates like fish and amphibians. There are two generally agreed methods by which organisms can become tolerant to a toxic contaminant. First, tolerance may be gained by physiological acclimation during the exposure of an individual organism to a sublethal bioavailability of the toxicant; this tolerance is not transferable to future generations. Second, tolerance may also be acquired as a consequence of genetic adaptation in populations exposed over generations to the toxic contaminant, through the action of natural selection on genetically based individual variation in resistance; this tolerance is transferable to future generations. This latter genetic adaptation may be lost in the absence of continuing exposure to the contaminant, again by natural selection, if, as appears to be usual, the genetically based tolerance has a metabolic cost that brings a selective disadvantage in the absence of contaminant. Indeed, the presence of a genetically tolerant population is direct evidence that the bioavailability of the toxic contaminant in the local environment is sufficient to be ecotoxicologically relevant.

So what? In fact, the gaining of tolerance, be it by physiological acclimation or genetic adaptation, can have great consequences for the local biodiversity, and hence the ecology and ecosystem functioning of many of the world's habitats. Contamination by toxicants can lead to decreased production of biological resources, including agricultural or fishery products, and the interruption of key ecological processes, such as decomposition and nutrient cycling. Tolerant species, particularly bacteria in sediments or primary producers like phytoplankton, may play key functional roles in ecosystems. Understanding the frequency of the occurrence of tolerance therefore has tremendous implications for the sustainability of biodiversity and ecosystem functioning. Metabolic processes involved in tolerance are energetically expensive, and thus may interfere with the allocation of energy in an organism, thereby governing the success of reproduction and growth. Reduction of the overall amount of genetic variation in populations exposed to a strong selective toxic pressure can result in increased sensitivity to new stresses in organisms otherwise tolerant to one source of stress. Thus, the adaptive benefit of being tolerant may have negative knock-on effects in the long term. Beyond effects on the crucial ecosystem functioning of the habitats around us, delivering the vital ecosystem services on which we depend (food, clean water, etc.), the acquisition of chemical tolerance may be a more direct source of concern to humans in that it allows the survival of harmful species (insecticide-resistant mosquitoes, antibiotic-resistant pathogenic bacteria) and the presence of highly contaminated links in food webs, including those leading to humans.

The book is an up-to-date compilation of the views of international experts on the phenomenon of tolerance of living organisms to toxic contaminants, usually of anthropogenic origin. The general principles governing the acquisition and biological consequences of tolerance, genetically or physiologically based, are examined at different levels of biological organisation, taxonomically from bacteria and archaea to flowering plants and vertebrates, and within organisms from molecular biology and biochemistry through physiology to whole organism, community, and ecosystem levels of organisation. Thus, part of the book is specifically devoted to mechanisms of defence involved in the acquisition of tolerance to different classes of environmental contaminants, taking into account the limits above which such mechanisms are overwhelmed. Another part of the book examines the ecological consequences of tolerance in terms of both positive (conservation of biodiversity in contaminated environments) and negative (physiological costs of tolerance with consequences on growth and reproduction, transfer of contaminants in the food webs) aspects. The final section of the book considers specific aspects of tolerance that can have major impacts for the environment and for society (tolerance in bacteria, plants, and insects).

Thus, this volume presents a state-of-the-art synthesis of the many aspects of the phenomenon of tolerance to environmental contaminants. Ecotoxicologists have made good progress in the understanding of the mechanisms that allow organisms to cope with pollutants in their environment, but the links with potential effects at higher levels of organisation need to be more strongly established. While the positive effects of tolerance at supra-organismal levels (population, community, ecosystem) for environment and health protection are often considered, the relative importance of any negative effects of tolerance are not typically fully assessed. The reviews offered in each chapter of this book contribute to the provision of tools to carry out relevant risk-benefit analyses in a more informed fashion. From an operational point of view, tolerance must be taken into account when biological responses (biochemical, behavioural, genetic biomarkers) are applied for environmental biomonitoring. Mechanisms of defence may be profitably used as biomarkers, revealing the exposure of organisms to contaminants but within limits that this book helps to define. The problem of over- versus underestimation of risk is also a core question for the development of toxicity reference values. The contaminant exposure history of populations, and whether the local biota have acquired tolerance or not, are clear confounding factors in the interpretation of bioassays that must be understood and taken into account. The reviews presented here can only assist ecotoxicologists to produce more informed and therefore more reliable risk assessments when assessing the ecotoxicological risks to life in any of the contaminated habitats that now surround us in our industrialised society.

We have deliberately sought to put together a synthesis that takes a multidisciplinary approach across contaminant types, habitats, organisms, biological levels of organisation, scientific disciplines, and approaches. The volume presents science at the frontier of research in the subject compiled by international experts from across the world. It is our aim that the book has relevance to environmental scientists and other stakeholders from government to the public. It should also prove invaluable to final-year undergraduate and master's students across the world, and contribute to graduate students in PhD programs, under a wide range of subject heads that include

ecotoxicology, ecology, marine and freshwater biology, microbiology, environmental management, and environmental regulation. The book has great relevance, both to readers in developed countries seriously addressing problems of environmental contamination, including North America, Europe, Asia, Australia, and New Zealand, and to those in developing countries with industrial expansion and associated real and potential problems of environmental contamination (Central and South America, Eastern Europe, Africa, India, China, East and Southeast Asia). It is our hope that we have succeeded in our objectives, and that this book serves as an important taking-off point for further understanding of the very wide significance of the phenomenon of tolerance to environmental contaminants.

Claude Amiard-Triquet

Philip S. Rainbow

Michèle Roméo

About the Editors

Dr. Claude Amiard-Triquet is a Research Director in the CNRS (French National Research Center), based at the University of Nantes, France. She was awarded the degree of DSc in 1975, for her research in radioecology at the French Atomic Energy Commission. Dr. Amiard-Triquet's topics of research interest include metal ecotoxicology, biomarkers and, more recently, emerging contaminants (endocrine disruptors, nanoparticles). As the head of multi-disciplinary research programmes, she has managed research collaborations between specialists in organic and inorganic contaminants, and chemists and biologists involved in studies from the molecular to ecosystem levels, with a constant concern for complementarity between fundamental and applied research. Dr. Amiard-Triquet regularly acts as an expert for the assessment of scientific proposals (e.g., the European Framework Program for Research and Development, the International Foundation for Science, and the Sea Grant Administration, Oregon State), and is also in demand as a referee for a dozen or so international journals. She has authored or co-authored more than 170 research papers, and has authored 7 chapters in books. Dr. Amiard-Triquet has also co-authored one book, *La Radioécologie des Milieux Aquatiques* with J.C. Amiard, and co-edited two books, *L'Évaluation du Risque Écologique à l'Aide de Biomarqueurs* with J.C. Amiard and *Environmental Assessment of Estuarine Ecosystems: A Case Study* with P.S. Rainbow. She has given or contributed to more than 90 presentations at international meetings.

Professor Philip Rainbow is the Head of the Department of Zoology at the Natural History Museum, London, leading a staff of more than 100 working scientists in one of the premier museums of the world. He holds the degrees of PhD (1975) and DSc (1994) from the University of Wales. Philip Rainbow was appointed (1994) to a personal chair in the University of London, where he was Head of the School of Biological Sciences at Queen Mary (1995–1997) and is now a Visiting Professor. Professor Rainbow has been an editor of the *Journal of Zoology* and is on the editorial boards of *Environmental Pollution, Marine Environmental Research* and the Journal of the Marine Biological Association, UK. In 2002 Philip Rainbow was invited to give the Kenneth Mellanby Review Lecture by the journal *Environmental Pollution* at the Society of Environmental Toxicology and Chemistry annual meeting at Salt Lake City, Utah. He has more than 200 peer-reviewed publications including 5 co-edited books and two co-authored books. The first (*Biomonitoring of Trace Aquatic Contaminants*, with DJH Phillips) went to two editions. The second has recently been published (2008) by Cambridge University Press, co-authored with Professor Sam Luoma — *Metal Contamination in Aquatic Environments: Science and Lateral Management*. Philip Rainbow's recent research has focused on the factors affecting the bio-

availability of trace metals to aquatic invertebrates from both solution and the diet, and the biodynamic modelling of trace metal bioaccumulation.

Michèle Roméo earned her PhD at the University of Nice–Sophia Antipolis in 1975 and has belonged to the French National Institute for Health and Medical Research (INSERM) since that time. She has been working as a researcher in the Marine Biological Station of Villefranche-sur-Mer and, since 1992, in the University of Nice–Sophia Antipolis and in the laboratory ROSE (Responses of Organisms to Environmental Stress), which became ECOMERS (Ecology of Marine Ecosystems and Response to Stress), where she is presently the head of the ecotoxicology team. Her general field of research concerns the response of aquatic organisms to chemical stress (metals and persistent organic pollutants) considered in terms of biomarkers (biomarkers of oxidative stress and of general damage leading to behavioural alterations). The chosen models are bivalve molluscs: mussels and clams and their larvae. Since 2004, research has evolved to the cloning of some genes coding for proteins used as biomarkers and to the use of a DNA microarray technique (genomics) and proteomics. These last two techniques allow measuring of the simultaneous expression of hundreds of genes (genomics) or proteins (proteomics) with very high sensitivity.

Roméo has been the head of the research project PNETOX with the French Ministry of the Ecology and Sustainable Development, and participated in another directed by Dr. Amiard-Triquet (University of Nantes, France). She has authored and coauthored around one hundred papers. She has participated in writing chapters for several books (for Elsevier, Lavoisier Tec and Doc, Taylor and Francis, and Humana Press). She is referee for more than ten journals. Her teaching activities concern ecotoxicology lectures in the master of applied biology of the University of Corsica and the master of environment management and sustainable development of the University of Nice–Sophia Antipolis.

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1 Pollution Tolerance
From Fundamental Biological Mechanisms to Ecological Consequences

Claude Amiard-Triquet

CONTENTS

1.1 INTRODUCTION

Tolerance may be defined as the ability of organisms to cope with stress, either natural, such as temperature changes, salinity variations, oxygen level fluctuations, and plant toxins, or chemical, depending on anthropogenic inputs of many different classes of contaminants into the environment. Resistance is frequently used in the scientific literature as a synonym for tolerance. Several authors have tried to clarify these terms (Lotts and Stewart 1995; Morgan et al. 2007). However, the definitions they proposed were strongly different, and none of them is currently generally adopted. In this book, most of the authors use the term *tolerance* in acceptance of the

general definition above. However, the use of the term *resistance* has been preferred here by some authors, particularly those interested in the genetic basis of an organism's ability to survive in a contaminated environment. In these cases, the authors will clearly specify their choice of terminology in their chapters.

Tolerance has been described in many taxonomic groups exposed to toxicants at sublethal levels. Tolerance may be achieved by many biological processes responsible for physiological acclimation or genetic adaptation. Among tolerant species, some have a key role in ecosystems. Understanding the frequency of occurrence of tolerance has tremendous implications for the sustainability of biodiversity. Processes involved in tolerance are energetically expensive, and thus may interfere with the allocation of energy, thereby governing the success of reproduction and growth. Reduction of the overall amount of genetic variation in populations exposed to a strong selective pressure can result in increased sensitivity to new stresses in tolerant organisms. Thus, the adaptive benefit of being tolerant may have negative counterparts in the long term. On the other hand, tolerance may be a source of concern in that it allows the survival of harmful species (mosquitoes, pathogenic bacteria) and the presence of highly contaminated links in food webs. From an operational point of view, mechanisms involved in tolerance may be a source of biomarkers of exposure. On the other hand, the history of experimental populations, either tolerant or sensitive, may be a confounding factor in the interpretation of bioassays. This volume brings together reviews on these several aspects of the tolerance of organisms to pollutants, with the ultimate aim of understanding the ecological consequences of such tolerance.

