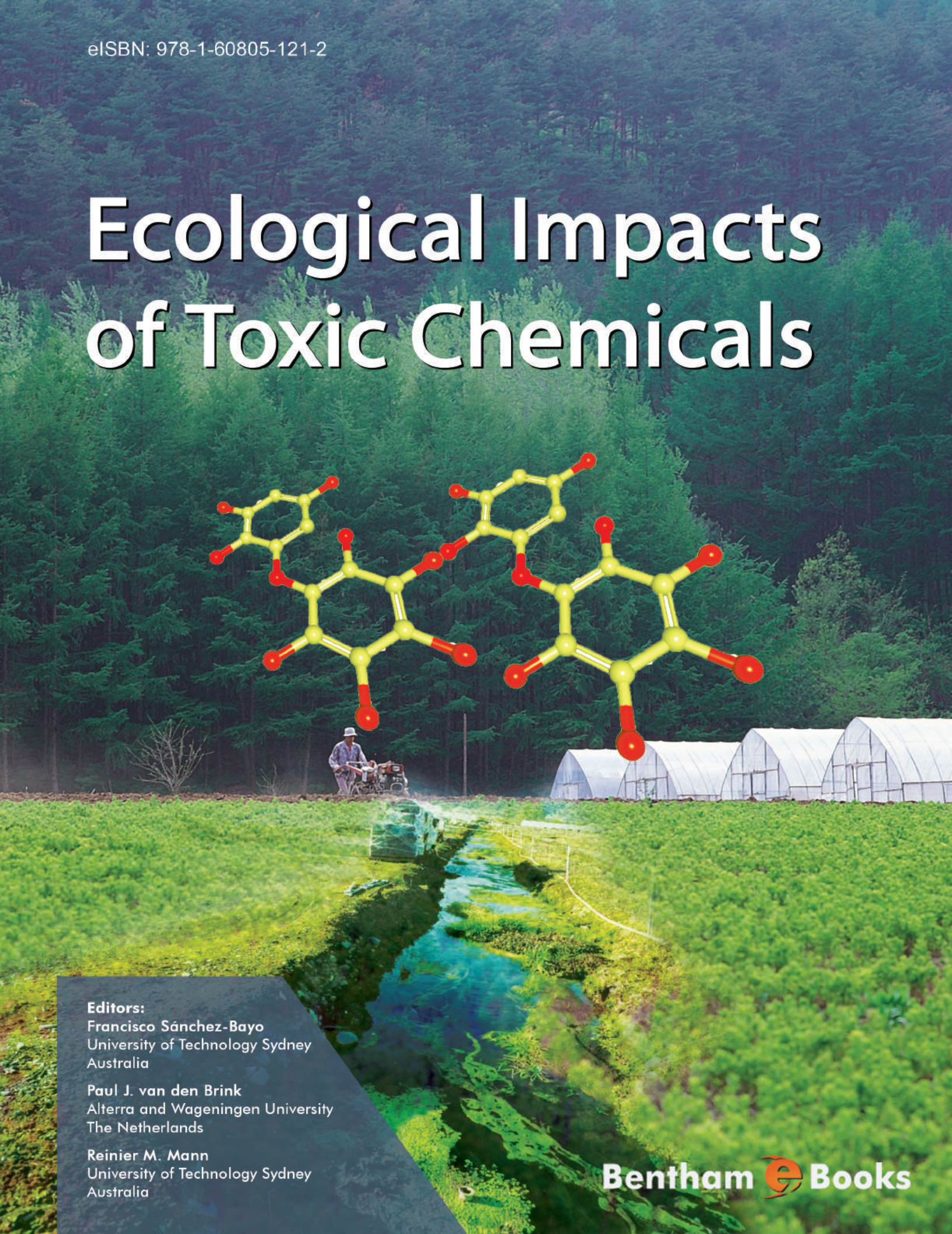
eISBN: 978-1-60805-121-2

Ecological Impacts of Toxic Chemicals



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Bentham eBooks

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FOREWORD

"Ecological Impacts of Toxic Chemicals" is a long-overdue, comprehensive coverage of chemical fate and effects in terrestrial and aquatic environments. The editors Sánchez-Bayo, van den Brink and Mann have brought together an excellent group of international experts to systematically cover this complex topic from the source of organic and metal compounds, to their fate and impacts on land and in our freshwater and marine ecosystems. The book is very readable, serving as an excellent introduction to the topic or as a useful supplement to courses and readings in the environmental sciences at any level. Indeed, it is appropriate for the general public, students, or scientists from outside the field of ecotoxicology.

The first two chapters, by Sánchez-Bayo (Chapter 1) and van de Meent, Hollander, Peijnenburg and Breure (Chapter 2) introduce the theme of the book, covering the sources and mode of action of environmental contaminants and the toxicity of various common pollutant categories: mining wastes, sewage, industrial and metropolitan discharges. The transport and fate of metal and organic pollutants in the environment is described from a modeler's perspective. The processes governing the movement of chemicals between air, land and water are described, along with biological transformations, including degradation and bioaccumulation. The understanding of the fate and ultimate exposure to biota is essential in ecotoxicology and risk assessment and management.

The following three chapters deal with terrestrial ecosystems. In Chapter 3, Mann, Vijver and Peijnenburg explain how naturally-occurring metals and metalloids can become contaminants when they bioaccumulate and result in sublethal to lethal effects on populations and food chains. They cover the key metals of toxicological concern which continue to be a problem world-wide: arsenic, cadmium, copper, lead, mercury, molybdenum, selenium and zinc. Agricultural pesticides have been widely used in developing and developed countries and because they are biocides, have resulted in a range of unintended adverse effects on non-target biota. Sánchez-Bayo discusses fungicides, insecticides and herbicides and how they have impacted virtually every level of the food chain, from the microbial level to birds and mammals. Thompson focuses on the forest industry's use of pesticides (herbicides and insecticides) and case examples of lab to field studies that have assessed the risk of these widely used compounds in pest management in the forest sector. These studies are then linked to the risk assessment and management process providing for a comprehensive perspective of multiple stakeholder concerns.

The final six chapters address the many issues of chemicals in marine and freshwater environments. Schäfer, van den Brink and Liess have an excellent review of pesticide impacts on freshwater ecosystems, from primary producers, up the food chain, to fish. They explain the many complex interactions that must be considered regarding pesticide mode-of-action, exposure (particularly consideration of peak concentrations), indirect effects, and the potential for recovery of populations and communities. They describe a range of useful techniques and approaches for assessing pesticide risk from the broad to local scales, and the need for incorporating ecological knowledge into the risk assessment process. A growing concern exists for the impacts of other, non-pesticide, organic chemicals in freshwater ecosystems which is dealt with in Chapter 7 by Sibley and Hanson. Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), polychlorinated dioxins and furans, polycyclic aromatic hydrocarbons (PAHs), and emerging contaminants such as pharmaceuticals, polybrominated diphenyl ethers and perfluorinated surfactants are becoming common in freshwaters throughout the world. This is due to their resistance to degradation and ability to be transported between water, soil, air and biota. Their bioaccumulation through the food chain presents recognized risks, but these risks are difficult to ascertain from studies at the lower end of the food chain. Chemicals tend to accumulate in sediments, hence biota associated with sediments, the benthos, are particularly susceptible. Borja, Belzunce, Garmendia, Rodríguez, Solaun and Zorita describe this complex issue in Chapter 8, for coastal and marine benthic communities. Their coverage begins at the molecular effect level and progresses up the ladder of biological complexity to populations and communities, and the need for integrative assessments. They document how important it is to understand biological effects by looking at the different levels of biological organization. Dying coral reefs have been documented throughout the world. They are impacted by nutrients, metals, organic chemicals, climate change and ocean acidification. In Chapter 9, van Dam, Negri, Uthicke and Mueller explain the severity of this phenomenon and the tools available for evaluating adverse effects. Of critical importance is their coverage of how adverse effects and risk is tied to exposures, which vary from short-term, to pulse-like spills, to recurring incidents from effluent discharges to river flooding. These later, chronic and repetitive

events are likely to decrease the resilience of reef organisms making them more susceptible to climate change and acidification. In contrast to the previous two chapters, Hylland and Vethaak in Chapter 10 focus on contaminant effects on water column organisms, often referred to as pelagic organisms which fuel the world's ocean ecosystems. The various ways of assessing pelagic effects are reviewed, along with the unique strengths and limitations in the context of making environmental management decisions. Better monitoring of the pelagic zone is critical for long term monitoring programs and effective ecosystem management. Finally, in Chapter 11, Moore, Kröger and Jackson inform the reader of how aquatic ecosystems are so efficient at transferring, transforming and sequestering pollutants, thus reducing their risk to organisms and ecosystems. They focus on the successful use of phyto-remediation of organic and inorganic pollutants.

Together, these chapters provide a broad, timely and comprehensive review of the potential effects of chemical pollutants in terrestrial and aquatic ecosystems. Readers new to this field will not be disappointed and quickly made aware of the critical issues affecting our current and near-future world.

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PREFACE

Ecotoxicology is a multidisciplinary science that examines the effects of toxic chemicals on individual organisms, populations, communities and ecosystems. However, with a 40-year history, ecotoxicology is still in its infancy. Up until recently a lot of work has been done to describe the fate and effect of chemicals in the environment, but most of it has been performed in the laboratory, usually with a narrow suite of test organisms. However, over the last two decades more and more experiments and monitoring have been performed in man-made (so called microcosms and mesocosms) as well as natural aquatic and terrestrial ecosystems. Also the use of modelling has allowed us to predict the behaviour of chemicals and their consequent effects in the environment. Impacts of pollutants at an ecosystem level, however, are reported mostly in the specialized journal literature as scattered pieces of a larger puzzle. To date, no systematic work bringing all the information on this subject together is available, neither to researchers nor the general public. This book was conceived to fill this gap.

Ecological Impacts of Toxic Chemicals presents a comprehensive, yet readable account of the known disturbances caused by all kinds of toxic chemicals on both aquatic and terrestrial ecosystems. Topics cover the sources of toxicants, their fate and distribution through the planet, their impacts on specific ecosystems, and their remediation by natural systems. Each chapter is written by well-known specialists in those areas, for the general public, students, and even scientists from outside this field. The book intends to raise awareness of the dangers of chemical pollution in a world dominated by industry and globalization of resources. Because the problems are widespread and far reaching, it is hoped that confronting the facts may prompt better management practices at industrial, agricultural and all levels of management, from local to governmental, so as to reduce the negative impacts of chemical contaminants in our Earth.

The editors would like to thank Bentham Science Publishers for providing this opportunity to bring this science to the general public.

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CHAPTER 1

Sources and Toxicity of Pollutants

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Abstract: Modern living standards depend largely on the production and usage of thousands of chemicals, many of which are toxic and synthetically produced. These substances are discharged into the air, soil, water bodies and the sea through a variety of ways, becoming pollutants of our environment. The investigation of their fate and impacts they have on ecosystems is called ecotoxicology, a multidisciplinary science which intends to evaluate the nature of the discharge, the transformation and distribution of toxicants in the environment, exposure, lethality and sublethal effects on organisms, population responses, and changes in community structure and ecosystem function. The sources and mode of action of some of the most common groups of toxicants are described in this chapter, leaving their fate and effects in organisms and ecosystems for the subsequent chapters.

INTRODUCTION

We are living in the Chemical Era. Indeed, the most distinctive characteristic of our modern society is the production and use of an enormous amount of chemical products. Currently, some 70,000 chemicals are utilised worldwide, while the rate of introduction of new substances can be estimated between 200 and 1000 each year [1]. Our civilization depends to a large extent on the search for new materials that are employed to develop technology, medicines, textiles and construction materials of all kinds. What would happen to us if such production were to stop suddenly?

Throughout history, civilizations have relied on the use of natural materials to manufacture tools, clothing and furnishings, while poisons and medicinal plants must have been known to the first humans. The development of agriculture during the Neolithic (11,000 BP) brought with it the manufacturing of textiles as well as dyes and paints made from minerals, plant and animal products, some of which are quite toxic. With the discovery of metals during the Bronze and Iron Ages (ca. 5000 and 3000 BP, respectively) came mining and consequently pollution by toxic metals. Alchemy started in Persia about 2500 BP, and since then each civilization in Asia looked to develop new substances, mainly for medicinal purposes, using the diverse array of natural products available to them. In Europe, alchemy laid the foundations of toxicology and modern chemistry in the 16th and 17th centuries respectively, which would result in the discovery and manufacturing of hundreds of entirely new substances. During the industrial revolution of the 19th century, mining and chemical companies were created specifically to exploit natural resources and create new products. The discovery of large deposits of crude oil in Baku (Azerbaijan) and North America in the 1850s [2], together with the realisation that petroleum could be used as fuel for combustion engines, boosted the mechanised and oil-dependent society we still live in. As a result of these activities, pollution on a large scale began at that time and still continues despite amelioration efforts by governments and industries in most developed countries.

The technological race that started in the 20th century, particularly since the end of World War II, included chemicals as an essential part of modern development. For instance, communications and transport have benefited enormously from the use of new metals and alloys to make transistors, batteries and more durable metallic products. Synthetic organic compounds, mostly derived from petroleum, underwent a revolution of their own: polychlorinated biphenyls (PCBs), used as insulating fluids in the electrical industry since their introduction in 1929; chlorofluorocarbons (CFCs) used in refrigeration and air conditioning systems; plastics to serve a wide range of uses, from building materials to household items and toys; pesticides to control insect and rodent pests, weeds and plant diseases; and the immense array of chemicals used to make paints, cleaning products, cosmetics and pharmaceuticals. Many of these new substances are toxic, have become environmental pollutants in air, water and soil, and created unforseen problems related to their waste and disposal.

Although the discovery of toxic substances dates from ancient times, their systematic study or toxicology began during the European Renaissance with Paracelsus (1493-1541), a medical doctor and alchemist who sought to understand the effects of toxicants and drugs used in medicine. However, it wasn't until the effects of new pollutants

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Francisco Sánchez-Bayo, Paul J. van den Brink and Reinier M. Mann (Eds)

from the industrial revolution started to take a toll on ecosystems that people realised the dangers they posed to our environment and our own health. In Japan, a country which experienced the fastest transformation from a feudal to an industrial society, Tanaka Shozo (1841-1913) appealed to the Meiji Emperor in protest against fish kills due to careless discharges from the Ashio copper mine north of Tokyo [3]. It might have been the first time that a local politician tried to protect the environment and the lives of his community by demanding regulation of indiscriminate exploitation of resources. Japan would suffer dearly the consequences of such a rush for industrial development, with Minamata and '*itai-itai*' being added to the infamous list of modern diseases caused by pollutants [4]. These unintended problems prompted a rethink of treating the natural environment as a receptacle for untreated waste, and yet many other industrialised societies would have to endure a large human toll from *smog* before taking any action to regulate the burning of fossil fuels in their cities [5]. In this climate, the publication of *Silent Spring* in 1962 [6] brought to the attention of ordinary people in the street what scientists were still trying to comprehend: the negative effects that pesticides can pose to the environment. Such a book would mark the start of the environmental movement in America and the rest of the world.

The investigation of the ecological impacts that toxic pollutants have on ecosystems constitutes a new science, emerged in the 1970s, called ecotoxicology [7]. Its approach is multidisciplinary, combining the knowledge from chemistry, toxicology and ecology to reach an understanding of the complex interactions of toxicants in the environment. In a broader sense, ecotoxicology has the role of assessing, monitoring and predicting the fate of foreign substances in the environment [8], with the ultimate end of helping the regulatory authorities establish limits that protect human health and nature.

This first chapter aims at providing the reader with a glimpse of the different kinds of pollutants currently in existence, where they come from and how they exert their toxic effects on organisms. Subsequent chapters will examine the ways these chemicals move between air, soil, rivers and oceans, with special attention given to the overall impacts on specific communities and types of ecosystems.

TYPES AND SOURCES OF TOXICANTS

Toxic chemicals that pollute the environment can be called ecotoxicants [1]. They can be natural or man-made substances, but a common characteristic to all of them is that they can exert a deleterious effect on living organisms at relatively small doses, measured in milligrams or micrograms per litre or per kilogram [9]. An important aspect to consider with ecotoxicants is whether they are available to organisms (see risk assessment below). Indeed, pollutants are discharged into the air and water or disposed of in or on the ground, where they may be absorbed by plants or taken up by animals, which may in turn be affected by their toxic activity. By contrast, the vast majority of naturally occurring toxicants (e.g. plant poisons) are stored in tissues that are only available to animals if eaten. In the case of crude oil, natural deposits are many metres underground and out of reach…except to humans! For this reason, biological toxins very rarely become pollutants – these originate mostly from human activities of our modern society (Table **1**). The following is a brief description of the most common types.

Toxins of Biological Origin

Although our knowledge is still limited, the variety of plant and animal poisons is staggering [10], with many of them being utilised as medical drugs or in the production of pesticides – the toxins of the soil microbe *Bacillus thuringiensis*, for instance, are used for pest control, either directly or through transgenic plants [11]. Most natural toxins produced by organisms are used as defence tools in mechanisms that evolved over millions of years, but some animals produce toxic venoms to capture and kill their prey [12]. In any case, very few of these toxins are ecotoxicants: botulin produced by the soil bacterium *Clostridium botulinum*, mycotoxins produced by some species of fungi, cyanotoxins and microcystins produced by certain blue-green algae [13], and saxitoxins and brevetoxins produced by several species of dinoflagellates (e.g. *Alexandrium* sp.) are the most notorious [14], as they can cause fish deaths through algal-blooms and serious intoxication or health problems in humans.

Waste Products

Natural ecosystems recycle the elements through a variety of pathways which end up in mineralization, thus ensuring that all organic wastes in soil, water and sediments are eliminated as soon as possible. Raw sewerage is processed by naturally occurring micro-organisms in waters provided its volume is within the capability of aquatic ecosystems, but

large cities discharge excessive volumes of refuse into rivers, lakes and coasts, which if insufficiently treated can lead to eutrophication of the waters and foster toxic algal blooms [15]. Moreover, stormwater runoff and waste discharges from cities often contain a variety of toxic chemicals, including metals, petroleum hydrocarbons, pharmaceuticals, pesticides [16,17], phenols, steroids and many others which have endocrine disrupting activity [18].

Table 1: Sources of toxic pollutants and their mode of action.

Types of toxicant	Chemical groups	Common sources	Mode of action
Natural	Polycyclic aromatic hydrocarbons (PAH)	Bushfires, fuel emissions	Carcinogens (DNA adduct formation)
	Biological toxins	Micro-organisms (bacteria, fungi, dinoflagellates, blue-green algae)	Gastrointestinal, narcotic, neurotoxic
	Inorganic (SO_2, CO, CO_2, NO_2 SH_2, NH_3, etc)	Volcanic eruptions, coal and vehicle emissions, fertilisers	Blocking of biochemical pathways
	Metallic (As, Cd, Cr, Cu, Hg, Pb, Sn, Sb, Zn)	Mining, smelters, metallurgic and transport industries, electronics, batteries, paints, herbicides	Several modes of action: neurotoxic, blocking respiration and biosynthesis
	Phenolic compounds	Plant wastes, disinfectants	Antioxidants, cell inhibitors, EDC
Artificial	Anilines*	Dyes (textiles, paints), rubber and pharmaceutical industries	Oxidation, dehydrogenation, *etc.*
	Antibiotics*, sulfonylureas and sulfonamides	Biocides, herbicides, pharmaceuticals	Amino acids and/or protein inhibitors
	Benzoylureas	Insecticides	Chitin synthesis inhibitors
	Carboxamides, phthalimides, pyrroles, strobilurins	Biocides	Respiration inhibitors
	Chlorofluorocarbons (CFC)	Coolants, propellants, fire-suppressants, solvents	Greenhouse gasses; ozone depletion
	DDT and synthetic pyrethroids*	Insecticides	Neurotoxic - alter Na^+ channels
	Dibenzodioxins (PCDD), dibenzofurans (PCDF)	Pesticide industry, waste-burning emissions	Disruption of Ah receptor; teratogenic
	Imidazoles, morpholines, triazoles	Fungicides	Ergosterol biosynthesis inhibitors
	Narcotics*	Pharmaceutical industries	Neurotoxic
	Neonicotinoids	Insecticides	Neurotoxic - nicotinic receptor inhibitors
	Nonylphenols, nonylphenol ethoxylates, steroids	Detergent and pharmaceutical industries	Endocrine disrupters (EDC)
	Organochlorines	Insecticides and fungicides	Neurotoxic - GABA receptors; respiration
	Organometallic* compounds	Biocides, antifoulants	Chelating, oxidants, respiration inhibitors
	Organophosphorous and carbamate pesticides	Insecticides, herbicides	Neurotoxic - acetyl-cholinesterase inhibitors
	Perfluorinated compounds (PFOS, etc)	Semiconductors, oil/water-proof materials	Unknown

	Phenoxy and pyridine herbicides	Herbicides	Control/stop plant growth
	Phthalates	Plasticizers, surfactants, pharmaceutical industry	Endocrine disrupters (EDC)
	Polychlorinated biphenyls (PCB)	Electrical insulators, lubricants, paints	Disruption of Ah receptor; EDC
	Polybrominated diphenyl ethers (PBDE)	Fire retardants	Unknown
	Solvents	Chemical industry, paints, cleaning products	Anesthetic or narcotic action
	Synthetic coumarins* and indandiones	Rodenticides	Anticoagulants
	Thiocarbamate, chloroacetamides, dinitroanilines and benzimidazoles	Herbicides and fungicides	Germination inhibitors - microtubules, lipid, fatty acid or cellulose biosynthesis
	Triazines and urea derivatives	Herbicides	Photosynthesis inhibitors

* Some compounds occur naturally as well

Metals and other Elements

All elements, whether they are toxic or not, occur naturally in soils, air, oceans and sediments, usually at very low, non-toxic levels. Some of them are called micronutrients, since they are essential for the synthesis of certain biomolecules: iron, cobalt, chromium, copper, iodine, manganese, selenium, zinc and molybdenum. However, human activities such as mining, manufacturing and transport have produced abnormally elevated levels of many toxic elements, thus causing detrimental effects in the environment [19]. For example, urban soils throughout the world contain high concentrations of lead due to the intense use of leaded-fuel in motor vehicles for many decades. Mine tailings contain very high concentrations of residual metals and constitute a high risk to the surrounding environments, with their accidental release causing enormous damage to aquatic ecosystems and associated fishing industries [20]. Smelters and factories that process large amounts of metals may also be sources of metal pollution through smoke stacks and discharges into waterbodies.

Synthetic and Natural Organic Toxicants

Although the majority of organic toxicants that contaminate the environment are man-made, some can be produced by natural events. Bushfires, for instance, are major sources of air and water contamination by polycyclic aromatic hydrocarbons (PAHs), which are present in nature at very low concentrations – background or baseline levels [21]; however, their environmental levels have increased in recent years due to the large increase of fossil fuels usage worldwide. Crude oil, composed mainly of a cocktail of aliphatic hydrocarbons of biological origin, has been stored safely underground for millions of years [2]; the petroleum spillages that result from the accidental break up of oil-tankers or oil-field shafts, even if they are not very toxic, may have other temporary impacts on the ecosystems exposed [22].

In addition to these natural ecotoxicants there is an immense range of synthetic compounds, man-made for a variety of purposes: pesticides and fertilizers used in agriculture, industrial chemicals such as PCBs and CFCs, chemical reagents, solvents, plasticizers, dyes, surfactants, detergents, pharmaceutical drugs and explosives used in warfare and industrial activity. All these chemicals can be released into the environment through manufacturing, usage, accidental spillage or inefficient disposal. For example, chlorodibenzodioxins (PCDD) and chlorodibenzofurans (PCDF) are by-products formed in the manufacturing of some chlorinated pesticides and also from incineration of chlorophenolic wastes [23].

Inorganic Toxicants

Volcanic eruptions discharge enormous volumes of sulphur dioxide, carbon monoxide and other inorganic poisons into the air (Table **1**), where in addition to their inherent toxicity are subsequently transformed into acids by reacting either with atmospheric water vapour or in direct contact with water [24]. Even carbon dioxide can be toxic at concentrations higher than 2%. Once again, the levels of these toxicants in the environment have increased due to

human activities such as fertiliser usage or burning of coal and petroleum fuels. On a different matter, they can affect the global weather patterns, with CO_2 and NO_2 contributing to the warming of the atmosphere while SO_2 forms aerosols which have the opposite effect [25].

MODE OF ACTION OF TOXICANTS

Toxicants are chemicals that have toxic effects on organisms. Such effects occur when the toxicant, after being taken up through the roots or leaves in plants, or through the skin, digestive or respiratory systems in animals, is transferred by the circulatory fluids to the site of action within the plant or animal body. Some of the original compound, or its biotransformed active products, may reach the site of action, while the remainder may be excreted and metabolised, usually to non-toxic products, or stored in lipid tissues [26].

For most chemicals, the site of toxic action is at the cellular level, and their activity translates into one or more physiological effects which are manifested as several toxicity symptoms in individual organisms. For example, mercurial compounds block the degradation pathway of catecholamines in neuronal cells, so the resulting excess of epinephrine (adrenaline) in the blood stream causes profuse sweating and hypersalivation, whereas excess of dopamine in the brain induces tachycardia and hypertension. But organisms are rarely affected in isolation; since toxic pollutants are spread over certain areas, they usually affect a number of organisms at the same time – effects at the population level can translate into reduction of numbers (mortality) or decreased reproduction success in certain species but not in others. In turn, these changes typically result in altered communities of animals and/or plants, which impact the ecosystem functionality (e.g. biomass productivity, nutrient and predator-prey dynamics) to a lesser or greater degree [27]. Effects at different levels of organization are not always observed: while all ecotoxicants have effects at the individual level, or at most at the population level in acute exposures, impacts on communities and ecosystems depend mainly on the persistence and/or bioaccumulation of the chemicals concerned. However, short pulses can also have a large, long-lasting impact when the recovery of the communities affected is slow or when the ecosystem has been pushed to an alternative stable state.

Inorganic and Elementary Compounds

Inorganic toxicants usually disable the functionality of essential biomolecules; for example, CO binds to haemoglobin and prevents it from transporting oxygen in the blood. Even if some are essential micronutrients, toxic metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Sn, Zn) tend to form covalent bonds with organic molecules and effectively disable their funcionality:

- Arsenic and antimony compete with phosphorus in several phosphorylation processes, disrupting ATP production; some organoarsenic compounds are neurotoxic [12].
- Cadmium replaces zinc, magnesium and calcium in some metabolic systems; if inhaled through smoking it can induce cancer [28].
- Hexavalent chromium (Cr^{6+}) is mutagenic, and nickel is carcinogenic.
- Copper ions (Cu^{2+}) from copper salts (e.g. $CuSO_4$) accumulate in certain cells such as algae and fungi spores and prevent their germination; in fish, copper ions disrupt the sodium regulation [29], and in mammals can also produce cirrhosis (liver damage), but the mechanism of toxicity is poorly understood.
- Mercury binds to sulfide groups in proteins, but organomercurial forms are much more toxic because they penetrate the tissues and reach the nervous system [12].
- Lead inhibits several enzymes involved in the synthesis of haemoglobin; it also interferes with calcium ions during nerve conduction.
- Zinc competes with copper in the uptake and synthesis of biomolecules; its free ion is a corrosive acid with broad-spectrum biocidal activity. However, organotin compounds inhibit the oxidative phosphorylation and ATP production in mitochondria [30]; tributyltin (TBT) also inhibits the P450 mono-oxygenase detoxification system in fish and marine invertebrates and causes imposex in gastropods [31].

Organic Compounds

Within the enormous variety of organic toxicants, the following are some of the most common mechanisms of toxicity:

a. *Anticoagulants*. Inhibit the regeneration of vitamin K, leading to dysfunctional hepatocytes that prevent blood clotting and, in certain conditions, can cause haemorrhaging. Exclusively used as rodenticides, but they also affect birds [32] and possibly other vertebrates.

b. *Auxin-type disrupters*. Phenoxy and pyridine herbicides (e.g. 2,4-D and triclopyr respectively) stop plant growth by mimicking plant hormones (auxins).

c. *Chemicals destructive of tissues*. Reactive substances such as chemical reagents (acids, formalin, strong bases), anilines and some herbicides (e.g. paraquat and diquat), cause either oxidation or reduction of tissues and consequently their destruction.

d. *Endocrine disrupting chemicals (EDCs)*. Some toxic chemicals mimic the role of hormones that control specific physiological processes in animals. For instance, PCBs disrupt the aryl hydrocarbon (Ah) receptor in the cytosol, triggering the induction of the detoxification complex P450; in some cases, the metabolites thus produced compete with thyroxine, reduce the level of retinol in blood and lead to vitamin A deficiency [12]. Certain phthalates, organochlorines, phenolic and organometallic compounds, and other toxicants described above act in similar ways [33]. Human hormones (synthetic and natural) can be discharged into sewage and cause endocrine disruption in other organisms.

e. *Inhibitors of biosynthetic processes*. Usually selective toxins for some taxa, as they target specific metabolic processes. Antibiotics inhibit the synthesis of specific proteins in bacteria; sulfonamides (e.g. flusulfamide) inhibit specific protein or amino acid synthesis in fungi; sulfonylurea herbicides (e.g. chlorsulfuron) act the same way in plant cells. Glyphosate also inhibits the biosynthesis of essential aromatic amino acids (histidine, phenylalanine, tryptophan, tyrosine), which cannot be produced by animals. Benzoylurea insecticides (e.g. lufenuron) inhibit the production of chitin in arthropods, effectively impeding moulting and stopping development in those organisms.

f. *Germination inhibitors*. Broad-spectrum herbicides and fungicides disrupt cell division. Thiocarbamates (e.g. thiobencarb), chloroacetamides (e.g. metolachlor) and dinitroanilines (e.g. trifluralin) inhibit the synthesis of certain proteins, lipids or fatty acids required for plant germination, while dichlobenil inhibits the production of cellulose. Triazoles (e.g. difenoconazole), imidazoles (e.g. imazethapyr) and morpholines (e.g. fenpropidin) inhibit the synthesis of ergosterol, an essential component of the membranes of fungi; the fungicide benomyl inhibits the synthesis of microtubules, and consequently stop cell division.

g. *Mutagenic, carcinogenic and teratogenic*. Aromatic hydrocarbons (e.g. benzene) and PAHs (e.g. anthracene) form adducts with the DNA, thus causing mutations in the genome that lead to cancers and malformations. In contrast, the aliphatic hydrocarbons (e.g. butane) are not as toxic and can be readily metabolised by bacteria [34]. Dioxins and related compounds act like PCBs but are more toxic and also have teratogenic effects [12].

h. *Neurotoxic*. Most insecticides are included here. Organochlorines (e.g. lindane) inhibit the GABA receptors in neuronal cells, whereas DDT, pyrethrum and synthetic pyrethroids (e.g. cypermethrin) act upon the axonal sodium channels that are voltage dependent [35], altering the nervous impulse and causing convulsions and paralysis. Organophosphorous (e.g. chlorpyrifos) and carbamate (e.g. carbaryl) insecticides inhibit the acetyl-cholinesterase receptor at the neuronal synapses, while nicotine and synthetic neonicotinoid insecticides (e.g. imidacloprid) inhibit the nicotinic receptor, as a result of which the transmission of the nervous impulse is blocked, thus causing hyper-excitability [36]. Narcotic drugs (e.g. morphine) activate the opioid receptors in the brain and spinal cord, causing analgesic and sedative effects.

i. *Photosynthesis inhibitors*. The photosystem II electron transport process, carried out in the chloroplasts within plant cells and algae, can be inhibited by many herbicides: triazines (e.g. atrazine), urea-derivatives (e.g. diuron), bromoxynil, *etc.*

j. *Respiratory inhibitors*. Most biocides are included in this category. The complex III (cytochrome C — oxidoreductase or cytochrome BC_1 complex) involved in electron-transfer mechanisms to produce ATP in the mitochondria and bacteria can be inhibited by some organometallic compounds (e.g. TBT), strobilurin fungicides and other toxicant groups (Table **1**).

BASIC TOXICOLOGICAL PRINCIPLES

Paracelsus established that "All things are poison and nothing (is) without poison; only the dose permits something not to be poisonous" [37]. In toxicology, the degree of exposure or dose is as important as the nature of a chemical. Common salt (NaCl), for instance, can be toxic to most aquatic organisms at concentrations above 6%. For a typical toxicant, the response is represented by a sigmoid curve which indicates increasing toxic effects at increasing doses, usually on a logarithmic scale (Fig. **1**). For essential elements (*i.e.* copper), the relationship is parabolic: at lower than normal doses for growth and development, the organisms may die of nutrient deficiency; as the levels increase within a short range there are not harmful effects, whereas higher doses produce the normal toxic response. In aquatic environments the dose is replaced by the concentration of the toxicant in water, since the internal dose (which causes the effect) is a function of the uptake rate by the organism and the external concentration [38]. The toxicity of a compound is usually evaluated as the dose required to cause a 50 percent effect in an organism (EC50), which in the case of mortality is the median lethal dose or concentration: LD50 or LC50 respectively. The no-observed effect level (NOEL) and lowest-observed effect level (LOEL) are also used, but they are less reliable measures and sometimes difficult to obtain.

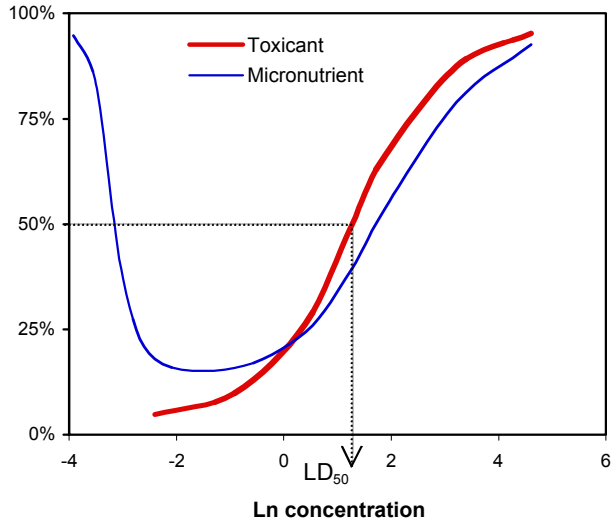

Figure 1: Dose-effect relationships.

In addition to dose, time is an important factor to be considered in toxicology. Indeed, the same effect can be produced by a large dose applied to an organism at once than by repeated small doses applied over a certain period of time (chronic exposure), as long as the toxicant is not degraded (Fig. **2**). The exposure time is in fact related to the actual internal dose, and both are linked by a linear relationship in the logarithmic scale [39]. Obviously this relationship is more important where a continuous input occurs or in the case of persistent ecotoxicants, which can linger in the environment for months or years, and can be accumulated in the tissues of the organisms affected. For example, many chlorinated organic toxicants (PCBs, PCDDs, DDT and other pesticides) are recalcitrant and non-polar compounds with a tendency to be stored in fatty tissues because they are lipophilic; the remobilization of these toxins from the body fat during periods of starvation or exertion, such as long migration, can take them to the sites of action (e.g. neuronal system) and produce a toxic effect [40].

Finally, sublethal effects – those effects caused by doses smaller than the LD50 or acute NOEL – are commonly found after chronic exposure of organisms to low levels of ecotoxicants. These effects are usually unrelated to the specific mode of action of the chemicals, and therefore are unpredictable. For example, some organochlorine insecticides which are neurotoxic at relatively high doses (mg/kg body weight), can also produce endocrine disruption when present at very low doses (µg/kg body weight) [41]. In other instances, such as DDT, the metabolite DDE causes the thinning of egg-shells and consequently can lower the hatching success of many bird species [42].