1.2 HOW MAY TOLERANCE BE ASSESSED?

The existence of tolerance in a given species or in one or more of its constituent populations may be revealed in many different biological responses to stress, the common feature being that higher levels of stress are necessary to induce an impairment of response in tolerant organisms than in their nontolerant (more sensitive) counterparts. Comparative survival to acute toxicity doses has been used in a number of studies with various species and contaminants, such as in the case of metal exposure of the worm *Nereis diversicolor* (Ait Alla et al. 2006 and literature cited therein), and different fish species (Lotts and Stewart 1995; Hollis et al. 1999; Chowdhury et al. 2004). Differential survival has also been observed in organisms exposed to organic compounds, for instance, in the decapod crustacean *Palaemonetes pugio* exposed to fluoranthene (Harper-Arabie et al. 2004) or the European eel *Anguilla anguilla* exposed to pesticides (Peña-Llopis et al. 2001, 2003).

Less harsh and simplistic experimental approaches to detect the presence of tolerance are frequently preferred, based on sublethal doses of exposure. Under these conditions, the toxicological parameters of interest may be measured in the medium or long term and include longevity or functional impairments. For instance, in the crustacean *Daphnia magna* exposed to the herbicide molinate for two generations, Sánchez et al. (2004) have observed increased longevity and reproduction in specimens belonging to the second generation compared to their parents. In the fish species *Catostomus commersoni*, the fertilisation rate and the quality of gametes were

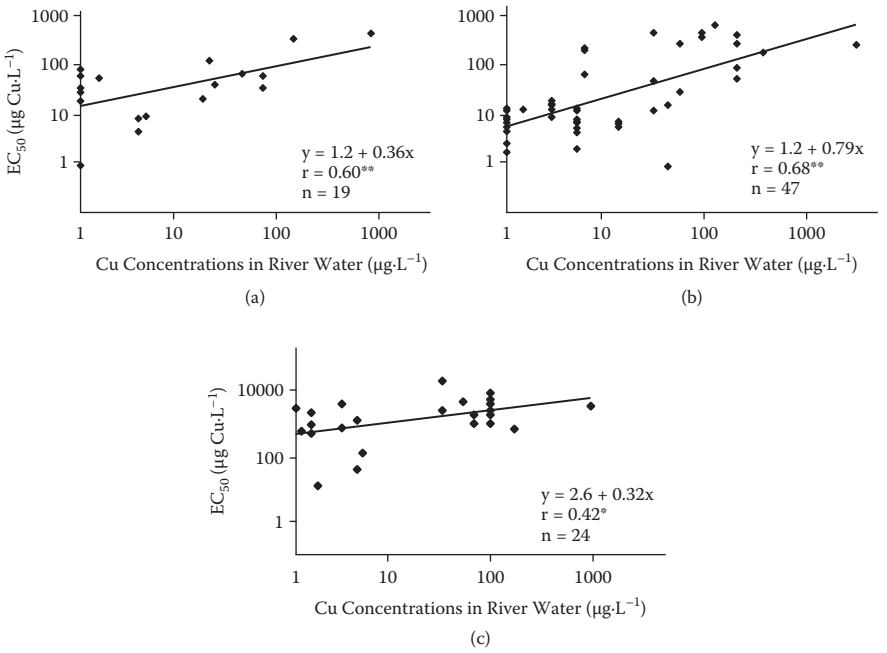

FIGURE 1.1 Cu concentrations in riverine waters giving 50% inhibition of photosynthesis in microalgae belonging to three different taxa: (a) Cyanophyceae, (b) Bacillariophyceae, and (c) Chlorophyceae (Chlorococcales). *, significant at the 95% level; **, significant at the 99% level. (After Takamura, N., et al., *J. Appl. Phycol.*, 1, 39–52, 1989.)

used to reveal tolerance to metals in field populations living in an area impacted by mining activities (Munkittrick and Dixon 1988). Burlinson and Lawrence (2007) based a comparison of tolerant and nontolerant populations of the worm *N. diversicolor* on behavioural disturbances.

In primary producers, growth is commonly used as a toxicological parameter to determine the noxious effects of contaminants. In addition, photosynthesis inhibition is considered a valuable measure to compare tolerance between taxa or populations within the same species, as exemplified in Figure 1.1. Takamura et al. (1989) have determined the effective concentrations of copper that reduce photosynthesis (EC_{50}) in many different strains of microalgae originating from rivers that are relatively clean or impacted by mining activities.

In any case, when the genes for resistance are well known, tolerance may be assessed by quantifying these genes. In populations or communities exposed to pollution in their environment, an increase of the tolerant genes is expected. However, high gene flow from neighbouring populations less exposed to contamination may be responsible for a moderate increase of the suspected tolerant genes, as suggested, for instance, in the European flounder, *Platichthys flesus*, along the French Atlantic Coast (Marchand et al. 2004). In some cases, for instance, in bacteria, the genes involved in resistance are well known, such as the merA gene, which encodes for a protein involved in the reduction of the toxic mercuric ion Hg^{2+} into the volatile and

less toxic elemental mercury Hg^0. Thus, in water, soil, and sediment, the merA gene is a relevant model in ecological studies for assessing relationships between microbial mercury resistance and bioavailable mercury contamination of the environment (Ramond et al. 2008).

1.3 INTER- AND INTRASPECIFIC VARIABILITY OF TOLERANCE

The existence of tolerant versus sensitive species may be exemplified in many taxa from microorganisms to invertebrates and vertebrates. It is necessary to keep this in mind when extrapolating from ecotoxicological data determined in a small number of standard species in the laboratory to the huge number of animals in the real environment. In addition to this inherent tolerance, populations within a species, exposed to environmental contaminants, can develop tolerance, based on either acclimation or adaptation. Among specialists, *acclimation* is the term used when the organisms are able to cope with pollutants as a result of nonheritable physiological processes. When the mechanisms involved in tolerance are genetically based and can be transferred to the progeny, the term *adaptation* is preferred. The latest thinking on physiological acclimation and genetic adaptation is reported in Chapter 2.

1.3.1 Interspecific Variability of Tolerance

In microalgae, the effects of metals have been studied considering many species belonging to different taxa, some of them represented by a consistent number of species (Figure 1.1). Among Chlorophyceae, the EC_{50} for copper was generally between 100 and 10,000 $\mu g \cdot L^{-1}$ in river waters with low levels of copper, whereas it was between 1 and 100 $\mu g \cdot L^{-1}$ for species belonging to Cyanophyceae and Bacillariophyceae. For cadmium and zinc, the differences of sensitivity between species belonging to these taxa also differed by orders of magnitude (Takamura et al. 1989).

In invertebrates, it has been demonstrated that important differences occur even between species belonging to a restricted taxonomic group—the bivalves—which in addition share the same mode of feeding (filter feeders). The oyster *Crassostrea gigas* and the scallop *Chlamys varia* are strong accumulators of silver, whereas mussels from both freshwater (*Dreissena polymorpha*) and seawater (*Mytilus edulis*) are weak accumulators. Among the strong bioaccumulators, one of the species is tolerant (*C. gigas*), whereas the other is sensitive (*C. varia*). Similarly, among weak accumulators, the freshwater mussel is tolerant, whereas the marine mussel is sensitive (Berthet et al. 1992). In addition, the impairments are very different for each species, being limited to metabolic effects in oysters, whereas in marine mussels, gill cells were damaged, and in scallops, byssus secretion was inhibited with consequences on behaviour, leading eventually to death.

Biomonitoring programmes implemented after the numerous oil spills that have impacted coastal areas have revealed consistent features concerning the relative sensitivity of invertebrate taxa to petroleum hydrocarbons (Gómez Gesteira and Dauvin 2000). Because there has been a very low impact of the spills on polychaetes, but a high one on amphipod crustaceans, these authors have proposed the use of a polychaete/amphipod ratio to monitor temporal changes of macrofauna in soft-bottom

communities. Similarly among species from the meiobenthos, nematodes show a greater tolerance to petroleum hydrocarbons (and also to hypoxia) than copepods.

In invertebrates, interspecific differences in tolerance are generally attributed to the variety of the processes developed to cope with stress. In vertebrates, regulation physiology is better developed and less diversified, with a greater homogeneity of physiological strategies allowing survival in a contaminated environment. However, even in fish, interspecific differences are well documented, such as differences in the behavioural responses of marine teleosts to the presence of copper (Scarfe et al. 1982), or differences in the toxicity of organophosphorus pesticides to freshwater fish, which can reach one to two orders of magnitude (Keizer et al. 1995). Oliveira et al. (2007) have carried out an extensive comparison of the responses of more than twenty species of marine fish to methyl paraoxon with a view to the use of acetylcholinesterase activity (an enzyme activity that is affected by exposure to organophosphorus and carbamate pesticides) as a pesticide biomarker. Their results suggest a possible evolutionary linkage of AChE sensitivity to methyl paraoxon.

Interspecific variability of tolerance is at the basis of the pollution-induced community tolerance (PICT) concept proposed by Blanck et al. (1988) and revisited in this book (Chapters 4 and 14). A biological community is composed of different species, the inherent sensitivity of which toward a given toxicant is highly variable, as documented above. Thus, in a contaminated environment, the most sensitive organisms are lost as a consequence of pollutant pressure, whereas tolerant organisms are maintained. Consequently, the new community as a whole is more tolerant to the toxicant responsible for selection than another community, initially identical, but which has never been exposed to this toxicant. Such a PICT has been demonstrated in many studies on periphyton (e.g., Blanck et al. 1988, 2003) and nematodes (Millward and Grant 1995, 2000), and the same philosophy is behind the use of lichen communities in the monitoring of air pollution (Hawksworth and Rose 1976, quoted in Millward and Grant 1995).

1.3.2 Tolerance Acquired in Populations Previously Exposed to Pollutants

In addition to the tolerance characteristics of different species, it is well established that, within the same species, populations previously exposed to chemicals in their environment are able to cope more efficiently than "naive" individuals. Carbamate resistance in mosquitoes was described as early as 1966 (Georghiou et al. 1966), and the importance of insecticide resistance has been recognised for ecological and evolutionary aspects as well as for management (McKenzie 1996; Denholm et al. 1999; Ishaaya 2001; Hemingway et al. 2004; Coleman and Hemingway 2007; Labbé et al. 2007). For decades, plant tolerance to herbicides (LeBaron and Gressel 1982) and metals (Shaw 1989) has been recognised, and because tolerant plants are often strong accumulators, their role in remediation of a metal-contaminated environment has given rise to many studies (Li 2006). Among the best known examples, the resistance of bacteria to antibiotics (and other chemicals) (Ramos et al. 2002; De 2004) has been a topic of major interest because of the consequences for human health

(limiting the efficiency of many drugs) and ecosystem functioning (allowing subnormal biogeochemical cycles and also remediation) (De 2004). Individual chapters in this book are devoted to the responses of bacteria (Chapter 14), metal-tolerant plants (Chapter 15), and resistant insects (Chapter 16). Metal tolerance in aquatic organisms is well established after a review by Klerks and Weis (1987). More recently, Amiard-Triquet et al. (2008) drew attention to acquired tolerance in microalgae and Cyanobacteria after exposure to metals, polychlorinated biphenyls (PCBs), and different classes of pesticides; in different taxa of annelids exposed to several metals; in crustaceans exposed to metals and pesticides; and in fish exposed to metals, PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and polyaromatic hydrocarbons (PAHs).