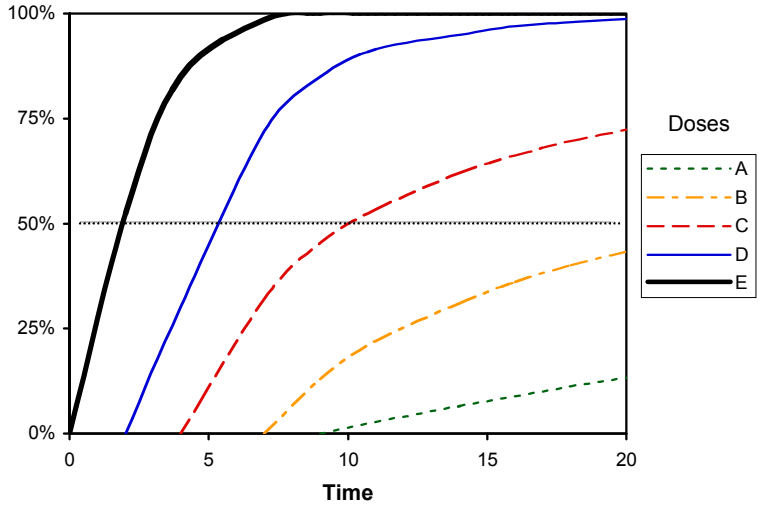

Figure 2: Time-to-effect relationships.

ECOLOGICAL RISK ASSESSMENT

The hazardous substances that pollute our environment (Table **1**) can cause detrimental effects only when organisms are exposed to them at sufficient, toxic doses. Ecological risk assessments aim at determining the extent of harm caused to the environment by the release of toxic chemicals. Such assessments consider the exposure of organisms and inherent toxicity of the substances as the two main components, and usually indicate the impacts in terms of probabilities [43]. Most risk assessments refer to individual toxicants discharged over specific areas either in one event (e.g. accidental spills) or repeatedly (e.g. continuous industrial effluents, pulsed pesticide applications), and consequently tend to be site-specific.

The exposure of organisms to toxicants depends on many factors: distribution and concentration of the chemicals in water, air, soil and sediment; uptake through the roots and/or leaves in plants or through contact, ingestion and inhalation in animals; persistence of the residues in the environment and bioaccumulation in tissues; proximity to the source of emissions and probability of being affected. The toxicity component is based on experimental laboratory data, which derive the LD(C)50 or NOEL (acute or chronic) of the chemical for certain species of organisms such as algae, *Daphnia*, worms, insects, fish, birds and mammals. Typically, endpoints include mortality as well as sublethal effects. A chemical's hazard indicates the danger it may pose to organisms; risk implies the probability of being affected. The simplest way to assess the risk is by comparing the amounts of chemical present in water, air, soil and sediment with the toxic endpoints to each organism [44]:

$$HQ = \frac{\text{Predicted Environmental Concentration (PEC)}}{\text{Toxicity endpoint (LD(C)50 or NOEL)}}$$

If the hazard quotient (HQ) between the two numbers is greater than 1 the organisms would be at high risk, meaning that more than 50% of the individuals of a given species may die or experience sublethal effects. Obviously, the PEC can be replaced by actual measured concentrations. Based on years of experience and numerous field trials – mainly with insecticides – it is concluded that for an ecotoxicant to be considered 'safe' to organisms, the HQ should be smaller than 0.1 [45]. Since the toxicity endpoints are fixed for each chemical and species the main variable of the quotient is the environmental concentration – this forms the basis for establishing contaminant guidelines for air, soil/sediment and water quality, to ensure that levels of pollutants are below harmful thresholds. In any case, such quotients only indicate potential hazards to certain species, while additional information on the chemical's persistence, bioaccumulation factors and probability of actual exposure to a community of species, under normal and worst-case scenarios, are needed for a more comprehensive risk assessment to ecosystems [46].

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CHAPTER 2

Fate and Transport of Contaminants*

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Abstract: Release of toxic chemicals into the environment cannot always be avoided completely. As a result organisms, man included, will be exposed to chemicals *via* the environment. Given the release of certain chemicals into the environment, their exposure concentrations in air, water and soil would depend on the rates at which they are removed from the environment. This chapter deals with the transport and transformation processes that affect concentrations in the environment, with emphasis on the modeller's perspective. Being interested primarily in the effects that processes have on concentrations of chemicals in environmental media, we focus on a quantitative description of the rates at which losses from the environment take place, and on how these rates differ for different chemicals. We systematically formulate process rate constants for each transport or transformation process. Eventually, the rate constants combine into a mass balance model which allows us to describe and predict how releases into the environment result in exposure concentrations of organisms.

PROCESSES AND MECHANISMS

After entering the environment, chemicals are transported, distributed over the various environmental compartments and may be transformed into other chemicals. Transport can occur within a compartment, such as in air or in soil, or between compartments (e.g. between air and water, air and soil or water and soil). Transformation processes in the environment involve chemical degradation or biodegradation.

Process rates (*i.e.* the mass flows of substance that result from them) generally depend on two independent factors: (i) the concentration of the substance in the environmental medium (driving force) and (ii) the likelihood of occurrence of the process (rate constant). When process rates are directly proportional to concentrations, process kinetics are called first order (first power concentration). Non-linear relationships apply in cases of higher or lower order kinetics. In the case of first-order kinetics, the mass M of chemical in the environmental compartment of origin falls exponentially with time t:

$$\frac{dM}{dt} = -k_{loss} \cdot M \quad or \quad M = M_0 e^{-k_{loss} \cdot t} \tag{1}$$

The first-order loss process is characterized by one single parameter: the loss rate constant k_{loss}. The loss mechanism causes the mass of chemical in the compartment to fall from its original value M_0 to half of that value in a constant time period: the half-life time $t_{1/2}$ of the chemical in the compartment. Mass keeps falling at the same half-life until the chemical has disappeared entirely. It can be deduced from Eq **1**, or seen from its graphical representation (Fig. **1**), that in case of first-order kinetics the half-life time $t_{1/2}$ has a constant value throughout the loss process:

$$t_{1/2} = \frac{\ln 2}{k_{loss}} \tag{2}$$

Throughout this chapter, we shall assume first-order kinetics to apply to all transport and transformation process. Although in reality this is certainly not always true, usually too little information is available to better describe the process rate-concentration dependence. Moreover, even in cases where higher order kinetics apply, assuming first-order kinetics will not always lead to dramatically erroneous predictions, as often concentrations do vary only slightly, so that so-called pseudo-first-order kinetics apply.

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*This text is taken largely from chapters 3 and 4 of the textbook by Van Leeuwen and Vermeire [1], the authors of which are kindly acknowledged.

Francisco Sánchez-Bayo, Paul J. van den Brink and Reinier M. Mann (Eds)

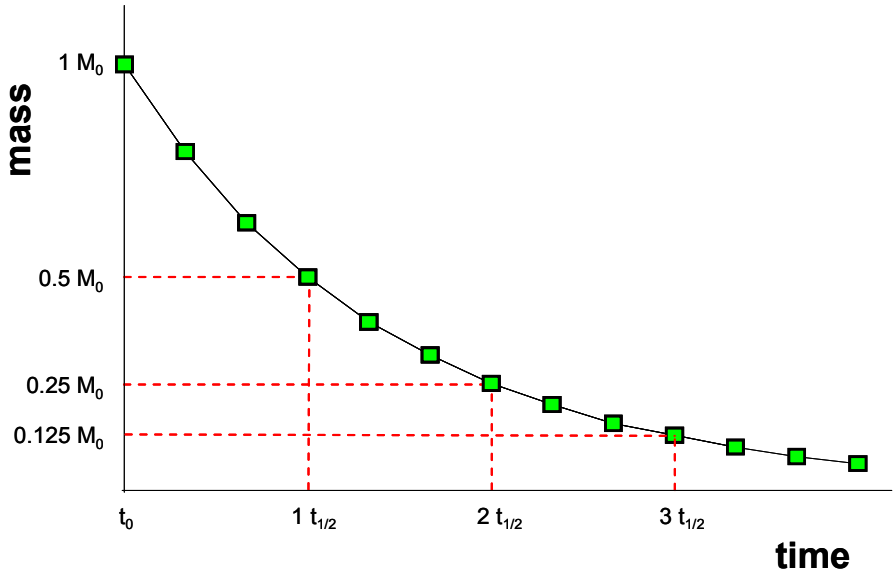

Figure 1: Loss of chemical from the environment through first-order kinetics results in constant half-life.

Equilibrium Partitioning Between Phases

Obeying general laws of thermodynamics, chemicals tend to spontaneously migrate from one phase (environmental medium: air, water, soil) to another if the phases are not in equilibrium. Migration in multi-phase systems continues until equilibrium has been reached. In thermodynamics, equilibrium is characterized as the state in which the chemical potential, and the chemical's activity and fugacity have the same value in the different phases. This principle has successfully been applied [2] in mass balance models of environmental fate of chemicals, that have become known as "fugacity models" or "Mackay models". For most practical situations, the equilibrium condition can be expressed by stating that chemicals are driven towards equilibrium until the ratio of concentrations (C_1 and C_2) is equal to the intermedia equilibrium constant K, also known as partition coefficient:

$$K_{12} = \frac{C_1}{C_2} \qquad \textbf{(3)}$$

Departure from thermodynamic equilibrium forms the main driving force of intermedia transport of chemicals. This is why intermedia equilibrium constants play such an important role in quantitative mathematical descriptions of transport and fate of chemicals in the environment.

Solids-Water Equilibrium

Equilibrium partitioning between water and solids is the result of adsorption of the chemical onto the surface of particles. For low concentrations of the chemical in water, the equilibrium ratio is usually a constant: K_{12} of Eq **3** is independent of the concentrations of the chemical. For higher concentrations, it is often observed experimentally that the equilibrium ratio does depend on the concentrations. In such cases, the equilibrium relationship between the concentrations is given by a non-linear sorption isotherm. The Freundlich-isotherm equation is often used (without making assumptions about the nature of the underlying mechanism) to fit experimentally observed non-linear sorption.

Commonly used estimation methods for solids-water partition coefficients K_p are based on the assumption that there is a "hydrophobic sorption" mechanism. This mechanism is generally modelled based on the organic carbon content of the soil, sediment or suspended solids f_{oc} and the octanol-water partition coefficient of the chemical K_{ow}, using simple regression equations:

$$\log K_p = \log(K_{oc} \cdot f_{oc}) = a \log K_{OW} + b + \log f_{oc} \qquad \textbf{(4)}$$

where

K_{oc} = organic carbon referenced solids-water partition coefficient (L/kg)

a, b = constants, specific for chemical classes

Given a value of K_p, the extent to which partitioning from water to solids occurs depends on the amount of solids present. At equilibrium, the mass fraction of chemical dissolved in water $\Phi_{dissolved}$ can be calculated as

$$\Phi_{dissolved} = \frac{1}{1 + K_p \cdot TSS} \quad \textbf{(5)}$$

where

TSS = mass concentration of suspended solids in water (= ~ 10^{-5} kg/L)

Normalization to the organic carbon content of particulate matter has become standard procedure in this field of research. This procedure is based on the experimental observation that the K_p of organic chemicals is often proportional to the organic matter content of the solid phase. The estimation method was derived originally for hydrophobic chemicals [3]. The method was extended to apply to various other classes of non-ionic organic chemicals [4] and, more recently, to ionizing organic acids and bases [5]. The method cannot be applied to metals and other inorganic ionizing substances.

Solids-water partition coefficients are commonly reported in units L/kg. The physical meaning of this dimension can be understood by reading it as "the volume of water (L) which contains the same amount of the chemical as one kilogram of solid material does". For many purposes, however, we are not just interested in the concentration ratio, but also in the mass distribution of the chemical over the phases. Obviously, this distribution depends on both the partition coefficient and the relative volumes of the phases. In surface water, the solids-water ratio is much smaller than in sediment and soil systems. As a result, the extent of partitioning of a certain chemical into the particle phase of sediment or soil is much greater than in surface water. Solids-water partition coefficients of chemicals K_p range from < 1 L/kg to > 10^5 L/kg, with resulting extents of partitioning into the solid phases ranging from negligible in surface water to near-complete in soil (Table **1**).

Air-Water Equilibrium

Equilibrium between air and water is given by Henry's law, which states that in equilibrium, the partial pressure of a chemical in the gas phase is proportional to its concentration in water. The ratio of these, Henry's law constant H, can be obtained as the ratio of the saturated vapour pressure P^s and water solubility S of the pure compound, provided that P^s and S refer to the same physical state (liquid or solid) and to the same temperature T. The air-water concentration ratio K_{AW} can be derived from Henry's law constant by reworking it into a "dimensionless" partition coefficient. Dimensionless air-soil concentration ratios can be obtained in the same way:

$$K_{AW} = \frac{C_{air}}{C_{water}} = \frac{H}{RT} = \frac{P^s_{L,S}}{S_{L,S} \cdot RT} \quad \textbf{(6)}$$

where

R = gas constant (8.314 Pa.m^3/mol/K)

T = temperature at the air-water interface (K)

Air-water equilibrium constants of chemicals K_{AW} range from < 10^{-10} to > 1 (Table **1**).

Table 1: Typical environmental values of intermedia partition parameters for selected chemicals.

	units[1]	Dichloroethane	Dieldrin	Benzo[a]pyrene	Cadmium^{2+}
$K_{aerosol\text{-}gas}$	-	6.2E+02	3.5E+08	5.8E+10	>>[3]
$K_{air\text{-}water}$	-	4.9E-02	4.5E-04	1.9E-05	<<[3]
$K_{susp\text{-}water}$	-	5.0E+00	2.1E+03	2.5E+04	2.5E+04
$K_{sed\text{-}water}$	-	2.5E+00	1.0E+03	1.2E+04	1.3E+04
$K_{soil\text{-}water}$	-	1.0E+00	4.1E+02	4.9E+03	5.0E+03
$K_{bio\text{-}water}$	-	3.8E+00	2.0E+04	1.4E+05	1.7E+02
Φ_{gas}(air)[2]	-	100% (100%)	99.6% (97%)	25% (15%)	<< (<<)[3]
$\Phi_{dissolved}$(water)	-	100%	98%	83%	87%
$\Phi_{pore\ water}$(sediment)	-	62%	0.4%	0.03%	0.03%
$\Phi_{pore\ water}$(soil)	-	25%	0.1%	0.01%	0.01%

[1] "Dimensionless" ratios to be read as $m_{medium1}{}^{3}.m_{medium2}{}^{-3}$; for K-values, and as "$mol_{phase1}/mol_{phase2}$" for Φ-values;

[2] numbers in parentheses calculated according to Eq **8**; [3] >>: very large; <<: very small

Air-Aerosol Equilibrium

The extent of association of chemicals with the aerosol phase of air is known to be inversely related to the chemical's vapour pressure. The fraction associated with the aerosol phase $\Phi_{aerosol}$ has successfully been described by Junge [6] with

$$\Phi_{aerosol} = \frac{c\Theta}{P_L^s + c\Theta} \quad \textbf{(7)}$$

where

Θ = aerosol surface area per volume unit of air (m^2/m^3)

P^s_L = vapour pressure of the pure compound in the liquid state (Pa)

c = constant (Pa.m)

The constant c depends on the heat of condensation and molecular weight for many organics, its value being approximately 0.17 Pa.m. The local pollution climate determines the aerosol surface density. A typical value for aerosol surface area under rural conditions is 3.5 x 10^{-4} m^2/m^3. For more polluted urban or industrialized areas Θ is estimated to be 1.1 x 10^{-3} m^2/m^3. Substitution of these values in Eq **7** shows that gas-particle partitioning is important for organic compounds with a P^s_L lower than approximately 10^{-3} Pa. Since P^s_L is strongly temperature dependent, the fraction of a substance absorbed to particles will also be temperature dependent. For certain organics this may imply that in tropical regions the pollutant will be in the gas phase, whereas in arctic regions it will be in the particle phase. More recently, it has been shown that for many (hydrophobic) chemicals, the octanol-air partition coefficient K_{OA} is a more accurate descriptor of aerosol-air partitioning [7].

$$\Phi_{gas} = \frac{1}{1 + K_{OA} \cdot (B \cdot TSP)} = \frac{1}{1 + (K_{OW} / K_{AW}) \cdot (B \cdot TSP)} \quad \textbf{(8)}$$

where

B = chemical- specific constant (= ~$2x10^{-12}$ $m^3/\mu g$)

TSP = mass concentration of aerosol in air (= ~50 $\mu g/m^3$)

Eq **8** predicts significant partitioning to the aerosol phase for chemicals with K_{OA} greater than approximately 10^{10}, which is the case for many polyaromatic cyclic hydrocarbons PAH (Table **1**).

TRANSPORT MECHANISMS

Two kinds of transport mechanisms may be distinguished: (1) *intramedia transport*, which is transport away from a source in one environmental medium, and (2) *intermedia transport*, which is transport from one environmental medium to another. Intramedia transport is important in relation to the mobile environmental media: air, water and groundwater; intermedia exchange takes place between all media, but is most important for transport of chemicals to and from the stationary media: sediment and soil.

Intramedia transport takes place through the mechanisms of advection and dispersion. Advection causes a chemical to travel from one place to another as a result of the flow of the medium in which it occurs; locally emitted packages or "puffs" of a chemical are carried as far as the wind or water current can take it during the residence time in that medium. Dispersion mechanisms (molecular diffusion, eddy diffusion) make the chemical move down concentration gradients until the gradients have disappeared. The residence time of the chemical in the medium is an important factor since besides this other removal processes occur at the same time. If, for example, a chemical is emitted into air and its degradation in air is rapid, the effective residence time of the chemical in air is short. Consequently, there is little time for the advective and dispersive processes to take place. In one medium, advection and dispersion always operate together. If a chemical is emitted continuously into air or water, the combined operation of advection and dispersion results in the formation of a plume. At short distances from emission sources, concentrations are usually affected most by intramedia transport. Intramedia transport of chemicals is observed as dilution, which in many situations is the most important process affecting environmental concentrations of chemicals. In this chapter, we shall account for the effect of intramedia dilution on the concentrations of chemicals by lumping it into rate constants for advective and dispersive loss of chemicals due to transport. However, explanation of the complex aerodynamic and hydrodynamic processes of advective and dispersive spread of chemicals within air, surface water and groundwater will not be discussed here. Interested readers are referred to specialized text books on mathematical modelling of air, surface water and groundwater [8].

Intermedia transport (air-water, water-sediment, *etc.*) also takes place by advective and dispersive mechanisms. Advective intermedia transport takes place if a chemical is transported from one environmental compartment to another by a physical carrier. Examples are deposition of fog, raindrops and aerosol particles from air to water or soil, sedimentation and resuspension of particulate matter across the water-sediment interface, and percolation of water through soil. Advective transport is a one-way phenomenon: the chemical is carried by the medium in which it resides in the direction of the medium flows. Intermedia dispersion is also diffusive in nature and follows concentration gradients. Examples are volatilization and gas absorption (air-water and air-soil), the direction depending on the concentration difference between the media, and diffusive exchange of chemicals between sediment and water. The driving force of intermedia transport is the tendency of chemicals to seek equilibrium between different phases. Transport from one environmental medium to another is commonly described by taking the box/compartment modelling approach. Theoretical backgrounds and detailed quantitative descriptions of intermedia exchange processes can be found in other texts [2, 9-13]. The most important interfaces are described below.

Air-Water and Air-Soil Exchange

Atmospheric deposition and volatilization processes transport chemicals between air and the earth's surface. It is customary to distinguish between wet (precipitation-mediated) deposition mechanisms and dry deposition mechanisms (Fig. **2**). Wet deposition is further split into rain-out (in-cloud processes) and wash-out (below-cloud processes). Dry deposition is the sum of aerosol deposition and gas absorption. In multimedia environmental

chemistry, the latter mechanism is usually treated as one part of a bi-directional exchange mechanism. Rain-out, wash-out and aerosol deposition are one-way advective transport processes: the chemical is carried from the atmosphere to water and soil. This is true even if the chemical has a greater fugacity in water or soil. Gas absorption is a diffusive mechanism. There is only net absorption of chemicals from the gas phase by water or soil if the fugacity in air is greater than the fugacity in water or soil. If the fugacity in water or soil is greater, the result will be the reverse: net volatilization. This will generally be the case if a chemical is emitted to water or soil, in which cases fugacities in these media will be highest of all. Deposition from air to water and soil occurs at all times, even when net volatilization occurs (see below). It should be noted that absorption and volatilization occur simultaneously, and it is the net difference that accounts for the effective intermedia transport.

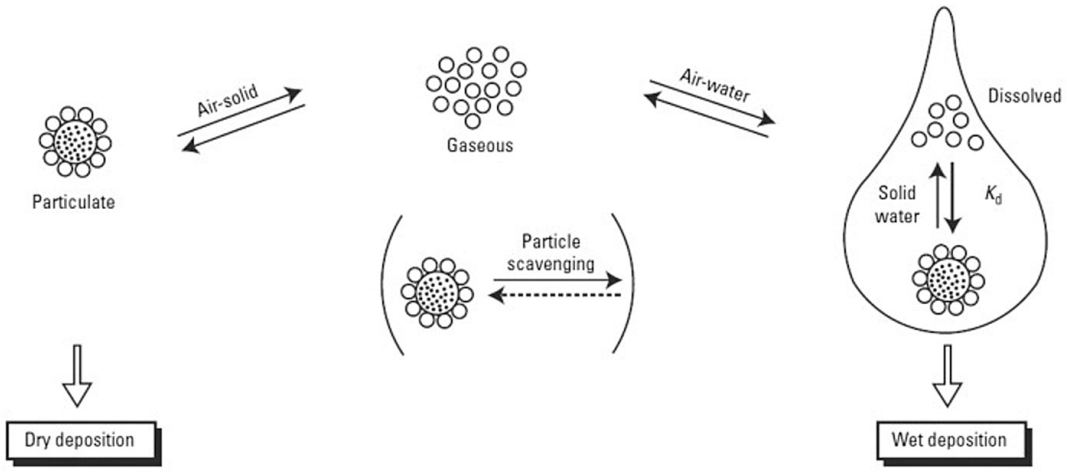

Figure 2: Exchange mechanisms between atmosphere and the earth's surface. From Schwarzenbach [14], as referred to by Sijm *et al.* [15].

Deposition with Aerosol and Rain

Chemicals adsorbed to aerosol particles are carried from the air compartment to the earth's surface by dry particle deposition. Aerosol particles can also be scavenged by rain drops as wet particle deposition. In addition, rain drops absorb chemicals from the gas phase and carry chemicals to the earth's surface by rain-out and wash-out.

Deposition rates depend on the physical parameters of the particle, of which the size is most important. Small particles tend to behave like gases; larger particles (> 2 μm) are efficiently removed from the atmosphere by deposition under the influence of gravity. Inertial impaction is important for particles with a diameter of between 0.1 and 10 μm. This effect greatly depends on the velocity of the air and the intensity of the turbulence, which varies with the properties of the landscape. Larger particles (> 10 μm) are deposited primarily by sedimentation and chemicals associated with them will, in general, be deposited close to the source. Typical aerosol deposition velocities range from 10^{-4} to 10^{-2} m/s. Some chemicals are associated predominantly with the larger, rapidly depositing particles, whereas other chemicals bind predominantly to the smaller particles and stay airborne for much longer times.

The efficiency of wet deposition varies greatly. It depends on meteorological factors such as the duration, intensity and type of precipitation (snow, rain, hail), as well as on the size and the number of droplets. Other specific parameters, like solubility in rain and snow, are important too. Wash-out is an efficient removal mechanism for chemical substances with low Henry's law constants, and for aerosols with a diameter greater than 1 μm. For less volatile chemicals (high Henry's law constants) the falling droplet will absorb only a very small amount of the compounds below the cloud. Wash-out plays an important role when concentrations below the cloud are much higher than the concentrations in the cloud, e.g., for plumes close to the source. In clouds the uptake of aerosols by cloud droplets is a very efficient process. For most purposes, it is sufficient to assume that the rain phase is in equilibrium with the gas phase. The extent of gas scavenging by falling rain drops can then be calculated from the air-water distribution ratio K_{AW} and the rain intensity. As a practical approach to estimating the extent of aerosol

scavenging, Mackay [2] has suggested that during rainfall in the atmosphere, each drop sweeps through a volume of air about 200,000 times its own volume. First-order rate constants for removal of chemicals from air by atmospheric deposition are given in Table **2**.

Table 2: Influence of transport- and transformation processes on concentrations in AIR for selected chemicals, after a steady state has been reached.

	Dichloroethane		**Dieldrin**		**Benzo[a]pyrene**		**Cadmium^{2+}**	
	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)
advection	7.1E-06	1.1	7.1E-06	1.1	7.1E-06	1.1	7.1E-06	1.1
deposition	9.1E-10	8852	1.2E-07	67	4.7E-06	1.7	5.5E-06	1.5
gas absorption	2.7E-06	3.0	2.9E-06	2.7	9.3E-07	8.6	3.8E-09	2089
volatilization	2.7E-06	3.0	2.0E-06	4.0	1.1E-07	72	<<	-
degradation	1.0E-07	79	3.1E-06	2.6	2.5E-07	32	<<	-
total loss	7.2E-06	1.1	1.1E-05	0.71	1.3E-05	0.63	1.3E-05	0.64

Volatilization and Gas Absorption

Transport of a chemical from water and soil into the gas phase of air and vice versa is commonly described by a two-resistance approach, as originally introduced 110 years ago by Whitman [16]. In this concept, the resistance to intermedia transfer is considered to be concentrated in two thin films on either side of the interface. Transport through this interfacial double layer has to take place by molecular diffusion and is, therefore, slow in comparison with transport to and from the interface. This concept was used by Liss and Slater [17] as a basis for modelling the transfer of gases across the air-sea interface. The direction of transport depends on the concentrations in air and water. In fugacity terminology: the net diffusion is from the compartment with the highest fugacity to the compartment with the lowest fugacity. The rate mass-transfer (depending on the direction referred to as either gas absorption or volatilization) is expressed by means of an "overall" mass-transfer coefficient k^{OV}, in which transfer resistances on either side of the air-water interface are accounted for, can be expressed in terms of air- or water concentrations. The mass-transfer coefficient (m/s) can be looked upon as the velocity of a piston, pushing the chemical through the interface. The driving force can be positive or negative, leading to absorption or volatilization. The overall mass transfer coefficients k^{OV} represent the resistance to mass transfer: the greater k^{OV}, the smaller the resistance. The magnitude of k^{OV} derives from the partial mass transfer coefficients of the stagnant films at either side of the air-water interface, through which the chemical must diffuse, and the intermedia equilibrium constant K_{AW} can be estimated as:

$$k_{air}^{OV} = \frac{kaw_{air} \cdot kaw_{water}}{kaw_{air} \cdot K_{AW} + kaw_{water}}$$
$$k_{water}^{OV} = \frac{kaw_{air} \cdot kaw_{water}}{kaw_{air} + kaw_{water} / K_{AW}} \quad , \qquad \textbf{(9)}$$

where

kaw_{air} = partial mass-transfer coefficient air side of the air-water interface (m/s)

kaw_{water} = partial mass-transfer coefficient water side of the air-water interface (m/s)

The partial mass transfer coefficients *kaw* represent the resistances of the stagnant air and water films. Thick films have greater resistances than thin films; substances with large diffusivities (small molecules) have smaller

resistances than substances with small diffusivities (big molecules). Since diffusivities of substances do not differ much, differences in *kaw* originate mainly from differences in thickness of the stagnant film through which the molecules must diffuse. Film thicknesses vary from water body to water body due to differences in turbulence. Typical values of *kaw* are 10^{-3} and 10^{-5} m/s for air and water films, respectively. As a result, differences in volatilization between substances arise from differences in the air-water equilibrium constant K_{AW}. It can be seen from Eq **9** that k^{OV} of water-loving chemicals ($K_{AW} << \sim 10^{-2}$) is proportional to K_{AW}; resistance to volatilization for such chemicals originates entirely from slow diffusion through the air film. Air-loving chemicals ($K_{AW} >> 10^{-2}$) volatilize independently of K_{AW}; resistance to volatilization for such chemicals is limited only by slow diffusion through the water film. Advanced readers are referred to specialized textbooks on this subject [9, 10].

When the concentration in air is negligibly small, the net rate of volatilization depends only on the concentration in water, so that volatilization acts as a first-order removal process from water. Applying the mass balance concept of Eq **1**, it follows that

$$VOL \approx A \cdot k^{OV}_{water} \cdot C_{water} = k_{vol} \cdot V_{water} \cdot C_{water},$$
$$\text{with} \quad k_{vol} = \frac{A \cdot k^{OV}_{water}}{V_{water}} = \frac{1}{D_{water}} \cdot \frac{kaw_{air} \cdot kaw_{water}}{kaw_{air} + kaw_{water} / K_{AW}}, \qquad \textbf{(10)}$$

where

k_{vol} = first-order rate constant for removal from water by volatilization (1/s)

V_{water} = volume of the water compartment (m^3)

D_{water} = depth of the water compartment (m)

Note that, while the volatilization rate (mol/s) depends on the area A of the water compartment, the effect that this volatilization has on the concentration C_{water} depends on the depth D_{water} of the water compartment. Similarly, gas absorption with negligible concentration in water acts as a first-order removal process from air. The rate constant for removal from air by gas absorption is left to be worked out by the reader. Volatilization from and gas absorption to soil can be deduced along the same lines and is not treated here. First-order rate constants for exchange of some chemicals between air and the Earth's surface are given in Tables **3** and **4**.

Table 3: Influence of transport and transformation processes on concentrations in WATER for selected chemicals, after a steady state has been reached.

	1,2-Dichloroethane		**Dieldrin**		**Benzo(a)pyrene**		**Cadmium^{2+}**	
	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)
advection	6.7E-08	119	6.7E-08	119	6.7E-08	119	6.7E-08	119
deposition	8.6E-12	936753	6.8E-11	118770	1.2E-10	68120	<<	>>
gas absorption	2.1E-09	3795	1.5E-09	5218	2.3E-11	348458	<<	>>
volatilization	1.8E-06	4.4	2.0E-07	40	1.0E-08	774	<<	>>
sedimentation	1.2E-10	69613	1.2E-07	69	5.1E-07	16	1.3E-06	6.4
resuspension	2.5E-10	31566	9.4E-08	86	4.3E-07	19	1.1E-06	7.1
adsorption	9.2E-09	875	9.1E-09	884	8.3E-09	965	8.1E-09	996
desorption	9.0E-09	896	1.6E-08	496	1.6E-08	514	1.6E-08	502
degradation	4.6E-08	174	4.5E-09	1779	4.1E-08	194	<<	>>
total loss	1.9E-06	4.1	2.9E-07	28	1.9E-07	42	1.9E-07	42

Table 4: Influence of transport and transformation processes on concentrations in SOIL for selected chemicals, after a steady state has been reached.

	Dichloroethane		**Dieldrin**		**Benzo[a]pyrene**		**Cadmium^{2+}**	
	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)	k (1/s)	$t_{1/2}$ (d)
deposition	2.2E-12	10036	1.0E-11	2167	1.5E-11	1504	<<	>>
gas absorption	1.8E-12	12432	2.1E-11	1059	9.0E-13	24311	<<	>>
volatilization	3.5E-08	0.63	3.7E-09	6.0	1.6E-10	136	<<	>>
run-off	1.3E-08	1.8	4.4E-10	50	1.1E-10	194	2.8E-12	7847
infiltration	1.3E-08	1.8	4.2E-10	52	9.4E-11	233	1.8E-12	11883
degradation	1.4E-08	1.5	4.6E-09	4.8	4.6E-09	4.8	<<	>>
total loss	3.9E-08	0.56	5.5E-09	4.0	4.8E-09	4.6	4.7E-12	4726

Soil Run-off

Part of the rainwater that reaches the soil runs off to surface water, *i.e.* rivers, estuaries and coastal waters. In urban areas, where most of the surface is paved, nearly all the precipitation is collected in sewerage systems, from where it may either be redirected to a waste water treatment facility or discharged into surface water. In rural areas the rainwater runs off directly into the surface waters. With the run-off, soil particles are washed away (eroded). Chemicals dissolved in water or associated with the soil particles, are transported by these mechanisms from soil to water. Assuming the water which runs off from soil is in equilibrium with the soil, the mass flow of a chemical resulting from run-off can be quantified. However, for most chemicals it is often more practical and accurate to conduct field measurements on contaminated sites than applying models.

Rates of net precipitation and fractions of water that run off and infiltrate into the soil are often well known from meteorological monitoring. Rates of soil erosion are much harder to obtain. There is extensive literature on the dependence of soil erosion on rainfall and terrain conditions (e.g. slope), which is not treated here [18]. First-order rate constants for removal of some chemicals from soil by run-off to surface water are given in Table **4**.

Deposition and Resuspension of Sediment Particles

The transport of chemicals across the sediment-water interface can be treated in the same manner as air-water and air-soil exchanges. In this case there is an advective transport component *i.e.*, sedimentation and resuspension, and a diffusive transport component, *i.e.*, direct adsorption onto and desorption from the sediment. To estimate the rate of advective transport from water to sediment by sedimentation of suspended particles, we need to know the concentration of the chemical on the particles and the rate at which they settle. For most purposes it is sufficient to assume equilibrium between the suspended particles and water phase. The concentration in the particles is then proportional to the concentration in water and the can be derived by means of Eq **5.** Settling rates of sediment particles can be obtained from field- or laboratory measurements, or can be estimated by theoretical means. Resuspension of freshly deposited sediment counteracts this removal from water. Resuspension rates are usually not known and must be derived as the difference between the sedimentation rate and the net sediment growth rate, which can be measured in the field, or deduced from mass balance calculations of incoming and outgoing sediment loads.

Exchange between Water and Sediment by Direct Adsorption and Desorption

Diffusive transport between sediment and water, by direct adsorption and desorption across that interface, is analogous to diffusive transport across the air-water and air-soil interfaces and can be described with a two-film resistance mechanism. A value of ~3 x 10^{-6} m/s (0.01 m/h) may be taken for the mass-transfer coefficient on the waterside of the sediment-water interface $kwsd_{water}$ [19]. The mass-transfer on the pore water side of the sediment-

water interface $kwsd_{sed}$ can be treated as molecular diffusion in the aqueous phase of a porous solid material, characterized by an effective diffusivity of 2 x 10^{-6} m^2/h and a diffusion path length of 2 cm. This gives kws_{sed} a value of ~3 x 10^{-8} m/s (0.0001 m/h). It should be noted, however, that additional processes that are typically of a non-equilibrium nature, may greatly affect the net mass-transfer of all kinds of chemicals. For instance bioturbation and shipping can play a key role in the sediment side resistance, essentially eliminating it in some cases. As the extent of bioturbation is not governed by thermodynamic principles, and as, in general, very limited information is available on this and similar topics, it will not be extensively discussed here. Instead, readers are referred to the textbook by Thibodeaux [10]. First-order rate constants for exchange of some chemicals between water and sediment are given in Table **3**.

Removal by Transport

Transport processes result in translocation of chemicals in the environment, but do not lead to their elimination. The advective exchange processes between environmental media as discussed above (e.g., sediment-water exchange by sedimentation and resuspension), are examples of such non-eliminating transport processes. Advective transport also occurs within environmental media (air, water). It is common to consider only a part of the environment, e.g., a world region or a layer of air, water or soil. In these open systems, transport does remove chemical from the environment. Transport across the system boundaries has the same effect on the concentration of chemical inside the system as real elimination (e.g., by chemical reaction).

Wind carries airborne chemicals out of the region considered to other parts of the world; water currents do the same function for waterborne chemical. First-order removal rate constants for the transport by advection processes that cause concentrations in air and water to decrease can be formulated by considering all incoming and outgoing air and water flows in relation to the volumes of their compartments.

Transport from the upper layer of the soil to the groundwater takes place through leaching with percolating water. If we choose to exclude groundwater from the system considered, soil leaching should be regarded as elimination from the system. Background information on transport in porous media can be found in Spitz and Moreno [20] and will not be considered in detail here. In many approximations, the process of soil leaching is simplified by assuming equilibrium between the solid phase and pore water phase at all times and in all places. It is clear that leaching is an important factor for chemicals with a small K_p value. Analogous transport phenomena take place in sediment. Surface water may seep into the sediment, thereby carrying the chemical from the upper sediment layer down and vice versa.

An additional phenomenon occurs in areas where there is continuous sedimentation. In this situation sediment is continuously being buried under freshly deposited material. It is common practice (e.g., in water quality management) to consider the (mixed) top few centimetres of sediment only. Regarded this way, the concentration of chemical in this sediment top layer can best be understood by regarding sediment burial as a mechanism that removes chemical from the top layer, transporting it to the deeper sediment. First-order rate constants for removal of some chemicals from air, water, sediment and soil by advection, burial and leaching are given in Tables **2-4**.

Effect of Transport Processes on Concentrations of Chemicals in the Environment

As explained earlier (Fig. (**1**), Eqs **1** and **2**), concentrations of chemicals in the environment are controlled entirely by the transport and transformation mass flows of chemical into and out of environmental compartments. As discussed above, transport rates vary greatly between chemicals in a way that can be understood and predicted from the differences in physical-chemical properties. In addition, transport rates vary with environmental conditions (geometry, temperature, wind, precipitation). The combined effect of all transport and transformation processes on concentrations of chemicals can be evaluated by comparing the rate constants for net transport from the compartment of interest. Tables **2-4** list the most important first-order rate constants for removal from (and addition to) air, water and soil, respectively, for a selection of different chemicals, together with the half-lives of change in concentration due to the process. For comparison, rate constants and half-lives for degradation (to be discussed in the following section) and rate constants and half-lives for all combined (net) losses are given. The process data in Tables **2-4** were derived from calculations with the multimedia fate model SimpleBox [12, 13], parameterized to reflect the regional spatial scale of EUSES.

It can be seen that

- The greatest rate constants are found in Table **2**, for the air compartment. Half-lives for change in the concentrations in air are in the order of magnitude of days. The smallest rate constants are found in Table 4 for soil compartment. Note that $t_{1/2}$ values are expressed in years! Rate constants for water (Table **3**) take positions in between those for air and soil, with half-lives of concentration changes in the order of weeks.
- Transport by advection out of the system takes a dominant position for all chemicals in air (Table **2**) and to a lesser extent, also in water (Table **3**). Note that removal by advection is independent of the properties of the chemical. One should bear in mind that the data given here reflect the relatively small regional spatial scale of the EUSES model. The effect of advection on concentrations at larger spatial scales (continental, global) is much smaller. There is no removal by advection of soil.
- The relative importance of the various processes on concentrations varies between chemicals and between environmental media.

TRANSFORMATION PROCESSES

Following its release into the environment, a chemical may undergo various biotic and abiotic processes which modify its chemical structure. Degradation or transformation of a compound refers to the disappearance of the parent compound from the environment by a change in its chemical structure. When this change is brought about by micro-organisms, the degradation process is called primary biodegradation or biotransformation. When chemicals are converted entirely to simple molecules and ions, such as carbon dioxide, methane, water and chloride, biodegradation is referred to as mineralization. Transformation of chemicals in the environment can also occur by abiotic processes. Four categories of abiotic transformation processes are distinguished:

- *Hydrolysis*: alteration of the chemical structure by direct reaction with water.
- *Oxidation*: a transformation process in which electrons are transferred from the chemical to the oxidant species accepting the electrons
- *Reduction*: the reverse of oxidation; electron transfer takes place from a reductant to the chemical to be reduced.
- *Photo degradation*: transformation due to interaction with sunlight.

Transformation and mineralization processes alter the physicochemical and toxicological properties and can reduce exposure concentrations of chemicals in the environment. The rate of degradation of a specific chemical depends on its intrinsic sensitivity to undergo chemical transformation (reactivity), the presence of reactants and the availability of the chemical to undergo reaction, *i.e.* the presence of the chemical in the gas phase of air or dissolved in water. Generally, the availability and reactivity of both the chemical and the reactant depend to a large extent on environmental conditions like pH, temperature, light intensity and redox conditions.

Hydrolysis

In a typical hydrolysis reaction a hydroxyl group replaces another chemical group in a molecule. However, certain functional groups, including alkanes, alkenes, benzenes, biphenyls, (halogenated) polycyclic aromatics (e.g., PAHs and PCBs), alcohols, esters and ketones, are often inert to hydrolysis.

The importance of hydrolysis stems from the fact that the products formed are more polar and, consequently, more water soluble and less lipophilic than the parent compound. Hydrolysis reactions are commonly catalysed by hydrogen or hydroxide ions. Because the concentrations of hydrogen ion $[H^+]$ and hydroxide ion $[OH^-]$ change with the pH of the water, the rate of hydrolysis directly depends on the pH. Hydrolysis rate constants k_h, which generally obey pseudo first-order kinetics, are measured experimentally in laboratory tests, in which a known quantity of the compound is introduced into a solution of fixed pH and the disappearance of the compound is followed over time. As in Eq **1** and Fig. (**1**), the mass (and hence the concentration) of the chemical typically declines exponentially with increasing time. When plotted logarithmically, the loss rate constant is observed as the slope of the concentration-time plot

$$\ln\left(C_t / C_0\right) = -k_h \cdot t \quad \textbf{(11)}$$

where

C_t = time-dependent observed concentration of the chemical (mol/m^3)

C_0 = concentration of the chemical at the beginning of the experiment (mol/m^3)

k_h = observed pseudo first-order hydrolysis rate constant (1/s).

From the results of a series of such experiments at different pH levels, a pH rate profile can be constructed by plotting the logarithms of the observed rate constants as a function of the pH of the experimental solutions. Fig. (**3**) shows the pH rate profile of the hydrolytic transformation of phenyl acetate to yield acetic acid and phenol. Under acid conditions (pH < 3), specific acid catalysis is the predominant mechanism. In this pH region, the logarithm of k_{obs} decreases by a unit slope -1 with increasing pH. At less acidic pH (pH > 4), the hydrogen ion concentration is so small that the specific acid catalysed hydrolytic reaction is too slow to be seen in the profile. Between pH 4 and 6, the neutral mechanism (independent of pH) predominates. Finally, at pH > 8, due to base catalysis, an increase of k_h directly proportional with increasing OH^- concentration, becomes visible.

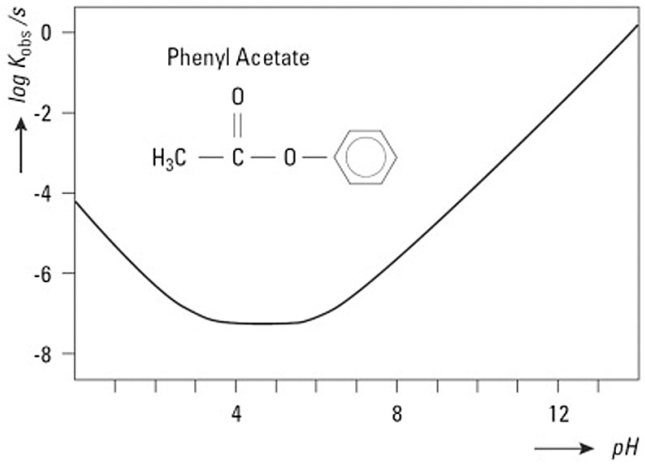

Figure 3: Hydrolysis pH rate profile of phenyl acetate. From Burns and Baughman [21] and Mabey and Mill [22], as referred to by Sijm *et al.* [15].

Oxidation

Oxidation is the chemical process in which an electron-deficient particle (the oxidant) accepts electrons from the compound to be oxidized. Examples of oxidants that occur under environmental conditions in sufficiently high concentrations and also react rapidly with organic compounds are: alkoxy radicals ($RO^{\cdot}$), peroxy radicals ($RO_2^{\cdot}$), hydroxyl radicals ($HO^{\cdot}$), singlet oxygen ($^1O_2$) and ozone (O_3).

Most of these oxidants are directly or indirectly generated from chemicals that interact with solar radiation, forming an “excited state” of the molecule; oxidation with photochemically formed reactive oxidants is usually referred to as photo oxidation. Oxidations are the main transformation routes for most organic compounds in the troposphere and also transform various micro pollutants in surface waters [23]. Most radical oxidants exhibit similar chemistry for aliphatic and aromatic structures.

Although many different kinds of $RO_2^{\cdot}$ or $RO^{\cdot}$ radicals may be present in a natural system, the simplifying assumption can be made that the structure of R has little effect on its reactivity [24]. Rate constants for reactions of most radical oxidants are known for a large number of organic molecules. The concentrations of the major oxidants

in less heavily polluted aquatic and atmospheric systems are also known. By combining these data it can be derived that, in general, the hydroxyl radical is the only oxidant of importance in atmospheric systems. In aquatic systems the concentration of.OH is so low that its contribution is negligible compared with $RO_2^{\cdot}$ or $RO^{\cdot}$. To illustrate the differences in reactivity of the hydroxyl radical to various organic chemicals, the half-lives for gas-phase oxidation of various classes of chemicals in the northern hemisphere are given in Table **5**.

Table 5: Half-lives (days) for tropospheric oxidation of various classes of organic compounds in the northern hemisphere.

Alkanes	1 – 10
Alcohols	1 – 3
Aromatics	1 – 10
Olefins	0.06 - 1
Halomethanes	100 - 47,000

From this table it is clear that chlorofluorohydrocarbons (CFCs or halomethanes), in particular, may remain in the troposphere for prolonged periods of time. This enables them to reach the stratosphere, where they pose a threat to the ozone layer.

Reduction

Reduction is the chemical process by which electrons are transferred from an electron donor (reductant) to the compound to be reduced. The redox half-reactions leading to reduction of a 1,2-substituted alkane are shown as a diagram in Fig. (**4**). In this example, Fe^{2+} is used as the reductant. Following the transfer of 2 electrons from 2 molecules of Fe^{2+} to the halogenated compound, Fe^{3+}, the free halide ion and the product of reduction (in this case ethene) are formed.

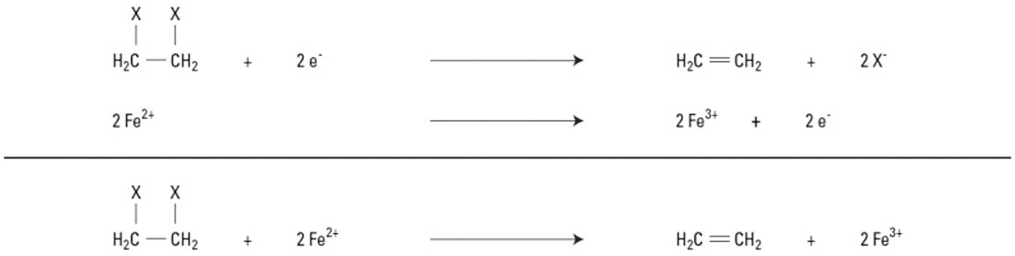

Figure 4: Example of a reductive transformation: electron transfer from Fe^{2+} to 1,2-dihalogen substituted ethane (X denotes a halogen atom).

It has been shown that reductive reaction pathways can contribute significantly to the removal of several micro pollutants. Nitro aromatics, azo compounds, halogenated aliphatic and aromatic compounds (including PCBs and even dioxins) can be reduced under certain environmental conditions [25].

Reduction can take place in a variety of reducing (non-oxic) systems, including sewage sludge, anaerobic biological systems, saturated soil systems, anoxic sediments, reducing iron porphyrin systems, solutions of various chemical reagents, as well as in the gastronomic tract of invertebrate species. It has also been shown that the reduction rate of specific halogen compounds depends on environmental factors, such as the prevailing redox potential, temperature, pH and the physical and chemical properties of the micro pollutant to be reduced. As in hydrolytic transformation, usually more polar products are formed from the parent compound by reduction, which makes them more susceptible to further chemical attack and less likely to accumulate.

At present, insufficient information is available on the nature of the reductants responsible for the main reductive transformations in natural systems. Nevertheless, it has been shown in most studies that reductive transformations generally follow pseudo first-order reaction kinetics.

Photochemical Degradation

Interaction with sunlight can initiate a wide variety of photolytic processes. The primary requirement for photochemical processes is the penetration of radiation (light, in particular UV light) in aqueous and atmospheric environments. Various categories of photochemical conversions can be distinguished:

- Direct photoreaction, in which the reacting molecule itself directly absorbs light;
- Indirect or sensitized photolysis, in which a light-absorbing molecule transfers its excess energy to an acceptor molecule causing the acceptor to react;
- Photo oxidation, in which molecules react with photochemically formed oxidative species (see above).

Following absorption of a photon by a compound, the photon energy either needs to be transferred to the reactive site within the molecule or transferred to another molecule, which may subsequently undergo a photochemical transformation. Not every photon that is absorbed by a molecule induces a chemical reaction. The proportion of absorbed photons which causes reaction is the quantum yield, a number between 0 and 1. Quantum yields may vary largely, depending on the chemical structure of the molecule. In direct photoreactions, the reaction rate is proportional to the absorption of light at a specific wave length and the quantum yield. The rate of light absorption depends on the light intensity and the specific absorptivity (molar absorption coefficient) of the chemical. Both the molar absorption coefficient and the quantum yield are intrinsic properties of the chemical; light intensity is a property of the environment. Since the rates of all photochemical reactions are proportional to light intensity, it is evident that the significance of the photo-transformation of a certain chemical will change with time and place. In this process factors such as time of the day or year, location (climate) and weather (cloud cover) play a major role.

In the aquatic environment, an important fraction of sunlight is absorbed by dissolved and particulate matter. This clearly reduces the rates of direct photo-transformation, and changes the solar spectrum in deeper water layers. However, this dissolved and particulate matter is also capable of initiating indirect photo-conversions. Given the complexity of these indirect conversions, and the many variables that influence the rate of indirect photolysis, it has so far only been possible, to a limited extent, to derive general, mathematical equations for rate constants in natural water systems.

Given the various direct and indirect transformations that can take place due to interaction with solar radiation, a variety of primary and secondary photoproducts is often observed. Since penetration of light is usually only possible in oxic systems, most photo-products formed are in an oxidized state, compared with the parent compound.

Biodegradation

For most xenobiotic organic chemicals, microbial degradation plays a key role in their removal from the environment. By contrast with non-biological elimination processes such as hydrolysis or photochemical degradation, biodegradation in the oxygen-containing biosphere is, generally, equivalent to conversion into inorganic end-products, such as carbon dioxide and water. This has been named ultimate biodegradation or mineralization and may be regarded as a true sink in aerobic compartments. In the anaerobic environment, microbial degradation processes are generally much slower and may not always result in complete mineralization. Transformation of the parent compound into another organic product (metabolite) is often referred to as primary degradation.

Heterotrophic micro-organisms are characterized by a high catabolic versatility. Mixed micro floras, rather than monocultures, are responsible for the elimination of substances from the biosphere, and because adaptation of the microbial ecosystem to a xenobiotic compound is so important, a more operational definition would be useful. Adaptation can be described as a change in the microbial community that increases the rate of biodegradation of a chemical as a result of prior exposure to that compound. This definition does not distinguish between mechanisms such as gene transfer or mutation, enzyme induction and population changes. The enzymatic machinery of micro-organisms consists of constitutive enzymes, which are involved in fundamental metabolic cycles (e.g., hydrolysis), and adaptive or induced enzymes. These enzymes enable bacteria to utilize organic compounds which are not appropriate for immediate use.

Environmental factors affect the population distribution and biochemistry of bacteria. Sediment and soil are more or less aerobic unless the oxygen consumption by micro-organisms, due to an abundance of substrate, is higher than the oxygen supply by diffusion. Aerobic bacteria use oxygen both as a reactant for the oxidation of organic compounds, and as a terminal electron acceptor. The latter is necessary for the conversion of the organic compound into carbon dioxide. This reaction, also known as dissimilation, produces the energy required during the formation of biomass from the organic compound (assimilation). Facultative anaerobic bacteria use oxygen but have the capability to change to another electron acceptor if their environment turns anaerobic. Other electron acceptors are nitrate, utilized by denitrifying bacteria and sulphate, used by sulphate-reducing bacteria particularly in marine and wetland environments. Oxygen is very toxic to the obligate anaerobic bacteria, which can only use alternative electron acceptors. The methanogens (methane-producing bacteria) derive energy from the conversion of hydrogen and carbon dioxide into methane. The considerable decrease in energy supply by the different electron acceptors from oxygen to the organic compound itself explains why microbial processes are faster in the aerobic world.

Biodegradation of synthetic chemicals does not always result in bacterial growth. When exponential growth does not occur the degradation process is called co-metabolism, in which micro-organisms - while growing on another, widely available, substrate - also have the capacity to transform other compounds (xenobiotics) without deriving any benefit from that transformation [26].

Biodegradation Kinetics

In first approximation, removal of chemicals from the environment by microbial degradation can be treated similar to other removal processes, *i.e.* by describing the process as following pseudo first-order kinetics and by formulating pseudo first-order reaction rate constants for it. In fact, the so-called second-order rate concept of microbial degradation in water was proposed as early as 1981 [27]. More recently, this almost forgotten concept has been re-introduced to quantitatively treat the subject of persistence of chemicals in the environment [28]. Following the assumption that the removal by microbial degradation obeys pseudo first-order kinetics, the pseudo first-order rate constant postulated to be proportional to the concentration of bacteria in the system:

$$k_{bio} = k^{2nd} \cdot [Bact] \qquad \textbf{(12)}$$

where

k_{bio} = pseudo first-order rate constant for biodegradation (1/s)

k^{2nd} = second-order rate constant for biodegradation (L/CFU/s)

$[Bact]$ = number of colony-forming units of bacteria in water (CFU/L)

Although of attractive conceptual simplicity, this approach has limited predictive power. Quantitative bacterial counts can be made, but the degrading power of the microbial colonies is hard to asses or predict; the second-order rate constants obtained from field observations or laboratory experiments are not nearly as constant as required for extrapolating observed biodegradation rates to other environmental situations, let alone to other chemicals. Reality of biodegradation kinetics is complex.

Biodegradability and Biodegradation Rates

Biodegradation rates are hard to predict. Despite major efforts, it has so far proved difficult to formulate generally applicable predictive theory, even for aerobic biodegradation in water and soil. At present, the re-interpretation of experimental studies is the only way to estimate rates of aerobic biodegradation. Most experimental data on microbial degradation originate from the standard tests of biodegradability of chemicals, as required by many regulatory agencies, e.g., the European Chemicals Agency ECHA. Biodegradability testing is commonly done according to standard methods published by the OECD [29, 30]. In the OECD hierarchy, three different levels of testing are distinguished as follows:

- Ready biodegradability tests (RBT), are designed for a quick selection of "soft" chemicals to avoid further costly and time-consuming research. To meet the demands of simplicity and cost efficiency, there are six different methods in the OECD scheme [30], which are all based on the principle that biodegradation is monitored as the degree of mineralization.
- Inherent biodegradability tests (IBT) are designed to demonstrate the potential biodegradability of a compound, using much higher population densities. IBT methods have a screening function as persistent chemicals are also detected. A negative result indicates that a chemical is clearly persistent and, tentatively, that no further research on biodegradation has to be done.
- Simulation tests (aerobic and anaerobic) provide data for biodegradation under specified environmentally-relevant conditions. These tests simulate the degradation in a specific environment used by indigenous biomass, media, relevant solids (*i.e.*, soil, sediment, activated sludge or other surfaces) to allow sorption of the chemical, and a typical temperature which represents that particular environment.

Recalcitrance

So it seems that microbial communities in the natural environment are catabolically so versatile, that always one or more species capable of degrading any chemical is present in a specific environment. Why then do some man-made chemicals persist in the environment for such a long time? The rate and extent of biodegradation of a chemical depends on both its chemical structure and the prevailing environmental conditions. In general, the following properties or conditions have a significant influence on the biodegradation of synthetic chemicals:

Chemical structure. Type, number and position of substituents on aliphatic or aromatic structures may cause "violation of comparative biochemistry and enzyme specificity", as described by Alexander [31]. Effects of substitution of radicals have already been discussed in the three examples of major metabolic pathways for biochemical oxidation; aromatic rings, however, are hard to break and substitute. The influence of the molecular structure on its biodegradability in the aerobic environment is clear.

Environmental conditions. Temperature is an important factor and especially around and below 4°C, microbial processes become very slow. The optimum temperature for psychrophilic (cold-loving) bacteria is between 0 and 20°C and for mesophyllic (moderate temperature loving) bacteria it is between 20 and 40°C. In seawater 15°C is the borderline between different microbial ecosystems. The inorganic nutrient status of the surface water affects the biodegradation rate and in some coastal waters may even exceed the temperature effect. The presence of auxiliary organic nutrients may also play a role, and the occurrence of co-metabolism has already been mentioned. Failure of biodegradation may be due to the presence of other, more easily degradable compounds used in preference to the specific xenobiotic compound. This phenomenon is known as diauxism. Unlike seawater, which is a well-buffered system of pH 8, inland waters can vary up to 5 pH units in acidity, thereby determining the form in which some chemicals exist. The availability of some natural organic substrates may also facilitate co-metabolism of the pollutant. However, even if it were possible to find two aquatic ecosystems characterized by similar environmental parameters, the outcome of a biodegradability experiment might be quite different for the same chemical. The presence and influence of high population densities of "specialized" degraders is evident. Some aquatic ecosystems may have been previously exposed to a chemical or another pollutant which shares a common enzyme system of such a specific degrader. The presence and density of specific degraders is often highly decisive for biodegradation to occur within a limited period of time.

Bioavailability. If a chemical is trapped in micro sites, e.g., in inorganic material such as clay minerals or the organic matrix of sediment or soil, interaction with micro-organisms may be physically impossible, which impedes biodegradation.

Biodegradation in Sediment and Soil

Biodegradation in sediment or soil is commonly reported to obey first-order kinetics. Experimentally observed half-lives and first-order rate constants can be found in the literature for many chemicals in many sediment and soil systems. It is often claimed that biodegradation in sediment- and soil systems is described and explained best from the theory that degradation takes place entirely in the water phase; chemical bound to the solid phase is considered

unavailable for attack by microbes and, therefore, non-reactive. Several studies have provided evidence that a chemical associated with sediment or soil particles is not available for biodegradation because micro-organisms only utilize dissolved chemicals [32]. Therefore, the overall rate of biodegradation in a solids-water system greatly depends on the extent of partitioning to the solid phase:

- Partitioning of a compound between the particle and the aqueous phase is governed by a thermodynamic equilibrium occurring at a fast rate with respect to degradation processes. The rate of elimination will then become strongly dependent on the organic carbon-water partitioning constant (K_{oc}) of a substance (see Eq 4). With increasing K_{oc} the concentration of a substance in the pore water subsequently becomes very low, and hence the elimination rate due to biodegradation becomes proportionally low.
- Biodegradation in the aqueous phase is relatively fast but overall elimination (and hence the biodegradation kinetics) from the solids-water system is controlled by slow desorption.

This again illustrates the difficulties associated with extrapolation of a laboratory-derived degradation rate to an environmental half-life of a laboratory-derived degradation rate. When assessing the environmental risk of a chemical, it is important to realize that even a relatively easily biodegradable chemical can become more or less persistent when it ends up in an environmental compartment where its bioavailability becomes limited.

MODELLING CONCENTRATIONS IN THE ENVIRONMENT

Distribution of chemicals in the environment can be determined by measurement of concentrations in air, water, soil and sediments. Such measurements are expensive, since they usually include analyses of many chemicals at different places and usually over long periods of time. Modelling the fate of chemicals in the environment is a feasible alternative which renders similar results at much cheaper cost. Moreover, modelling is necessary when measurement is no option, e.g., when predictions are to be made of expected results of environmental management measures, or when making predictions for new chemicals that have not been released yet, so they can be used in regulatory risk assessment.

Many of the models used in risk assessment of toxic substances are compartment models, also referred to as box models or mass balance models. The environment is thought to be made up of homogeneous, well-mixed compartments. Compartments can represent segments of the environment, or even entire environmental media. Examples of the former are the spatially segmented air and water transport models and layered soil models. The latter is used in multimedia (air, water, soil, *etc.*) fate models and in physiology-based pharmaco-kinetic models (blood, tissue, *etc.*). Compartment models apply the principle of mass conservation: the mass of a substance in a compartment appears or disappears only as a result of mass flows of a substance into or out of the compartment. What compartment models have in common is that the mass balance equation is used as their basic instrument. Because mass balance modelling is used so widely in the environmental risk assessment of toxic substances, its principles will be explained here. We shall first derive a mass balance equation for one compartment, then a mass balance model for more compartments.

One Compartment

If a substance is added to or taken from a compartment, the mass of that substance in the compartment changes. This change can be quantitatively expressed in a mass balance equation, in which all incoming and outgoing mass flows of the substance are accounted for

$$\frac{\Delta M}{\Delta t}\left(= \mathrm{V}\frac{\Delta C}{\Delta t}\right) = \text{gains} - \text{losses} = \sum \text{mass flows}, \qquad \textbf{(13)}$$

where ΔM and ΔC and are changes in mass and concentration within a time interval Δt, respectively, and V is the (constant) volume of the compartment. Note that the change is in unit mass per unit time (e.g., kg/s): a sum of mass flows. If nothing is added or taken away, or if gains and losses match exactly, the mass of substance in the compartment does not change: a steady state. If ΔM, ΔC and Δt are infinitesimally small, Eq **13** becomes what is

mathematically known as a differential equation. Differential equations describe at what rate a variable (here: mass of a substance in a compartment) changes. If the mass at starting time (t=0) is known (the initial condition), a differential equation can be used to derive the mass at other times. The art of mass balance modelling is thus to properly quantify the mass flows of a substance going into and out of the compartments. For the purpose of mass balance modelling it is useful to distinguish between mass flows that take place independently of what happens in the compartment and mass flows that do depend on the conditions within the compartment. Emissions and imports are examples of the first category. The rate at which mass is brought into the compartment by these processes may be constant or time-dependent, and may relate to the mass of a substance outside the compartment, but bears no relationship to the mass of a substance within the compartment. These mass flows need to be specified to the model as so-called "forcings". If a constant emission of E (kg/s) is forced upon a compartment, which contains M_0 kg of the substance at t = 0, and nothing else happens, the mass balance equation becomes:

$$\frac{dM}{dt}\left(= \mathrm{V}\frac{dC}{dt}\right) = \mathrm{E} \quad , \tag{14}$$

of which the integral form or solution is

$$M = \mathrm{M}_0 + \mathrm{E}\cdot t \quad . \tag{15}$$

How this solution is obtained is not further explained here. Readers may want to refresh their knowledge of this mathematical calculation method by reviewing a standard text on differential calculus, e.g. Wikipedia [http://en.wikipedia.org/wiki/Differential_equation]. The result of a constant inflow of a substance is that its mass in the compartment continuously increases. Note that this occurs at the constant rate of E kg/s (Fig. **5**).

Loss rates generally depend on the mass of a substance in the compartment (see Eq **2** and accompanying text). It should be noted that first-order reaction kinetics (see Eq **1**) are the exception, rather than the rule. Zero-order kinetics, in which the reaction is independent of C (formally proportional to C^0), second-order kinetics (reaction rate proportional to C^2) and broken order kinetics (proportional to $C^{1.5}$) commonly occur. Second-order kinetics will generally apply when a substance reacts with a chemical agent: the reaction is first-order in relation to both the substance degraded and the reactant. It is only because the concentration of the reactant is often approximately constant that the reaction appears proportional only to C^1. This is called pseudo first-order reaction kinetics. For instance, the loss due to reaction with chemical or microbial agents (degradation) is often characterized by pseudo first-order kinetics.

If degradation is the only process, the mass balance equation becomes:

$$\frac{dM}{dt}\left(= \mathrm{V}\frac{dC}{dt}\right) = -\mathrm{k}\cdot M \quad , \tag{16}$$

the solution of which results in an exponential decrease of mass in the compartment (Fig. **5**):

$$M = \mathrm{M}_0 \cdot e^{-\mathrm{k}\cdot t} \quad . \tag{17}$$

If both emission and degradation act on a compartment, the combined result will be:

$$\frac{dM}{dt}\left(= \mathrm{V}\frac{dC}{dt}\right) = \mathrm{E} - \mathrm{k}\cdot M\,; \quad M = \mathrm{M}_0 \text{ at } t = 0 \quad , \tag{18}$$

the solution of which is:

$$M = \mathrm{M}_0 \cdot e^{-\mathrm{k}\cdot t} + \frac{\mathrm{E}}{\mathrm{k}}(1 - e^{-\mathrm{k}\cdot t}) \tag{19}$$

(see Fig. **5**). Eq **18** and **19** illustrate how the mathematical solution of the mass balance equation yields a mass-time profile of a substance in a compartment as a function of the initial conditions (here: mass M_0 at $t = 0$), forcings (here: emission rate, E) and the parameters of the mass flow rate equations (here: the degradation rate constant, k). Note that eventually (at $t = \infty$), the mass of substance in the compartment will reach a level at which the loss by degradation, k.M (kg/s), exactly matches the constant emission, E (kg/s), so that the mass of substance in the compartment is maintained at the steady-state level of E/k (kg).

There are many other loss mechanisms that need to be accounted for in the mass balance equation, such as such as advective or diffusive outflow. Because losses due to all mechanisms i are proportional to M, and can each be represented by a first-order rate constant k_i (1/s), the full mass balance equation keeps the same simple format of Eq **18**:

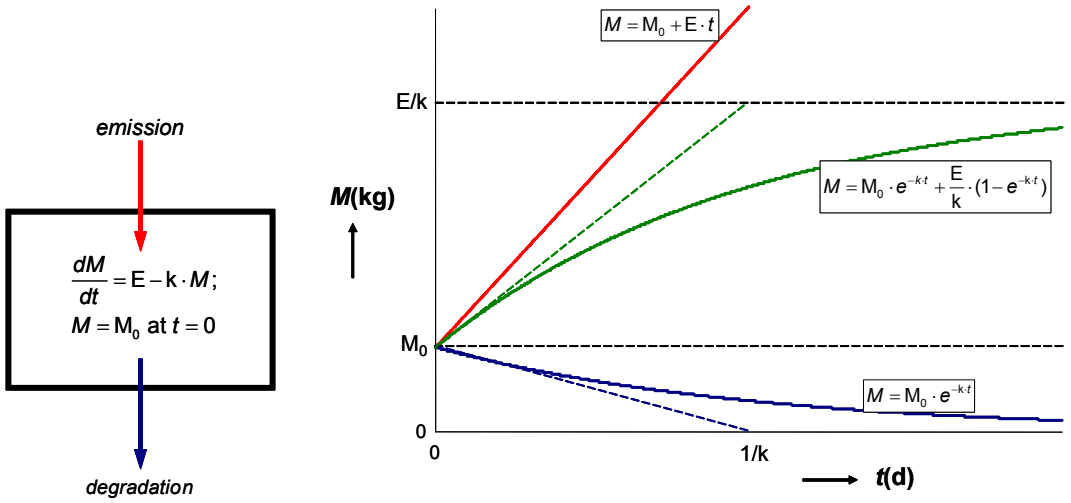

Figure 5: Elementary form of a one-compartment mass balance model, showing the differential mass balance equation and its solution for the cases of emission only (red), degradation only (blue) and both (green).

$$\frac{dM}{dt}\left(= V\frac{dC}{dt}\right) = \text{gains} - \text{losses} = E - \sum_i k_i \cdot M; \quad M = M_0 \text{ at } t = 0 \tag{20}$$

and its solution takes the same format as Eq **19**:

$$M = M_0 \cdot e^{-\sum_i k_i \cdot t} + \frac{E}{\sum_i k_i}\left(1 - e^{-\sum_i k_i \cdot t}\right) \tag{21}$$

.

More Compartments

Models usually comprise many compartments and describe the transport of a substance in and between these compartments. Such multicompartment mass balance models contain one mass balance equation for each compartment in the model. As in the above situation for one compartment, losses are all assumed to obey first-order kinetics. Where more than one compartment is involved, losses may be due to degradation or export, but losses may also represent mass flows from one compartment to another.

For a set of n compartments, this leads to a set of n mass balance equations, all of which will have the same format as Eq **20**, with n unknown masses M_i and a suite of first-order rate constants which describes the losses from the compartments. An example for three compartments is shown in Fig. (**6**). Each of the compartments receives an emission – for the sake of simplicity, emissions will be assumed to be constant and imports considered to be included in the emission flows. The emission flows into the compartments i are denoted by E_i (kg/s). Degradation

occurs in the three compartments – again, in the interests of readability, the degradation flows will be considered to include possible exports. The resulting mass flows from the compartments i, out of the system are characterized by pseudo first-order loss rate constants k_i and denoted by $k_i.M_i$ (kg/s).

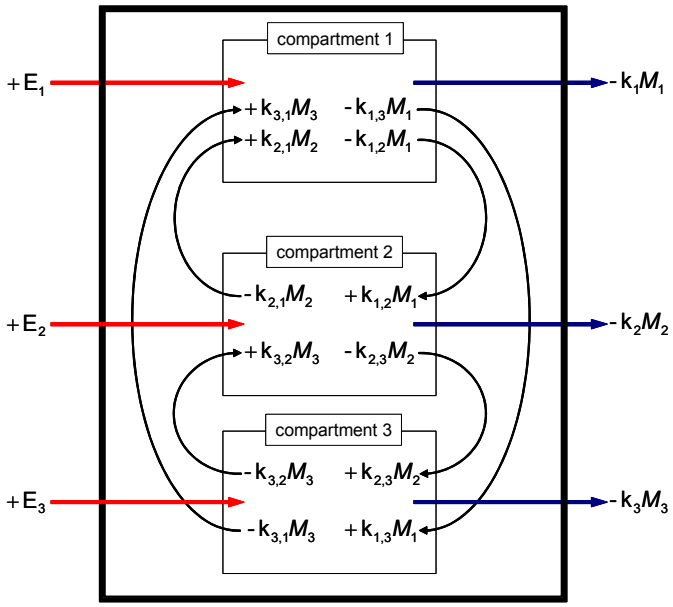

Figure 6: Diagram of a three-compartment mass balance model. Intercompartment mass-transfer represents a loss to the source compartment and a gain to the receiving compartment.

There are six intercompartment mass-transfer flows, each proportional to the mass in the source compartments denoted by $k_{i,j}.M_i$ (kg/s). On this basis, and assuming all initial masses to be zero, the three differential mass balance equations become:

$$\frac{dM_1}{dt} = E_1 - (k_1 + k_{1,2} + k_{1,3}) \cdot M_1 + k_{2,1} \cdot M_2 + k_{3,1} \cdot M_3; \quad M_1 = 0 \text{ at } t = 0$$
$$\frac{dM_2}{dt} = E_2 + k_{1,2} \cdot M_1 - (k_2 + k_{2,1} + k_{2,3}) \cdot M_2 + k_{3,2} \cdot M_3; \quad M_2 = 0 \text{ at } t = 0$$
$$\frac{dM_3}{dt} = E_3 + k_{1,3} \cdot M_1 + k_{2,3} \cdot M_2 - (k_3 + k_{3,1} + k_{3,2}) \cdot M_3; \quad M_3 = 0 \text{ at } t = 0 \qquad \textbf{(22)}$$

For this system of three compartments there is an equation equivalent to Eq **19**, *i.e.* the analytical solution of the one-compartment system, which expresses the mass of the substance at all times. It is not possible to formulate precisely how the three masses in the three compartments change with time. Solutions can be approximated quite well, however, with computer-based numerical techniques which will not be described here. As in the one-compartment system, the three-compartment system will eventually (at $t = \infty$) reach to a steady state in which emission is equally balanced by degradation ($dM_i/dt = 0$) and masses reach their constant steady state level, M^*_i:

$$\text{balance}_1 = E_1 - (k_1 + k_{1,2} + k_{1,3}) \cdot M_1^* + k_{2,1} \cdot M_2^* + k_{3,1} \cdot M_3^* = 0$$
$$\text{balance}_2 = E_2 + k_{1,2} \cdot M_1^* - (k_2 + k_{2,1} + k_{2,3}) \cdot M_2^* + k_{3,2} \cdot M_3^* = 0$$
$$\text{balance}_3 = E_3 + k_{1,3} \cdot M_1 + k_{2,3} \cdot M_2^* - (k_3 + k_{3,1} + k_{3,2}) \cdot M_3^* = 0 \qquad \textbf{(23)}$$

The set of steady-state masses for which the mass balance equations become zero can be derived directly from Eq **23** quite easily through simple algebraic manipulation. Solving sets of equations algebraically becomes increasingly tedious for larger sets, so linear algebra (matrix calculus) is used to obtain solutions to large sets of linear equations, as follows:

$$\mathbf{m} = \begin{bmatrix} M_1 \\ M_2 \\ M_3 \end{bmatrix}, \quad \mathbf{e} = \begin{bmatrix} \mathrm{E}_1 \\ \mathrm{E}_2 \\ \mathrm{E}_3 \end{bmatrix}, \quad \mathbf{A} = \begin{bmatrix} -(\mathrm{k}_1 + \mathrm{k}_{1,2} + \mathrm{k}_{1,3}) & \mathrm{k}_{2,1} & \mathrm{k}_{3,1} \\ \mathrm{k}_{1,2} & -(\mathrm{k}_2 + \mathrm{k}_{2,1} + \mathrm{k}_{2,3}) & \mathrm{k}_{3,2} \\ \mathrm{k}_{1,3} & \mathrm{k}_{2,3} & -(\mathrm{k}_3 + \mathrm{k}_{3,1} + \mathrm{k}_{3,2}) \end{bmatrix} \tag{24}$$

Using this, the three mass balance equations of Eq **23** can be rewritten into a one-line linear-algebraic equation:

$$\mathbf{m} = -\mathbf{A}^{-1} \cdot \mathbf{e}\,. \tag{25}$$

Various standard software packages, such as Microsoft Excel, can be used to carry out matrix inversion.