Many studies on contaminant effects focus on acute exposures and short-term effects. In these studies, the detoxificatory processes, which will be reviewed below, are immediately overwhelmed (see Figure 1.3) and do not allow organisms to cope with contaminants as they do in the field. However, at sublethal doses, these mechanisms are functional, and many of them respond on the scale of days or weeks. For instance, in worms *Nereis diversicolor* exposed for ninety-six hours to sediments from reference and contaminated sites in the Mira and Sado estuaries (Portugal), Moreira et al. (2006) examined a number of biochemical parameters involved in detoxification processes, antioxidant defence, and an indicator of oxidative stress. Even after this relatively short duration of exposure, compensation mechanisms were already activated, enzyme activities showing generally higher values for worms from the contaminated sites. Preexposure to chemicals is responsible for the induction of detoxification processes, subsequently allowing a different response between preexposed and naive individuals, as shown, for instance, in the mussel *Perna viridis* exposed to cadmium for only one week (Ng et al. 2007). Lotts and Stewart (1995) have demonstrated that minnows were able to acclimate to total residual chlorine (TCR) within a short period of exposure (seven to twenty-one days). Their study may explain why several species of fish were observed in aquatic systems where TCR concentrations were large enough that fish kills would have been expected. Previous papers reported by these authors indicated similar fast acclimation to TCR in crayfish or copepods. However, the protection provided by acclimation is limited since de-acclimation of minnows can also occur over a short period (seven days). In the fish *Catostomus commersoni* living in copper- and zinc-contaminated lakes, the tolerance of larvae at the yolk-supported stage is lost when larvae begin feeding, twenty-four days after hatching (Munkittrick and Dixon 1988).

When animals are chronically exposed to contaminants in the field or over several generations in the laboratory (particularly in this case, when many generations are produced over a short duration, as known for microorganisms or small invertebrates like *Daphnia*, mosquitoes, etc.), there is a possibility that they acquire tolerance as a result of genetic adaptation. This phenomenon represents a protection both for individuals living in an impacted environment and for their progeny, allowing the durability of local populations in contaminated environments.

1.3.3 Choice of Tests Organisms and Sentinel Species

Sentinel species may be used for different objectives, including (1) risk assessment based on bioassays developed in agreement with national and international regulatory bodies; (2) monitoring of biodiversity, including the use of indicator species, the presence or absence of which reveals environmental changes at work; and (3) the use of sensitive and early biological responses (biomarkers) able to reveal noxious effects in biota well before local extinction of species occurs (Berthet 2008). In all these three cases, intra- or interspecific tolerance must be considered for a relevant choice of sentinel species (Chapter 3).

To assess environmental risk in habitats exposed to chemicals, the following procedure is often recommended. After bioassays on a number of species (for instance, the European Community Water Framework Directive recommends the use of (1) algae or macrophytes, (2) *Daphnia* or marine organisms, (3) fish) have been carried out, security factors are applied and guidelines are established, indicating the maximum concentrations (in water, sediments or soils, food species) below which the environmental quality is considered good. These guidelines are published for individual contaminants, neglecting the fact that in natural environments, living organisms are generally exposed to complex mixtures of contaminants that can interact with each other, but also that changes in natural conditions can interfere deeply with the ability of biota to cope with chemical stress. For decades, it has been hypothesised that those species that are able to cope with natural stress in their environment (e.g., estuarine species exposed to large and fast fluctuations of salinity, oxygen, and temperature) are able to tolerate additional stress due to chemical inputs linked to anthropogenic activities. This assumption has been revisited, for instance, by Hummel et al. (1997) and Heugens et al. (2001), and their conclusions are not so optimistic. Thus, it is indispensable to discuss the state of the art in this field (Chapter 5).

1.4 MECHANISMS OF DEFENCE INVOLVED IN TOLERANCE TO CHEMICAL STRESS

Biochemical mechanisms allowing aquatic biota to cope with the presence of chemicals in their environment have been recently reviewed (Amiard-Triquet et al. 2008). Many of them are based on processes involved in defence against natural substances. It is particularly evident in the case of metals (Chapter 6) that are normal constituents of the earth's crust and are present everywhere in our environment as traces, some of them (essential metals such as copper or zinc) even being indispensable for a number of vital functions. However, at very high doses, even essential metals can turn toxic, and anyway, this class of contaminants includes nonessential metals (e.g., mercury, lead) that are known only for their toxicity.

Organisms are also exposed to natural organic compounds, such as plant toxins, and a number of species are well equipped to face the challenge of deriving energy from food containing molecules that are highly toxic for others. Some examples include coniferyl benzoate, a secondary metabolite of aspen buds, which are commonly used as food by grouse *Bonasa umbellus* (Guglielmo et al. 1996); terpenoids,

alkaloids, and ranunculin present in the plants preferentially consumed by the tortoise *Testudo horsfieldi* (Lagarde et al. 2003); terpenes in eucalyptus, an important food items for possums (Sorensen et al. 2007); etc.

In addition to these biochemical mechanisms, behavioural responses can contribute to the defence of organisms in environments submitted to toxic chemical inputs. Detection of a toxicant (in water, soil or sediment, food) can induce an avoidance response. Avoidance may be a strategy to escape exposure, sometimes in the short term only (e.g., valve closure in bivalves), but in certain cases also in the long term (e.g., food selection).

1.4.1 Biochemical Mechanisms

The cytochrome P450s (CYPs) are a group of enzymes responsible for the oxidative metabolism of a wide range of organic compounds. The CYPs are a well-supported counterdefence mechanism employed by herbivores to metabolise, and subsequently eliminate, ingested plant secondary metabolites (PSMs). Specifically, CYP3A is an important metaboliser of PSMs in a variety of herbivores (Sorensen et al. 2007). Biotransformation of organic xenobiotics such as hydrocarbons, organochlorine insecticides, and polychlorinated biphenyls (PCBs) also involves the cytochrome P450 monooxygenase system (Newman and Unger 2003). Metabolites resulting from this phase I reaction can be acted on in phase II reactions that involve conjugation with endogenous compounds (carbohydrate derivatives, amino acids, glutathione, or sulphate). The biotransformation of a highly lipophilic compound into a more water-soluble metabolite, more prone to elimination, is very often termed detoxification (Newman and Unger 2003). However, it must be kept in mind that biotransformation can also result in the production of reactive compounds that can be responsible for toxicity to cellular macromolecules, leading to toxification instead of detoxification, particularly by producing oxidative stress (Chapter 8).

Oxidative stress, i.e., damage to biomolecules from free oxyradicals (oxygen peroxide (H_2O_2), superoxide radical ($O^{\bullet}{}_2^-$), and hydroxyradical ($^{\bullet}OH$)), is experienced potentially by all aerobic life. Reactive oxygen species (ROS) are produced in organisms as a consequence of processes involving both endogenous and xenobiotic compounds. Because oxyradical-generating compounds are normally produced by aerobic metabolism, organisms are well equipped to face oxidative stress (Chapter 7), being able to produce antioxidants that react with oxyradicals (vitamins C and E, β-carotene, glutathione, etc.) and enzymes that reduce the amount of oxyradicals (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase) (Van der Oost et al. 2005). However, when antioxidant defences are overcome by prooxidant forces, increased concentrations of free radicals can cause a number of dysfunctions, including lipid peroxidation and changes in the structure and function of biomolecules, including DNA, with potential consequences such as genotoxic effects and increased risk of cancer (Newman and Unger 2003).

Lesions of biomolecules induce stress proteins able to recognise denatured or aggregated proteins that are then unfolded and refolded properly in order to restore their functions. When the damage is too great to be repaired, stress proteins ensure the breakdown and elimination of nonfunctional proteins. Because of their role,

stress proteins are also termed *chaperones* (Frydman 2001). They were first recognised in organisms submitted to drastic temperature changes, the reason why they were initially known as heat shock proteins. In fact, such cellular stress responses may be elicited by both physical (temperature, ultraviolet radiation) and chemical (metals, organic xenobiotics) agents. Stress proteins have evolved very conservatively, and their induction has been recognised in many bacterial, plant, and animal species. They appear as parts of a universal process, able to contribute to tolerance in biota exposed to environmental contaminants (Chapter 9).

Because the study of the ecotoxicology of metals has taken advantage of efficient analytical procedures long before they were reliable for organic xenobiotics, the knowledge of adaptive strategies adopted by organisms exposed to metals in their environment is particularly well developed. Briefly, they include the control of metal uptake (for instance, by binding metals at the surface of the cell as a result of the secretion of mucus), the control of intracellular metal speciation (mainly by biomineralisation or binding to detoxification proteins such as metallothioneins in animals and phytochelatins in plants), and the elimination of excess metals (Chapter 6). In the case of organic xenobiotics, little is known about the first process. Contrary to what happens with incorporated metals, detoxification of organic compounds is based mainly on biotransformation, even if certain molecules such as organochlorines are not metabolically active when stored in reserve lipids.

A transmembrane P-glycoprotein (P-gp) has been recognised as responsible for the resistance of some tumour cells to anticancer drugs (multidrug resistance (MDR)). It prevents the accumulation of cytotoxic drugs, by acting as an energy-dependent efflux pump. P-gp-like proteins have been described in many nonmammalian organisms, including even invertebrates (sponges, mussels, oysters, clams, worms). In addition to natural products, environmental contaminants may be translocated, thus preventing cellular accumulation in exposed biota. With reference to MDR, this phenomenon has been termed *multixenobiotic resistance* (MXR). As early as 2000, Bard suggested that the induction of a multixenobiotic defence mechanism may explain why some species are able to face the challenge of surviving in polluted environments. However, it is necessary to examine if it is a general phenomenon and what are the limits of MXR as a protective system against environmental contaminants (Chapter 10). For instance, in the case of metals, the detoxificatory role of MXR is not clearly established. In the Gironde estuary strongly contaminated by metals (Cd, Cu, Pb, Zn), no influence on the expression of a MXR-type system was observed in oysters (Minier et al. 1993). In addition, the protective role of MXR may be counteracted in the presence of inhibitors (emerging contaminants, natural substances produced by certain invasive species) at doses that may be encountered in the natural medium (Smital et al. 2004; Luckenbach et al. 2004). In the Seine estuary, despite enhanced levels of MXR proteins being determined in the freshwater mussel *Dreissena polymorpha*, impaired condition index and decreased lysosomal stability were also observed (Minier et al. 2006).

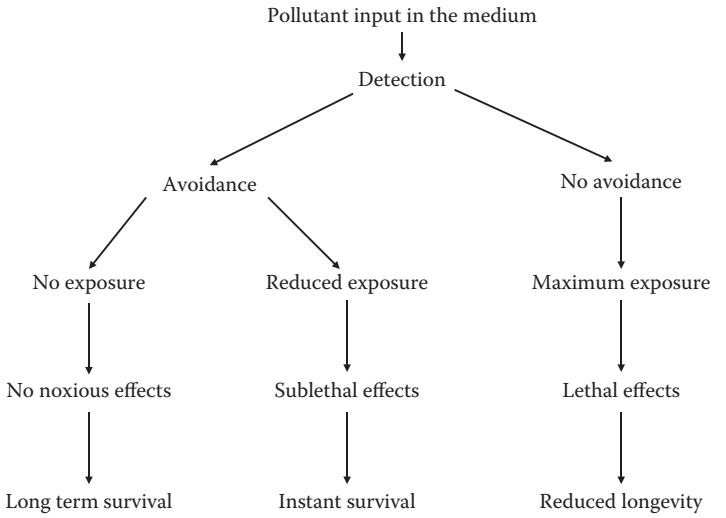

FIGURE 1.2 Different types of animal responses to the presence of contaminants and consequences for the fate of individuals. (After Amiard, J. C., *Océanis Doc. Océanogr.*, 9, 465–80, 1983.)

1.4.2 Behavioural Responses

Facing a chemical input in its environment, an animal will react differently, depending on its ability to detect or not a pollutant (Figure 1.2). If the toxic chemical is not detected, no avoidance will take place and the organism will be submitted to maximum exposure with drastic potential effects. If the pollutant is readily detected, the animal will be able to avoid at least partly the contaminated water mass, sediment/soil, or food, thus leading to a reduced exposure with limited effect on long-term survival when the animal leaves the contaminated area.

Avoidance reactions have long been studied, and the capability of avoiding metals and organic compounds is well known in both invertebrates and vertebrates (for a review, see Amiard-Triquet and Amiard 2008). Biological early warning systems have taken advantage of avoidance responses for monitoring water quality, particularly in freshwaters, but some of them are also applicable in estuaries and coastal waters. Fish monitors have been developed for the protection of potable water intakes and are sensitive devices particularly for the detection of a wide range of metals and some polyelectrolytes. In bivalves, the closure of the shell is a typical example of an escape behaviour response under stress. Warning systems have been developed using the valve movement response of both marine mussels and the freshwater mussel *Dreissena polymorpha* (see review by Baldwin and Kramer 1994).

Food selection is an important component of defence against toxic molecules. Plant secondary metabolites (PSMs) are a major constraint to the ingestion of food by herbivores. Recent reviews indicate that herbivores can use diet choice and the rate and amount of PSM consumption to prevent the concentration of PSM in blood

from reaching levels able to produce significant adverse effects (Marsh et al. 2006; McLean and Duncan 2006). Avoidance of metal-enriched food has been recognised in the freshwater crustacean *Gammarus pulex* exposed to zinc (Wilding and Maltby 2006). In the terrestrial isopod crustacean *Oniscus asellus*, individuals from a control population ingested artificially metal-enriched leaf litter material at the same rate as unpolluted food. In contrast, a population from a site in the vicinity of a mine was able to distinguish between metal-enriched and clean food, reducing food intake drastically during exposure when only contaminated food was available. In the field, this selective feeding enables *O. asellus* to survive under high metal concentrations (Köhler et al. 2000).

In sediment-dwelling species, different types of environmental contaminants can disturb burrowing behaviour (Amiard-Triquet 2009; Bonnard et al. 2009). Such behavioural impairments have been observed even in organisms (the ragworm *Nereis diversicolor* and the clam *Scrobicularia plana*) exposed to naturally contaminated sediments. Cross experiments between specimens and sediments originating from contaminated versus reference sites have allowed us to distinguish between physiological disturbance and avoidance. In both species, animals are able to recognise contaminated sediments, and in this case, decreased burrowing speeds were registered (Mouneyrac et al. 2010; Boldina-Cosqueric et al. 2010). However, this avoidance behaviour has a poor adaptive value since disturbed burrowing facilitates predation, as demonstrated for the littleneck clam *Protothaca staminea*, allowed to burrow in clean sand or in sand mixed with Prudhoe Bay crude oil. Shallow burial and slow reburrowing in oiled sand were responsible for increased predation as a consequence of increased accessibility of clams to the Dungeness crab *Cancer magister* (Pearson et al. 1981). Nevertheless, when the conditions get particularly harsh, even species considered sedentary can get involved in migrations, such as *N. diversicolor* observed by Essink (1978, reported in Essink 1985) as they escape a confined area after the onset of organic waste discharge.

Anyway, it would be wrong to consider that all living organisms are able to escape polluted environments. The ecological context is indeed very important in determining whether or not pollutant-induced avoidance will occur in the wild. For instance, freshwater fish *Coregonus clupeaformis* avoid metals at low concentrations under standardised conditions of light, but in the case of competing gradients of light and metals, the fish prefer the contaminated shade to the uncontaminated high light intensity, except at the highest concentration tested (Scherer and McNicol 1998).

1.4.3 Limits of Defence Mechanisms

Mechanisms of defence provide useful biomarkers that have been widely studied in the laboratory and in the field (Van der Oost et al. 2005; Amiard-Triquet et al. 2008). However, low concentrations of metallothioneins or ethoxyresorufin O-deethylase (EROD; a phase I biotransformation enzyme) in contaminant-exposed organisms do not necessarily indicate stress insensitivity. Although many heavy metals have been shown usually to induce metallothioneins in many organisms, including both vertebrates and invertebrates (Amiard et al. 2006), the concentrations of these proteins

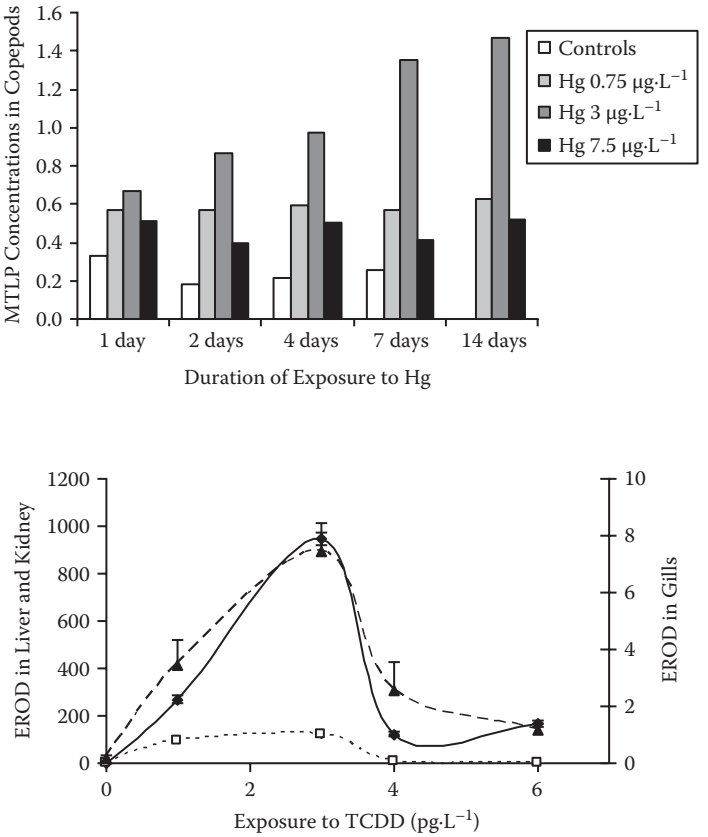

FIGURE 1.3 Different responses of biomarkers along a pollution gradient. Top: Metallothionein-like protein content (MTLP in $\mu g \cdot g^{-1}$ wet weight) in the copepod *Tigriopus brevicornis* exposed for 1 to 14 days to mercury; EROD (ethoxyresorufin O-deethylase in pmol $res^{\cdot -1}$ $min^{\cdot -1}$ mg protein) in kidney (dotted line), gills (dashed line), and liver (solid line) of the fish *Sparus aurata* exposed to TCDD for 20 days. (Data kindly provided by Barka, S., et al., *Comp. Biochem. Physiol.*, 128C, 479–93, 2001; Ortiz-Delgado, J. B., et al., *Ecotoxicol. Environ. Saf.*, 69, 80–88, 2008.)

follow a curve with a maximum at rather high but not extreme concentrations (Figure 1.3). Despite it being considered a reliable marker of exposure to dioxin, PCBs, and PAHs in fish, a similar bell-shaped relationship may be observed for EROD (Figure 1.3). Similar patterns have been mentioned by Dagnino et al. (2007) for GST (a phase II biotransformation enzyme) and catalase (an enzyme involved in antioxidant defence). In response to very high toxicant concentrations, the protein levels or the enzymatic activities decrease, most probably due to severe pathological impact upon target organs as explained by Köhler et al. (2000) in the case of hsp 70. Consequently, interpreting these biochemical indices in terms of organism health is not a simple task.

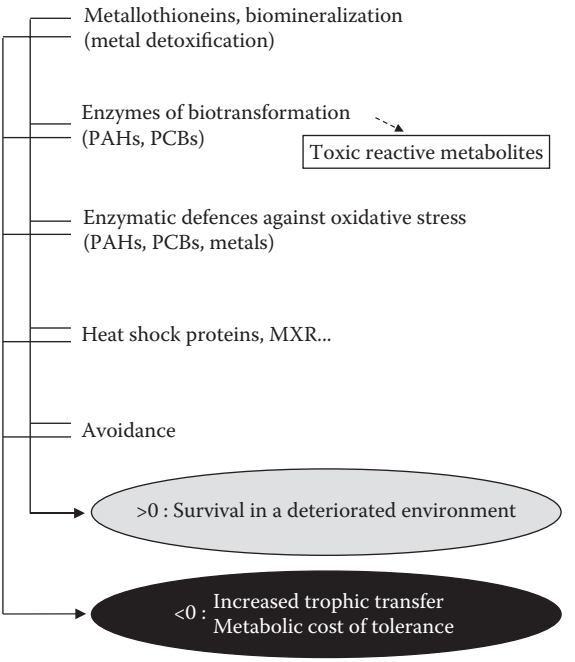

FIGURE 1.4 Some major causes and consequences of tolerance.

1.5 ECOLOGICAL AND ECOPHYSIOLOGICAL ASPECTS OF TOLERANCE

All the mechanisms of defence described above allow the survival of the most tolerant species and, within each species, the populations that have been able to acclimate or adapt to the presence of environmental contaminants (Figure 1.4). Thus, tolerance has an obvious positive aspect by contributing to the (partial) conservation of biodiversity—at least species diversity (Chapter 11)—since adaptations are suspected of being a source of reduction of genetic diversity (Chapter 2). On the other hand, the constitutive formation of mucus, enzymes, and other proteins at a high rate would be an energy-intense strategy to counteract the symptoms of toxicity, and would be likely to alter processes of energy allocation with potential consequences on fitness (Chapter 12). Significant modification of a population's genetic composition, including the selection of resistant genotypes, can reduce mean fitness, erode evolutionary potential, and contribute to the likelihood of local or even global extinction (cited opinion, Laroche et al. 2002). However, because a number of resistant species are able to cope with environmental pollution, they can constitute highly contaminated links in the food web, thus inducing a potential risk of trophic transfer (Chapter 13).

1.5.1 Conservation of Biodiversity

In some cases, tolerance to chemical stress is efficient only for a short period, for instance, when an animal is able to isolate itself from its medium when it has detected

the presence of a toxic chemical. In bivalves, valve closure is an efficient behavioural response at least for short-term exposure, but the bivalves suffer respiration disturbances that can become lethal within days, whereas in the long term, partial closure results in a reduction of food intake and absorbed energy, with potential effects on fitness. In mobile species, avoidance can result in animals' flight out of polluted media, a situation that is beneficial at the individual and species level, but from an ecosystem point of view, it plays the same role as a local extinction, decreasing local biodiversity. Other examples have been cited (see Section 1.2.2) showing a temporary acclimation that permits survival in the short or medium term, for instance, in the cases of a pollution accident or pulse inputs of effluents.