Multimedia Modelling

If a chemical is released into one medium and resides there until it is removed by degradation or advection, single-media models may be perfectly suitable for estimating the environmental concentration. If, however, a chemical is released into several compartments simultaneously, or after release into one compartment is transported to other compartments, it becomes necessary to account for the intermedia transport processes so that its ultimate fate in the overall environment can be assessed. Multimedia models are specifically designed to do this. This section on multimedia models starts with a short description of their features and the explicit and implicit assumptions usually made. The use of these models in exposure assessment is described together with their limitations. Subsequently, some information on data requirements and on the different models available is given, following which a number of sample calculations are presented to illustrate the use of these models.

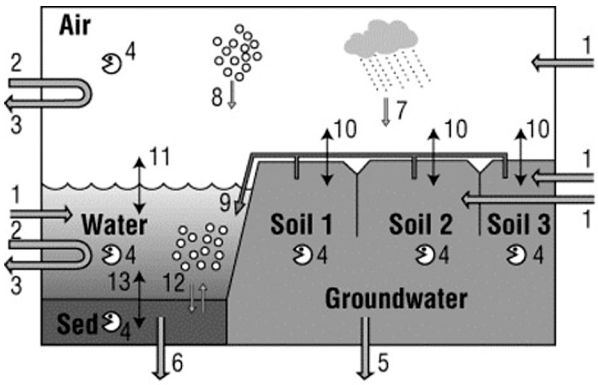

Figure 7: Diagram of a multimedia mass balance model concept. 1 = Emission, 2 = Import, 3 = Export, 4 = Degradation, 5 = Leaching, 6 = Burial, 7 = Wet deposition, 8 = Dry aerosol deposition, 9 = Run-off, 10, 11 = Gas absorption and volatilization, 12 = Sedimentation and resuspension, 13 = Sorption and desorption.

Multimedia fate models are typical examples of compartment mass balance models. The total environment is represented as a set of spatially homogeneous (zero-dimensional) compartments; one compartment for each environmental medium in which the chemical is assumed to be evenly distributed (Fig. **7**). Typical compartments considered in models are: air, water, suspended solids, sediment, soil and aquatic biota. Multimedia mass balance modelling was initiated in the early 1980s by Mackay and co-workers [2, 33-36]. The example was soon followed by others [11, 37-39]. In Europe, the model SimpleBox used in The Netherlands was adopted as the basis for the risk assessment model EUSES [12, 13]. While the early models described a fixed, "unit world", which was meant to represent a global scale, later models have enabled users to customize the environment and define smaller and more open spatial scales. More recently, the use of spatially resolved multimedia fate models has become more common [40-50].

A typical regional multimedia model describes a region between 10^4 and 10^5 km^2. In this generic form, the models can account for emissions into one or more compartments, exchange by import and export with compartments "outside" the system (air and water), degradation in all compartments and intermedia transport by various mechanisms (Fig. **7**). Mass flow kinetics, formulated slightly differently in models by different authors, are usually defined as simply as possible: mass flows are either constant (emission, import) or controlled by (pseudo) first-order

rate constants (degradation, intermedia transport), as in Eq **15**. In all the models, the user has to set parameter values for these mass flows to provide input for the model.

Using a number of criteria, such as equilibrium or non-equilibrium, steady-state or non-steady-state, and based on whether to take the degradation of the chemical into account in the calculation or not, Mackay and Paterson introduced a classification of multimedia models [34]. This classification begins with a Level I model which describes the equilibrium partitioning of a given amount of a chemical between the above media. The Level II model simulates a situation where a chemical is continuously discharged into a multimedia environment in which partitioning, advection and degradation take place. Transport between the media is assumed as infinitely rapid, so that thermodynamic equilibrium between the media is maintained. At Level III, realistic intermedia transport kinetics are assumed, so that media may not be in thermodynamic equilibrium. Level III models calculate steady-state concentrations in all compartments. Finally, Level IV models assume a non-steady-state and yield time-related chemical concentrations.

Level I calculation requires knowledge of intermedia partition coefficients (air-water, water-solids) only. Calculation at level II and above requires additional knowledge of degradation rate constants in air, water, sediment and soil. Unfortunately, measured partition coefficients and rate constants are not always available. In the absence of measured data, partition coefficients can be estimated from basic substance properties, using quantitative structure-activity relationships (Q)SAR. Easy to use software is available to support such estimates. The consequence of using estimated model input data is that the accuracy of the model output will also depend on the quality of the (Q)SAR methods that have been used. Very often biodegradation rate constants are extrapolated from standard degradation tests, or even estimated using (Q)SARs (e.g., BIOWIN). This may introduce another uncertainty into the outcome of the calculation, especially if precise data is not available for the degradation rate constants in compartments that serve as a "sink" for a specific chemical.

The principal utility of multimedia models, as a first step in exposure assessment, is to determine to what extent intermedia partitioning may occur. If it appears that no significant partitioning into secondary compartments is expected, further exposure assessments may focus on the primary compartment(s) only. As intermedia transfer is usually relatively slow, its effect on the fate of chemicals is significant only over longer periods of time, *i.e.* if the spatial scale is large or the chemical does not degrade rapidly. This brings us to one of the major applications of these models, which is the exposure assessment of chemicals on regional (usually 10^4 to 10^5 km^2) and larger spatial scales. These models are particularly useful for calculating the predicted environmental concentration (PEC) especially of chemicals with a very diffuse release pattern. Results from Level III multimedia models are used in EU risk assessments for new and existing chemicals. In addition to calculating the regional concentration of a chemical, the results of Level III models can also be used as input for local models. When using such models, the actual concentration is greatly underestimated if the concentration of the chemical in air or water from "outside" is set to zero, especially in relation to high production volume chemicals with a widely distributed use pattern. Regional concentrations estimated from the release rates for a larger region fed into a regional multimedia model can then be used as boundary concentrations in local model calculations.

One of the key processes in multimedia models is the partitioning between aqueous and solid phases. Most models follow in the footsteps of the original Mackay models and estimate solids-water partitioning from the octanol-water partition coefficient K_{OW}. This means that the models are particularly useful for organic chemicals whose K_{OW} values can be accurately measured or estimated. Applying these models to ionisable compounds, surface-active chemicals, polymers, or inorganic compounds (including metals) should be done with great care. However, the models can be used for these chemicals, provided certain adaptations to specific physicochemical properties are made. Mackay and Diamond, for instance, used an "equivalent" based model to describe the fate of lead in the environment [50], while in the example calculation for cadmium parameters such as soil-water and sediment-water partition coefficients or the fraction of the chemical associated with aerosols, must be specifically entered by the user in order to overrule the standard estimation routines.

Naturally, representing the environment in the form of a unit world or unit region with homogeneous boxes is a major simplification of reality. However, this extreme degree of simplification in this model concept is both a weakness and a strength at the same time. By disregarding spatial variation, the modelling effort can focus on intermedia distribution and

understanding the ultimate fate of a chemical. The concentrations calculated with multimedia models should therefore be interpreted as "spatially-weighted averages" of the concentrations that would be expected in real situations. However, the assumption of homogeneity brings with it a considerable risk that potentially more localized effects may be overlooked. The disadvantage of zero-dimensionality becomes evident with larger areas since, other than for air, it is difficult to identify any large-scale situations where the homogeneity of compartments would seem to be a realistic assumption. To overcome this problem the SimpleBox has introduced the concept of "nesting" [12]. In a nested model the input and output flows of a regional or smaller scale model are connected to a continental scale model which in turn, is connected to a global scale model. In this way, the specific environmental characteristics of the region can be taken into account when the overall fate of the chemical is assessed. While spatial scale nesting was originally introduced as a tool for assessing the overall persistence of a chemical in the environment, the concept soon found wider application in regional exposure assessment in EUSES [13].

Testing the validity of multimedia models is difficult and, until recently, had not been seriously addressed [51]. If a common evaluation environment with agreed fixed environmental characteristics is used, validation of the outcome becomes almost paradoxical since this generic environment does not actually exist in reality. However, the regional generic characteristics can be modified at a later stage and region-specific information on environmental parameters, as well as information on specific discharge rates can be introduced in order to "validate" a specific model setting [35, 52].

Multimedia Models in Use

Multimedia fate models of the Mackay type have been produced by different authors, most of them for their own scientific use. Many of these have been documented and made available for end users, e.g., HAZCHEM [53], SimpleBox [12, 13] CemoS [38], CalTOX [39], ChemCAN [40], EQC [41], ChemRange [42], ELPOS [43], Globo-POP [44], CliMoChem [45], BETR North America [46], BETR World [47], IMPACT 2002 [48] and MSCE-POP [49]. The similarities between these models are more striking than the differences. When fed the same input, the models were shown to yield the same results [51]. The main differences lie in the number of compartments or sub-compartments included and how they are handled in terms of computer calculation.

Table 6: Parameters used for steady-state calculations with SimpleBox

Parameter	Value in SimpleBox	Parameter	Value in SimpleBox
Area of the system	3.8×10^4 km^2	Organic carbon content in suspended matter	0.1
Area fraction of water	0.125	Atmospheric mixing height	1000 m
Area fraction of natural soil	0.415	Mixing depth of water[a]	3 m
Area fraction of agricultural soil	0.45	Mixing depth of sediment[a]	0.03 m
Area fraction of industrial/urban soil	0.01	Average annual precipitation	792 mm/year
Mixing depth of natural soil[a]	0.05 m	Wind speed	5 m/s
Mixing depth of agricultural soil[a]	0.2 m	Residence time air[b]	0.40 days
Mixing depth of industrial/urban soil[a]	0.05 m	Residence time water[b]	54.5 days
Organic carbon content in soil	0.029	Fraction of rain water infiltrating soil	0.4
Organic carbon content in sediment	0.029	Fraction of rain water running off soil	0.5
Concentration suspended solids	15 mg/L	Temperature	285 K (12°C)

[a] The mixing depth represents the thickness of the soil, water or sediment box.

[b] Residence time for air or water represents the time needed for air or water to flush through the air or water compartments, respectively.

ENVIRONMENTAL FATE OF CHEMICAL SUBSTANCES

Examples of how to perform Level I, II and III calculations for a range of different chemicals have been presented by Mackay and others [2, 35, 36, 40, 41, 54]. To illustrate the utility of Level III and IV type multimedia modelling, let us consider the use of three chemicals, 1,1,1-trichloroethane, dieldrin and cadmium, in a system resembling The Netherlands, as simulated with SimpleBox [12, 13].

The system parameters are summarized in Table **6**. Let us assume that the background concentrations of these chemicals in air and water outside The Netherlands are equal to the quality standards or objectives set for environmental protection. After 10 years, with these background concentrations, domestic emissions of 1000 tonnes/year for each chemical start to occur: dieldrin to water, cadmium to air, and 1,1,1-trichloroethane to air, water and soil simultaneously (ratio 1:1:1). This situation continues for 40 years and then suddenly stops. What concentrations may be expected in the different environmental compartments, how are the chemicals distributed, and how long does it take to return to the original situation after the emissions stop? In order to evaluate the change in concentrations of the three chemicals in the different environmental compartments some chemical-specific information is needed. This is summarized in Table 7.

Table 7: Input parameters used in the multi-media model calculations for 1,1,1-trichloroethane, dieldrin and cadmium

		1,1,1-Trichloroethane	**Dieldrin**	**Cadmium^{2+}**
Background (air)	g/m^3	10^{-8}	10^{-9}	10^{-9}
Background (water)	g/L	10^{-8}	10^{-7}	10^{-7}
Emission (air)	tonnes/year	333	-	1000
Emission (water)	tonnes/year	333	1000	-
Emission (soil)	tonnes/year	333	-	-
K_h (air-water)	-	1.1	$1.7x10^{-4}$	10^{-10a}
Frac (aerosol)	-	0.0	0.25	0.9
Scavenging ratio	-	0.96	$5.5x10^4$	10^5
K_p (susp.solids)	L/kg	$3.1x10^1$	$6.3x10^2$	10^4
K_p (sediment)	L/kg	$1.6x10^1$	$3.2x10^2$	10^4
K_p (soil)	L/kg	$1.6x10^1$	$3.2x10^2$	10^3
Half-life (air)	days	200	200	∞
Half-life (water)	days	1000	1000	∞
Half-life (sediment)	days	1000	1000	∞
Half-life (soil)	days	2000	100000	∞

[a] Substitute for zero-value.

The Level III mode of the SimpleBox program is then used to generate the concentrations and intermedia distribution at steady-state. The concentrations in and distribution over the environmental compartments at steady-state are summarized in Table 8. The mass flows that support these steady-states are also shown in Fig. (**8**). The model calculation emphasizes the high volatility of 1,1,1-trichloroethane. Approximately all emissions to soil and water go to air through diffusive transport. Of the total mass in the system, however, a high percentage still resides in the soil.

Table 8: Steady-state distribution of 1,1,1-trichloroethane, dieldrin and cadmium in The Netherlands, calculated with SimpleBox [12, 13]. Numbers in parentheses represent a percentage of the total mass in the environment at steady-state.

	1,1,1-Trichloroethane	**Dieldrin**	**Cadmium^{2+}**
Air (g/m^3)	3.9×10^{-8} (19%)	1.5×10^{-8} (0%)	2.7×10^{-8} (0%)
Water (g/L)	4.5×10^{-8} (8%)	5.1×10^{-6} (3%)	2.1×10^{-7} (0%)
Suspended matter (g/kg)	1.2×10^{-6} (0%)	2.8×10^{-3} (0%)	2.1×10^{-3} (0%)
Sediment (g/kg)	7.5×10^{-7} (1%)	2.1×10^{-3} (7%)	2.1×10^{-3} (0.5%)
Soil (g/kg)	1.6×10^{-6} (73%)	6.1×10^{-4} (90%)	9.2×10^{-3} (99.5%)

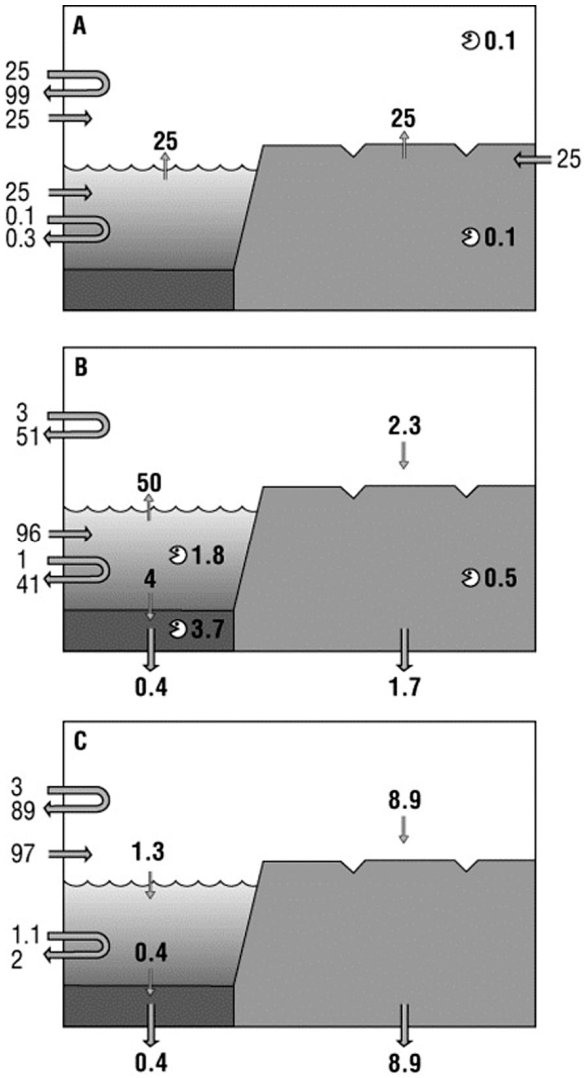

Figure 8: Steady-state mass flows of trichloroethane (A), dieldrin (B) and cadmium (C), as a percentage of the total throughput of the system.

Remarkably, the relatively high volatility of dieldrin causes more than half of the total load of the water compartment to be transported to air, from where it is exported out of the system. The high hydrophobicity and low biodegradation rates of the chemical produce relatively high concentrations in sediment and soil. Cadmium does not

degrade at all. When emissions go to air the most important fate process is advection out of the air compartment. However, due to atmospheric deposition, some 10% of the total load of the atmosphere is transported to soil and water. Atmospheric deposition to soil leads to a build-up of cadmium in the soil, from where it is eventually leached to the ultimate sink: the deeper groundwater. It should be borne in mind that this build-up may be slow. If, as in the case of cadmium in soil, all mass flows are small, it may take an extremely long time before the steady-state is achieved. This can be demonstrated with Level IV calculations using the SimpleBox model. Fig. (**9**) shows the change in concentrations in the different compartments according to the above emission scenario relative to the background concentrations which result when there are no domestic emissions. For cadmium, the compartments air, water and sediment are expected to respond relatively quickly, whereas a near linear increase in the concentration in soil is predicted over the 40-year exposure period.

After reducing the emissions, the soil concentration of cadmium shows little response (Fig. **8C**). For dieldrin exposure, for 40 years is almost long enough to reach a steady-state, even in the "slow" soil compartment; after reducing the emission to 10% of its original value, the concentrations decrease at the same rate (Fig. **8B**). For trichloroethane the situation is completely different. The steady-state situation is reached so quickly that plotting the concentrations against time on a 100-year scale would yield a block diagram. Therefore, the Level IV calculation was repeated over a time-scale of one year. The results as presented in Fig. (**8A**) show that concentrations in air, water and soil reach steady-state within one month. For sediment this takes a little longer, though probably not much longer than a year. These results demonstrate the usefulness of Level III and Level IV multimedia box model calculations. Where steady-state calculations can give information on the concentrations and distribution in the environment at a constant emission scenario, the results of a Level IV calculation elucidate the time scale in which this situation may be reached. In addition, changes in the emission scenario as a result of evolving risk reduction strategies can be evaluated in this way.

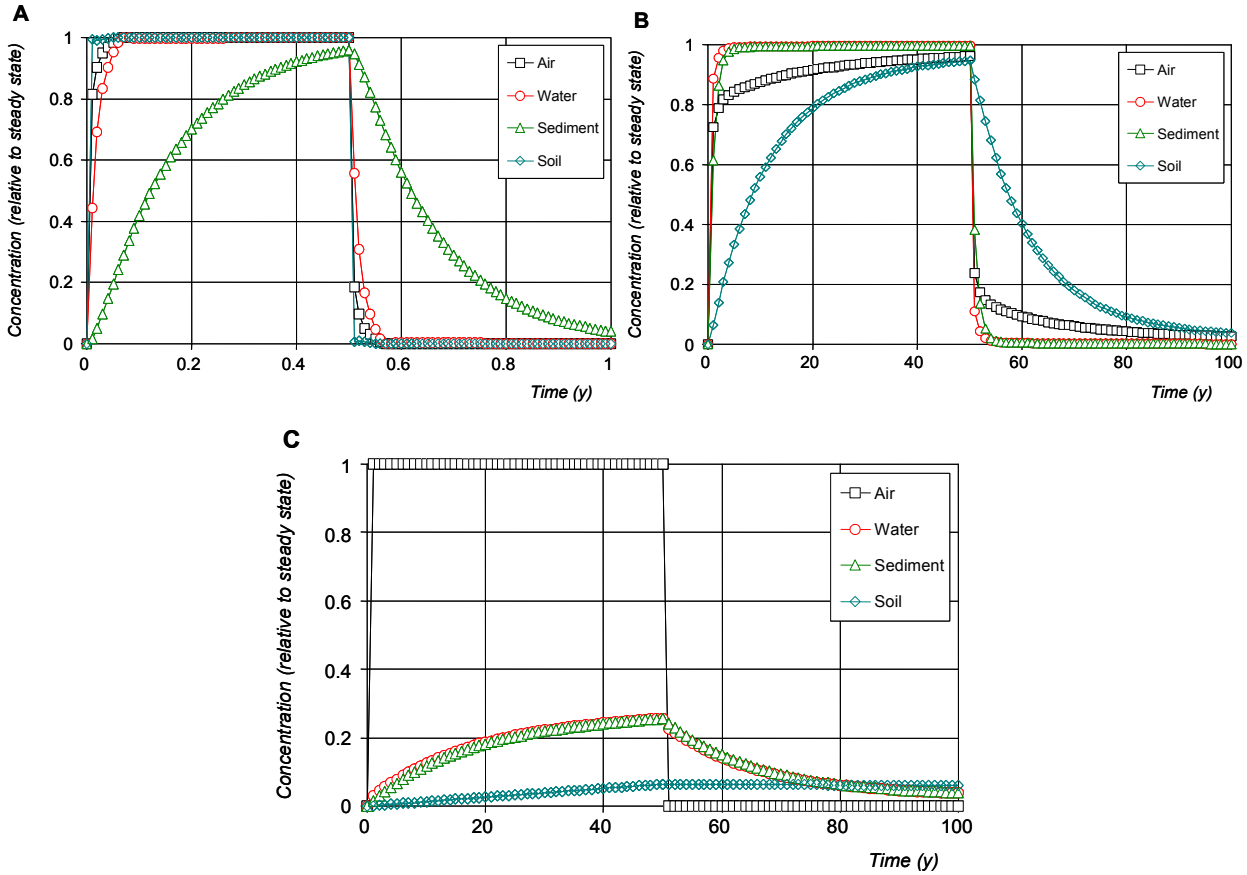

Figure 9: Change in concentrations of trichloroethane (A), dieldrin (B) and cadmium (C) after a change in emission rates. Note the shorter time scale in graph A.

Calculation of Overall Persistence in the Environment and Long-Range Transport Potential

It is clear that the physical and chemical properties of substances greatly influence their concentrations and distributions in the environment. Not only does this have implications for the risks posed to humans and ecosystems, there are other ethical and scientific consequences to be considered [55]. Slow degradation and great mobility mean that substances disperse throughout the entire globe. This has been recognized internationally. Two international conventions: the UNEP Stockholm Convention [56] and the UN ECE POP protocol [57] now regulate substances on the basis of their persistence in the environment and their long-range transport potential. Both of these are indirect or "derived" substance properties.

Persistence reflects the resistance of a substance to degradation. This is indicated by the dynamic response to changes in emissions, as shown above. Alternatively, persistence can be quantified by the degradation half-life or reactive residence time during an emission episode [58, 59]. As degradation half-lives in air, water and soil differ greatly, it needs to be decided how to combine the different single-medium half-lives. Calculation of overall persistence in the environment P_{ov} as the reciprocal of the overall degradation rate constant k_{ov}, or the mass-weighted average reactive residence time in the environmental media M_i, has been proposed for this purpose [58-60]:

$$P_{OV} = \frac{1}{k_{OV}} = \frac{\sum_i M_i}{\sum_i M_i \cdot k_i} \quad \textbf{(26)}$$

In this derivation of P_{OV}, k_i's are the first-order degradation rate constants in pure media and M_i's are the masses in the media at steady-state. According to this derivation, substance properties other than degradation half-lives (partition coefficients and mass-transfer velocities) play a role in determining the "derived property" P_{OV}. Applied to the calculation results of the previous paragraph, this would yield P_{OV} values of 2.8 years, 20.8 years and ∞ for trichloroethane, dieldrin and cadmium, respectively.

The long-range transport potential (LRTP) reflects the tendency of a substance to be transported away from the location where it was emitted. There are different ways to capture this in a "derived property" [59, 60]. One is to take the fraction of the total emission exported out of an open regional environment, as shown in the previous paragraph:

$$LRTP = \frac{adv_{air} + adv_{water}}{E}, \quad \textbf{(27)}$$

with adv_{air} and adv_{water} denoting the advective mass flows by air and water, respectively and E the sum of emissions. The LRTP values (dimensionless) for trichloroethane, dieldrin and cadmium would be 0.99, 0.92 and 0.91, respectively, based on example model used. Another method is to use the Lagrangian characteristic travel distance. The distance travelled by a parcel in the period that the original mass is reduced exponentially to 37% (=1/e) of its original value is calculated as [59, 60]

$$LRTP = \frac{u}{k_{ov}^*}, \quad \textbf{(28)}$$

in which u is the average velocity at which the parcel travels. Here, k_{OV}* considers non-reactive losses to ultimate sinks such as sediment burial, groundwater or deeper ocean layers as well as abiotic and biotic degradation processes.

What P_{OV} and LRTP have in common is that they cannot easily be determined by observation, but must be calculated from substance properties that can be measured (degradation rate constants, partition coefficients, mass-transfer velocities), using a multimedia environmental fate model. This has raised the concern that the choice of model could play a role in the calculation result, which would be undesirable if P_{OV} and LRTP are to be used as a property of the substance in a regulatory context. This issue has been thoroughly studied by an international group of modelling experts for the OECD [60]. The experts concluded that indeed the absolute values of P_{OV} and LRTP obtained from different models differ greatly, as a result of different modelling objectives and model

parameterization. However, the rankings of substances obtained appeared to be relatively insensitive to the model choice: models tend to put chemicals in roughly the same order of P_{OV} and LRTP. If properly processed, output of any well-designed multimedia model can be used to derive P_{OV} and LRTP [61, 62]. This was concluded from a comparison of the performance of existing models with respect to P_{OV} and LRTP calculation, which demonstrated that a simplified version of existing models could be constructed that differed as little from the existing models as the models differed among themselves. This consensus model is available from the OECD on their website [63].

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CHAPTER 3

Metals and Metalloids in Terrestrial Systems: Bioaccumulation, Biomagnification and Subsequent Adverse Effects

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Abstract: Metals and metalloids are elemental substances that occur naturally in the Earth's crust, and are variously incorporated into biological systems as structural components or proteins. Imbalances in the environmental concentrations of several metals present a challenge to ecosystems because the species that form part of these ecosystems are often not equipped to regulate internal concentrations of these elements, or employ detoxification mechanisms that serve to biomagnify these elements in the food chain. This review examines the trophic movement of metals and metalloids within terrestrial ecosystems and the consequences of biomagnification and toxicity on populations. Several elemental contaminants are given special emphasis, including copper, zinc, arsenic, selenium, molybdenum, cadmium, mercury and lead. All these elements are of high historical importance and continue to be deposited within the biosphere.

INTRODUCTION

Elemental chemicals have a tendency to stick to clayish, peaty or organic-rich materials, and contamination of terrestrial systems generally occurs because soils are capable of acting as a sink for metals and other elemental chemicals [1]. The elemental contaminants of most concern are predominantly metals such as cadmium, lead, mercury or copper, among others. However, a few, like selenium and arsenic are metalloid elements. For the sake of simplicity in this discussion we have used only the term 'metal' to describe both types, even though examples may include metalloid elements like selenium.

Metals in soils originate from two separate sources: from geogenic processes related to the occurrence of metal-bearing geological formations, and from anthropogenic sources. Information on the sources, fate, transport and toxicity of metals and metalloids can be found in Chapter 1 and 2 of this book. Once bound within soils, metals are persistent, because elemental contaminants cannot degrade further (unlike complex organic pollutants), although they can undergo various reversible changes in speciation depending on the chemical environment [2].

Many trace elements are essential for life functions [3], and plants and animals possess various mechanisms for the accumulation of sufficient amounts of trace elements from their environment. These same mechanisms can also facilitate the uptake of non-essential metals [4, 5]. The uptake and retention of a metal (or any other chemical) by an organism is termed bioaccumulation. Bioaccumulation of essential as well as non-essential elements is dependent on both the chemical availability of the metals within the environment and the organism's capacity for uptake and subsequent excretion. A full overview on bioaccumulation is given in Hodson *et al.* [6], in which the different definitions of bioavailability currently in use are reviewed.

The severity of impact on ecosystems will reflect the concentrations of bioavailable metals in the soil. The concentrations of individual metals are dictated by the source of each contaminant. For example, mercury (Hg) contamination occurs predominantly as a consequence of atmospheric deposition, and is therefore rather diffuse. By contrast, a major source of cadmium (Cd) contamination has historically been through the application of rock-phosphate fertilizers, thereby selectively elevating Cd concentrations in agricultural soils. Very high levels of soil contamination usually only occur in the proximity to metal smeltering activities, and an examination of studies conducted in these environments are instructive about the relative movements of metals within local ecosystems.

One such study was conducted by Hunter *et al.* [7-9] in the vicinity of a copper refinery within Merseyside in north-west England. Copper (Cu) and Cd content of the soils within a 1 km radius of the refinery typically exceeded 500

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and 5 mg/kg, respectively [7]. In this highly impacted area, floral diversity was reduced to a few metal tolerant species compared to a reference site. However, invertebrate diversity, as represented through pitfall trapping, was not greatly affected, with the exception of a reduction in abundance of isopods (woodlice) and oligochaetes (earthworms) within a 1 km radius of the refinery. The site also supported small mammals; specifically field voles (*Microtus agrestis* L.), wood mice (*Apodemus sylvaticus* L.) and common shrews (*Sorex araneus* L.).

All organisms within this contaminated site accumulated metals to varying degrees, and following the ratio of Cu:Cd through the various trophic levels illustrates the variability in accumulation potential for different metals. The Cu:Cd ratio in the soil close to the refinery was 716:1. Vegetation at the refinery bioaccumulated both Cu and Cd, however the ratio was reduced to 37:1. The ratio of Cu:Cd in the herbivorous field voles was reduced further to 5:1. Among the various herbivorous, detritivorous and predatory invertebrate taxa the Cu:Cd ratio varied markedly, but within the diet of the carnivorous common shrew the ratio averaged 10:1 and was reduced further to 1:3 in the shrew itself [8, 9]. These changes in Cu:Cd ratios illustrate the element specific mobility of Cu and Cd within terrestrial food chains. In this example the change in ratio of Cu:Cd occurs because copper is an essential element that can be regulated by homeostatic mechanisms. In the study cited here [9], shrews within the vicinity of the refinery accumulated large body burdens of Cd, whereas their Cu burdens remained low. Unlike copper, cadmium is a non-essential metal and organisms have only limited capacity to eliminate it from their bodies and tend to pass it on to consumers/predators. It is notable that despite the large body burdens of Cd, shrews persisted in the contaminated environment, and this will be discussed further later in the chapter.

This chapter will examine the metal- and species-specific movements of metals in terrestrial ecosystems, and where examples exist, the consequences of bioaccumulation and biomagnification of metals for populations of terrestrial organisms. Illustrations of metal transfer through the aquatic food chain are included to provide a complete picture, thereby improving our ability to make general statements or to fill gaps of knowledge for terrestrial ecosystems.

What is Biomagnification?

The process whereby pollutants are transferred from food to an organism resulting in higher concentrations compared with the source is called biomagnification. There are two main groups of substances that biomagnify:

1. Novel (synthetic), lipophilic organic substances that are not easily degraded or metabolized because organisms lack previous exposure and therefore, have not evolved specific detoxification and excretion mechanisms. These substances are consequently known as 'persistent organic pollutants' or POPs.
2. Metals which, by definition, are not degradable because they are elements. Because metals are a natural part of the environment, organisms, particularly those subject to naturally high levels of metal exposure, have developed mechanisms to sequester and excrete them. Problems arise when organisms are exposed to higher concentrations of metals than usual, and which they cannot excrete or detoxify rapidly enough to prevent damage. Some of these metals are transferred in organic forms, like methylmercury, organoselenium and organotin, and like POPs will readily bioaccumulate.

These pollutants biomagnify along food chains because successive trophic levels consume relatively large quantities of biomass (food) to obtain the resources required for metabolic functioning. If that biomass is contaminated, the contaminant will be taken up in large quantities by the consumer. Lipophilic contaminants within consumed biomass are subsequently absorbed and stored in the bodies of the consumers rather than eliminated along with other waste products. If the consumer is eaten by another consumer organism, the fat tissue is digested and the contaminant is then stored in the tissues of the latter consumer. In this way, the contaminant builds up in the fatty tissues of the subsequent consumers and the concentration of the contaminant in their tissues becomes higher with each trophic level. Water-soluble pollutants usually do not biomagnify in this way because they would dissolve in the bodily fluids of the organism and be excreted. Thus the principle of biomagnification is based on the fact that the mass of the contaminant is largely conserved along the food chain, while the biomass decreases [10].

The extent to which biomagnification occurs between a consumer and its food/prey can be expressed as the biomagnification factor (BMF). The BMF can be used to predict ecological risk of chemicals [11, 12]. Determining the

biomagnification for a food chain experimentally, by studying the transfer within a chain of prey and predators, is rather simple, although it may not be very practical as all possible chemical exposure routes to organisms (water, food and soil/sediment) must also be taken into account [13]. The total bioaccumulation of a metal in species of each trophic level within a specific food chain corresponds to the chemical concentration in the organism relative to the concentrations in its surrounding environment and in its diet, respectively. Hence, bioaccumulation (expressed as the bioaccumulation factor (BAF)) is the sum of two processes: bioconcentration, which is accumulation *via* the exposure medium (expressed as the bioconcentration factor (BCF)) and biomagnification which is uptake *via* food only (expressed as the biomagnification factor (BMF)). Although BAF is the sum of BCF and BMF, it should be noted that summing both factors in a numerical way requires much care because of the differences in units for BCF and BMF [14].

Are Metals Biomagnified?

Hendriks and Heikens [15] modelled metal kinetics in a food chain by means of empirical regressions based on mean values. In this modelling, it was concluded that despite taxonomic variability, metal concentrations diminish with increasing trophic levels. Also for marine ecosystems, Gray [16] concluded that biomagnification of metals is not a universal rule. This is in agreement with earlier conclusions of Laskowski [17], who proposed that, based on the mean concentrations that accumulate in successive trophic levels, biomagnification of Cd and Cu does not lead to high concentrations in carnivorous predators. In contrast to this view, van Straalen and Ernst [18] suggested that trophic movement of metal cannot be examined by generalizing about the body burdens of different trophic levels (*i.e.* using the statistical means of body burdens), but must be examined by following the path of each metal through each species, because different species will have different capacities to accumulate metals. The difference between these two views is illustrated in Fig. (**1**). Scheme A (Fig. **1A**) provides a simplistic hierarchical view of a trophic cascade in which all consumers can be allocated to discrete trophic levels. Alternatively, scheme B (Fig. **1B**) provides a model with more complicated interactions between different trophic levels, and when examined in this way, metal biomagnification may be manifest in some trophic pathways, but not in others.

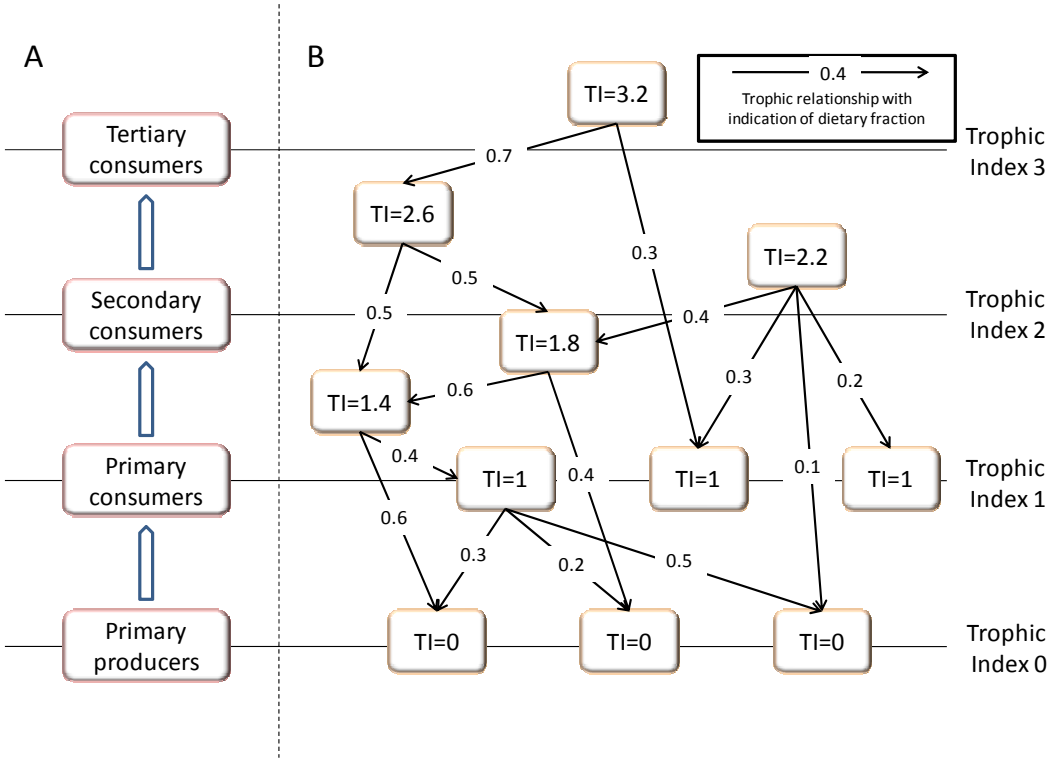

Figure 1: Schematic diagram of two biomagnification models. **A** provides a simplistic hierarchical scheme which places all consumers in subsequent trophic levels. **B** provides a more realistic "food web" representation where each organism has an associated trophic index (TI). TI is calculated as $TIi = 1 + \Sigma (TIj \times DFi,j)$, where TIi is the Trophic Index of the species i, TIj is the Trophic Index of the species j and DFi,j is the fraction of the species j in the total diet of species i. The TI for primary producers is set at 0. Redrawn and modified from Alonso [21].

Croteau *et al.* [19] concluded that predictive relationships between metal concentrations in predators and prey was only possible if their prey can be identified, and if the concentrations of metals in their prey is known. This information can subsequently be used in a bioaccumulation model. In the field situation, stable isotope ratios of carbon and nitrogen can be used to ascribe trophic positions of species within a food web. These ratios provide insights into time-integrated energy flows and food web structures [20]. The isotope ratios of carbon can be used to identify food sources, whereas the nitrogen isotopes can be used to infer the trophic position of an organism. After defining these relationships, pollutant concentrations can be compared to trophic levels inferred with the isotopic techniques, and a better understanding of habitat-specific food webs can be gained.

There is ongoing discussion as to whether biomagnification of metals occurs within aquatic and terrestrial systems, and more importantly, whether it can occur to the point that there is a detrimental effect on middle or top-order predators. Food web complexity (*i.e.* as represented in Fig. **1B**) makes predicting the trophic movement of metals rather difficult. Up to now, most information about biomagnification in field situations, especially quantitative relationships, is limited to comparative assessments between food chains, because the complexity of the process and the variability of data increase when moving to food-chain assessments.

TROPHIC MOVEMENT OF METALS AND FACTORS INVOLVED

Partitioning of metals is dictated by the environmental compartments present (especially solid and liquid phases) and the size of those compartments. Within the compartments, various environmental factors affect the bioavailability of metals for bioaccumulation [22-24] The environmental factors that influence the fate and partitioning of metals include pH, sorbing ligands in the exposure matrix, and the amounts of competing ions. Also, metal specificities affect bioaccumulation [25], as do species-specific characteristics such as the excretion capacity of organisms [10, 26] and the trophic level of the species.

Metal Specificity

Some metals are known to biomagnify. The best understood example is that of mercury (Hg), which biomagnifies to a great extent, but only when present in the lipophilic organic form, methyl mercury (MeHg). Methyl mercury is formed under anoxic conditions through microbial methylation [27], is readily bioaccumulated by algal species [28] and fungi [29] and subsequently biomagnified through trophic transfer [28, 30]. High body burdens of Hg are usually found in top-order marine predators [31] like the toothed whales. Among terrestrial fauna, particularly in birds, high body burdens in Hg usually occur as a consequence of consumption of aquatic invertebrates [32-34] or fish [34]. Similarly, semi-terrestrial mammalian predators in aquatic ecosystems (e.g. mink, otters, polar bears) also bioaccumulate Hg [35, 36], as do wholly terrestrial carnivores for which fish forms a large proportion of the diet [37].

The extent to which Hg biomagnification occurs in these food chains requires knowledge of the dietary preferences of the species involved. For example, polar bears are known to bioaccumulate Hg from their prey. However, the concentrations of Hg reported in polar bear liver, although high (between 1 and 200 μg/g dry wt) is rarely higher than in their prey food (seals) [38]. The reason for the apparently low level of trophic transfer of Hg between seals and polar bears likely lies in the polar bear's dietary preference for the skin and fat which have relatively little of the bioavailable MeHg, and the low bioavailability of inorganic Hg in seal liver [39].

Biomagnification of other metals is more difficult to demonstrate, although there are theoretical reasons to suspect that Se and Cd could biomagnify under some circumstances [40]. Gray [16] reviewed 35 papers on the subject of biomagnification of metals in aquatic systems and judged that there was little evidence, with the exception of MeHg, for the biomagnification of metals, despite the fact that 28% of papers (2 of 7) that examined biomagnification of organotin, did demonstrate biomagnification. Recently Se biomagnification has been demonstrated in a short aquatic food chain in the field [41]. In terrestrial systems, Se has not been demonstrated to biomagnify [42] although elevated tissue concentrations of selenium have been found among small resident mammals and birds nesting in the vicinity of a Se contaminated site [43, 44].

In aquatic systems, and contrary to prevailing views, Croteau *et al.* [40] demonstrated that Cd is progressively enriched among trophic levels in discrete epiphyte-based food webs composed of macrophyte-dwelling invertebrates

(the first link being epiphytic algae) and fishes (the first link being gobies) [40]. In the same food web, Cu was not similarly enriched. Biomagnification of Cd has also been demonstrated in terrestrial food chains (see below).

Nickel (Ni) and thallium (Tl) also have the potential for transference along aquatic food chains. Dumas and Hare [45] demonstrated that the majority of both metals (58 to 83%) was assimilated by predatory alderflies (*Sialis velata*) feeding on aquatic invertebrates that had previously accumulated Ni and Tl from contaminated sediment, and indicates that these metals are easily transferred along the aquatic food chain and that food is an important source for biomagnification of these elements. Thallium in particular is known to bioaccumulate in plants grown in contaminated soils [46, 47]. However, very little information is available about the biomagnification potential of Tl in higher trophic fauna, although high levels of Tl were reported in greater white-toothed shrew (*Crocidura russula*) 19 months after the collapse of a tailing dam at the Los Frailes Mine in Aznalcóllar that resulted in extensive contamination with various metals in Doñana National Park, Spain [48].

Dietary Exposure to Metals

In aquatic systems, all organisms are subject to the diffusive mechanisms that allow metals to passively enter tissues. Bioconcentration occurs when organisms sequester those metals internally (*i.e.* when assimilation is higher than excretion), and thereby maintain an inward diffusion gradient. Bioconcentration is distinct from bioaccumulation. Bioaccumulation takes into account the internalization and retention of contaminants *via* all routes including ingestion and absorption across membranes such as gills, whereas bioconcentration is particular to aquatic organisms that accumulate contaminants across exposed membranes. All aquatic organisms, including fish, possess membranes that are exposed to the water column (e.g. gills) where diffusion of metals can occur. With this distinction in mind, Gray [16] observed that with passive uptake (e.g. fish) biomagnification does not occur as opposed to dietary uptake (e.g. birds) where biomagnification may be observed. In aquatic systems, up to the trophic level of fish, there is usually no need to assume that food is the major route for contaminant intake and therefore, that biomagnification is not so important. However, Gray [16] also observed that organisms that have aerial respiration (e.g. sea birds, reptiles and marine mammals) must take in contaminants *via* food rather than their body surface and are likely to show biomagnification. Therefore it can be concluded that aquatic systems react differently on the transfer of metals through the food chain than terrestrial food chains.

Transfer from Soil to Plant to Grazing Fauna

Many plants are able to bioaccumulate metals and some plant species are even able to hyperaccumulate various metals at levels exceeding their concentrations in ores [49]. It has been suggested that metal accumulation by plants may be a defence strategy to discourage consumption by herbivores [50, 51], although it may be more accurate to say that avoidance of plants with high metal burdens establishes an evolutionary selection pressure for hyperaccumulation among plants [51]. Similarly, some species of herbivores have evolved to utilise metals bioaccumulated in the ingested plant biomass as a defence against subsequent predation [52]. The implication here is that some animal consumers/predators are able to detect and selectively ingest or avoid metals in prey/food items.

However, in the absence of metal avoidance behaviours, trophic biomagnification of metals might be expected. In a study examining a floodplain area in The Netherlands with elevated metal concentrations [53], the most dominant plant species was the stinging nettle *Urtica dioica*. The stinging nettle contained only very low metal concentrations, far below the maximum values found in plants from non-polluted sites. Nevertheless, the main herbivore feeding on these plants, the snail *Cepaea nemoralis*, did contain metal concentrations that were much higher than background values [53]. Cadmium in particular was accumulated to very high levels, with consequent negative effects on reproduction [53, 54]. Similarly, substantial Cd accumulation was also reported among snails (*Helix aspersa*) in mesocosm studies [55, 56]. However, these studies indicated that up to 40% of the accumulated Cd, and even higher proportions of accumulated lead (Pb) and zinc (Zn) are bioconcentrated directly from the soil [56]. Exclusively dietary accumulation of Cd has been demonstrated in aphids. In an examination of the trophic movement of Cd and Zn between wheat grown on Cd-contaminated soils, and aphids (*Rhopalosiphum padi* and *Sitobion avenae*), aphids were demonstrated to bioaccumulate both Cd and Zn up to ten times the concentrations in wheat [57, 58].

Bioaccumulation of metals through grazing is not only dependent on metal levels in the plants consumed, but effectively depends on a delicate interplay between internal processes regulating concentrations of both essential and

non-essential elements below the concentrations at which toxicosis occurs. A well known example is a two-stage process leading to copper toxicity in sheep and cattle. Copper toxicity among domestic ruminants generally occurs as a consequence of consumption of Cu-contaminated water, vegetation contaminated with Cu-based insecticides or fungicides or pasture that has been top-dressed with Cu salts or swine and poultry manure [59]. Initially, in the first stage, there is the steady accumulation of copper in the liver over time. Under normal circumstances, Cu is absorbed from the diet and transported in the bloodstream to the liver for storage. Excess Cu from the diet is stored in the liver and is released into the blood as needed for regular body functions. The circulating Cu level tends to remain constant regardless of the amount of excess Cu accumulating in the liver. When dietary intake of Cu is high, Cu can build up in the liver over a matter of weeks, months, or more than a year depending on a variety of factors without any clinical signs. However, with increased accumulation above the detoxification capacity of the animal, there is a sudden release of copper from the liver into the bloodstream. This rush of Cu into the sheep's bloodstream causes causing massive hemolysis, renal and liver failure and ultimately death in two to five days [59]. Copper toxicity among ruminants is usually recognised following veterinary examination of domestic stock, whereas copper toxicity among wildlife is seldom documented.

Despite the capacity of most animals to obtain and regulate Cu within narrow limits, Cu deficiency has also been observed among wild ruminants and domestic stock as a consequence of the presence in the diet of elements like molybdenum (Mo) and sulphur (S). Copper deficiency occurs because of the formation of insoluble Cu-Mo-S complexes that are excreted by the grazers [60]. Molybdenosis, or molybdenum induced Cu deficiency is likely the cause of death and disease among wild moose (*Alces alces*) in Sweden [61]. In such cases, total Cu levels in blood plasma are an unreliable guide to Cu status. Similarly, Cu levels in plants or in soil in themselves are also not reflective of actual bioaccumulative levels of essential elements for grazers as levels of other elements affecting the effective Cu dose also need to be taken into account.

Transfer to Higher Predators

Cadmium is the one metal that has been demonstrated to be biomagnified along terrestrial food chains. As indicated above, herbivorous invertebrates can biomagnify the Cd ingested from their food plants. High body burdens among herbivorous/detritivorous invertebrates occur because of high dietary-Cd assimilation efficiencies [up to 100%, 62, 63, 64] and low rates of elimination [e.g. 65]. With successive trophic levels in terrestrial food chains, the occurrence of biomagnification becomes less predictable. Some invertebrate predators have developed physiological mechanisms that allow them to avoid accumulating Cd from their prey. Using the example of aphids cited above, Merrington et al. [66] and Green et al. [67] demonstrated that two predators of aphid, lacewings (Mallada signata) and ladybird beetles (Coccinella septempunctata), did not biomagnify Cd contained in their aphid prey. In the case of the beetles, Cd was assimilated by the larvae, but was subsequently sequestered in pupal exuviae. Another example is the spider Dysdera crocata, which preys upon isopods. Isopods are known to accumulate large body burdens of metals, including Cd; however, when maintained exclusively on a diet of isopods with high body burdens of Cd, D. crocata did not assimilate Cd [68]. The absence of net assimilation in this spider occurs because of the breakdown of the digestive cells in the midgut diverticulae where metals temporarily accumulate, with subsequent release of metals into the lumen of the midgut prior to excretion [69]. In contrast, wolf spiders (Pirata piraticus), when provided with Cd contaminated fruit flies, assimilated nearly 70% of Cd from its prey without any elimination [70].

Among terrestrial vertebrates, Cd assimilation efficiencies are relatively low [<10%, 71, 72, 73], indicating that vertebrate digestive physiology presents an efficient barrier against Cd assimilation [74, 75]. However, overall bioaccumulation can still be expected to be high among some taxonomic groups, particularly homeothermic animals with high metabolic demands (and high food intakes) and long-lived animals. The species-specific differences in capacity to biomagnify Cd is best illustrated by an examination of the numerous studies that have reported accumulation of Cd among carnivorous shrews (*Sorex araneus*) and herbivorous voles (*Microtus agrestis* and *Myodes* [syn. *Clethrionomys*] *glareolus*). Shrews are particularly interesting because their high metabolic rates require them to consume >80% of their body weight each day. The field data presented in Fig. (**2)** comprise several studies and locations, mainly diffusively polluted floodplain soils and former mining areas. Studies were selected for inclusion if Cd concentrations in food items, *i.e.* earthworms and plants, were measured. As small mammals predominantly accumulate Cd *via* ingestion, absorption from water and inhalation could be disregarded as negligible. Binding of Cd to the metal-binding protein metallothionein, with subsequent storage in the liver and kidney is the

main detoxification mechanism of small mammals and results in very low elimination rates. Any decrease in the concentration of Cd in the body could be attributed to elimination *via* growth dilution only.

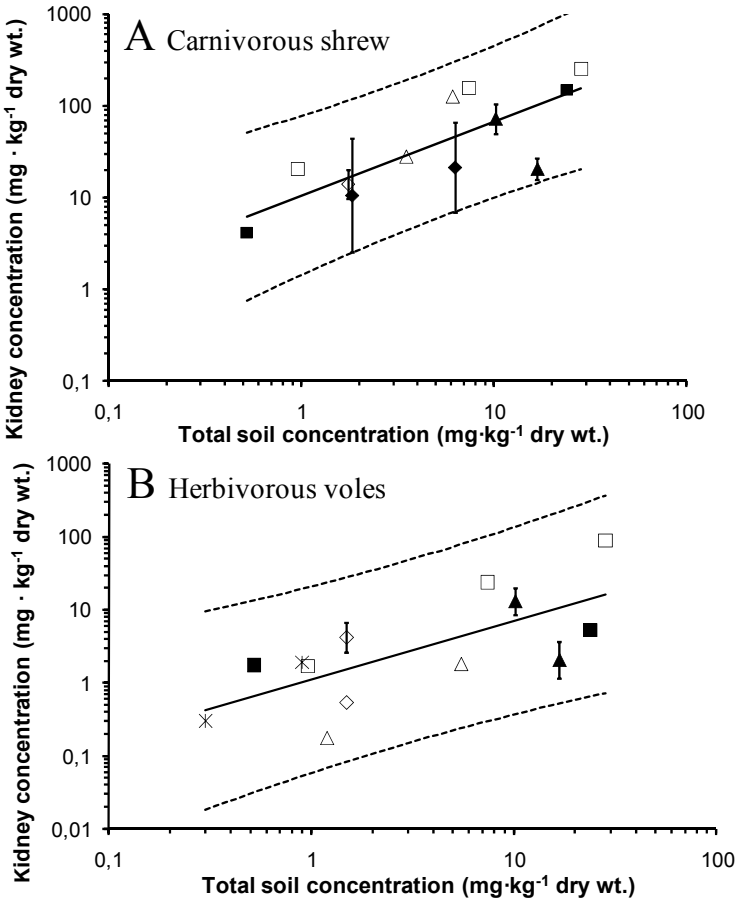

Figure 2: Cadmium accumulation in kidneys of carnivorous shrews (A) and herbivorous voles (B) (mg/kg dry weight) compared to total metal concentrations (mg/kg dry weight) in soils of various origins. ▲ = Biesbosch [76], ◆ = Rhine [77], ◇ = ADW [78, 79], △ = near closed smelter (Budel) and industrially polluted area (Arnhem) [80], □ = near Cd / Cu refinery, 1 km from refinery and reference location [9, 81], ■ = near mine and reference location in UK [82, 83], ✱ lead mine (Frongoch) and reference site [84]. Full line represents empirical regression. Upper and lower dashed lines represent the 97.5th and 2.5th percentile of the field data, respectively. The error bars represent the 95% confidence intervals and were plotted where possible. Figure adapted from Veltman *et al.* [85].

Linear regression analysis was performed to relate Cd concentrations in kidney, liver and whole body of small mammals to total soil concentrations. Results show a significant relationship between total soil concentrations and Cd concentrations in kidney and liver of carnivorous shrew and herbivorous voles (Fig. (**2**), only kidney data shown). Cadmium concentrations in above ground parts of plants were generally lower than concentrations in earthworms when exposed to similar soil-Cd concentrations. This was in agreement with the observation that Cd concentrations in voles were generally lower than in the shrew. Additionally, a large variation in Cd levels in plants was observed when related to total soil concentrations. As a consequence, regressions of Cd accumulation in herbivorous voles have a lower explained variance compared to carnivorous shrews based on total soil concentrations.

Transfer from Soil to Higher Fauna

Direct uptake of metals from soil by wildlife may be an important pathway for metal accumulation as well. Wildlife may ingest soil deliberately, or incidentally when they ingest soil-laden forage or organisms that contain soil in their intestine (e.g. earthworms) [e.g. 86]. Because the concentrations of metals in ingested soil may be higher compared to the metal concentrations in prey items, the soil can be an important pathway of exposure to predators as well.

Beyer *et al.* [87] provided an overview on the estimates of soil ingestion by wildlife based on experimental data, field captured animals and modelling. The authors found that between 3% and up to 30% of the ingested diet of wildlife consists of soils and/or sediments. Examples of carnivorous predators are raccoons (*Procyon lotor*) with 9% direct soil ingestion and red fox with 3%, whereas sandpipers consumed sediments at a rate of 7 to 30% of their diets. Highest rates among the herbivorous eaters studied were Canada geese (*Branta canadensis*) and the black-tailed prairie dog (*Cynomys ludovicianus*) (up to 8%).

Excretion of Metals by Predators

The capacity for excretion differs between predatory species. A high excretion capacity implies that biomagnification is lower. Reinfelder *et al.* [88] suggested that trophic transfer potential could be described from biodynamic parameters - weight-specific ingestion rate, assimilation efficiency (AE), and a rate constant of loss. Hendriks and Heikens [15] based their studies on these parameters as well. Higher concentrations at higher trophic levels can be explained by the fact that elimination rates decrease with increased body size [12, 89, 90]. The main reason for an absence of biomagnification among aquatic trophic chains is the observation that most marine organisms can fairly easy eliminate metals [16].

Predator Specificity (Gender & Life-Span)

Some authors indicate that females accumulate higher concentrations of metals than males of the same species. For example, some studies suggest that male and female moles (*Talpa europaea*) accumulate different heavy metal concentrations in their tissues. Kormanicki [91] observed that females have higher levels of Cd in the femur and stomach and higher levels of Zn in the gonads, spleen and skin. Pankakoski [92], in an examination of the same species in Finland indicated that Pb accumulation was also higher in females than males. The consequences of female biased accumulation for local population viability are not studied and remain unknown.

Gender variation of metal burdens was also observed by Deng *et al.* [93] in a study on metal levels in great tit (*Parus major*) and greenfinch (*Carduelis sinica*) in the western mountains of Beijing (China), but the differences did not follow a specific pattern. In general, trace metal concentrations in different body parts were similar between males and females in both species. However, in the liver and feathers, there were significant gender related differences, and the pattern was species specific. In the great tit, males possessed higher chromium (Cr) and Ni in the liver and Se in the feather than females, while females had higher levels of Cd in these body parts. In the greenfinch, males had higher concentrations of arsenic (As) and Zn than females in the feather, while females had higher concentrations of Zn and Se in the liver. Janssens *et al.* [94] found no general age- or gender-related differences in metal levels across a pollution gradient except for As and iron (Fe), where a significant interaction between site and gender was observed. Actually, the results of these authors suggest that feathers of great tits might be useful biomonitoring tools because they reflect the environmental contamination by heavy metals well.

Nam *et al.* [95] on the other hand observed no gender-related variation in two populations (Lake Biwa and Mie) of great cormorants (*Phalacrocorax carbo*) from Japan for most of the trace elements that they studied, except for higher hepatic strontium (Sr) concentrations in males from Lake Biwa. In both populations, some elements revealed tissue-specific accumulation. For example, most of the burden of Mo, silver (Ag) and Cd was in liver, Tl and Cd in kidney, Cu, rubidium (Rb) and caesium (Cs) in muscle, and vanadium (V), Sr and barium (Ba) in bone. Hepatic V, muscular Hg and Tl, and Cd in liver, kidney and muscle increased with growth. There was little gonad-specific accumulation of any metal. Thus this study countered the hypothesis that enhanced excretion of metal in the eggs laid by females reduces female metal burdens. Site specific variation in elemental concentrations in stomach contents also indicated that dietary sources tended to be the main factor in regional variations observed between the two colonies studied. Concentrations of toxic Hg and Cd in the liver of cormorants from the two colonies were lower than those from other areas, implying relatively low exposure to these metals. Concentrations of V, Co, Ag, Cd, Cs, Hg, Tl, Pb and Bi in liver remained more or less at the same level between 1993 and 2003, while hepatic Cr, Mn, Cu, Zn, Se, Rb, Sr and Ba showed apparent decrease.

The life span of organisms is also important when considering the bioaccumulation of metals because it affects the total period of time that an animal is exposed to contaminants. The older an organism is, the longer it has been exposed to any contaminant. Bioaccumulation will have a linear relationship with the exposure time of an organism,

particularly when excretion is negligible. Overall, the life-span of an animal is likely to be a more important factor affecting metal burdens in specific predators than gender variation [96-98, but see 99].

Metal Sequestration in Prey

The transfer of metal from prey to predator is dependent on two highly variable factors. First, the capacity of predators to minimize net assimilation of metals contained within their prey is variable. As indicated above, the digestive physiology of vertebrates and some invertebrate predators provides an effective barrier against metal assimilation and accumulation. Second, the bioavailability of metal sequestered in the prey is also variable. Some plants (e.g. members of the family Brassicaceae) and animals (e.g. crustacean, oligochaete species) are able to accumulate large tissue burdens of metal. They are able to avoid the damaging effects of reactive forms of essential and nonessential metals and to selectively control utilization of essential metals, by sequestering them in non-toxic forms. This is effectively a kind of elimination. In the case of plants, metals appear to be sequestered predominantly as granular deposits in cell vacuoles [100]. Similarly, soil invertebrates are able to store metals such as Cd in special hepatopancreatic cells as inert granules [101, 102].

The form in which metals are stored in prey species has implications for bioavailability of metals to higher trophic levels (predators) [103-105]. In an attempt to discriminate between the various forms of sequestered metal, Wallace *et al.* [106, 107] defined various sub-cellular partitions based on a centrifugal protocol that partitioned animal tissue into five separate fractions: metal rich granules (MRG), cellular debris, organelles, heat stable proteins (HSP) and heat denatured proteins (HDP). Using this protocol in an examination of the relationship between sub-cellular Cd distribution in an oligochaete and its trophic transfer to a predatory shrimp [108], the authors proposed that only metal present in the soluble fraction (*i.e.* organelles and protein fractions) of prey is available to the predator. Similar conclusions were drawn in a study using bivalves as prey; the authors again concluded that the metal partitioning to organelles, HDP and HSP, comprise a sub-cellular compartment that is "trophically available" to predators [106]. This model seems to have established a degree of acceptance and provides a pragmatic approach to resolving sub-cellular metal distribution, and has been shown to yield useful insights with a variety of species: e.g. bivalves, perch and earthworms. However, refinements to the protocol will be required before it can be used to predict bioavailability of prey-bound metal because subsequent studies have demonstrated that the soluble fractions were not always 100% bioavailable and that the insoluble fractions were not always 100% non-bioavailable [105, 109].

CONSEQUENCES OF BIOMAGNIFICATION FOR POPULATIONS DECLINES/SHIFTS OR ECOSYSTEM EFFECTS

Direct Poisoning by Metals

Occasionally, human activities can lead to deposition of metal in such high concentrations that biomagnification is not required before symptoms of toxicity can be observed in wildlife, and result in the direct poisoning of higher vertebrates with metals. The most obvious example of this is through the use of lead shot for hunting and lead fishing sinkers. A recent study by Mateo *et al.* [110] reported up to 148 lead shot pellets per square meter in wetlands in southern Spain. Poisoning occurs when animals ingest spent shot. Waterfowl can ingest lead shot directly while feeding, and galliform birds can ingest shot as grit for their gizzards. Also, predators and scavengers ingest lead shot or lead shot fragments that are embedded in carcasses or that persists in the gizzards of birds [111, 112].

The first studies of Pb poisoning in waterfowl were conducted in the USA in as early as 1959 [113]. Since then, various scientific studies have examined Pb pollution in wetlands and the possible consequences of direct or indirect Pb poisoning on water birds. In a laboratory study, a single lead shot experimentally imbedded into the crop of ring-necked ducks (*Aythya collaris*) caused mortality or severe symptoms of debilitating toxicosis among 87% of birds [114]. Lead is a non-specific poison affecting numerous body systems, and sublethal exposure has variously been reported to result in (i) weakness of contaminated birds making it vulnerable to predation; (ii) alteration of energy requirements that will consequently be a handicap for birds on migration; (iii) reductions in clutch size; and (iv) result in eggshell thinning and embryo malformations [111, 115]. Most of these toxicological studies were conducted on species of ducks and swans.

Lead poisoning among waterfowl has resulted in several population declines. Population declines of mute swans (*Cygnus olor*) in the UK between the 1960s late 1980s were associated with ingestion of lead sinkers. Their numbers

subsequently increased following the ban on sales of small lead sinkers in the late 1980s. Similarly, up to 50% of recorded mortalities among common loons (*Gavia immer*) in Canada were also associated with ingestion of lead fishing sinkers [116]. Additional investigations to see if Pb poisoning could have a similar effect on waders (common snipe *Gallinago gallinago* and jack snipe *Lymnocryptes minimus*) concluded that Pb poisoning also affects waders to a similar extent to that found in ducks, thus confirming previous studies on aquatic birds [117]. The use of lead sinkers for recreational fishing and lead shot for waterfowl hunting has been restricted in the USA and various other countries since the 1980s/1990s. However the incidence of Pb poisoning among raptors has not decreased in the USA and it seems likely that the continued use of lead shot for upland hunting has shifted the emphasis from water birds to predatory birds [116, 118].

Mercury poisoning among mammals is exemplified by Minamata disease. Minamata disease is manifested as severe neurological pathologies among victims exposed to Hg-contaminated food. In Minamata, Japan (the location from which the syndrome takes its name), poisoning resulted among human inhabitants following consumption of fish contaminated with biomagnified Hg (see below). However, similar neurological pathologies have been observed among people and birds following the use of organo-mercurial fungicides for the protection of grains and as a consequence of ingestion of contaminated seed [31].

Arsenic poisoning in animals and humans is caused by several different types of inorganic and organic arsenical compounds. Toxicity varies with factors such as oxidation state of the As, solubility, species of animal involved, and duration of exposure. Organic forms appear to have a lower toxicity to mammals than inorganic As. Research has shown that arsenites (trivalent forms) have a higher acute toxicity than arsenates (pentavalent forms) [119]. In mammals, As is known to promote cancer of the bladder, lung, and skin. Arsenic poisoning among the human population of Bangladesh occurs as a consequence of direct consumption of ground water, which has naturally high concentrations of As. However, because groundwater is also used for irrigation, contamination of rice (the staple crop in Bangladesh) is also a likely source of As [120]. Many plants are able to accumulate As, and some are able to hyperaccumulate this metalloid. For example, the Chinese brake fern (*Pteris vittata*) is able to hyperaccumulate As (more than 1000 mg As/kg of shoot dry weight) as As(V), reduce it to As(III), translocate it through the xylem with water and minerals as an As(III)-S compound, and then store it as As(III) in the fronds [121], thus making trivalent As available to herbivores. It is not known to what extent As poisoning occurs in animals that feed on plants that accumulate As, but because there is no threshold below which As intake is regarded as safe for humans, there is clearly potential for poisoning among other herbivorous species following the consumption of plants.

The occurrence of arsenic poisoning in Bangladesh is a similar scenario to that of *itai-itai* disease in post-WWII Japan. *Itai-itai* (meaning 'painful' in Japanese) disease was the name given to cases of cadmium poisoning among the human population living around the Jinzu River that had been contaminated with Cd originating from a zinc mine further upstream. *Itai-itai* disease is characterized by symptoms of osteoporosis and osteomalacia associated with renal dysfunction resulting from chronic Cd poisoning. Poisoning occurred because people either drank or cooked with the river water, or because they ate rice that had been irrigated with Jinzu River water [122]. Kobayashi *et al.* [122] performed multiple regression analyses, and found a strong correlation between consumption of Cd-contaminated rice and the occurrence of renal tubular dysfunction, indicating that Cd poisoning in the human population has occurred as a consequence of biomagnifications of Cd through rice.

Populations Under Threat from Biomagnification

Bioaccumulation of metals as a consequence of biomagnification or bioconcentration is frequently observed among lower trophic levels; however, elevated tissue burdens of metals do not necessarily translate into shifts in community or population structure. Again the studies by Hunter *et al.* [7, 8] are instructive. As indicated earlier, the vegetation structure was only altered in the highly metal-contaminated zone around the copper refinery, which was dominated by a few metal tolerant plant species. Although de-vegetation and dominance of contaminated soils by metal-tolerant plant species has been demonstrated in other sites with high levels of metal contamination [e.g. 123, 124, 125], moderate levels of metal contamination generally have little effect on plant communities.

Another notable difference between the highly contaminated zone near the refinery and less contaminated areas studied by Hunter *et al.* [8] was the lower abundances of isopods and oligochaetes in the most highly contaminated

zone. Other studies have also shown earthworms to be sensitive to high levels of metal contamination in soils [126]. However, at sites where metal concentrations are moderate, soil community shifts are often subtle or masked by other environmental factors [127] and indicates that bioaccumulation of metals in lower trophic levels does not necessarily impact on invertebrate community structure [128].

However, impacts in vertebrate consumers/predators might be expected to be more pronounced because of the large volumes of prey taken. For example, godwits (*Limosa limosa*) are migratory waders that breed and feed at numerous contaminated sites in The Netherlands [129]. Although their residency at contaminated sites is temporary, godwits feeding on worms that were known to accumulate metals, in turn accumulated Pb, Hg and Cd from worms. *Limosa limosa* is listed in the so-called Dutch Red List (a list of endangered species that are protected by special legislation) and is therefore a conservation priority species. Numerous factors are implicated in the population declines of this species and it is not possible to isolate metal contamination as an important factor.

Arsenic poisoning has been implicated in declines of small mammals in alpine regions of the Snowy Mountains of Australia, although the causal links remain speculative. Bogong moths (*Agrotis infusa*) migrate annually from the agricultural plains to alpine regions to estivate and carry with them large body-burdens of As. Between the time when arsenic was first reported from two mountain estivation sites in the summer of 2000/2001 [130] and the following summer, the amount of arsenic found in moths increased by at least an order of magnitude [131]. Over this period, populations of the herbivorous broad-toothed rat (*Mastacomys fuscus*), the insectivorous dusky antechinus (*Antechinus swainsonii*) and the omnivorous mountain pygmy possum (*Burramys parvus*) declined, and As was detected in the faeces of *A. swainsonii* and another non-declining species, *Rattus fuscipes*. Because the declines in mammals were across several trophic groups (*i.e.* herbivores and insectivores), As poisoning is not likely to be the predominant causal factor, but may be a co-factor that needs to be considered in the declines of rare species like the mountain pygmy possum (*B. parvus*). The source of As in the moths was not known [130, 131], but it is presumed to come from the breeding grounds of the moths in the agricultural plains of southern Queensland and northern NSW [130]. As-based pesticides have been used in Australia since the early 1900s [132, 133] and continue to be used to a lesser extent in the form of the organoarsenic herbicides (e.g. monosodium methylarsonate; MSMA).

MSMA has also been applied widely in British Columbia, Canada to control outbreaks of Mountain Pine Beetle [134]. As a consequence, beetle larvae accumulated between 1.3 and 700 μg As/g (dry weight). Subsequent feeding by insectivorous woodpeckers and other forest passerines breeding within 1 km of MSMA stands contained elevated blood concentrations of total arsenic (geometric mean = 0.18 μg/g; range = 0.02 to 2.20 μg As/g) [135]. This range of whole blood concentrations is similar to the range of concentrations found among zebra finches provided orally with monomethylarsonic acid (MMA(V)) at doses between 8 and 72 μg/g/day over 14 days [136]. Oral doses of this magnitude were found to cause weight loss among adult finches after 14 days [136] and mortality among nestling zebra finches after 20 days [137], indicating that natural field populations of passerine birds may be affected in MSMA treated areas.

As indicated in the introduction, various small mammals are able to persist in highly contaminated sites. Of particular note is the persistence of common shrews (*Sorex araneus*) in sites with high levels of Cd contamination. Shrews are higher order predators in terrestrial environments, and because of their high metabolic demands, they consume large quantities of prey. In Cd-contaminated sites, shrews accumulate very large body burdens of Cd. In the study by Hunter *et al.* [81], shrews in the vicinity of a cadmium/copper refinery accumulated from their prey Cd residues in excess of 200 μg/g and 500 μg/g (dry weight) in the kidney and liver, respectively. Similar liver-Cd concentrations have also been reported by other authors [80, 138, 139]. It remains unclear if the health of shrews in these studies was compromised by the high body burdens of Cd. In an earlier report, Hunter *et al.* [140] described lesions in the kidney and liver of shrews from the same location and with similar Cd burdens. Later laboratory studies [73, 141] demonstrated reduced weights (but no mortality) among shrews exposed to Cd-contaminated diets for a period of 12 weeks. In those studies, test animals accumulated kidney and liver Cd burdens in excess of 1000 μg/g. It seems likely that shrews have evolved to tolerate high levels of metals [142].