On the other hand, when the acquisition of tolerance results from genetic adaptation in populations that have been exposed for generations to selection pressure due to the presence of toxicants in their environment, it represents an efficient protection for the local population of a species, allowing survival and reproduction, even if tolerance is not an all-purpose remedy (see Section 1.5.2 about the cost of tolerance and major problems associated with cross-tolerance of bacteria to antibiotics and contaminants (Chapter 14) and insecticide tolerance in insects (Chapter 16)). Many, but not all, populations in polluted areas do have an increased tolerance, and in particular, there is more evidence for the evolution of tolerance in microorganisms and small invertebrates than in macrofauna. In addition, Klerks and Weis (1987) have identified a potential bias in the reporting of field examples of tolerance since it is questionable whether negative results have an equal chance of being published in the relevant scientific literature.

Ascending from population to species level, it is clear that acute pollution resulting from accidents will be responsible for the local loss of the most sensitive species. Even in the case of chronic contamination, when chemical stress increases, sensitive species will disappear first, followed by less sensitive species. For instance, in a river impacted by historical mining activities, lotic insect species that store metals in nondetoxified form were rare or absent in the most contaminated sections, whereas tolerant species, which have efficient detoxification strategies, remained present (Cain et al. 2004). As mentioned above (Section 1.2.1), interspecific variability of sensitivity is at the origin of communities showing pollution-induced community tolerance (PICT). PICT is an important phenomenon in the conservation of ecosystem functioning even in areas submitted to toxicant pressure high enough to provoke local species extinction. For instance, in the Seine estuary, one of the largest estuaries in France, diatom assemblages—which are the main constituents of the microphytobenthos on the surface of the mudflats—were consistently different from those observed in a small reference estuary (Sylvestre 2009). Nevertheless, the photosynthetic performances of the two microphytobenthic communities, measured under the same environmental conditions (light and temperature), were quite similar, as was also the gross community primary production in $mgC.m^{-2}.h^{-1}$ (Migné et al. 2007; Amiard-Triquet et al. 2009).

Depending on the functional role of different species in the community, indirect effects of tolerance will appear following different patterns (Fleeger et al. 2003). If a sensitive species is a prey or a host species, its extinction will lead to a depletion of its predator or symbiont populations (Figure 1.5). On the contrary, if a sensitive species

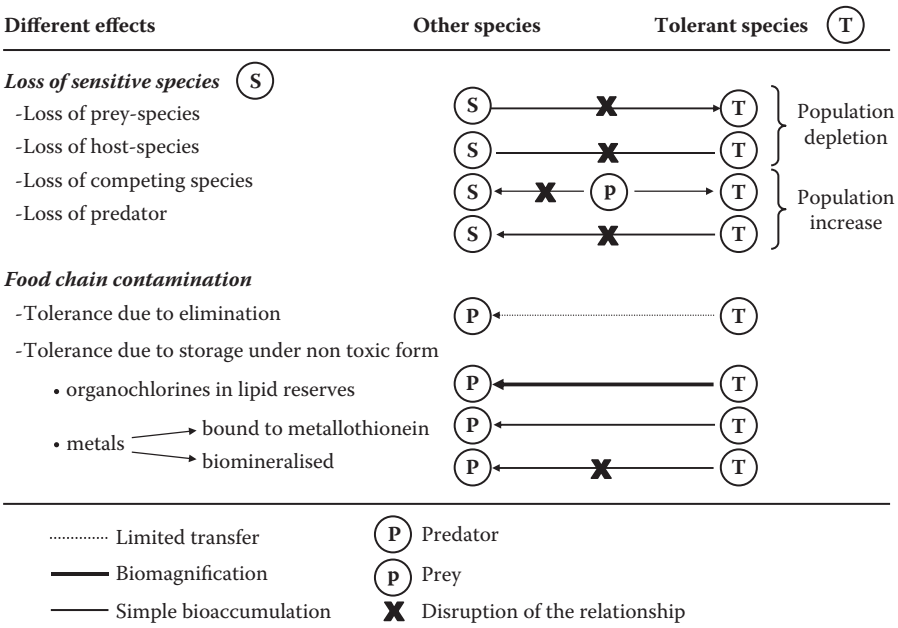

FIGURE 1.5 Indirect ecological effects of tolerance. (After Moore, N. W., in *Advances in Ecological Research*, ed. J. B. Cragg, Academic Press, New York, 1967, Vol. 4, pp. 75–129; Amiard-Triquet, C., et al., in *Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques*, ed. J. C. Amiard and C. Amiard-Triquet, Lavoisier, Paris, 2008, pp. 55–94.)

is a competitor or a predator of a tolerant species, the extinction of the former will favour the latter, the populations of which will increase.

Among organisms that have strategies (at the individual, population, or species level) allowing them to cope with chemical stress, some of them are keystone species, with important roles in ecosystem functioning, on which numerous species will depend. Thus, resistant bacteria will be able to intervene in biogeochemical cycling of nutrients. Primary producers using these nutrients will contribute to the normal functioning of food webs, the basis of which they constitute, and so on to successive trophic levels. Earthworms in terrestrial environments or endobenthic organisms in aquatic environments will remain able to rework soil or sediment, an important process for the oxygenation of porewater and fate of organic matter and nutrients. Within a given type of ecosystem, several species able to fulfil the same functional role are often present concomitantly. However, in other media, the natural conditions are harsh enough to limit the number of such equivalent species even in the absence of any anthropogenic stress. For instance, it is well established that estuarine species are much less numerous than freshwater or marine species (McLusky 1989). Thus, in estuaries that are among the most polluted areas across the world, it is evident that the extinction of even a small number of species is sufficient to affect ecosystem function.

1.5.2 The Cost of Tolerance

Combating chemical toxicants has physiological costs and subsequent ecological implications. Since Calow (1991) conceptualised these cascading effects, his paper has been quoted in numerous studies to explain the increase of metabolic rates in organisms exposed to stress, resulting in the induction of metallothioneins, heat shock proteins, biotransformation enzymes, or the implementation of antioxidant defences (Amiard-Triquet et al. 2008). In several species (the freshwater decapod *Palaemonetes paludosus*, the banded water snake *Nerodia fasciata*, the frog *Rana catesbeiana*) differing deeply in their phylogeny, exposure to trace element-rich coal combustion waste induced elevated maintenance costs, a similarity that suggests that this may be a general response to metal contaminants (Rowe 1998). Leung et al. (2000), similarly, have suggested that metabolic depression in cadmium-exposed specimens of the marine gastropod *Nucella lapillus* may be a strategy to minimise the uptake and toxicity of cadmium while meeting the extra energy demand for detoxification (mucus production and metallothionein synthesis) and maintenance (e.g., repair of cellular damage). The energy cost of biochemical defences is an even more general phenomenon since it has been also shown for various organic substances in different taxa (biotransformation of plant toxins or pyrene, respectively, by the grouse *Bonasa umbellus*, studied by Guglielmo et al. (1996), and in hepatocytes of the trout *Oncorhynchus mykiss* studied by Bains and Kennedy 2004). In the fish *Leporinus elongatus*, it appears that a significant part of the energy available under severe O_2 restriction may be directed preferentially to the synthesis of antioxidants at the expense of food consumption and weight gain, which consistently decreased (Wilhelm Filho et al. 2005). Even if the cost of tolerance is well documented, only a few studies have allowed a precise quantification of the fraction of total absorbed energy, which is devoted to defence mechanisms. In addition, when impairments (changes in oxygen consumption, levels of energy reserves, condition indices, growth, reproduction) occur in organisms exposed to chemical stress, separating the relative contribution of the cost of tolerance and direct toxic effects of contaminants is far from clear. Thus, it is crucial to determine the ramifications of pollution-induced modifications in energetics on individual- and population-level parameters before importance can be ascribed to the cost of tolerance (Chapter 12).

Genetic adaptation evolved under the pressure of environmental contaminants can be assimilated to a maladaptation in another context. For instance, recent studies with laboratory lines of fish have shown that increased resistance to chemicals has a cost, consisting of a higher susceptibility to other stress factors, such as UV, hypoxia, or increasing temperatures (Meyer and Di Giulo 2003; Xie and Klerks 2004). This may be a major concern in the context of climate change in the near future.

1.5.3 Trophic Transfer of Environmental Contaminants

The trophic transfer of environmental contaminants is partly linked to mechanisms involved in the tolerance of prey species (Figure 1.5). For instance, organochlorines stored in lipids have no toxic interference with animal metabolism until reserves are remobilised (for instance, during sexual maturation or migrations over long distances),

but during digestion by a predator or human consumer, they can be transferred to the next trophic level. Biotransformation efficiency and the chemical characteristics of the resulting metabolites are also crucial factors controlling the bioaccumulation and biomagnification of lipophilic environmental contaminants along a food chain. When metabolicity is low, or when it is high but leads to the formation of hydrophobic metabolites, high food chain bioaccumulation is likely to occur. When phase I metabolism is important but results in highly polar metabolites, the latter are easily excreted, thus limiting bioaccumulation in the food web (Chapter 13). Metals detoxified in the cytosol by binding to metallothioneins are more prone to assimilation by a predator than biomineralisation products such as crystals of silver sulphide or mercury selenide, which are very stable compounds resistant to degradation during digestion, whereas the fate of metals associated with other inorganic granules, for instance, may be more questionable (Chapter 13).

Fish are considered a very healthy food because they are rich in proteins, poor in saturated fats, and can be protective against coronary heart disease (CHD), since marine fish oils are rich in omega-3, an essential fatty acid, known to reduce CHD risk. However, lipophilic contaminants (such as mercury as methyl mercury, dioxins, PCBs, and brominated flame retardants) with high K_{ow}s are easily bioaccumulated (Chapter 13) and are present at the highest concentrations in the most lipid-rich fish. Intake of these environmental contaminants from fish may counterbalance beneficial effects. Virtually all humans who consume fish and shellfish have at least trace quantities of methyl mercury present in their tissues. Health effects at current levels of exposure in populations showed various effects with most evidence for neurodevelopmental and cardiovascular symptoms. A major outbreak of severe methyl mercury poisoning occurred in Minamata, Japan, caused by the presence of mercury in effluent from a chemical factory that contaminated the surrounding bay and its living resources; led to very high human exposure to methyl mercury via fish consumption; and resulted in severe injuries to people, of whom hundreds died (Harada 1995). Within the past decade there have been clinically obvious cases of mercury poisoning in the Songhua River region of China (Chun et al. 2001, cited by Mahaffey 2004). Artisanal gold mining has been a source of mercury exposure in the Brazilian Amazon and other countries in South America (Venezuela, Colombia, Bolivia, French Guyana, Guyana, Ecuador, and Peru) since the 1980s. Inorganic mercury, subsequently methylated and incorporated into fish, has produced severe exposures and neurological symptoms among people routinely consuming fish from these waters. Many reviews have described the nature and extent of this environmental pollution problem (e.g., Malm 1998; Maurice-Bourgoin et al. 2000). Other important human exposures are well documented among the Faroese population and the Inuit of Nunavik, as a consequence of the consumption of whale meat in addition to fish (Dewailly 2004).