Other species do not appear to be so tolerant. Damek-Poprawa and Sawicka-Kapusta [143] described histopathological changes in the liver and kidneys of herbivorous bank voles (*Clethrionomys glareolus*) with much lower tissue burdens of Cd in kidney (33 μg/g dry weight) and liver (16 μg/g dry weight). Cadmium is also known to

compromise reproduction [144], and accumulation of heavy burdens of Cd may be limiting recoveries of European badgers (*Meles meles*). Van den Brink and Ma [145] found that the quantities of Cd and Zn in the kidneys of badgers in the different regions of The Netherlands were negatively correlated with the increase in the number of breeding dens (setts) in these regions and hence with the number of cubs born. The highest kidney Cd burdens (101 to 405 μg/g dry weight) were found among adult female badgers living close to rivers where earthworms (a major prey species) accumulated large burdens of Cd.

Although studies like those conducted by Hunter *et al.* [9, 81] and Mertens *et al.* [138] provide evidence that small mammal communities can persist even in highly contaminated environments without apparent detriment, other studies show that community structure is likely to be altered in the face of severe metal contamination. The Tar Creek Superfund Site in Oklahoma, USA, has a long history of metal contamination as a consequence of mining activities, and elevated tissue concentrations of Cd have been documented in several non-mammalian inhabitants [146, 147]. Although tissue-metal concentrations were not reported, Phelps and McBee [148] reported reduced species diversity among small mammal assemblages at Tar Creek compared to reference locations. At reference sites, several small mammals were recorded; however, in the contaminated sites, white-footed mice (*Peromyscus leucopus*) predominated, and in greater numbers than found in reference sites. White-footed mice have previously been demonstrated to dominate small mammal assemblages in disturbed environments [149, 150].

The capacity for white-footed mice to dominate in disturbed environments is note-worthy. In the case of the study by Levengood and Heske [150], the authors cited very high soil concentrations for Cd, Hg and Se (as well as other metals) in a wetland site that had received sediments dredged from Lake DePue, Illinois, in 1982. The wetland is periodically inundated as a management strategy for the conservation of waterfowl. White-footed mice, because of their arboreal habit, were able to utilise this habitat and persist with tissue burdens of Se that have been shown to be toxic to rats in laboratory studies. The white-footed mouse is an omnivore, and likely consumes invertebrates as well as plant seeds. Tissue metal (Cd and Pb) concentrations in grasshoppers and crickets were high (2.5 to 4.8 μg/g), and it seems likely that mice were exposed to relatively high levels of metal in their diet. However, apart from Se, kidney- and liver-metal concentrations remained comparatively low, indicating that the white-footed mouse was not under threat as a consequence of biomagnification of toxic metals. The mechanism by which white-footed mice are able to avoid bioaccumulation of metals is unknown, although tolerance to high levels of metals and other contaminants is likely related to the efficiency by which this species can detoxify reactive oxygen species as well as highly efficient DNA repair mechanisms [151].

Some metals only enter the higher terrestrial food chain after commencing their trajectories through aquatic food chains. Selenium and Hg are two such metals. Selenium is bioaccumulated in aquatic habitats and organoselenium compounds can be bioconcentrated over 200,000 times by zooplankton when water concentrations are in the 0.5 to 0.8 μg Se/L range. Inorganic selenium bioaccumulates more readily in phytoplankton than in zooplankton. Phytoplankton can concentrate inorganic selenium by a factor of 3000. Further biomagnifications occurs along the food chain, as predators consume selenium rich prey. A water concentration of 2 μg Se/L is considered highly hazardous to sensitive fish and aquatic birds. Compounding this trophic biomagnification, selenium poisoning can also be passed from parents to offspring through the egg, and selenium poisoning may persist for many generations [152]. As indicated above, selenium migrates into terrestrial ecosystems when birds or other taxa feed on aquatic organisms. The best studied example comes from California in the USA.

In the 1980s Kesterson National Wildlife Refuge, CA was contaminated with Se as a consequence of subsurface irrigation drainage. Selenium and other trace elements were leached from agricultural soils in the San Joaquin Valley, and the excess water was transported to Kesterson as a wildlife management strategy. Elevated body burdens of selenium were found in all animal taxa examined, including mammals, birds and reptiles [152]. The concentrations of Se in livers taken from waterfowl typically ranged between 20 and 100 μg/g and were significantly higher than in similar birds sampled from a reference site [153]. Selenium, although an essential element required for normal development, is toxic even when exposure is only slightly higher than essential requirements. Among waterfowl in Kesterson, particularly among eared grebes (*Podiceps nigricollis*), very low hatching rates were ascribed to selenium-induced embryotoxicosis [154]. More recent studies in Kesterson have continued to report elevated level of Se in passerine species such as starlings (*Sturnus vulgaris*) and small mammals, but without associated effects on hatching success or population declines [43, 44].

As indicated above, mercury enters the terrestrial food chain when predators eat aquatic species that have bioaccumulated the metal. The best known example of Hg poisoning in a terrestrial mammal is that of Minamata disease among human inhabitants of Japan following consumption of contaminated fish downstream of a Hg-contaminated river. Minamata disease is characterized by severe neurological disorders, and similar neurological symptoms of poisoning among mammalian predators such as predatory birds and mink have also been reported [31, 39]. Among species of mink (*Mustela* sp.), a lowest observable adverse effects level (LOEL) for Hg of 5 μg/g (wet weight) in the brain has been established in laboratory studies [36]. Brain Hg concentrations of this order of magnitude (0.5 to 5.0 μg/g wet weight) are common among wild mink [36], and some authors have speculated that mercury poisoning may be responsible for declines in mink (*Mustela vison*) in Georgia, North Carolina, and South Carolina [155], although the co-occurrence of chlorinated organic compounds in mink tissues was also implicated.

Biomagnification of metals in higher predators, such as predatory birds, large carnivorous mammals and reptiles [156-158], is more difficult to demonstrate. Predatory birds and large mammals are more sparsely distributed and far more mobile, having home ranges that go well beyond localised contamination. Contaminated prey, though likely forming part of their diets, will be diluted with prey from less contaminated sites. In the case of reptiles, a combination of low metabolic rate typical of poikilothermic animals and relatively low assimilation efficiencies [72, 159], is likely to reduce the risk of metal bioaccumulation. However, reptiles in general are under-represented in the ecotoxicology literature [160] and it would be premature to suggest that reptiles were not at risk from exposure to metals.

CONCLUSION

Direct metal poisoning of higher vertebrates is found sporadically, and examples thereof are mostly found for Pb. Magnification in the food chain *via* prey having elevated body burdens of metals is shown more often. The level of biomagnification that can be expected within the field situation is difficult to predict, and depends on five factors, namely: (i) metal specificity; (ii) exposure route of the predator; (iii). excretion possibilities of organisms; (iv) predator specificity such as gender and life span, and (v) metal sequestration in the prey organism.

In general, carnivorous populations show higher biomagnification compared to herbivorous organisms. Aquatic food chains are less at risk than terrestrial food chain when it comes to biomagnification of metals and metalloids. To assess biomagnification effects at community level, unraveling ecosystem complexity is necessary before species most exposed and at risk can be identified. Therefore, for the purpose of setting environmental quality objectives, it is important to include biomagnification as well.

The final objective of studies that examine biomagnification and toxicity of metals is to provide sufficient protection to all biological organisation levels. Observations in the laboratory and field have demonstrated that secondary poisoning of, for example, worm-eating birds and mammals may be more critical than direct exposure of soil organisms. In such cases, quality criteria that are protective of lower trophic levels, may not provide protection for top predators.

FUTURE PROSPECTS

Several metals are familiar as common contaminants in our environment by virtue of the fact that they have been used for many decades or centuries for industrial and agricultural purposes. Indeed, the various phases in human civilization are characterised by our use of specific metals. The Copper Age (c. 3200–2300 bc), the Bronze Age (2300–700 bc) and the Iron Age (700–1 bc), mark the discovery and adoption of these metals. The more recent industrial ages have seen the use of many other metals including those which now present a pollution hazard, such as Cd, Pb and Hg. Much of our knowledge about the environmental risks posed by metals comes from research on these metals. However, the industrial age is far from spent, and recent decades have seen increased reliance on several other metallic elements. In the immediate future the continued and increased reliance on coal combustion for electricity production will result in increased diffuse contamination of soils and oceans with various elements, including Hg, As, Cd, Cu, Pb, Se, Zn and various others because they are volatile species which are not retained by flue gas filtration systems [161]. Therefore, it can be expected that the environmental consequences of bioaccumulation and subsequent biomagnification of various metals, particularly Hg, Se and Cd are yet to be fully realised.

Other, less abundant metals are also increasing in our environment, due to use of a multitude of metals in innovative new applications like the broad area of nanotechnology and related fields (nanofood, nanocosmetics,

nanopharmaceuticals, etc). Some of these new metal-based products will follow well understood metal uptake kinetics. However others may potentially find entry into biological systems *via* yet to be discovered modes of bio-uptake. Only time will tell if biomagnification and subsequent toxicity of these innovative combinations of metals and metalloids will occur.

ACKNOWLEDGMENT

Martina Vijver was supported by a NWO VENI-grant, project number 863.08.023.

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CHAPTER 4

Impacts of Agricultural Pesticides on Terrestrial Ecosystems

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Abstract: Pesticides are toxic chemicals used to control pests, weeds and pathogens. Three quarters of all pesticides are employed in agricultural production, particularly in developed countries, in an effort to mitigate crop damage endured by intensive agriculture. However, after more than 60 years of worldwide usage, their side-effects on terrestrial ecosystems – even when applied as recommended – are obvious. This chapter examines the ecological problems caused by specific chemicals/groups, so that this awareness may help improve agricultural practices through appropriate risk management. Fungicides alter the microbial-fungi communities responsible for the recycling of nutrients in the soil, and copper fungicides are toxic to earthworms and other animals. The routine application of herbicides has produced a net loss of plant biomass and biodiversity in many landscapes, which indirectly reduces the associated arthropod communities and leads to population declines in many species of birds, and possibly amphibians too, due to lack of food. Insecticides are very toxic to most invertebrates in the soil, birds and small mammals, causing significant reductions in their populations and disturbing the trophic structure of their communities. Persistent pesticides accumulate in soil and concentrate through the trophic chain, causing a plethora of sublethal effects which are negative for the survival of individuals as well as the viability of their populations; the long term effects of DDT and cyclodiene poisoning in birds is still an ecological issue despite more than 30 years of not being applied in most developed countries. While pesticides have increased our agricultural productivity and helped feed the current human population, the price of this productivity is being paid by the Earth's ecosystems at large.

INTRODUCTION

Since Neolithic times, humanity has learnt to use agriculture to supply the food needed for its own sustenance. Agricultural practices first started with cereal crops in the Fertile Crescent about 11,000 years ago, and subsequently developed in other regions of the world, although a rather small suite of 35 domesticated plants and seven animals ended up established over the world because of their yield and nutrient characteristics [1]. For centuries, most of the staple plant foods have been cultivated as monocultures: unusual ecosystems in which no diversity of plants other than the crop is allowed to grow on the same land in order to maximize crop yields, and where all means possible are used to ensure this is the case; the unwanted, competing plants are called weeds. Because of this feature, monocultures are ideal targets for specialized consumer animals (usually insects, birds and rodents) that feed on them. Once such animals find a crop that suits them, they multiply explosively and become pests. With the exception, perhaps, of locust plagues all other agricultural pests are a product of monocultures, and from early times humanity has struggled to keep at bay the pest species that decimated our crops.

As with agriculture, the story of pesticides – the substances used to control and kill pests – started in the Middle East. The Persians found that the extract of certain chrysanthemum flowers (known as pyrethrum) was very effective in killing flies and other insects, so they used it to control agricultural pests [2]. Late in the 18th century, Erasmus Darwin found nicotine (the extract of Nicotiana tabacum) to be a powerful insecticide, and early in the 1900s arsenic salts were also used to control a wide range of pests, particularly in orchards. However, it wasn't until the 1940s that a revolution in pesticides took place, when the chemical industry started to mass-produce synthetic toxic substances that were effective, not only in killing insects (insecticides) and other animal pests (rodenticides), but also weeds (herbicides) and fungal diseases (fungicides). The rapid development that ensued, especially in North America, Europe and Asia-Pacific, led to the establishment of a new kind of agriculture based on chemistry. The so-called Green Revolution involves the use of chemical pesticides and fertilizers together with increased irrigation and genetic improvement for agricultural production. Hailed as the saviour of human starvation, the Green Revolution practices were quickly adopted worldwide, particularly in densely populated countries of South East Asia such as Indonesia and the Philippines, where food shortages were soon replaced by bumper crop yields [3]. Indeed, the use of pesticides in agricultural production became so widespread that the term 'conventional agriculture' indicates a cropping system where the Green Revolution tools are applied routinely.

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Francisco Sánchez-Bayo, Paul J. van den Brink and Reinier M. Mann (Eds)

While the Green Revolution was producing 'miracles' everywhere, the newly developed pesticides applied to an increasing variety of crops started to have side effects in the surrounding natural ecosystems. Bioaccumulation of DDT and cyclodiene insecticides was first noticed in bird predators like the peregrine falcon (*Falco peregrinus*) despite the fact they had little relation to the sprayed crops [4]. Through a long and painstaking research that involved many experts in the areas of environmental chemistry, toxicology and agriculture [5], it was eventually revealed how these chemicals had secondary and indirect effects on non-target organisms, and their impacts on the structure and functionality of natural ecosystems rang the alarm in environmental circles. Even the direct effects of insecticides on arthropod communities, and the birdlife that depended on them, was brought into question by Rachel Carson as early as 1962. The birth of the environmental movement and ecotoxicology was thus linked from its very beginnings to the widespread use of synthetic pesticides in agriculture, forestry, and urban pest control. It was realised that all pesticides are toxic to a greater or lesser degree, so their release could not be without risks to some kind or other of organisms.

Pesticides are the only man-made contaminants released into the environment deliberately, for a purpose; whereas industrial chemicals, mining wastes, pharmaceutical residues and the large list of pollutants that humanity produces find their way into the air, rivers and oceans either unintentionally or because our technology is still unable to reduce their emissions, avoid accidents, and too inefficient to recycle the wastes.

PESTICIDES IN AGRICULTURE

There are currently 835 chemical compounds used in all sorts of agricultural enterprises [6], comprising some 1300 registered products, of which 31% are herbicides, 21% insecticides, 17% fungicides, 9% acaricides and 2% rodenticides; the remaining 20% of products include a plethora of biocides for control of snails (molluscicides), algae (algicides) and nematodes (nematicides) as well as plant growth regulators (6%) and natural or artificial pheromones (5%). In addition, 610 products, including most of the infamous organochlorine (OC) insecticides, were used in the past but not nowadays – they were banned for safety and environmental reasons or because they were no longer efficient (due to resistance) and have been replaced by newer products. Despite using so many chemicals, world crop losses are estimated at 37% of agricultural productivity: 13% due to insects, 12% to weeds and 12% to diseases [7].

The toxicity and specificity of pesticides depends on the mode of action of the active ingredients (a.i.), while the effects on organisms depend on the dose they are exposed to (see Chapter 1). Thus, organochlorine, cholinesterase inhibitors (organophosphorus (OP) and carbamates), synthetic pyrethroid and neonicotinoid insecticides are neurotoxic substances that disrupt the nervous system of arthropods and other animals. Given the similarities in neuronal physiology among all kinds of animals, it is not surprising that insecticides are also toxic to aquatic and terrestrial arthropods and, to a lesser extent, vertebrates, whereas they are harmless to plants and the majority of microbial organisms. Other insecticides affect cellular or physiological mechanisms of animals (e.g. chlorfenapyr, arsenic salts). Herbicides are very toxic to plants and algae, as they target physiological pathways specific to plants such as the photosynthesis; however, herbicides can interfere with metabolic and reproductive processes in animals as well, often in ways that are unrelated to their specific mode of action in plants. Fungicides are considered in some countries to be medicine for the crops as they control fungal infections of the roots or other parts of the plant; many of them are antibiotics or metabolic inhibitors of certain fungi, while organomercurial compounds are neurotoxic and poisonous to many animals. Rodenticide poisons are usually anticoagulants, and consequently are very dangerous to humans and all vertebrates alike. Thus, the specificity of action of pesticides is not restricted to the target pest or weed species, but it is rather general, affecting large taxonomic groups often at the order or class level, even though within the same class of organisms some species are more susceptible than others due to differences in body size and/or physiological traits [8].

Pesticide Usage

Global pesticide usage is estimated at 4 million tons per year [9], although its distribution throughout the world is very uneven [10], with Europe using one third and North America a quarter of the total market until recently (Table 1). Herbicides account for nearly half of the pesticides used in North America, insecticides 19%, fungicides 13%, with the remaining 22% including a variety of other products [11], whereas insecticides are prevalent in developing countries. Agricultural industries, i.e. crops and livestock, are the main users of pesticides in the USA and other countries (74% of

the annual consumption), with gardening, golf courses, industry and urban uses making up 25% of the total amount whilst only 1% is being used in forestry [7], mainly in Canada and Scandinavian countries. DDT and lindane are still used in countries like India [12]; by necessity most of the DDT is to control mosquito-vectors of malaria and tse-tse fly in tropical countries and South Africa, where no other cost-effective chemicals are available. The distribution of pesticide types among crops differs widely: corn, soybean and cotton crops are the main users of herbicides in the USA (75%); orchards use mainly insecticides, while vineyards and vegetables use most of the fungicides [7].

Table 1: Annual pesticide usage in the world up to 1996. Source: [10]

	Millions of kg	**% Total**
Europe	800	32
Asia-Pacific	800	32
North America*	600	24
South America	200	8
Africa	100	4
Total	2500	100

* USA and Canada only

Average pesticide application in developed countries is 4.4 kg/ha per year. Since almost one fifth of the Earth's land area is dedicated to agriculture (12% as cropland and 6-8% as pastureland [13]), the impact of agrochemicals on ecosystems is quite significant at a global scale. However, not all agricultural land is treated with pesticides: in the USA, for instance, some 38% of the acreage is not treated with chemicals [7].

Application of Pesticides

Agricultural pesticides are typically applied directly on to the crop plants or fruit trees by spraying them in a liquid carrier (oil or water mixed with surfactants) that can be delivered by plane, helicopter, ground machinery or simply by hand-operated sprayer-guns. Some pesticides are applied as granules buried in the soil, or as seed-dressings to protect the growing seedlings.

The method of application greatly determines the exposure of non-target organisms to pesticides. For instance, 25-50% of the pesticide sprayed from aircraft reaches the crop, or 65-90% if sprayed with ground machinery [14]; the remainder is scattered around the target crop/orchard, with the spray droplets reaching distances up to 1.5 km under established conditions for application, *i.e.* low flying path, wind speeds between 3 and 15 km/h and no air inversions. Further drift can occur whenever these requirements are not met, as often happens with inexperienced personnel especially in developing countries. Not surprisingly, wildlife populations are systematically being affected every year by direct exposure to insecticide sprays, specially birds that are present in agricultural areas at the time of insecticide spraying [15] and receive a high dose *via* droplets or concentrated toxic vapours [16]. Exposure of terrestrial animals to herbicide sprays is less hazardous because of their lower toxicity. However, aquatic ecosystems and susceptible crops in nearby land can be affected as well, so the adoption of buffer zones around the crops can substantially mitigate the drift onto surrounding areas. For example, unsprayed strips 3 m wide around agricultural fields in the Netherlands reduced drift onto irrigation ditches by 95% [17]. Under present management practices in that country using narrow unsprayed buffer zones and other measures, the impact of sprays on non-target insects are down to 41% for herbicides, 21% for insecticides and 14% for fungicides compared to impacts in the past [18].

Granular pesticides are designed to avoid the risks of spray drift to farmers/applicators and wildlife. Also, the granules release the active ingredient over time, thus increasing the efficacy of the product. Many water soluble herbicides and fungicides are applied as granules, as well as some OP (fensulfothion, terbufos, parathion, fonofos, disulfoton, phorate, diazinon) and systemic insecticides (aldicarb, bendiocarb, carbofuran, imidacloprid). Special

machinery is used to bury the granules in the soil, but inevitably some granules remain exposed on the surface (from <1 to 50% depending on conditions), where birds and other animals may ingest them [19]. Birds are particularly fond of such granules, which they take as grit for their gizzards or simply mistake them as food, and consequently are more at risk from this formulation than small mammals [20]. In the case of insecticides, a single granule may contain a lethal dose (up to 20% a.i.), so the consequences are often dramatic: in North America, waterfowl were poisoned by eating fonofos granules they sifted from waterlogged fields six months after they were applied [21]. Seed-dressing was a common practice with OC insecticides such as aldrin, dieldrin and lindane, as well as organomercurial fungicides, and it poses similar risks as the granules, *i.e.* granivorous birds and mammals ingest the treated seeds often spilt around the edges of the crop and farm buildings. Poisoning incidents with seed dressings of cholinesterase inhibitors are still relatively frequent, especially in Europe [22]. The systemic insecticide imidacloprid is often used as a dressing for maize, sunflower and rape seeds; when the plants grow the insecticide is still present at concentrations ranging from 4.1 mg/kg in stems to 2.1 mg/kg in pollen, thus causing a great risk to honeybees [23]. Rodenticides are applied as baits spread around the farm buildings or near the crops where pest mice or voles congregate, posing a risk to other non-target vertebrates.

In irrigated crops, herbicides are often poured into the water channels either to allow an even distribution of the chemical throughout the irrigated field or simply to eliminate aquatic plants that may clog the channels and use up the water. Treated waters such as these invariably affect aquatic communities in agricultural landscapes (see Chapter 6), and are a constant source of contamination for many birds, frogs and mammals that bath in or drink from them.

Finally, some insecticides are used to control ectoparasites in domestic animals. In the 1950-60s it was common practice in many places to drench farm animals with solutions of DDT to combat cattle ticks. Today, the OC insecticides have been replaced by OPs (e.g. famphur), pyrethroids (e.g. cypermethrin), spinosad, cyromazine, avermectins and insect growth regulators (e.g. fluazuron) to control ticks, lice and blowfly maggots. Despite their lesser persistence and greater specificity, residues of the latter chemicals in dung from treated livestock affect dung-breeding insects and the degradation of faeces [24].

EXPOSURE OF ORGANISMS TO AGRICULTURAL PESTICIDES

Animals and plants are exposed to all these toxicants in a variety of ways. It is important to realise that just as the target pests and weeds are killed by the pesticides, all other non-target organisms may suffer deleterious or deadly consequences when exposed to the same doses of those chemicals.

Animal Exposure

The first route of pesticide exposure for most animals, vertebrates and invertebrates alike, is by direct deposition of the sprayed products on them, which is equivalent to a topical application on their skins/epidermis. Spray droplets are made of concentrated active ingredient in an oily or water-based carrier solution that sometimes contains an adjuvant. The tiny droplets (100-200 μm in diameter [25]) deliver a concentrated dose of toxicant to the skin, hair and feathers of animals they fall upon. Thus, liphophilic insecticides are quickly absorbed through the skin, and the ensuing acute dermal toxicity is often enough to kill the animal. In fact, dermal deposition has been recognized as one of the most crucial routes of exposure in birds [26]. Animals that die as a consequence of direct pesticide spray deposition do so because they happen to be at the wrong place at the wrong time [15], but it is hard to imagine how this could be avoided since agricultural land and surrounding landscapes are the natural home to countless species of non-target organisms of all kinds. Inevitably, pesticides and fertilizers are applied during the crop growing season, which coincides with the breeding of insects, nesting of birds and breeding/metamorphosis of amphibians. Although application schedules are dictated by crop pest/weed infestation levels and other management practices, the timing of application can have different impacts. For instance, the OP insecticide dimethoate applied early (spring) to barley crops at maximum rates (0.4 kg/ha) was very harmful to seven non-target soil-dwelling breeding beetles, but the same rate has a reduced impact on populations of old beetles when sprayed in autumn [27].

Concomitant with the deposition of spray droplets, inhalation of the misty and vaporized pesticides brings the active ingredients directly into the lungs and bloodstream of terrestrial vertebrates, even if they were initially sheltered from the spray deposits. Volatilisation of lipophilic insecticides from soil and other surfaces is a source of constant

air contamination in agricultural areas [15] even years after they were applied. For example, fluxes of DDE, toxaphene, dieldrin and trans-nonachlor from cotton soils in Alabama (USA) have been estimated between 325 and 7000 kg annually or 0.07-1.56 mg/kg per day for each of the respective chemicals [28]. Animals with a high rate of ventilation such as birds are at the highest risk. Nevertheless, it is difficult to separate the two kinds of exposure mentioned here – direct contact and inhalation – when an animal has been found paralysed or dead in the field. Most of the time it is the combination of several routes of exposure that accounts for the fatalities observed.

The third route of exposure is by direct consumption of contaminated plants, fruits, granules and coated seeds. This is known as primary poisoning to distinguish it from the secondary poisoning that occurs when a predator eats contaminated prey, insects or worms containing pesticide residues. Primary poisoning also occurs through drinking of contaminated waters from irrigation channels, drains, farm reservoirs, puddles, streams, rivers and lakes, which may contain high levels of pesticide residues, especially when they are in or near the agricultural fields that act as their source. A typical example is the case of DDT and cyclodiene insecticides used lavishly in the past; the persistence and lipophilic characteristics of these OCs resulted in their accumulation in granivorous birds and rodents that consumed seeds dressed with aldrin or dieldrin, in caterpillars that fed on leaves, and in worms of the treated soil – exposure through primary poisoning. In turn these animals were eaten by insectivorous birds and small predators, so the residues accumulated in their bodies as well. Larger predators such as falcons and eagles ate the contaminated prey and ended up with insecticide concentrations in their bodies which were several thousand times those found in the original seeds or treated plants – secondary poisoning. A parallel chain of contamination events occurred in the aquatic ecosystems where residues of these insecticides found their way through washoff from plants, runoff and drift [29]. Fortunately, most modern pesticides do not accumulate in organisms because they are either metabolized readily or eliminated in the urine and faeces. This does not mean they are all safe in regard to trophic contamination; *i.e.* woodlice consuming litter materials contaminated (0.1-500 μg/g food) with parathion-ethyl and endosulfan-sulfate take up these insecticides and experience their toxic effects [30]. Nor does it mean that secondary poisoning is a phenomenon relegated to past use of OC insecticides; it still occurs wherever the land was treated with arsenates [31] and OCs, as well as in tropical regions where they are still in use. Evidence of regular wildlife contamination by ingestion has been demonstrated by analyzing the gut contents of passerine birds in Australia during the agricultural season; sublethal levels of OC insecticides were found in 41-63% of the birds sampled, the OP parathion-methyl in 22% and the herbicide diuron in 78% of the birds [32]. The distribution of residues among trophic levels suggests that insecticides were obtained through ingestion of food whereas the herbicide was acquired by drinking from polluted waters. The highest residues were DDT (35-1980 μg/L) and its metabolite DDE (2-21 μg/L) even if it had not been used in that country for 20 years. Although the bioavailability of such old residues in soil and sediment decreases considerably with time [33], the fact that many animals continue to show DDT/DDE in their body tissues decades after they were applied indicates that the movement of this insecticide through the food chain is still a current issue in ecotoxicology.

Organisms exposed through primary consumption of highly toxic insecticides, rodenticides and fungicides usually experience acute effects, which may result in death if sufficient amounts are ingested. There are numerous examples of this, including the squirrels, raccoons and white-tail deer that have died over the years in the state of New York as a consequence of ingesting anticoagulant rodenticide baits [34], or the geese poisoned by ingesting heptachlor and chlordane treated seeds, and the countless songbirds killed in similar circumstances [15]. However, for the majority of pesticide products in the market, chronic and sublethal effects are more common because of the low level of residues (see Chapter 1). Secondary poisoning typically leads to chronic toxicity and unforeseen side-effects, as in the case of eggshell thinning in birds of prey and fish-eating birds contaminated with OC insecticides [35]. Nonetheless, secondary poisoning can be lethal to the predator even at normal rates of application if the chemical is very toxic (e.g. OP and carbamates) [36], or when the contamination is severe due to misuse; for example, the inappropriate spraying of monocrotophos over alfalfa fields to control voles in Israel resulted in the killing of hundreds of kites, eagles, buzzards and owls in a few days because they fed on voles that had been affected by this OP insecticide [37].

Exposure of Plants to Pesticides

Plants are affected by herbicides and fungicides only when these products are deposited directly onto them (contact) or are taken up through the roots. To avoid damaging the crop they intend to help, herbicides are usually applied prior to

planting. Herbicide drift on to non-target areas may affect other crops and wild plants alike, and is a common cause of economic injury to neighbouring farmers, which can reach up to 10% yield losses in the case of canola [38]. For this reason, aerial sprays of 2,4-D on fields of cereal crops must be carefully planned to avoid drift onto nearby sensitive crops like cotton [10]. Granular formulations of herbicides are otherwise preferred. Irrigation waters containing residues of unwanted herbicides and other pesticides may also affect the performance of rotational crops grown on the same fields. However, water-borne residues of herbicides in runoff are more likely to affect aquatic plant communities growing along streams, rivers and marshes since their levels are at most sublethal to animals.

Effects from Exposure to Pesticides

Toxicological effects depend on the doses exposed to, and such effects may occur at individual, population and community levels (see Chapter 1). The focus of this chapter is on the latter two effects, since they define the impacts on the ecosystem more clearly than any sublethal effect manifested on particular individuals. Besides, standard measurements of toxicity (e.g. LD50, EC10, NOEL) are determined with reference to populations. Community effects are typically described by the proportion of species eliminated or severely reduced in numbers within a collective group of species, but there is no standardized measurement to express this kind of impacts.

Dose is the amount taken up by the organism, which can be taken either all at once or through several episodic events. This distinction is important, particularly when dealing with pesticides, as most agrochemical products are recommended to be applied once or twice within the growing season of a crop; orchards usually require several applications. When a pesticide is applied only once, all non-target animals and plants that are directly exposed to it may experience short-term, acute toxic effects. In ecotoxicology this is called pulse exposure to distinguish it from constant exposure to pollutants in a given environment. After an initial shock, the affected organisms will be subject to decreasing exposure as the pesticide disappears progressively by natural decay, microbial degradation, and other dissipation routes (see Chapter 2). However, residues remaining in the plants, soil and water of the agricultural fields and surroundings can be taken up by animals moving into those areas any time after application. For non-persistent and biodegradable pesticides, those residual amounts are sufficiently low to ensure the LD50s for most species are not reached, although there is no guarantee they won't have any impact whatsoever – sublethal effects on some individuals may still take place.

In a different situation, when a pesticide persists in the environment for longer than one season (which occurs whenever half-lives are over 3 months) its residues are expected to build-up between consecutive annual applications. That is the case with most 'old' pesticides like OC insecticides and copper fungicides. In such circumstances, all organisms chronically exposed are at risk of accumulating the toxicant in their tissues, and with time the internal doses may be sufficient to cause either sublethal or lethal effects – the eggshell thinning due to DDE residues in birds is a classical example of this problem [5].

Mortality is the most obvious consequence of direct pesticide toxicity, reducing the populations of both target and non-target species affected. Such reduction in numbers is directly proportional to the toxic potency of the chemicals involved as measured by their LD50s. Since species live in communities rather than in isolation, the decrease in numbers of one species inevitably affects the other species with which it interacts. The resulting imbalance of populations is the most apparent direct effect of pesticides in biological communities. This usually takes place in the agricultural fields and small surrounding areas affected by drift and volatilization, whereas direct effects on aquatic ecosystems may take place beyond these boundaries since water-borne residues can be transported long distances (see Chapter 2). It is important to bear in mind that populations can recover once the toxicant levels drop or disappear, so the direct ecological disturbances caused by pesticides are temporary, not permanent.

It is also important to consider that organisms are not exposed to a single agricultural pesticide alone but rather to a suite of insecticides, herbicides and fungicides that are routinely applied to the crops, sometimes on the same day or even at the same time. Evidence that the combination of several toxic substances produces synergistic effects on the organisms exposed was first reported for mosquito larvae (Aeddes aegypti) and fruit flies (Drosophila melanogaster) exposed to the OP insecticide parathion and the herbicide atrazine [39]; the addition of the herbicide enhanced the lethal effect of parathion by a factor of 2 to 12 depending on the soil type used and other factors. The fungicide propiconazole enhances the activity of neonicotinoids [40], but the best known synergism is the enhancing effect of piperonyl butoxide on pyrethroids and cyano-substituted neonicotinoid insecticides, because the synergist inhibits the P450 enzymatic

detoxification mechanism. Estrogenic effects of mixtures of OC insecticides which are innocuous individually, are also examples of synergism that may have profound environmental implications [41]. The synergistic interaction of atrazine has also been proven in combination with some OP insecticides applied to house flies (Musca domestica), and other interactions between different types of pesticides are well documented in aquatic ecosystems (see Chapter 6); however, most of the mixture effects of pesticides are additive rather than synergistic [42].

Finally, sublethal doses of pesticides may cause enough stress in the organisms exposed so as to trigger anomalous behaviour. Examples are the reduced predatory skills in frogs exposed to malathion [43], negligence of female starlings exposed to OP insecticides in looking after their nestlings [44], as well as depressed immunological responses that may result in higher than normal rates of parasitic infection [45].

Persistence of Residues and their Bioavailability

Persistence indicates the ability of a toxicant to remain intact and active over long periods of time. The half-life is a useful measure of persistence: it is the time required for half of the chemical to disappear, usually by transformation into a non-active degraded product (metabolite). However, some metabolites can also be toxic (e.g. endosulfan sulphate, dieldrin, aldicarb sulfoxide and sulfone, heptachlor epoxide), in which case the total persistence of parent compound and metabolites should be considered in assessments of ecological impact.

Apart from a few exceptions, modern pesticides are not as persistent as those used in the past, and this together with specificity of action is a prominent feature of modern agrochemical products. Compared to the arsenates and OC insecticides of old, with half-lives in the environment of several years, most neurotoxic insecticides are easily degraded in the environment by chemical and biological processes. Modern herbicides and fungicides are also more degradable than their early products, even though these chemicals are generally more persistent than insecticides (Table **2**). Currently, over 50% of pesticides have half-lives in soil under a month, with only 14-20% having half-lives over three months either in soil or water.

Table 2: Persistence of pesticides according to their average half-life in soil, and their proportion among the total number of registered products of the same type. Source [6]

Type	Non-persistent	Moderate	Persistent	% products
Fungicides	64	31	20	59%
Herbicides	138	77	34	73%
Insecticides	82	41	19	58%
Rodenticides	1	1	1	18%

Non-persistent = half-life under 30 days, equivalent to 1% or less residues remaining after half a year

Moderate = half-life between 1 and 3 months, equivalent to 1-5% residues after 1 year

Persistent = half-life over 3 months, equivalent to 5% or more residues after 1 year

Persistent pesticides are more efficacious due simply to their prolonged action over time. From an environmental point of view this is undesirable because the longer the residues stay in the environment, the more chances of being dispersed and the higher risk they pose to organisms as a result of their prolonged exposure and accumulation. Indeed, persistence of agrochemicals poses as much concern as their acute toxicity. A highly toxic and degradable substance may have short-term lethal effects, but it usually allows recovery of populations after its disappearance, whereas a persistent substance of low toxicity will undoubtedly accumulate in the environment and in non-target organisms, in which case sublethal and unknown side-effects are likely to appear in the future. Obviously, when a pesticide is both persistent and very toxic the consequences can be disastrous, as happens with the 'old' OCs, arsenic insecticides and copper fungicides.

Residue accumulation in tissues of both plant and animals occurs whenever the degradation rate of a chemical is lower than its rate of uptake. Since toxic effects are related to the doses exposed, the bioavailability of the pesticide

residues is essential. For example, residues attached to soil particles may remain largely inaccessible to soil organisms, as if the residues were locked [33], and do not cause the effects one would expect. An extreme case is glyphosate: to be effective this herbicide must be absorbed by the plants, either by direct contact on the leaves or by uptake of the chemical in solution through the roots [46]. However, when glyphosate falls on bare ground it is immediately adsorbed onto the clay particles and humic substances in the soil, so it cannot be taken up by the plant roots – it remains effectively inactivated. In contrast, residues of most hydrophobic insecticides (e.g. pyrethroids, OCs and many OPs), systemic and soluble insecticides (e.g. imidacloprid; carbaryl) and herbicides (e.g. diuron) are adsorbed onto organic matter in the soil and remain available to earthworms and other soil microfauna even many years after being applied to the fields.

REVIEW OF PESTICIDE IMPACTS ON NON-TARGET COMMUNITIES

Soil Communities

The soil is a micro-ecosystem in its own right, and the organisms that make it or live in it play a crucial role in recycling nutrients, thus sustaining the soil fertility which allows ecosystem and agricultural productivity. Their diversity and heterogeneity are therefore necessary for long-term ecological resilience of the biosphere.

Micro-Organisms and Soil Metabolism

Fungi, bacteria and protists metabolize decaying plant and animal matter and convert it to either organic waste products (e.g. CO_2, methane and others) or minerals (e.g. nitrates, phosphates), which constitute the nutrients of plants. In addition, white-rot fungi have evolved to degrade lignin, an ability that enables them to degrade recalcitrant chlorinated pesticides such as toxaphene, lindane and pentachlorophenol [47].

Pesticides can affect these processes by altering the microbial composition of the soil. For example, applications of the systemic fungicide benomyl over many years reduced mycorrhizal root colonization by 80%, thereby indirectly reducing the abundance of fungal-feeding and predatory nematodes by 33% while increasing microbial substrate-induced respiration by 10% [48]. Generally, fungicides eliminate pathogenic root-rot or dumping-off fungi (e.g. *Pythium, Phythophthora, Rhizoctonia*), thus fostering the growth of competing bacteria while surviving and resistant strains of fungi become dominant (Fig. **1**). Among the latter are the actinomycetes *Aspergillus, Penicillium, Mucor, Pyrenochaeta* and *Trichoderma,* which are less susceptible [29]. Reduction of fungi affects negatively the decomposition of the surface litter by 25-36%, but increases the mineralization in the buried litter carried out mostly by bacteria [49]. This structural change is not always significant in single applications of chlorothalonil (15 g/kg soil) [50], and may be masked by quick recovery and other factors. Suppression of mycorrhizal symbiosis in crop plants treated with fungicides has been observed with normal rates of captan, carbofuran and mercury fungicides, resulting in stunted plant growth and yield reduction [51]. In contrast, typical application rates of some OP insecticides (trichlorfon, chlorpyrifos and quinalphos) may promote rhizosphere fungi temporarily until the suppressed bacterial populations recover in 45-60 days [52]. In soils contaminated with persistent arsenic and copper fungicides the regeneration of fungi is slow and takes many years [53], and this also reduces the ability of the indigenous soil microbial community to degrade DDT [54].

Soil basal respiration is generally reduced 30-50% after treatment with the fungicides benomyl and captan at field rates (51 and 125 mg/kg soil, respectively) [55], or under persistent residues (21-490 mg/kg) of copper fungicides [54], but carbendazim, even at dosages as high as 87.5 kg/ha, does not have significant impacts on soil nutrient cycling processes nor on soil microbial activity [56]. Suppressed basal respiration has also been observed after treatment with the herbicides 2,4-D, picloram and glyphosate, usually at concentrations higher than normally applied, whereas glyphosate applied at 2.2 mg/kg for several years in Brazil increased soil metabolism some 10–15% and fostered fungi while reducing bacterial counts [57]. Repeated application of the herbicides atrazine and metolachlor over 20 years altered the soil community structure in corn fields, in particular by reducing methanotrophic bacteria, but did not cause a decreased community function (methane oxidation) [29].

The mineralization of organic N to ammonium and then nitrate in soil, carried out by the nitrifying bacteria Nitrosomonas and Nitrobacter, is suppressed by the fungicide maneb and the herbicide picloram, but is unaffected by the continuous use of most pesticides either singly or in combination. However, the fungicides metalaxyl, mefenoxam,

mancozeb and chlorothalonil, and the herbicide prosulfuron, increase ammonium and nitrate levels by indirectly fostering nitrifying and denitrifying bacteria which inhibit N2O and NO production [29]. Nitrogen fixation in rice paddies by Azospirillum bacteria can increase following application of recommended doses of carbofuran insecticide (2-5 mg/L), but larger doses are inhibitory [58]. The herbicide glyphosate suppresses most soil bacteria, including nitrogen-fixing Rhizobium, because it inhibits the biosynthesis of aromatic amino acids. Susceptibility of plants to pathogens is also increased by glyphosate treatment as biosynthesis of the proteins phytoalexin and glyceollin, which normally block infection, is also inhibited. However, significant impairment is only observed at high concentrations, since glyphosate itself is completely degraded to CO2 by other micro-organisms living in the same soil [59]. A general inhibitory effect of phosphatase (5-98%) in the presence of glyphosate has also been observed [60], whereas the herbicides oxyfluorfen and oxadiazon at 0.4 and 0.12 kg/ha, respectively, stimulate the population and activities of phosphate solubilizing micro-organisms and also the availability of phosphorus in the rhizosphere [61].

Little is known about the impact of pesticides on soil protozoans, but it seems that they are just as sensitive as other soil micro-organisms, with insecticides being more toxic than herbicides. Soil protozoa can be critically disturbed as populations often do not fully recover within 60 days. Fungicides have rather varied effects: ciliates decrease slightly but testate amoeba species can be reduced by 50% in pesticide-treated agroecosystems, contrasting with the increased abundances and biomasses of soil protozoa found in ecofarming [62]. Transgenic Bt-crops, which produce the toxic Cry proteins from *Bacillus thuringiensis,* do not have much impact on microbial, nematodes and protozoan communities. Although some effects of Bt-plants on microbial soil communities have been reported, they were mostly the result of differences in geography, temperature, plant variety, and soil type and, in general, were transient and not related to the presence of the Bt-toxins [63].

Soil Mesofauna

The contribution of mesofauna to the recycling of total carbon has been estimated in the range 0.4-11% for surface litter from non-tillage fields and 6-22% in buried litter from pesticide treated fields [49]. Typical applications on crops, particularly of insecticides, can decimate the minute animals that carry out this essential task and disrupt the complex structure of the soil, which they effectively form. However, no matter how drastic their impact may be, all these effects can be reversed once the toxic activity has disappeared because populations of these small organisms recover very quickly [64]. The following is a summary of direct impacts on the most important taxonomic groups of soil fauna as affected by normal application rates used in agriculture, unless specified otherwise.

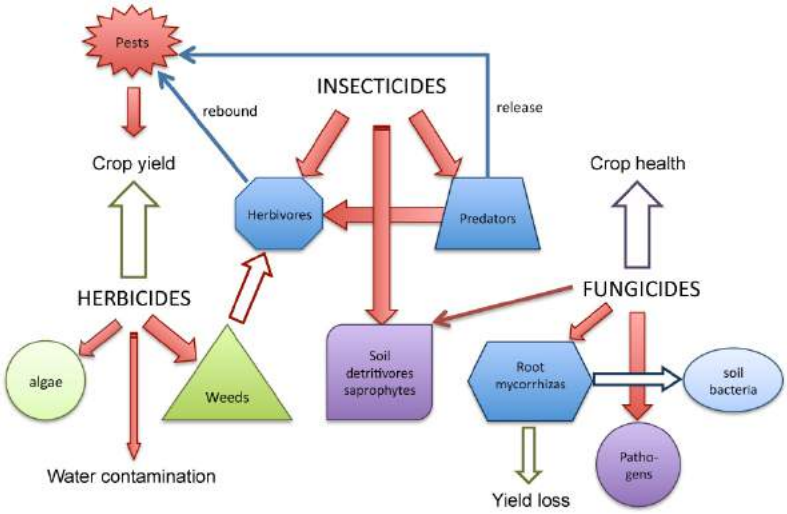

Figure 1: Diagram showing the main impacts of pesticides on soil, plant and arthropod communities. Red arrows indicate decreases and blue arrows indicate increases; empty arrows indicate indirect effects.

Arthropods

Since the early days of pesticide usage it was noted that OC insecticides had mixed effects on the animal communities of the soil [65]. On the one hand, aldrin, dieldrin, heptachlor, chlordane and DDT controlled well the insect pests of the

crops, but on the other hand their residues in soil greatly reduced most species of springtails (Collembola), saprophagous mites, symphylids and pauropods (Myriapoda). DDT was less toxic than aldrin and dieldrin but killed higher percentages of predatory mites than other insecticides, the destruction of the latter resulting in indirect increases of their Collembola prey species. All OC insecticides had little or no effect on earthworms, enchytraeid worms and nematodes at low application rates (*i.e.* aldrin 2.5 kg/ha), whereas five times that dose, as applied for the control of *Phyllophaga* larvae, affected several earthworm species. Many of these early reports refer to field observations that are difficult to evaluate, but proper assessments carried out later confirmed those findings [27].

Of special significance are the impacts on populations of mites because these tiny organisms are the most numerous arthropods in soil; many of them are predators, others are saprophytic while some *Tetranychus* are crop pests. Among the 84 studies in a variety of crops reported by Edwards and Thompson [66], 56 showed a decrease in mite densities, nine reported increases and 19 did not show significant changes. Impacts occur across all ecological types of mites, with populations of predatory mites being negatively affected more frequently when treated with OC insecticides (e.g. DDT, endosulfan, aldrin, chordane and heptachlor), most OP insecticides and carbamate biocides (*i.e.* aldicarb, carbofuran) [67]. Although mites recover within six weeks or a few months, a single exposure to aldicarb (25 kg/ha) resulted in different successional outcomes over the subsequent four years because of the elimination of many Gamasina predacious mites, which are often the most susceptible [68]. Even natural extracts like neem (from *Azadirachta indica*) are more detrimental to oribatid mites than other mites and spiders [69]. Fumigants (gaseous pesticides) have devastating effects: the D-D mixture eliminates all mite populations, does not allow their recovery until two years later and eventually decreases the soil biodiversity [66]. Some mites are susceptible to the herbicides simazine, atrazine, monuron and DNOC, but most of the population changes observed in fields treated with herbicides appear to be from indirect effects on the flora [70]. Apart from mites, predatory arthropods of the soil include carabid and staphylinid beetles, earwigs, centipedes and spiders, all of which control many pests and are, therefore, beneficial species to agriculture. Centipede populations were reduced by DDT and aldrin in the past, as subsequently did most OP insecticides and carbamates, but reports on impacts of modern pesticides on this group of animals are very few [71].

Saprophytic arthropods such as springtails (Collembola), Pauropoda, most millipedes (Diplopoda), woodlice (Isopoda), certain mites, symphylids and Diptera lavae help desintegrate plant material that many soil micro-organisms are unable to process directly [66]. Although the role of these soil organisms is not as important in agricultural fields as it is in forests and other ecosystems, the current agronomic trend of no-tillage draws its benefits in soil fertility mainly from the role of these animals. For instance, an 80% reduction of springtail numbers after applications of lindane (0.5 kg/ha) to corn crops in Africa resulted in reduced breakdown of organic matter by 45% [72]. Collembola species are not as susceptible to pesticides as mites are; in fact, their numbers usually increase when fields are treated with normal doses of insecticides, as these kill the predatory mites that prey on them [73], thus altering the dominance structure of the springtail community even if the species composition remains unchanged. Springtails are very susceptible to fumigants, carbamates and many OP insecticides [74]. The arsenic herbicides reduced springtail populations in barley by half [75], while DNOC, paraquat, dalapon-sodium and several triazines also reduce their populations when applied in large doses [76], but most herbicides affect springtail communities indirectly [70]. Only a few fungicides (e.g. benomyl) appear to impact negatively on populations of springtails and woodlice [77]. Among the tiny Myriapoda of the soil, the pauropods seem to be most susceptible to all kinds of insecticides, and some populations are completely eliminated by OP insecticides. Symphylids, by contrast, do not suffer drastic effects because they live buried in the deep soil layers where they feed on plant rootlets. Thus, non-leaching, hydrophobic insecticides (most OCs, pyrethroids and some OPs) hardly affect their populations, whilst systemic, hydrophylic insecticides and fumigants deeply penetrate the soil and cause serious population reductions in all taxa [66]. Millipedes are more tolerant, and even if their populations are reduced temporarily by OC and OP insecticides, the herbicide monuron, or the fungicide carbendazim, they recover within a few months [78]. However, persistent residues of DDT in soil of cabbage plots can progressively accumulate in millipedes and reduce their populations over the years [29].

Larvae of many Diptera species are agricultural pests, but the majority of them are not. In any case, they all play an important role in breaking down dead plant/animal matter, so the repeated application of insecticides and herbicides like simazine leads to a significant loss of Diptera larvae and a potential accumulation of dead organic material on the surface [66]. Larvae of dung beetles and flies in pastureland are also affected by residues of parasiticides found

in the faeces of treated livestock. For example, emergence of the dung beetle *Liatongus minutus* and eight species of flies from cowpats in the first two weeks following ivermectin treatment at normal rates (0.5 mg/kg body weight) was significantly reduced, while Ceratopogonidae and Psychodidae species prospered [79]. These impacts occur while lethal levels of residues persist in the dung – usually 1-3 weeks for most pyrethroids and avermectins in cowpats [80] but shorter times in sheep dung [81]. By contrast, insect growth regulators like fluazuron and methoprene appear to have no such effects at normal rates of treatment [82, 83].

Other Invertebrates

Parasitic nematodes are regularly controlled with fumigants, lindane, some OP and carbamate insecticides applied directly into the soil, but depending on the doses applied, populations of saprophytic and beneficial nematodes are also reduced [29, 66]. Most OC insecticides and fungicides do not affect nematode numbers. Among the latter chemicals, carbendazim increases omnivorous species and benomyl reduces them [66]. Under field conditions, the risk of indirect effects from fungicide application is usually much greater than that of direct effects. For example, by reducing total fungal biomass and activity, captan decreases the numbers of fungal-feeding nematodes [84]. Herbicides have mixed effects, and this is believed to result from the complex interplay of top-down and bottom-up forces in soil food webs. Another example: plant-root parasitic species increased in rice paddy plots treated with a mixture of thiobencarb and simetryne (2.8 and 0.6 kg/ha, respectively) while predaceous mononchids, which mostly live on the surface, were drastically decimated when chlormethoxyfen at 2.8 kg/ha [85] was added to that mixture.

More important, particularly in tropical agroecosystems, orchards and vegetable patches with litter, are the impacts on detritivorous earthworms, because they remove large amounts of leaves and stubble material, and in doing so increase soil fertility and lessen the ability of certain pathogens to overwinter in the fields [66]. Past applications of copper fungicides and arsenates have led to the formation of mats of undecayed organic matter on the surface of many orchards, because these highly toxic and persistent compounds decimate earthworms populations [86], increase their avoidance behaviour [53], and negatively affect their burrowing rate. The latter sublethal effects have also been observed with the insecticide imidacloprid at 0.5-1.0 mg/kg dry soil [87]. The majority of OC, OP and carbamate insecticides do not cause significant reduction of earthworm populations at normal application rates [66], but chlordane, heptachlor, phorate and carbofuran are extremely toxic to all worms and eliminate them completely [88]. Recovery times from carbofuran treatments can last 90-105 days, and that from the OC insecticide butachlor can be longer than a season. Phorate can also foster enchytraeid worms indirectly by eliminating their predators [89]. All fumigants are deadly to earthworms because they penetrate the deep layers of the soil [66]. Among fungicides, carbendazim at 1 kg/ha decreased the abundance of several *Lumbricus* species in terrestrial model ecosystem (TME) studies, as well as *Fridericia* enchytraeid worms and native earthworms in rubber plantations of the Amazonia [78]. Some herbicides (e.g. DNOC, chlorpropham, atrazine, simazine, monuron) reduce earthworm populations slightly, and paraquat appears to increase them [70], but most have no direct effect on them. In general, conventional agronomic practices in orchards seem to affect negatively detritivores such as earthworms and woodlice. However, some long-term studies have shown that insecticide-treated fields had no ecologically significant impacts in earthworm populations when compared to untreated fields, the differences being largely consistent with the expected effects of climate, soil types, crop types and cultivation practices [90].

Vegetation and its Arthropod Communities

The soil is the substrate and nutrient source for the growth of plants, and the vegetation provides the basic structure on which most species of arthropods live. Both weeds and macro-invertebrates provide many valuable services to the agroecosystem – nitrification; soil aeration and water percolation; recycling of litter, dung and decay materials; pollination; and vectors of mycorrhizal spores, among others.

Impacts on Vegetation

Weeds are the competitors of the crop for water and nutrients, and can reduce crop yields significantly. Broad-spectrum herbicides are toxic to all kinds of plants alike, usually by inhibiting the photosynthesis (e.g. urea herbicides, triazines) or any other essential plant metabolic pathway (e.g. glyphosate), but others inhibit seedling development from the seed (e.g. trifluralin and pendimethalin). Selective herbicides are designed to inhibit metabolic processes common to either grasses (monocotyledons) or broad-leaf plants (dicotyledons). This feature

allows them to be used on certain crops to control weeds of the opposite type; for example, 2,4-D is used in cereal crops because it only inhibits growth of broad-leaf plants. The effectiveness of herbicides in reducing plant biomass is often underestimated. They effectively exclude many annual plants from being established, and although vegetation communities may recover in the following season, the constant application of herbicides year after year leads to the depletion of soil seed banks. For example, it has been reported that after many years of intensive agricultural practices using a range of herbicides, the Hilly Country of Saxony has lost many landscapes and their associated flora diversity [91]. It appears that the time of their application in relation to plant seed production influences more the nature of vegetation changes than does the soil seed bank type. However, individual herbicides have minimal impacts; a review of the impacts of the broad-spectrum herbicide glyphosate on a variety of ecosystems found the shifts in species floral composition and structure of habitats were within the normal range of variation in natural ecosystems [92].

Indirect impacts of herbicides on soil fauna are often reported. Long-term studies carried out over several years in vegetable crops have revealed that the soil arthropod community structure is positively correlated with the weed community biomass, which varies with the use of specific herbicides and other management practices [93]. For example, the abundance and diversity of rove beetles (staphylinids) is dependent on weed community composition as well as ploughing, with the highest biodiversity being observed on fields with no-tillage and less pesticide use [94], whereas use of paraquat and trifluralin herbicides in tomato plots result in significant reductions in the density of ground beetles. The unintended consequences of such indirect impacts are illustrated by the reduction of weeds in orange groves in Spain: many years of herbicide applications have reduced the abundance and biodiversity of consumer ants to the point that fewer ant colonies made the soil progressively less porous and more compacted, thus enhancing rainfall erosion and slowly depleting the orchard's soil fertility [95].

Plant biodiversity is not considered to be important in crop monocultures, but it is relevant to the establishment of stable arthropod communities in or around the crop. These play an essential role in effective crop protection and also sustain populations of birds and other vertebrates. In many cases, the losses in yield caused by weed competition can be offset by the benefits that predatory arthropods bring to the crop. For example, cane and sugar yields averaged 19% higher in weedy sugarcane plantations than in the weed-free plantations in Lousiana, because broadleaf weeds enhanced the populations of beneficial carabids, ants and spiders that control the sugarcane borer (*Diatraea saccharalis*) [96]. Similarly, the combined use of Bt-cotton, lucerne strips and a nuclear polyhedrosis virus in Australian cotton farms reduced the use of synthetic pyrethroid insecticides by 50% without sacrificing yield and profitability [97]. Experience over the years in these and other crops have demonstrated the benefits of the appropriately named integrated pest management (IPM) strategies that promote the conservation of existing natural biological controls through major reductions in insecticide and herbicide use.

The introduction of recent transgenic herbicide-tolerant crops (TGHT) may encourage no-tillage practices which are beneficial for soil fertility, but there is concern that such crops may lead to a more intensive use of herbicides and the removal of many weeds that support populations of pollinators [98]. Pollination by bees is a very important ecological service provided to agriculture, as 25% of tropical crops and possibly up to 84% of temperate crops [99] depend on insect pollination. Thus, management and protection of pollinator populations and habitats of nectar-producing plants can be essential for some crops, and for plant biodiversity in the environment at large. However, there are no clear examples of low crop yields resulting from the effect of pesticides or transgenic Bt-plants on pollinators [98]. Although agricultural intensification and habitat loss are the most frequent cause of pollinator impoverishment (64% of cases), direct bee mortality by insecticides is evident and cannot be ignored either [100].

Arthropod Communities

Insecticide sprays can wipe out 99% of the population of target pests as well as those of non-target species, just as chemotherapy kills both bad and good cells alike. Since the early years of the Green Revolution entomologists realized the limitations of this approach and looked for alternative methods of pest control. In nature, predatory arthropods keep the populations of phytophagous insects (most pests) in check: ladybird beetles, dragonflies, earwigs, some ants and crab spiders predate on eggs of pest species, while parasitic Hymenoptera play an essential part in controlling numerous pest larvae, so they are being used in biological pest control. A recent review of 39 ecosystems found that agrochemical pollutants negatively affect these parasitoids in 46% of cases [101], with

persistent and systemic insecticides (e.g. cartap and imidacloprid) having the greatest impacts [102]. However, predatory arthropods are less susceptible than parasitoids and more variable in response to pesticides [103]. Although some predatory species are very tolerant to pesticides (e.g. the spider *Lycosa pseudoannulata*, the coccinellid *Cryptolaemus montrouzieri,* and the lacewing *Chrysopa carnea*), their initial elimination by insecticides and their slower recovery than that of the pest species they control often results in rebounds of pests (Fig. **1**) in the short and long term [104].

Early insecticide impacts in non-target arthropod communities were reported for orchards sprayed during three years with lead arsenate and nicotine. Ground-dwelling beetles, spiders and ants were reduced by 15%, and the proportion of eggs and larvae of the main apple pest – the coddling moth (*Laspeyresia pomonella*), which is parasitized by Hymenoptera species – decreased by 64-97%, allowing the moths to come back unopposed [29]. DDT sprays helped eliminate the coddling moth, but it created new pests among leaf-rollers, woolly aphids, red-spiders and *Tetranychus* mites that surged as a consequence of the lack of predators and the suppression of parasitism. Citrus orchards sprayed with DDT to control cottony-cushion scales and mealybug pests also eliminated the predatory ladybird beetles and parasites which control them – as a result, pest numbers not only did not decrease but rather surged exponentially [105]. Because of the persistence of DDT, restoration of a normal predator-prey relationship after cessation of sprays could take up to five years [106].

The annihilation of predatory and parasite arthropods in cotton, corn, rice and horticultural crops has created new community structures characterized by the absence of predator-prey relationships, one where pests species thrived for a while until the next insecticide spray decimates them, where resurgence became the norm and resistance to chemicals the final outcome [29, 107]. In America and Australia, early sprays of calcium arsenate to control the main cotton pest, the boll weevil (*Heliothis* spp.), boosted the populations of cotton aphids due to the elimination of predatory arthropods. Lindane was applied to control both the weevils and the aphids, but this resulted in outbreaks of *Tetranychus* mites, as more predators were also affected. To top it all, the application of OP and systemic carbamate insecticides to control leafworms (*Spodoptera* spp.) resulted in further outbreaks of boll weevils and mites due to a combination of two factors: total lack of predators and insecticide resistance developed within the pest species [29]. It is easy to understand that restoring these shattered communities usually takes a few years. Pest management plans in cotton agroecosystems continued to rely on the routinely, heavy use of pyrethroids, OPs, carbamates and new insecticides until the 1990s [108]. Recently, the introduction of transgenic Bt-cotton in some countries appears to have a positive effect on restoring the biodiversity of most predatory insects, spiders and birds in cotton fields, since insecticide applications are reduced 50% or more [109]. Similarly, the biodiversity of arthropods in Bt-corn crops is much higher than in fields treated with pyrethroids. Insecticide sprays on rice crops upset natural enemy control of pests such as plant hoppers (*Nilaparvata lugens*) and also create heavy selection pressure for strains of pests that can overcome previously resistant rice cultivars. Such circumstances create outbreaks of secondary pests and impair biological control of some key primary pests such as Pyralidae stem borers [104]. Typical applications of BHC and parathion significantly decreased densities of predatory dragonflies, spiders and parasitoids, thus increasing the herbivore:predator ratio among arthropods [110]. The insecticides imidacloprid and fipronil also change this ratio even if their main impact is on midge larvae (Chironomidae) [111]. In addition, herbicides applied to rice paddies foster the numbers of parasitic nematodes and alter the plankton communities [85]. Perhaps, the rich biodiversity of rice fields, with some 200 species of predatory arthropods, could be used in IPM programs to control the 55 species of pests found in this crop [112].

Ground-dwelling carabid beetles are essential in controlling many horticultural pests, and together with staphylinid beetles make up about 75% of the predaceous and/or parasitic insects on vegetable crops [113]. In the past, OC insecticides decimated their populations and allowed very slow recovery afterwards [29], whereas the OC endosulfan at 1 kg/ha appears not to cause major impacts on these arthropods [72]. The impact of cholinesterase inhibiting insecticides on carabid populations ranges widely among species [114, 115], but all allow their recovery within a few weeks [66], whereas pyrethroids and imidacloprid at recommended rates have minimal impacts in spite of their extreme toxicity to insects [72]. Most herbicides indirectly increase densities of carabids, ladybird beetles and linyphiid spiders [74], but 2,4-D and chlorpropham are toxic to carabids too [29]. TGHT sugar beet and Bt-canola crops do not appear to have any significant effect on carabids, staphylinids nor spiders, but rather reduce the overall arthropod abundance through indirect effects on weed biomass [116].

Spiders and phytoseiid mites are important predators in all kinds of crops. Applications of OP, carbamate and pyrethroid insecticides in vineyards, orchards and other crops usually result in increases of pest *Tetranychus* mites because of reductions in the more susceptible phytoseiid predators [117]. In experimental plots, spiders were three times less abundant in apple orchards treated with insecticides than in untreated ones, and spiders and ants were reduced in numbers in 53% of the corn crops in Africa treated with lindane (0.5 ka/ha), an effect that lasted 2-3 weeks [72]. Lycosidae and linyphiid spider populations undergo a similar pattern – they are initially eliminated from cereal fields treated with OP insecticides, but their abundance may increase subsequently in response to rebound densities of unaffected prey like springtails [29]. Indirect effects of herbicide application on field margins often reduces the habitat for lycosid and linyphiid spiders, as border crop fields and hedges act as refuges for these and many other beneficial predatory invertebrates [118]. No-tillage practices and TGHT crops enhance spider populations through a more heterogeneous and diverse vegetation structure [119].

The direct impact of insecticides on honey bees (*Apis mellifera*) was recognized a problem since the calcium arsenate dust sprays killed entire hive colonies in the past [29]. They also affect the performance of the colonies, with impacts ranging from odour discrimination to the loss of foraging bees due to disruption of their homing behaviour [120]. Pyrethroid and OP insecticides such as triazophos and dimethoate continue to be very toxic and hazardous to bees [121]. Spray drift and volatilization are responsible for most of the incidents reported on hives [100], while impacts on wild bumblebees (*Bombus* spp.) are likely underestimated and non-reported. All bees are also affected by the poisoned nectar and pollen taken from plants treated with systemic insecticides such as carbamates and imidacloprid. Typical concentrations of imidacloprid of 6 mg/kg in male flowers (panicles) and 2 mg/kg in pollen from maize, sunflower and rape plants are sufficient to decimate honeybee colonies [23], especially when the pollen contains higher residues of other pesticides that could act simultaneously or synergistically. Besides mortality, imidacloprid appears to affect the brain (memory) and metabolism in bees, with the resulting impairment in the workers activity [122].

Crop diversification in conventional farming can help increase the biodiversity of arthropods while significantly reducing the densities of phytophagous pests by 60-70% [123]. In tropical rice crops particularly, which sustain a large biodiversity [112], pest management is best achieved using natural controls rarely supplemented by insecticides [104]. In ephemeral annual crops such as cereals, sugar cane, alfalfa or even cotton, leaving strips of grass and weeds on field margins, woody borders and other practices that attract and provide refuge to many arthropods can increase both biodiversity and abundance of natural predators [118]. It should be borne in mind, however, that any efficiency in controlling the pest populations through natural enemies depends very much on the identity of both predator and prey species, not on the diversity of predators *per se* [124].

Vertebrates

Since invertebrates are small and not very mobile – except some insects –, pesticide impacts on their communities are restricted to the agricultural fields, orchards and the margins affected by spray drift. By contrast, vertebrates move around fields, nearby forests, wetlands, rivers, lakes and even far away places in the case of many bird species. Therefore, off-farm contamination is another source of exposure for vertebrates, even though it is much lower than on-farm exposure due to its lower residue levels [125]. For persistent chemicals, the possibility of bioaccumulation in the animal tissues introduces also a new and often unknown risk factor.

Direct Impacts

The susceptibility of vertebrates to agricultural doses of pesticides is typically lower than that of invertebrates simply because of their size difference. Vertebrates are more tolerant to synthetic pyrethroids, neonicotinoids and OC insecticides, but very susceptible to cholinesterase inhibitors, whereas amphibians are generally very sensitive to pyrethroids and more tolerant of cholinesterase inhibitors than birds and mammals [126]. Reptiles appear to have either less or similar sensitivities to mammals in regard to neurotoxic compounds [127]. Mammals are more tolerant to certain pesticide groups than other vertebrates because they posses active detoxification mechanisms. However, small insectivorous mammals, such as shrews and moles, are very sensitive to neurotoxic anti-cholinesterase insecticides because of their high feeding and metabolic rates. Birds are more tolerant of pyrethroid and neonicotinoid insecticides, but are very susceptible to chlorfenapyr.

Killing of non-target organisms such as birds, lizards and small mammals is often observed at the time of insecticide applications [128], but most incidents are probably not reported [129]. Bird mortalities from direct exposure to insecticides can range from a few birds to several hundreds. Indeed, OC insecticides were blamed for many bird fatalities in the past, and cholinesterase inhibitor insecticides were responsible for 25-50% of bird mortality observed in farmland of the United Kingdom between 1975-1990s, of 17% of all birds poisoned in agricultural lands of the Netherlands, and 3-12% of all birds of prey found poisoned in the USA [15]. Levels of inhibition of brain acetyl-cholinesterase in birds below 20% are associated with sublethal effects and levels above 70% result in death [130], whereas in lizards the levels are typically below 40% and above 50% for the respective effects [131]. In contrast to OC insecticides, carbamate and OP insecticides do not accumulate in vertebrates as they can be readily metabolized, so their potent effects are usually short-lived. Even so, mortality of magpies by direct poisoning with famphur, applied to cattle as parasitic treatment, has been reported [132]. Lizards suffer similar effects as birds and mammals when exposed to the latter insecticides, but impacts on their populations and ecology are unknown [127]. Frogs, toads and tadpoles are common inhabitants of rice paddies, irrigation ditches and farm ponds as well as in surrounding wetlands and riverbanks, and so are exposed to direct pesticide applications on farm and drift sprays into their habitats. Although pesticide concentrations in agricultural waters are insufficient to cause frog mortality, the development of tadpoles is usually affected by low concentrations of many OPs in water (e.g. 4-8 mg/L fenitrothion) and herbicides like triclopyr (2.4-4.8 mg/L) [133].

Apart from mortality, sublethal effects on birds and small mammals exposed to these insecticides are more common, including reductions in food consumption and drinking activity that leads to noticeable weight losses [44], lack of aggressive behaviour, memory impairment that can compromise their survival ability, immobility on the ground which puts them at risk of predation [134], apathy in bird hatching, nest defence and care for the nestlings [44] and reduced fertility [135]. In amphibians, stress [43], suppression of immunity, and susceptibility to parasite infections [45] have been reported. Most of these effects are transient, but those affecting reproduction impact on the long-term viability of a species, even if there might not be apparent short-term population reductions. For example, direct exposure to OC insecticides reduced the breeding success of songbirds in apple orchards [136] and the recovery of vole populations in experimental plots. This is of concern because wildlife species rely on tight net reproductive rates to maintain their populations and cannot cope with such adverse effects. Thus, it has been suggested that reduced egg weight and hatchling success in caimans as a result of typical exposures to atrazine (15 μg/egg) and endosulfan (0.15-1.5 mg/egg) may influence the populations of this species in the wild Amazon [137]. As the field assessment of such populations is difficult, models have been developed to predict the long-term effects caused by reproduction impairment. Balanced population densities are important in the case of rodents, where a delay in reproduction can give a competitive advantage to another species. In this regard, exposure to the OP azinphos-methyl applied on alfalfa at 0.9-3.6 kg/ha caused lower than normal pregnancy rates, or its delay, in both voles and mice [138], whereas similar rates on tall grasses did not have effect on populations of *Microtus canicaudus* voles [139]. Similarly, lack of aggressiveness after exposure to dimethoate (0.4-0.6 kg/ha) did not impede the populations of herbivorous prairie voles (*Microtus ochrogaster)* to increase five-fold because the survival of competitor, omnivorous deer mice (*Peromyscus maniculatus*) decreased significantly [140].

Endocrine disruption is another sublethal effect by which some pesticides and other contaminants may impair developmental growth and reproduction in vertebrates [141]. Altered thyroid hormone concentrations, which influence development and metamorphosis, have been observed in birds exposed to DDT, OP, carbamate and pyrethroid insecticides [142, 143], in goldfinches exposed to the herbicide linuron [144], in amphibians and fish exposed to endosulfan and other insecticides [145]. Abnormal sexual differentiation caused by herbicides like atrazine have been observed in frogs, although conflicting evidence also exists [146]. Confirmed cases of impaired reproduction refer to populations of bald eagles (*Haliaetus leucocephalus*) in the Great Lakes of North America [147] and alligators in Florida [148], both of them after many decades of exposure to DDE residues. In the second case, high residues of OC insecticides and other chemicals were found in alligator eggs from Lake Apopka, which was heavily contaminated by a spill of difocol and DDT in the nearby agricultural area, and though hatching success was lower than normal it appeared to be unrelated to the pesticide levels measured in eggs [149]. Subsequent studies found the levels of estrogen in female alligators from that lake were double than normal, while levels of testosterone in male alligators were three times lower than normal or similar to those found in females. In addition, males had poorly organized testes and abnormally small phalli and females exhibited abnormal ovarian morphology [148]. As a consequence, alligator populations in Lake Apopka are in decline.

Primary Poisoning

More common among vertebrates is the exposure to pesticide residues through ingestion of contaminated food. Granivorous birds and rodents often ingest large quantities of seeds that often contain pesticide residues; grazing mammals may consume pasture contaminated with herbicides or insecticide spray drift; and birds of prey and scavengers often consume the guts of their prey and/or carcass, so the undigested granules of cholinesterase inhibitors and rodenticides found in the prey can result in fatalities among raptors [150]. The extent of this contamination can be assessed by the relative amount of residues found in animal tissues. Based on the residue levels of mirex across a large number of non-target animals [151], we know that insects accumulate more residues than other invertebrates, and among the vertebrates amphibians and reptiles had lower levels than birds and mammals, which possibly reflect their differences in feeding rate and metabolism. While most residues are metabolized and/or excreted by the animals, persistent and recalcitrant chemicals may accumulate in organs such as the liver and kidney, whereas lipophilic residues usually are stored in fatty tissues. Modern biomarker techniques make it feasible to investigate the poisoning level of live animals in a non-destructive way, *i.e.* using small samples of blood serum from reptiles, birds and mammals [152, 153].