Still in the aquatic environment, the presence of dioxins and other persistent organic pollutants (POPs) in farmed salmon received great media coverage (Hites 2004). The contamination of prey species, particularly seafood, able to tolerate high levels of POPs is also at the origin of increasing levels of these pollutants in the polar bear, a talisman species for conservation, with possible effects at the population level (Derocher et al. 2003).

Itai-itai disease (IID) was officially recognised in 1968 as the first disease induced by environmental pollution in Japan. IID was found initially in the cadmium-polluted Jinzu River basin in Toyama Prefecture. The patients of IID suffered from renal anemia, tubular nephropathy, and osteopenic osteomalacia. The degree of pollution in different parts of the endemic area, determined by analysing Cd concentrations in the soils of the paddy fields, was correlated with the prevalence of IID, whereas no phytotoxicity occurred; thus, rice was able to play the role of a pollution vector.

Another talisman species is the bald eagle, officially adopted as the U.S. national emblem on June 20, 1782. Use of the pesticide DDT after World War II poisoned eagles' foods and weakened eggshells, making them too thin to support the weight of brooding parents. A 1972 ban on DDT led to gradual improvements in the bald eagle population. Bald eagles eat fish, waterfowl, and small to medium-sized mammals. As supercarnivores, they are at the top of the food chain and particularly exposed to lipophilic POPs such as DDT, bioaccumulated to a lesser degree, and therefore tolerable in their prey species. According to the U.S. National Parks Conservation Association (http://www.npca.org), PCBs are emerging as another chemical threat due to food chain contamination, but nonchemical threats such as habitat loss resulting from development in coastal areas must not be ignored.

1.6 CONCLUSIONS

It is important to establish the state of the art in the field of tolerance to environmental contaminants because of many important aspects for science, management, and society. In 1987, Klerks and Weis wrote: "It seems dangerous to relax water quality criteria on the assumption that all populations in polluted environments will evolve an increased resistance" (p. 173). Is it now possible to go further in risk assessment and the implementation of toxicity reference values by regulation bodies, taking into account our knowledge about tolerance? Ecotoxicologists have made good progress in the knowledge of mechanisms, allowing organisms to cope with pollutants in their environment, but the links with potential effects at higher levels of organisation need to be more strongly established (Amiard and Amiard-Triquet 2008). While positive effects of tolerance at supra-organismal levels (population, community, ecosystem) for environment and health protection are often considered, the relative importance of any negative effects of tolerance is not typically fully assessed. The reviews offered in each chapter of this book will contribute to the provision of tools to carry out the appropriate risk-benefit analysis.

REFERENCES

Ait Alla, A., et al. 2006. Tolerance and biomarkers as useful tools for assessing environmental quality in the Oued Souss estuary (Bay of Agadir, Morocco). *Comp. Biochem. Physiol.* 143C:23–29.

Amiard, J. C. 1983. Les tests de toxicité subléthale: Approches biochimiques et éthologiques. *Océanis Doc. Océanogr.* 9:465–80.

Amiard, J. C., and C. Amiard-Triquet. 2008. *Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques*. Paris: Lavoisier.

Amiard, J. C., et al. 2006. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquat. Toxicol.* 76:160–202.

Amiard-Triquet, C. 2009. Behavioral disturbances: The missing link between sub-organismal and supra-organismal responses to stress? Prospects based on aquatic research. *Hum. Ecol. Risk Assess.* 15:87–110.

Amiard-Triquet, C., and J. C. Amiard. 2008. L'écotoxicologie du comportement. In *Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques*, ed. J. C. Amiard and C. Amiard-Triquet, 211–40. Paris: Lavoisier.

Amiard-Triquet, C., et al. 2009. Tolerance in organisms chronically exposed to estuarine pollution. In *Environmental assessment of estuarine ecosystems. A case study*, ed. C. Amiard-Triquet and P. S. Rainbow, 131–53. Boca Raton, FL: CRC Press.

Amiard-Triquet, C., C. Cossu-Leguille, and C. Mouneyrac. 2008. Les biomarqueurs de défense, la tolérance et ses conséquences écologiques. In *Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques*, ed. J. C. Amiard and C. Amiard-Triquet, 55–94. Paris: Lavoisier.

Bains, O. S., and C. J. Kennedy. 2004. Energetic costs of pyrene metabolism in isolated hepatocytes of rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* 67:217–26.

Baldwin, I. G., and K. J. R. Kramer. 1994. Biological early warning systems (BEWS). In *Biomonitoring of coastal waters and estuaries*, ed. K. J. R. Kramer, 1–28. Boca Raton, FL: CRC Press.

Bard, S. M. 2000. Multixenobiotic resistance as a cellular defense mechanism in aquatic organisms. *Aquat. Toxicol.* 48:357–89.

Barka, S., J. F. Pavillon, and J. C. Amiard. 2001. Influence of different essential and non-essential metals on MTLP levels in the copepod *Tigriopus brevicornis*. *Comp. Biochem. Physiol.* 128C:479–93.

Berthet, B. 2008. Les espèces sentinelles. In *Les biomarqueurs dans l'évaluation de l'état écologique des milieux aquatiques*, ed. J. C. Amiard and C. Amiard-Triquet, 121–48. Paris: Lavoisier.

Berthet, B., et al. 1992. Bioaccumulation, toxicity and physico-chemical speciation of silver in bivalve molluscs: Ecotoxicological and health consequences. *Sci. Total Environ.* 125:97–122.

Blanck, H., S. A. Wängberg, and S. Molander. 1988. Pollution-induced community tolerance—A new tool. In *Function testing of aquatic biota for estimating hazards of chemicals*, ed. J. J. Cairns and J. R. Pratt, 219–30. STP 998. Philadelphia: American Society for Testing and Materials.

Blanck, H., et al. 2003. Variability in zinc tolerance, measured as incorporation of radio-labeled carbon dioxide and thymidine, in periphyton communities sampled from 15 European river stretches. *Arch. Environ. Contam. Toxicol.* 44:17–29.

Boldina-Cosqueric, I., et al. 2010. Biochemical, physiological and behavioural markers in the endobenthic bivalve *Scrobicularia plana* as tools for the assessment of estuarine sediment quality. *Ecotoxicol. Environ. Saf.*, submitted.

Bonnard, M., M. Roméo, and C. Amiard-Triquet. 2009. Effects of copper on the burrowing behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and the bivalve *Scrobicularia plana*. *Hum. Ecol. Risk Assess.* 15:11–26.

Burlinson, F. C., and A. J. Lawrence. 2007. Development and validation of a behavioural assay to measure the tolerance of *Hediste diversicolor* to copper. *Environ. Pollut.* 145:274–278.

Cain, D. J., S. N. Luoma, and W. G. Wallace. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environ. Toxicol. Chem.* 23:1463–73.

Calow, P. 1991. Physiological costs of combating chemical toxicants: Ecological implications. *Comp. Biochem. Physiol.* 100C:3–6.

Chowdhury, M. J., E. F. Pan, and C. M. Wood. 2004. Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: Respiratory, ionoregulatory, and stress parameters. *Comp. Biochem. Physiol.* 139C:163–73.

Coleman, M., and J. Hemingway. 2007. Insecticide resistance monitoring and evaluation in disease transmitting mosquitoes. *J. Pest. Sci.* 32:69–76.

Dagnino, A., et al. 2007. Development of an expert system for the integration of biomarker responses in mussels into an animal health index. *Biomarkers* 12:155–72.

De, J. 2004. Mercury-resistant marine bacteria and their role in bioremediation of certain toxicants. PhD thesis, Goa University.

Denholm, I., J. A. Pickett, and A. L. Devonshire. 1999. *Insecticide resistance from mechanisms to management*. Wallingford, UK: CAB International.

Derocher, A. E., et al. 2003. Contaminants in Svalbard polar bear samples archived since 1967 and possible population level effects. *Sci. Total Environ.* 301:163–74.

Dewailly, E. 2004. Le cas du mercure. In *Les valeurs toxicologiques de référence en santé environnementale et en santé au travail: les comprendre, les appliquer.* http://www.inspq.qc.ca/jasp/archives.

Essink, K., et al. 1985. Population dynamics of the ragworm *Nereis diversicolor* in the Dollard (Ems estuary) under changing conditions of stress by organic pollution. In *Marine biology of polar regions and effects of stress on marine organisms*, ed. J. S. Gray and M. E. Christiansen, 585–600. New York: John Wiley & Sons Ltd.

Fleeger, J. W., K. R. Carman, and R. M. Nisbet. 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci. Total Environ.* 317:207–33.

Frydman, J. 2001. Folding of newly translated proteins *in vivo*: The role of molecular chaperones. *Annu. Rev. Biochem.* 70:603–47.

Georghiou, G. P., R. L. Metcalf, and F. E. Gidden. 1966. Carbamate-resistance in mosquitoes: Selection of *Culex pipiens fatigans* Wield (= *Culex quinquefasciatus*) for resistance to Baygon. *B. World Health Organ.* 35:691–708.

Gómez Gesteira, J. L., and J. C. Dauvin. 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. *Mar. Pollut. Bull.* 40:1017–27.

Guglielmo, C. G., W. H. Karasov, and W. J. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by Ruffed Grouse. *Ecology* 77:1103–15.

Harada, M. 1995. Minamata disease: Methylmercury poisoning in Japan caused by environmental pollution. *Crit. Rev. Toxicol.* 25:1–24.

Harper-Arabie, R. M., et al. 2004. Protective effects of allozyme genotype during chemical exposure in the grass shrimp, *Palaemonetes pugio*. *Aquat. Toxicol.* 70:41–54.

Hemingway, J., et al. 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem. Mol.* 34:653–65.

Heugens, E. H. W., et al. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. *Crit. Rev. Toxicol.* 31:247–84.

Hites, R. A. 2004. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environ. Sci. Technol.* 38:945–56.

Hollis, L., et al. 1999. Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long-term sublethal Cd exposure in rainbow trout. *Aquat. Toxicol.* 46:101–19.

Hummel, H., et al. 1997. Sensitivity to stress in the bivalve *Macoma balthica* from the most northern (Arctic) to the most southern (French) populations: Low sensitivity in Arctic populations because of genetic adaptations? *Hydrobiologia* 355:127–38.

Ishaaya, I. 2001. *Biochemical sites of insecticide action and resistance*. Heidelberg: Springer.

Keizer, J., et al. 1995. Enzymological differences of AChE and diazinon hepatic metabolism: Correlation of *in vitro* data with the selective toxicity of diazinon of fish species. *Sci. Total Environ.* 171:213–20.

Klerks, P. L., and J. S. Weis. 1987. Genetic adaptation to heavy metals in aquatic organisms: A review. *Environ. Pollut.* 45:173–205.

Köhler, H. R., et al. 2000. Selection favours low hsp70 levels in chronically metal-stressed soil arthropods. *J. Evol. Biol.* 13:569–82.

Labbé, P., et al. 2007. Forty years of erratic insecticide resistance evolution in the mosquito *Culex pipiens*. *PLoS Genetics* 3:e205.

Lagarde, F., et al. 2003. Foraging behaviour and diet of an ectothermic herbivore: *Testudo horsfieldi*. *Ecography* 26:236–42.

Laroche, J., et al. 2002. Genetic and physiological responses of flounder (*Platichthys flesus*) populations to chemical contamination in estuaries. *Environ. Toxicol. Chem.* 21:2705–12.

LeBaron, H., and J. Gressel, 1982. *Herbicide resistance in plants*. Hoboken, NJ: John Wiley & Sons.

Leung, K. M. Y., A. C. Taylor, and R. W. Furness. 2000. Temperature-dependent physiological responses of the dogwhelk *Nucella lapillus* to cadmium exposure. *J. Mar. Biol. Ass. U.K.* 80:647–60.

Li, M. S. 2006. Ecological restoration of mineland with particular reference to the metalliferous mine wasteland in China: A review of research and practice. *Sci. Total Environ.* 357:38–53.

Lotts, J. W., and A. J. Stewart. 1995. Minnows can acclimate to total residual chlorine. *Environ. Toxicol. Chem.* 14:1365–74.

Luckenbach, T., I. Corsi, and D. Epel. 2004. Fatal attraction: Synthetic musk fragrances compromise multixenobiotic defense systems in mussels. *Mar. Environ. Res.* 58:215–19.

Mahaffey, K. R. 2004. Fish and shellfish as dietary sources of methylmercury and the [omega]-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: Risks and benefits. *Environ. Res.* 95:414–28.

Malm, O. 1998. Gold mining as a source of mercury exposure in the Brazilian Amazon. *Environ. Res.* 77:73–78.

Marchand, J., et al. 2004. Physiological cost of tolerance to toxicants in the European flounder *Platichthys flesus*, along the French Atlantic Coast. *Aquat. Toxicol.* 70:327–43.

Marsh, K., et al. 2006. The detoxification limitation hypothesis: Where did it come from and where is it going? *J. Chem. Ecol.* 32:1247–66.

Maurice-Bourgoin, L., et al. 2000. Mercury distribution in waters and fishes of the upper Madeira rivers and mercury exposure in riparian Amazonian populations. *Sci. Total Environ.* 260:73–86.

McKenzie, J. A. 1996. *Ecological and evolutionary aspects of insecticides resistance.* Austin, TX: Academic Press.

McLean, S., and A. Duncan. 2006. Pharmacological perspectives on the detoxification of plant secondary metabolites: Implications for ingestive behavior of herbivores. *J. Chem. Ecol.* 32:1213–28.

McLusky, D. S. 1989. *The estuarine ecosystem*. Glasgow: Blackie.

Meyer, J. N., and R. T. Di Giulo. 2003. Heritable adaptation and fitness costs in killifish (*Fundulus heteroclitus*) inhabiting a polluted estuary. *Ecol. Appl.* 13:490–503.

Migné, A., et al. 2007. Photosynthetic activity of intertidal microphytobenthic communities during emersion: *In situ* measurements of chlorophyll fluorescence (PAM) and CO_2 flux (IRGA). *J. Phycol.* 43:864–73.

Millward, R. N., and A. Grant. 1995. Assessing the impact of copper on nematode communities from a chronically metal-enriched estuary using pollution-induced community tolerance. *Mar. Pollut. Bull.* 30:701–6.

Millward, R. N., and A. Grant. 2000. Pollution-induced tolerance to copper of nematode communities in the severely contaminated Restronguet Creek and adjacent estuaries, Cornwall, United Kingdom. *Environ. Toxicol. Chem.* 19:454–61.

Minier, C., F. Akcha, and F. Galgani. 1993. *P*-Glycoprotein expression in *Crassostrea gigas* and *Mytilus edulis* in polluted seawater. *Comp. Biochem. Physiol.* 106B:1029–36.

Minier, C., et al. 2006. A pollution-monitoring pilot study involving contaminant and biomarker measurements in the Seine estuary, France, using zebra mussels (*Dreissena polymorpha*). *Environ. Toxicol. Chem.* 25:112–19.

Moore, N. W. 1967. A synopsis of the pesticide problem. In *Advances in ecological research*, ed. J. B. Cragg, 75–129. Vol. 4. New York: Academic Press.

Moreira, S. M., et al. 2006. Effects of estuarine sediment contamination on feeding and on key physiological functions of the polychaete *Hediste diversicolor*: Laboratory and *in situ* assays. *Aquat. Toxicol.* 78:186–201.

Morgan, A. J., P. Kille, and S. R. Stürzenbaum. 2007. Microevolution and ecotoxicology of metals in invertebrates. *Environ. Sci. Technol.* 41:1085–96.

Mouneyrac, C., H. Perrein-Ettajani, and C. Amiard-Triquet. 2010. Influence of anthropogenic stress on fitness and behaviour of a key-species of estuarine ecosystems, the ragworm *Nereis diversicolor*. *Environ. Pollut.* 158:121–28.

Munkittrick, K. R., and D.G. Dixon. 1988. Evidence for a maternal yolk factor associated with increased tolerance and resistance of feral white sucker (*Catostomus commersoni*) to waterborne copper. *Ecotoxicol. Environ. Saf.* 15:7–20.

Newman, M. C., and M. A. Unger. 2003. *Fundamentals of ecotoxicology*. Boca Raton, FL: Lewis Publishers.

Ng, T. Y. T., et al. 2007. Metallothionein turnover, cytosolic distribution and the uptake of Cd by the green mussel *Perna viridis*. *Aquat. Toxicol.* 84:153–61.

Oliveira, M. M., et al. 2007. Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes. *Mar. Environ. Res.* 63:303–12.

Ortiz-Delgado, J. B., et al. 2008. Tissue-specific induction of EROD activity and CYP1A protein in *Sparus aurata* exposed to B(*a*)P and TCDD. *Ecotoxicol. Environ. Saf.* 69:80–88.

Pearson, W. H., et al. 1981. Effects of oiled sediment on predation on the Littleneck Clam, *Protothaca staminea*, by the Dungeness Crab, *Cancer magister*. *Estuar. Coast. Shelf Sci.* 13:445–54.

Peña-Llopis, S., M. D. Ferrando, and J.B. Peña. 2003. Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by N-acetylcysteine. *Aquat. Toxicol.* 65:337–60.

Peña-Llopis, S., et al. 2001. Glutathione-dependent resistance of the European eel *Anguilla anguilla* to the herbicide molinate. *Chemosphere* 45:671–81.

Ramond, J. B., et al. 2008. Relationships between hydrosedimentary processes and occurrence of mercury-resistant bacteria (*mer*A) in estuary mudflats (Seine, France). *Mar. Pollut. Bull.* 56:1168–76.

Ramos, J. L., et al. 2002. Mechanisms of solvent tolerance in gram-negative bacteria. *Annu. Rev. Microbiol.* 56:743–68.

Rowe, C. L. 1998. Elevated standard metabolic rate in a freshwater shrimp (*Palaemonetes paludosus*) exposed to trace element-rich coal combustion waste. *Comp. Biochem. Physiol.* 121A:299–304.

Sánchez, M., E. Andreu-Moliner, and M. D. Ferrando. 2004. Laboratory investigation into the development of resistance of *Daphnia magna* to the herbicide molinate. *Ecotoxicol. Environ. Saf.* 59:316–23.

Scarfe, A. D., et al. 1982. Locomotor behavior of four marine teleosts in response to sublethal copper exposure. *Aquat. Toxicol.* 2:335–53.

Scherer, E., and R. E. McNicol. 1998. Preference-avoidance responses of lake whitefish (*Coregonus clupeaformis*) to competing gradients of light and copper, lead, and zinc. *Water Res.* 32:924–29.

Shaw, J. 1989. *Heavy metal tolerance in plants: Evolutionary aspects*. Boca Raton, FL: CRC Press.

Smital, T., et al. 2004. Emerging contaminants—Pesticides, PPCPs, microbial degradation products and natural substances as inhibitors of multixenobiotic defense in aquatic organisms. *Mutat. Res.-Fund. Mol. M.* 552:101–17.

Sorensen, J. S., et al. 2007. Tissue distribution of cytochrome P450 3A (CYP3A) in brushtail possums (*Trichosurus vulpecula*) exposed to *Eucalyptus* terpenes. *Comp. Biochem. Physiol.* 145C:194–201.

Sylvestre, F. 2009. Modern diatom distribution in the Seine and Authie estuaries. In *Environmental assessment of estuarine ecosystems: A case study*, ed. C. Amiard-Triquet and P. S. Rainbow, 241–54. Boca Raton, FL: CRC Press.

Takamura, N., F. Kasai, and M.M. Watanabe. 1989. Effects of Cu, Cd and Zn on photosynthesis of freshwater benthic algae. *J. Appl. Phycol.* 1:39–52.

Van der Oost, R., C. Porte-Visa, and N. W. Van den Brink. 2005. Biomarkers in environmental assessment. In *Ecological testing of marine and freshwater ecosystems*, ed. P. J. Den Besten and I. F. Munawar, 87–152. Boca Raton, FL: Taylor & Francis.

Wilding, J., and L. Maltby. 2006. Relative toxicological importance of aqueous and dietary metal exposure to a freshwater crustacean: Implications for risk assessment. *Environ. Toxicol. Chem.* 25:1795–801.

Wilhelm Filho, D., et al. 2005. Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongatus* (Valenciennes, 1847). *Aquaculture* 244:349–57.

Xie, L., and P. L. Klerks. 2004. Fitness cost of resistance to cadmium in the least killifish (*Heterandria formosa*). *Environ. Toxicol. Chem.* 23:1499–503.

2 Tolerance to Contaminants

Evidence from Chronically Exposed Populations of Aquatic Organisms

Emma L. Johnston

CONTENTS

2.1 INTRODUCTION

Contaminants may reduce the survival, recruitment, growth, and reproductive success of an organism, and hence they are potentially powerful agents of selection. Ecotoxicologists have long been interested in the rapid evolution of tolerance to contaminants (Luoma 1977), and it is generally accepted that this may occur after only a few generations (Klerks and Levinton 1989). There are substantial social and environmental consequences of the evolution of contaminant tolerance. The evolution of

pesticide and herbicide resistance is a costly concern for agriculture, while pollution-tolerant bacteria and plants are increasingly used in the remediation of contaminated waste. Moreover, if guidelines for the protection of the environment are based on sensitivity data from adapted populations, they may not be adequately protective. It is therefore crucial that we understand the frequency at which contaminant tolerance occurs and the extent to which this phenomenon modifies the response of plants and animals to contaminant exposure.

A number of excellent reviews have recently been published regarding various aspects of evolutionary ecotoxicology (e.g., Grant 2002; Klerks 2002; Nacci et al. 2002b; Hoffmann and Daborn 2007; Medina et al. 2007; Morgan et al. 2007; Janssens et al. 2009), and additional theses are published in this book. The purview of this chapter is to critically review and summarise the last decade of research on tolerance (resistance) to contaminants in chronically exposed field populations of aquatic invertebrates and fish. A systematic approach to literature selection was taken in order to describe the range of approaches and organisms tested, and the frequency at which differential tolerance has been observed. This review is deliberately constrained to studies that assess an endpoint of direct relevance to an organism's fitness (e.g., survival, recruitment, growth, or reproductive success). It does not review direct genotoxic effects of contaminants on the molecular structure of genetic material, which tend to result in detrimental effects on fitness. Nor does it describe the impacts of contaminants on biomarkers, bioaccumulation, or the genetic variability of populations *unless* such differences were also associated with a direct change in the fitness of the exposed populations.

2.1.1 Physiological Acclimation and Genetic Adaptation

Differential fitness in response to contaminant exposure may result from physiological acclimation or genetic adaptation, and the mechanism will vary with the organism and toxicant in question. This chapter will use the term *tolerance* synonymously with the term *resistance*, to include two subcategories: (1) heritable adaptive responses (genetic adaptation) that affect the mean tolerance to contaminants in a population, and (2) physiological acclimation of individuals. Tolerance will thus also include instances where the mechanism is unknown (Morgan et al. 2007). Physiological acclimation and genetic adaptation are not mutually exclusive, as the ability to acclimate is conferred through the genetics of a species. Some species are inherently capable of tolerating a contaminant through existing physiological acclimation mechanisms. In this case, initial exposure to a contaminant may reduce the effective toxicity of future exposures of that individual to the contaminant. The response is usually a physiological one that upregulates an existing detoxification mechanism, but it may also be a behavioural response that effectively reduces exposure. In this case, an observation of differential tolerance between populations may or may not be the result of anthropogenic contamination acting as a selection agent. Where the ambient contamination levels have been sufficiently different to trigger physiological acclimation at one site, differential tolerance to acute toxicant exposure should be observed in field-collected

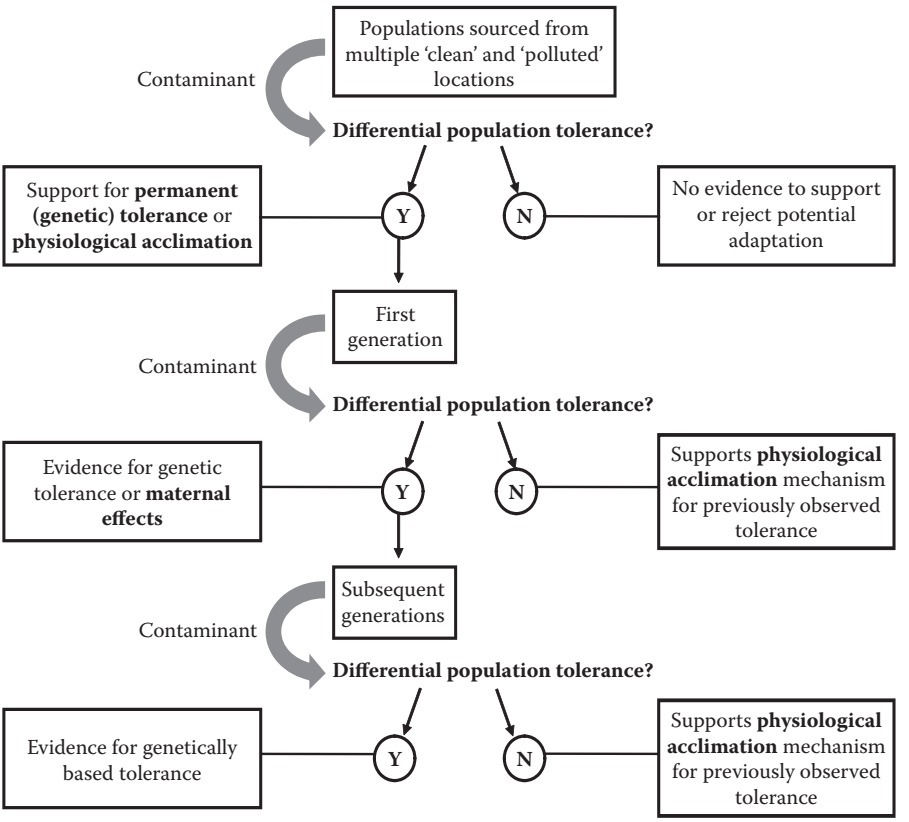

FIGURE 2.1 Process for assessing evidence for contaminant tolerance in chronically exposed field populations.

organisms (Figure 2.1). Tolerance should be rapidly lost if exposures are not maintained.

If the selective force applied by a toxicant is maintained across multiple generations, it may result in differences in fitness that are associated with changes to population genetics. Heritable adaptive responses of a population to a contaminant may manifest in different ways. There is potential for selection to increase the frequency of phenotypically plastic organisms that can rapidly upregulate a toxicant defence mechanism. This is the same effect as described above (physiological acclimation), but the frequency of its occurrence will be higher in adapted populations. Alternatively, selection may favour individuals with permanently increased tolerance to a contaminant. Distinguishing between selection for phenotypic plasticity in tolerance and selection for elevated mean tolerance requires bioassays on the offspring of individuals collected from the field and raised in a controlled environment. Preferably, testing should be done on the first and second generations so that maternal effects derived from the mother's response to a contaminant (essentially a form of physiological acclimation transferred to the first generation) may be explicitly

defined (Figure 2.1). This process also helps to identify any potential fitness costs to a tolerant organism that is raised in a clean environment.

2.2 LITERATURE SEARCH

A systematic literature review was conducted to capture a representative sample of the aquatic tolerance (resistance) literature. A systematic search methodology does not aim to capture every paper published on a particular topic. It is a repeatable sampling approach designed to capture a selection of studies demonstrative of the current trends and focus in a research field. I used specific search terms in the Scopus database, which covers >4,300 titles in the life sciences (peer-reviewed journals, trade publications, conference papers, or book series). Searches were limited to English language studies published between 1999 and 2009:

> Search: ALL ("contam*") AND ALL ("genetic adaptation") OR ALL ("physiological acclimation")

All of the abstracts of the papers that emerged in these searches were read (332), and I selected for review those that (1) had an aquatic invertebrate or fish focus and (2) tested the effects of a contaminant on the tolerance of at least two populations collected from the field. At least one of the test endpoints had to be a test relevant to the fitness of an individual (e.g., relative survival, reproduction, growth, or recruitment). In total, thirty-four research articles satisfied the criteria for inclusion in the review.

From these studies I extracted qualitative data relating to contaminant type, organism(s) studied, and endpoints assessed. I then collated data on the overall finding of the research (reduced, increased, or no difference in contaminant tolerance or resistance between the populations tested) as concluded by the authors and the direction and magnitude of the change. Many of the studies performed no formal tests of hypotheses, and I considered descriptive data from these studies.

2.3 RESULTS: SUMMARY OF LITERATURE SEARCH

Of the 332 abstracts examined, 106 studies dealt explicitly with tolerance (or resistance) to contaminants in aquatic vertebrates or invertebrates. Of these, thirty-four included a test directly related to fitness, i.e., relative survival, reproduction, growth, or recruitment, and hence were included in this review (summarised in Table 2.1). A further twenty-nine examined the relative response of a biomarker, bioaccumulation, or genetic variability between field-collected populations; thirty-two studies examined differential tolerance through laboratory breeding studies; and fourteen reviewed the literature regarding the evolution of contaminant tolerance (Figure 2.2a).

The average number of sites from which animals were collected and tested in the review studies was 3.4 (range 2–14; Figure 2.2b). Eight studies only tested populations from two sites, making it difficult to attribute differential tolerance to the contaminant exposure at the site. Contaminated habitats may differ from other locations in many environmental features; e.g., contaminated sites in estuaries tend to

TABLE 2.1
Summary of the Focal Species, Contaminant, Test Endpoints, and Evidence for Differential Tolerance for All of the Papers Selected for This Review (bold denotes significant difference between populations for that endpoint)

Taxonomic Group	Species	Contaminant	Differential Tolerance in Field-Collected Populations	Differential Tolerance in Subsequent Generations	Endpoints Directly Relevant to Fitness (Assayed on Field-Collected Populations)	Other Study Endpoints	Reference
Copepod	*Tisbe holothuriae*	Co, Cr	Y	Y	**48 h LC50**, reproductive output	NA	Miliou et al. 2000
Copepod	*Microarthridion littorale*	Contaminated sediment	N	NA	Mortality, reproductive output	Genetic relatedness greater between polluted and reference populations than between polluted populations	Kovatch et al. 2000
Cladoceran	*Daphnia magna*	Model pesticide	Y	Y	**48 h EC50**	Measurement of neutral genetic marker indicates genetic diversity positively correlated with land use intensity	Coors et al. 2009
Cladoceran	*Daphnia magna*	Cd	Y	Y	**48 h EC50**	Lower expression of stress protein hsp70 in more sensitive clones	Haap and Köhler 2009
Cladoceran	*Ceriodaphnia dubia*	Zn	Y	N	**48 h EC50, carapace length, reproductive output**	NA	Muyssen and Janssen 2001

(*continued*)

TABLE 2.1
Summary of the Focal Species, Contaminant, Test Endpoints, and Evidence for Differential Tolerance for All of the Papers Selected for This Review (bold denotes significant difference between populations for that endpoint) (Continued)

Taxonomic Group	Species	Contaminant	Differential Tolerance in Field-Collected Populations	Differential Tolerance in Subsequent Generations	Endpoints Directly Relevant to Fitness (Assayed on Field-Collected Populations)	Other Study Endpoints	Reference
Cladoceran	*Moinodaphnia macleayi*	Uranium	N	N	48 h EC50, LOEC, NOEC	NA	Semaan et al. 2001
Cladoceran	*Daphnia longispina*	Cu	Y	Y	**Mortality**, feeding inhibition	NA	Lopes et al. 2004
Cladoceran	*Ceriodaphnia pulchella*	Contaminated water	Y	Y	**Mortality, reproductive output**	Support for genetic erosion due to higher frequency of sensitive individuals in reference population	Lopes et al. 2005
Cladoceran	*Daphnia longispina*	Contaminated water, Cu	Y	Y	**Mortality, fecundity**	NA	Lopes et al. 2006
Amphipod	*Orchestia gammarellus*	Cd, Cu, Zn	N	NA	17 d LC50	Significant differences in MTLP levels in field populations, but response could not be elicited in lab	Mouneyrac et al. 2002
Caddisfly larva	*Hydropsyche betteni*	Zn	Y	NA	Mortality, **feeding, weight**	NA	Balch et al. 2000
Chironomid larva	*Chironomus riparius*	Cd	Y	Y	**4 d EC50**	Reciprocal crosses suggesting absence of maternal effects	Groenendijk et al. 2002

Flatworm	*Polycelis tenuis*	Cd	N	N	28 d LC50 and EC50	NA	Indeherberg et al. 1999
Oligochaete	*Tubifex tubifex*	Hg	Y	NA	**10 d LC50**	Elevated levels of accumulated tissue Hg levels in metal-exposed populations	Vidal and Horne 2003
Polychaete	*Nereis diversicolor*	Ag, Cd, Cu, Zn	Y	NA	**21 d LC50**	Lower levels of detoxificatory enzymes and MTLP in metal-exposed field populations	Mouneyrac et al. 2003
Polychaete	*Nereis diversicolor*	Cd, Cu, Zn	Y	NA	**LT50**	Stored energy reserves lower in metal-exposed populations	Durou et al. 2005
Polychaete	*Nereis diversicolor*	Cu, Zn	Y	NA	**Mortality**	Higher rate of Cu accumulation in heavily contaminated population	Zhou et al. 2003
Bryozoa	*Bugula neritina*	Cu	Y	Y	Mortality, **growth**, settlement	NA	Piola and Johnston 2006b
Snail	*Physella columbiana*	Cd, Pb, Zn	Y	Y	LC50, **avoidance behaviour**	NA	Lefcort et al. 2004
Benthic invertebrates		Cd, Cu, Zn	Y	NA	Presence/absence	NA	Courtney and Clements 2000
Sessile invertebrates		Cu, Zn, TBT	Y	NA	Presence/absence	NA	Dafforn et al. 2009
Sediment infauna		Contaminated soil	Y	NA	**Emergence**	NA	Bahrndorff et al. 2006

(continued)