Secondary Poisoning

Insectivorous birds, frogs, lizards and mammals often consume insects contaminated with pesticides [32]. The ecological consequences of secondary poisoning differ markedly among vertebrate taxa and the role each species plays in the trophic structure of the ecosystem, and obviously depend on the chemical nature of the poison. Build-up of insecticide residues in primary consumers can make them more susceptible to predators and scavengers. Birds of prey feeding on these animals accumulate even higher residue levels and often die as a result [36]. Most of the fatalities in raptors due to secondary poisoning are associated with the illegal use of insecticides and rodenticides (e.g. to eliminate wild carnivores), but some result from the normal use of pesticides by farmers [154]. Indeed, secondary poisoning by non-persistent carbamate and OP insecticides has been attributed as the cause of mortality in barn owls (*Tyto alba*), American kestrels (*Falco sparverius*), red-tailed hawks (*Buteo jamaicensis*), great horned owls (*Bubo virginianus*) and bald eagles [36, 150], and it is probably more common than we think because most of the time the victims die without being noticed. The removal of vertebrate predators from an ecosystem leads to similar imbalances as described above for the insect communities in (Fig. 1), encouraging pest rodent species to multiply unrestrained.

Persistent OC residues bioaccumulate in the fatty tissues of all organisms, and are released slowly during periods of fasting or intense flying activity such as during migration [155]. As they are passed on from consumers to predators at the top of the trophic chain, the biomagnification factors can be staggering – up to 10,000 times or more [156]. Not surprisingly, consumption of invertebrates contaminated with OC insecticides causes the death of many insectivorous birds and bats [157], but the sublethal effects from this poisoning are more damaging in the long term. One of the first known impacts of OC insecticides was the reproduction impairment they caused in birds of prey and fish-eating birds, which was felt worldwide in less than two decades, and put some species on the brink of extinction [4]. The case is well documented for DDT, though cyclodiene insecticides like dieldrin produced similar effects [158]. Persistent residues in soil, plant forage, seeds, earthworms and other invertebrates accumulate up the trophic ladder because vertebrates consuming such contaminated foods cannot excrete them. Consequently, predators and scavenger birds concentrate large amounts of DDT in their bodies, where it is transformed into DDE, an equally recalcitrant compound which causes eggshell thinning by altering the calcium metabolism in birds [5]. This unforeseeable effect produced a high mortality of embryos and chicks in birds of prey such as the peregrine falcon (*Falco peregrinus*), sparrowhawk (*Accipiter nisus*), kestrels (*Falco* spp.), Spanish imperial eagles (*Aquila adalberti*) [159] and many fish-eating birds like herons, cormorants and pelicans [160]. Initially, the reduction in juveniles was compensated by higher reproduction rates because there was less competition for food, until the introduction of cyclodienes years later dealt a fatal blow and populations of raptors started to decline [161]. DDT and many other OC insecticides were banned in most countries during the 1970-80s, but their residues are still out there. Wildlife feeding in areas where DDT was applied for agricultural pest control continues to be affected by the persistent residues [32], which fortunately are now reduced to the point that raptor populations are no longer threatened with extinction and, on the contrary, are slowly recovering [162, 163].

Rodenticides are one of the most common causes of secondary poisoning in bird and mammal predators that feed on the target rodents. In particular the second generation of anticoagulant coumarin rodenticides are very persistent, and

residues ingested with the carcasses of poisoned animals accumulate in the predators' bodies, causing internal or external bleeding and eventually death. Some 70% of the owls collected in Canada between 1988-2003 had residues of at least one rodenticide at levels up to 0.93 mg/kg (brodifacoum) or 1.01 mg/kg (bromadiolone) in their liver [164]. Birds of prey are being increasingly reported dead as a consequence of coumarin poisoning in America [34].

Indirect Effects

Insecticides directly affect insectivorous vertebrates by reducing the insect prey base available to them, whereas herbicides indirectly affect their populations through a variety of pathways, including 1) the direct removal of the food base of granivorous species, 2) reduction in invertebrate abundance by removing the plants that invertebrates depend on for food or habitat, and 3) reduction in vegetative cover necessary for nesting/breeding and reproduction [165].

The best documented evidence of indirect pesticide effects on insects and bird populations is found in the United Kingdom, where declines of grey partridge (*Perdix perdix)* had been noticed by game hunters and ornithologists for some time – it was rightly attributed to the combined indirect effect of herbicides and insecticides that resulted in breeding failure as a consequence of chick starvation and low survival [90, 166]. Even if other contributing factors such as worm parasites have added to the partridge demise [167], the fact that pesticides are routinely sprayed on cereal and other crops everywhere has indirectly affected the populations of many other bird species as well, which are declining in European countries and North America [168]. Declining bird species (e.g. skylark, corn bunting, *etc.*) are not associated with particular foods, but with overall reductions in abundance and diversity of plants, seeds and insects [169, 170] resulting from intensive agriculture [171]. Granivorous species feed on cereal grain and seeds of many 'weeds' like knotgrasses (Polygonaceae), chickweeds (*Stellaria* spp.), goosefoots (*Chenopodium* spp.), and others, so their decline has been driven primarily by herbicide use and the switch from spring-sown to autumn-sown cereals [172], both of which have massively reduced the food supplies of these birds [173]. However, herbicides are not the only culprits, as other intensive management practices (including TGHT crops) also reduce farmland food and biodiversity. During the breeding season, grasshoppers, sawflies, spiders, leaf-beetles, weevils, butterflies/moths and their larvae, aphids, and crane-flies and their larvae are important foods for insectivorous and omnivorous birds; the first four taxa (which are sensitive to insecticides) are associated with the diet of most declining bird species [174]. Recovery of plant and insect densities can be achieved in a few years once the intensive management practices are abandoned [174], offering hope for the recovery of birds as well. Hedgerows with bushes and trees may also provide protection and nesting places for birds, but first the food supply needs to be restored to levels capable of sustaining their populations. Thus, bird densities and biodiversity can double in corn organic farms compared to conventional corn farms [175], despite some organic crops providing only slightly better food supplies.

It is reasonable to assume a similar fate in small insectivorous mammal and reptile populations, but at present evidence from field studies on these animal taxa is lacking. The fact that many amphibian population declines occur in intensive agricultural areas [176] has alerted some researchers. It appears that a combination of indirect effects from insecticides and herbicides, which introduce a cascade of events affecting negatively the feeding and growth of tadpoles, plus sublethal effects involving trematode infection [45] and other intensive farming practices, such as the use of fertilizers, may account for such declines [146]. However, pesticide-treated rice paddies continue to be a valuable haven for many species of frogs, since herons are not interested in preying in conventional fields because they have less foraging value than organic ones [111].

Apart from farmlands, indirect herbicide impacts are observed in wetlands that receive the outflows of agricultural waters, which often contain residual concentrations of atrazine, diuron and other persistent herbicides. For example, the constant use of herbicides for intensive rice production is thought to have contributed to the elimination of macrophyte vegetation in the lagoons of Ebro delta (Spain) during the 1980s, consequently reducing the populations of diving ducks and coot (*Fulica atra*) that depend on vegetative cover for nesting and feeding [177]. In a controlled experiment, density reductions of cattails (*Typha* spp.) after glyphosate sprays (5.8 L/ha) were well correlated with parallel reductions in the abundance of insectivorous and granivorous birds that depend on those plants for nesting [178]. Many wetland plants can take up and metabolize certain herbicide and insecticide residues found in waters (see Chapter 11), but they are still susceptible to the harmful effects of others. A recent study indicates that even if concentrations of individual herbicides may have a low risk to macrophytes, mixtures of bromacil, diuron, and norflurazon have a high risk [179]. At present, more field data are needed to assess the extent to which submerged

and emergent (cattails, reeds, rushes and sedges) macrophytes in wetlands are exposed to harmful concentrations of herbicide from aerial spraying, drift from ground application, runoff or soil erosion.

CONCLUSION

Although evidence indicates that 'conventional' chemically-based agriculture renders higher yields per area than 'organic' traditional practices, this has come at a price – high costs due to chemicals and fuel inputs to produce them [180], and multiple environmental impacts which in the long term can be detrimental [10]. Indeed the 'chemotherapy' applied to agriculture has had many side-effects and one wonders if it can go on forever without destroying the fabric of the biosphere. Here I have focused only on the problems, but an overall assessment must consider the benefits pesticides provide to humanity and the negative environmental consequences of not using them. The latter actions would reduce crop yields and lead to further deforestation in developing countries just to produce enough food to feed us all [181]. In this dilemma, the search for alternative agricultural practices that reduce the ecological risks of pesticides is an urgent necessity [182, 183]. The use of pheromone traps is, for example, a very effective alternative to control most insect pests, one that does not impact on non-target organisms and cannot induce resistance [184, 185].

This review has shown that impacts of pesticides on soil fertility are almost neutral, although the long-term crop sustainability is questionable [7]. Truly, fungicides protect the crops against certain pathogens but may destroy the beneficial mycorrhizal symbioses that increase nutrient uptake by the plants. Copper fungicides and certain insecticides are detrimental to earthworms and reduce the recycling capacity of the soil; in the end, soil fertility decreases and yields drop slightly.

Impacts on the prevalence of weeds and pests are mixed and negative in many cases. On the one hand, herbicides increase crop yields, but on the other hand they indirectly reduce the biodiversity and abundance of beneficial arthropods that carry out pollination and keep most pest species at bay. Insecticides are then applied to decimate the pests arising naturally under these circumstances, but eliminate the predators and parasitoids; this causes serious destabilizing effects on invertebrate communities which result in the rebound, promotion and increased resistance of all pests. After a few years, such futile efforts to contain the pest populations reach an unbearable cost, which could be avoided if integrated management practices that rely on natural means of weed and pest control were put in place [184, 186]. On the positive side, these effects are short-lived for the majority of the agrochemical products currently in use, so the ecosystem can recover within a year or two following cessation of pesticide application.

Finally, the impacts on terrestrial wildlife vertebrates are clearly negative – the death toll that certain insecticides have annually on non-target bird and small terrestrial vertebrates cannot be overlooked, even if such mortality may not reduce their populations in the long term due to compensatory effects [187]. More serious is the indirect impacts of routinely applied herbicides that cause declining population densities and biodiversity of birds and possibly amphibians. Equally, the secondary poisoning of consumer and predatory birds, reptiles and mammals by ingestion of pesticide-contaminated food is a real and present worry affecting individuals in various ways; unfortunately, long-term impacts on their populations usually take years to be noticed. Significant changes in current policies, institutions and practices are necessary to reconcile biodiversity conservation and food security [183]. The contribution of DDT and other persistent OC insecticides to the local extinction of birds of prey is undeniable, and also a reminder that persistent toxic chemicals should have no place in this world. Indeed, the contamination of the planet's ecosystems with these and other persistent pesticides is an ecological tragedy that will take many decades to be cleaned up.

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CHAPTER 5

Ecological Impacts of Major Forest-Use Pesticides

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Abstract: Assessing the potential for ecological impacts of pesticides requires a hierarchical approach with research ranging from simple laboratory to complex field experiments and operational monitoring. While all levels of study provide useful information, higher tier research has inherently greater environmental relevance and inference potential. In this chapter, selected higher tier studies relating to the use of herbicides glyphosate and triclopyr, as well as the insecticides *Bacillus thuringiensis* var. *kurstaki* (Btk) and diflubenzuron in the forest sector are reviewed. These case examples illustrate scenarios in which higher tier studies either negate or support the presumptions of risk derived from results of lower tier experiments. Specifically, assessment of the cases for glyphosate and Btk support their continued judicious use as environmentally acceptable components of integrated vegetation and insect pest management strategies. In contrast, higher level studies confirm risk postulates associated with typical forest-sector use patterns for triclopyr ester and diflubenzuron. Mitigation measures are required to ensure that use of these latter compounds do not pose undue risk to sensitive non-target organisms. In a broader context, the ecological implications of pesticide use in the forest sector must be considered in light of the fact that any management action, including the "no intervention" option, carries both economic and ecological risk. Strict adherence to the weight of scientific evidence principle, incorporation of knowledge gained from all levels of investigation, and a balanced assessment of relative risks of all potential options are considered primary requisites of comprehensive risk analysis and effective decision making.

INTRODUCTION

Truhaut [1] described ecotoxicology as that branch of the discipline concerned with the toxic effects of natural or synthetic pollutants on the constituents of ecosystems. As noted by Butler [2], this concept carries the inherent requirement to consider how the toxicant is released, its potential transformation and its possible transport to other compartments, since these are the primary determinants of exposure and effect. Potential effects must be considered at multiple scales, including those of biological organization (organism, population, or community), space (local to landscape) and time (days to years). These concepts are particularly relevant to the assessment of ecological impacts of pesticides in the forest sector where they may be applied to assist in regeneration or protection of forest stands and where there is potential exposure of a diverse array of organisms within highly interconnected ecosystem compartments.

Ecotoxicological risks associated with modern forest-use pesticides are quite unlike those of historic compounds such as DDT. However, the potential for both direct and indirect effects exists, and such risks are often the dominant element of public concern and policies associated with this forest management practice, as well as with forest certification schemes. Forest pesticide use varies across the globe, principally in relation to the size and accessibility of the resource, primary crop species and the value of commodities derived there from. In some countries blessed with huge areas of natural forests (e.g. Canada, Russia, USA), pesticides are applied to only a very small proportion of the forest land base that is managed for commercial production of high value products such as sawn wood, panels or pulp and paper. In other countries (e.g. New Zealand, Australia, Finland, Sweden and south-eastern USA) relatively more intensive "plantation" management may be employed for the same general purpose and of course gradients of relative management intensity occur in most countries.

While the focus of this chapter is on ecotoxicological risks of forest-use pesticides, such risks must be considered within the broader context of assessing both risk and benefit of this or alternative forest management actions. Few, if any forest managers would choose to apply pesticides if there were not substantial benefits associated with such treatments. For example, herbicides are recognized as the most effective tool for controlling competing vegetation to favour partitioning of essential light, water, nutrients and growing space to the desired crop species rather than to weedy competitors [3]. Wagner *et al.* [4] recently reviewed results from 60 of the longest-term studies in Canada, the USA, South Africa, Brazil, New Zealand and Australia, documenting that the majority of studies show 30 to

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500% increases in wood volume as well as reduced rotation periods from effective vegetation control treatments. Positive outcomes are reflected in significantly enhanced regeneration success and overall sustainable management of forest resources. A diverse array of insect pest species are capable of causing significant economic or ecological damage in major plantations or natural forest stands [5, 6]. Both chemical and biological insecticides are applied to protect semi-mature or mature high value forest stands or to slow the spread of invasive species across the landscape and thus mitigate either economic or ecological losses. In cases where no effective chemical or biological controls are applied, devastating losses are typically the result. For example in Canada, no effective pesticides have been developed or applied to control the epidemic outbreak of mountain pine beetle in lodgepole pine stands. This single insect pest now affects a forest area in excess of 14.5 million ha in the province of British Columbia [7] an area essentially equivalent to that of England. As the beetle moves across the Rocky Mountain divide into Alberta it threatens stands of other pine species including jack pine that spans the boreal forest region across country with massive implications in terms of economic loss, carbon release to the environment and unknown ecological effects in a region not previously adapted to this pest species.

Pesticide risk should also be considered in relation to specific use patterns and proportional use. In comparison to agriculture, pesticide use in forestry involves substantially fewer active ingredients as well as dramatically lower use frequency and proportion of the total productive land area treated in any given year. For example, pesticide use in Canadian forestry accounts for only ~2% of total pest control products sold in that country. Only two active ingredients, the herbicide glyphosate and the microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (Btk) have any significant degree of use, each comprising more than 90% of the total forest area treated with a herbicide or insecticide respectively, a determination based on 2007 statistics for pest control product use in that sector [8]. Similarly, pesticide use in plantation forestry in Australia accounts for only 0.7% of the total annual national expenditures on pesticides [9]. The latter report presents detailed analysis of pesticide expenditures in agricultural crops as compared to forestry. Results emphasize the dramatically higher use frequency and hence expenditures associated with pesticide use in agricultural crop production. To a large degree, this reflects the common practice of multiple pesticide applications on an annual basis to much of the agriculture land base. In contrast, individual forest stands rarely, if ever receive annual pesticide treatments and frequency of use is typically quite low. Even under intensive forest management regimes, the total number of pesticide applications during a rotation period is unlikely to exceed four; that is two herbicide treatments in the early regeneration phase and two insecticide treatments when trees are semi-mature to mature. However, rotation periods vary markedly with forest crop species ranging from as little as 8 to 10 years for example in short rotation eucalypt plantations of Australia, to 80 years or more for spruce stands in the boreal forests of Canada. The total proportion of the productive forest land base treated is also an important consideration in ecotoxicological risk assessments. Again, on a comparative basis, agricultural food crop production often involves essentially 100% of the land base receiving at least one pesticide treatment each year, whereas production of fibre typically involves pesticide application to only a very small proportion of the commercial forest land base annually. However, exceptional cases have been documented historically, including for example massive spruce budworm outbreak in New Brunswick, Canada where almost 4 million ha of forest land was treated with insecticides in one year [5]. While these statistics vary with jurisdiction, year and pesticide type, the point is well exemplified by herbicide use in Canadian forestry where <1% of the commercial forest land base is treated in any given year [10].

Considered in combination, and particularly in relation to agricultural pesticide use, the few active ingredients employed in forest management, their relatively low use frequency, the minor proportion of total forest land area treated and the resultant lower environmental loadings (*i.e.* mass of total pesticide applied per unit area), public concern over pesticide use in forestry seems disproportionately high. For example a poll of 2500 Canadians indicated 71% opposed the use of chemicals in the forest [11]. As noted by Guynn *et al.* [12], public perception of risks may contrast significantly with scientific conclusions based on the weight of scientific evidence from the cumulative primary literature. However, under current socio-political systems in most countries, public opinion carries significant influence over decision making and management policy, thus controlling the "social license to operate" on publicly owned lands. Current examples include restrictions or outright bans on chemical pesticide use in the forest-sector in certain political jurisdictions of Canada and the USA, despite registration and approval for these specific uses by federal regulatory agencies. Another example is the mandatory requirement to reduce or eliminate the use of chemical pesticides as a forest management option in some forest certification schemes [13], presumably reflecting the wishes of a more environmentally conscious and engaged consumer base.

Scope Statement

Ecological risk estimation is generally considered as a tiered or hierarchical process which requires fundamental knowledge and data derived from scientific disciplines of environmental chemistry, biology, ecology and toxicology [14]. Production of these primary data is a legislative requirement of government regulatory bodies in many countries (e.g. the United States Environmental Protection Agency, the Canadian Pest Management Regulatory Agency and the Australian Pesticides and Veterinary Medicines Authority). Each of these regulatory agencies, as well as many other regional regulatory agencies, conduct independent reviews of the data prior to national registration and specific regional or sectoral use of pesticides. General discussion of the fundamental environmental fate and toxicology data requirements are discussed in chapters 1 and 2 of this text and will not be considered in detail here. Readers interested in more specific details on these fundamental toxicological data are directed to the Pesticide Information Profile briefs available on the EXTOXNET website [15], which provides convenient summaries for each pesticide. The United States Department of Agriculture – Forest Service documents also available *via* the internet [16] are another comprehensive source of data and information on how such data may be used directly in human health and environmental risk analysis.

Over and above these fundamental regulatory data requirements, numerous higher tier experiments and field investigations are conducted to inform the process. Among the various classes of pesticides that might be applied in forest management, herbicide and insecticide use predominates, with relatively minor amounts of fungicides being broadcast applied to plantations or natural forest stands [6]. As such, discussion in this chapter will be restricted to herbicidal and insecticidal compounds and based on four selected case examples (two for each pesticide class). Case examples were chosen as representative compounds most commonly used in the forest sector, or because they emphasize key ecotoxicological issues which are integral to the continuous debate over pesticide use both in the forest sector and more generally. The examples put forward in this chapter are intended to demonstrate the wealth of scientific information pertinent to possible ecological impacts of major forest-use pesticides and to emphasize the importance of higher tier manipulative field experiments and monitoring as critical components of the overall risk assessment process governing their regulation and use.

USE PATTERNS AND EXPOSURE ASSESSMENT FOR MAJOR FOREST-USE PESTICIDES

The critical determinant of any toxicological effect is the dose; that is the level of the toxicant which occurs at the physiological site of activity within the organism. As such, toxicological effects are often directly proportional to environmental exposure concentrations with due consideration for modulating effects associated with the fundamental biology or behaviour of the receiving organism. For example, the feeding rate and preferences of different insects may influence exposure, while seasonal development of plant cuticles may act as a barrier to herbicide uptake in plants. In the case of forest-use pesticides, which are intentionally applied to known areas for very specific purposes, typical use patterns and application rates (Table **1**) are, in turn, the key determinants of potential environmental exposures. The actual application rate employed is selected by experienced forest managers based on the degree of infestation, susceptibility of the pest problem and cost considerations. Often the rates employed operationally may be less than the maximum allowed.

As competing vegetation and insect pest problems in the forest sector often occur at very large spatial scales and with substantial infestation intensities, broadcast techniques are often the only practically feasible method for applying the chemical to target sites. Forest-use herbicides, excepting soil active compounds, are applied with the specific intent of impinging the maximum possible mass of active ingredient on foliage of the competing vegetation canopy. Similarly, insecticides are typically applied such that they impinge predominantly within the crop tree canopy upon which many insect pests feed. Thus, non-target organisms residing or foraging in targeted plant canopies have the greatest likelihood of direct exposure [17, 18]. However, since not all of the depositing spray cloud is impinged within the target canopy, exposures of ground dwelling, soil or aquatic organisms may occur to some extent through either direct or indirect mechanisms (e.g. by rain-wash) and cannot be completely disregarded. Such exposures may be of particular importance in cases where highly sensitive or rare species are known to occur. The development and use of various new technologies including low drift nozzles, electronic guidance systems on spray aircraft and geographic information system mapping of spray blocks have greatly improved control and optimization of spray deposition. When used in conjunction with recently developed decision support systems such

as SprayAdvisor, such advanced tools and techniques can substantially reduce the probability of depositing toxicologically significant levels of pesticide outside the targeted spray area[10].

Table 1: Comparative examples of maximal and typical use rates, as well as calculated and actual environmental concentrations observed in field research and monitoring studies for four major forest-use pesticides.

Active Ingredient	**Country**	**Registered end-use product examples**	**Typical use pattern**	**Use rates in forestry***	
				Label Max.	Typical
Glyphosate	Canada USA	Vision VisionMax Accord SP Roundup Original	Aerial -Conifer Release	2.14 2.16 11.2; 4.2	1.9[8,81] 2.7[15] 2.63[37]
Triclopyr butoxyethyl ester	Canada USA	Release® Garlon 4	Ground: foliar & woody weed control	3.84	2.3[15]
Bacillus thuringiensis var. *kurstaki* (Btk)	Canada USA	Foray 76 Foray 48B Dipel 8L	Aerial broadcast – Spruce and Jackpine budworms, Gypsy moth, Douglas Fir Tussock Moth, Spruce budworm, Painted Apple Moth	60 BIU/ha 60 BIU/ha	30-60 BIU/ha[8] 49-99 BIU/ha[18]
Diflubenzuron	USA	Dimlin 4L Dimlin 25W	Aerial – gypsy moth	0.07 0.035	0.009-0.070[125] 0.009-0.035[125]

Superscripted numbers in brackets correlate directly to references from which data were obtained.

* kg/ha unless otherwise noted

Accord®, Roundup Original®,Vision® and VisionMax® are registered products of the Monsanto Co., St. Louis, Missouri; Garlon 4®, and Release® are registered products of DowAgroSciences, Indianapolis IN; Foray 76®, Foray 48B® and Dipel® are registered products of Valent Biosciences, Toronto ON; Dimlin 4L® and Dimlin 25W® are registered products of Uniroyal Chemical Co., Bethany CT;

Table 2: Observed concentrations, primary mechanisms of degradation or dissipation and persistence estimates for major use pesticides in environmental compartments of various forest ecosystems.

Active Ingredient	**Environmental Compartment**	**Maximum Conc. (mg/L or ppm)**	**Primary Mechanisms of Degradation or Dissipation**	**DT50 (days)**
Glyphosate	Vegetation Litter Soil Water	529[59] 322[43], 8.3[60] 1.4[43], 1.5[60] 550[71]	Uptake & translocation Microbial Microbial Microbial, sorption	2[59] 12[60] 10[60] 4.2 to 26.4[70]
Triclopyr ester or acid	Target Vegetation Litter Soil Water	1630[59], <450[39], 127[35] 53[35] 0.73[35], 45.7[60] 0.35[76]	Uptake & translocation Photolysis Microbial Microbial Based-catalyzed hydrolysis, photolysis	4[59], 31-202[35] 31[35], 39[60] 60[60], 14[84] 4-8[93]
Bacillus thuringiensis var. *kurstaki* (Btk)	Vegetation Litter Soil Water	480[18] n/a n/a n/a	UV kill of endospores, alkaline or enzymatic hydrolysis of endotoxins[19]	1-64[20] 100-200[92] >70[21]
Diflubenzuron	Target Vegetation Litter Soil Water	n/a n/a n/a 0.006-0.014[112]	Photolysis Microbial Microbial Photolysis, hydrolysis, sorption	<21[22] n/a 2.1[117], 8.6[117] <14[23], 3-8[113]

Superscripted numbers in brackets correlate directly to references from which data were obtained.

Pesticides currently in widespread use in the forest sector may be generally characterized as non-persistent, susceptible to microbial degradation, photolysis, hydrolysis or other degradation mechanisms and non-bioaccumulatory. Extensive scientific knowledge on their fundamental physico-chemical properties and on their environmental fate under both laboratory and representative field scenarios exist. From field experiments under quasi-operational conditions, empirical estimates of initial concentrations observed in various environmental compartments as well as time to 50% dissipation (DT50 or half-life) estimates are also available as shown in Table **2**.

DT50 values provided in Table **2** indicate that residues of pesticides commonly used in the forest sector are relatively short-lived in all major environmental compartments. As such, exposure regimes are typically characterized by peak concentrations occurring shortly after application and with diminishing magnitude of exposure through time. The duration of exposures are often curtailed by the combined effect of environmental degradation and dissipation mechanisms which are active in these compartments. The resultant changes to chemical structure or bioavailability may significantly modulate exposure regimes and thus potential toxicological effect. Commonly, where wildlife exposures to pesticides occur, the exposure regime may be characterized as a pulse exposure of relatively short duration. In some cases, natural environmental exposure regimes differ markedly from those typically employed in standard tier 1 toxicity testing protocols in which test concentrations are artificially maintained at some constant high level. Considering all of the foregoing information, Tier 1 hazard quotient analyses, which are based on estimated exposure under the assumption of maximum labeled use rates and effect endpoints derived from atypical exposure regimes, should be considered as worst case risk estimates. Often the magnitude and duration of real world exposures, as well as toxicity observed in studies conducted *in situ*, are substantially lower than those predicted from simple hazard quotient analyses. Nonetheless, the majority of these types of risk analyses demonstrate that major forest-use pesticides do not pose substantial risks of direct toxicity to most wildlife species. Risks are generally greater in cases where the mechanism of activity is common to target and non-target organisms alike (e.g. acetylcholine esterase inhibition) and where both groups may be equivalently exposed (e.g. target and non-target insects in forest canopies) or where a particular group of organisms are uniquely sensitive to the pesticide or constituents of the pesticide formulation. While it is recognized that there are exceptions to most, if not all, generalities (some of which are described below), ecological impacts associated with the modern pesticides currently in widespread use for forest insect pest control and vegetation management are much more likely to occur through indirect mechanisms, such as changes in habitat or food availability, as opposed to direct acute toxicity.

FOREST-USE HERBICIDES

Among countries leading international trade in forest-resource based products, only a handful of herbicidal active ingredients are registered and commonly used to control competing vegetation as a means of enhancing forest regeneration (Table **1**). Given that vegetative competition is most critical during the early establishment phase of forest regeneration [24], herbicide applications are typically made to prepare the site just prior to planting or in the very early stages (1-3 years) subsequent thereto. It is important to recognize that herbicide treatments therefore follow shortly after the major physical disturbances which result from harvesting and planting operations. In ecological terms, this is a transient stage in the cycle characterized by dynamic change and relatively rapid vegetative succession. Immediately following the physical disturbance of harvesting, sites typically become dominated by pioneer plant species well adapted to the high light intensities, disturbed soils and fluctuating temperatures which are often characteristic. As such, the potential changes in ecological structure and function that may be induced by herbicide treatments, must be considered in the context of the typical ecological dynamics of the sites to which they are applied and with due consideration to the dynamics in the broader forest landscape to which that specific site is connected [25-27].

The environmental fate and effects of herbicides used in forest vegetation management have been extensively investigated at experimental scales ranging from small laboratory studies to whole ecosystem manipulations. Several directly relevant reviews have been published previously [28-37, 52]. Independent regulatory reviews conducted in several countries (e.g. USA, Canada and Australia) with significant herbicide use in the forest sector consistently conclude that when applied in accordance with their specific product labels, such uses do not pose a substantive risk to wildlife or general environmental health. A special issue of the Wildlife Society Bulletin considers the transport and direct toxicity of many of many herbicides noted here [29]. A discussion of indirect influences of herbicide products used predominantly in the south-eastern USA on forest biodiversity [30] and wildlife habitat [12] is also

included in the same publication. Collectively, the authors drew the following general conclusions from their review of the pertinent scientific literature:

- Herbicides most commonly used for vegetation management in forestry (glyphosate, triclopyr, imazapyr, sulfometuron, metsulfuron methyl, hexazinone) degrade quickly once they enter the environment and thus are neither persistent nor bioaccumulative.
- As modern herbicides have been designed to target biochemical processes unique to plants, they exhibit a low level of direct toxicity to animals.
- When used according to label instructions, modern silvicultural herbicides pose little risk to wildlife.
- Due to the high resilience of floral communities, plant species richness and diversity rebound rapidly after single herbicide treatments, with short- and long-term compositional shifts according to the selectivity and efficacy of the herbicide used.
- Under more intensive management regimes including multiple applications of herbicides, the shortened period of suitable habitat and reduction in habitat quality may reduce populations of disturbance-dependent species, however, the scale of application and the landscape context will determine the level of effects on local or regional populations.
- Detailed studies on influences of silvicultural treatments, including herbicides, on amphibian and reptile communities are especially needed.
- Despite these findings, public opinion against forest herbicides often has limited or restricted their use, likely due to common public values associated with forests and a lack of technical knowledge.

These conclusions are drawn largely from studies conducted in the south-eastern USA. However, they are further supported by results derived from several higher-tier studies conducted in Canada and in other major forest regions and may thus be considered as generally applicable. Below, case study examples for two different herbicides (glyphosate and triclopyr) are presented to illustrate scenarios in which ecotoxicological field studies demonstrated substantially differing levels of risk and the value of conducting detailed studies under real-world conditions as a critical component of a hierarchical approach to ecotoxicological risk assessment.

Glyphosate

As well as being the dominant herbicide in modern agriculture [31], glyphosate is also one of the most widely used herbicides in the forest sector around the globe including in Canada, the USA and Australia. For example, glyphosate-based products have accounted continuously for more than 93% of the total forest market in Canada for the 15 year period from 1992 through 2006 [8]. The knowledge base pertaining to the ecotoxicology of glyphosate is arguably the most extensive ever developed for a forest-use herbicide. The general environmental behaviour and toxicology of this herbicide has been the subject of several major independent reviews [32-35]. In addition, a seminal text presents much of the historical background and detailed information on all aspects of this unique compound in the early years post-discovery [36]. A search of several electronic databases provided several hundred records of primary scientific literature specific to the fate and effects of glyphosate in forest ecosystems. Many of these are field studies involving formulated end-use products applied at typical or maximal application rates and designed to examine the fate and effects under natural conditions typical of major forest uses. In addition, a number of other field studies are currently being conducted to address specific issues of scientific, public or operational forestry interests.

Since its discovery and introduction by Monsanto, numerous formulations of glyphosate have been registered and used in forest vegetation management globally. More recently, with the loss of patent control, multiple manufacturers are generating “generic” glyphosate products and more than 35 different formulations are used in the USA alone [37]. From both a use and ecotoxicological perspective, not all formulations are equivalent, largely owing to differences in the exact chemical composition of the products but also because of differences in application methods and rates as specified on the product labels. Several formulations contain different glyphosate salts or different surfactant blends which may significantly influence the uptake of the chemical in plants or potential ecotoxicological effects. However, in general, it is known that glyphosate is rapidly taken up by the plant following application of the formulated product and thereafter translocated to active growing tissues in both the aerial and root

structures. As such, it is particularly effective for control of biennial or perennial species which self-propagate from basal sprouts, roots or rhizomes. Plants with this type of reproductive strategy are often the most problematic in forestry, particularly because they tend to be very poorly controlled by mechanical techniques. Often mechanical cutting actually stimulates more extensive growth, thereby exacerbating rather than alleviating competition with more desirable crop species. The mechanism of action for glyphosate involves blockage of a specific enzyme (5-enolpyruvyl-shikimate-3-phosphate synthetase or EPSPS) in the synthesis of aromatic amino acids. This biosynthetic pathway exists in both plants and microorganisms but not in higher animals [38, 39]. Owing to its highly plant-specific mode of action, direct effects of glyphosate on animals generally require much higher dose levels than would be typically encountered in natural environments, thus conferring a substantial level of safety for many wildlife species that may be potentially exposed. The environmental fate and persistence of glyphosate has been examined in vegetation, litter, soil, and water compartments of forest ecosystems ranging from the Pacific coastal forests in both the USA [40, 41] and in Canada [42, 43], to high latitude coastal and interior forest sites in Alaska [44], in southern and northern deciduous forests of the USA [45], in boreal forest sites of central Canada [46-48] and in the Acadian forest region of eastern Canada [49, 50]. The results of these extensive field studies allow for broad inferences on the environmental fate of glyphosate in forest ecosystems. In general, it is known that glyphosate is effectively impinged within the target canopy, with relatively low residues in ground vegetation or in soils. In all compartments, glyphosate is susceptible to rapid microbial degradation and thus non-persistent. It binds strongly to essentially any organic substrate including organic matter and clay particles of sediments and soils, and thus shows essentially no tendency to leach or move laterally with surface runoff even though it has relatively high solubility in water. The time to 50% dissipation for glyphosate in these various environmental compartments is provided in Table **2**. The primary degradation product is aminomethylphosphonic acid (AMPA) and several studies indicate that AMPA is also non-persistent under typical forest environmental conditions. At least one assessment [51] has focused specifically on AMPA which suggests that it provides little risk to aquatic organisms.

The United States Department of Agriculture – Forest Service [52] provided the first comprehensive review on glyphosate fate and effects related to forest uses in 1984, with a subsequent workshop proceedings pertaining to uses in coastal forests of western Canada constituting a second review [53]. Both documents provide detailed estimates of environmental exposures following normal use and concluded that such levels would be expected to have neither acute or chronic toxic effects, nor reproductive effects in animals. Durkin [37] published a more recent review and risk assessment in 2003, pertaining to typical ground-based backpack spraying of glyphosate at rates of 2.24 kg a.i./ha. The risk assessment generally supported the conclusions reached by the U.S. EPA, indicating that based on the currently available data, effects on birds, mammals, fish and invertebrates are minimal. Sullivan and Sullivan [54] provided another review of more than 60 published studies on glyphosate in forestry, considering potential effects of this management practice as a disturbance agent in forest ecosystems and focusing on aspects relating to biodiversity. These authors concluded that species richness and diversity of vascular plants, songbirds and small mammals were either not affected or affected to only a minimal degree by glyphosate treatments. The degree of change observed in all cases was considered to be within natural fluctuations. For both avian and small mammal species, temporary declines did occur in some species, whereas in other species, abundance actually increased in treated sites. Such differential responses are largely attributable to the specific habitat preferences of the species in question. For those species whose preferred habitat is removed by the herbicide treatment the typical response is transient reduction in populations in these specific treated sites, followed by return when these habitat features become re-established on the site. The impact of glyphosate on large mammalian herbivores was measured by abundance of animals and food plants and by habitat use. Hares (*Lepus* spp.) and deer (*Odocoileus* spp. and *Capreolus capreolus*) were little affected, whereas reductions in plant biomass and related moose (*Alces alces*) forage and habitat use generally occurred for 1 to 5 years after treatment. Studies on terrestrial invertebrates covered a wide range of taxa with variable responses in abundance to glyphosate treatments. The authors noted that management for a mosaic of habitats, which provides a range of conditions for plant and animal species, are likely to ameliorate any short-term changes in species composition which might occur on specific sites treated with glyphosate to enhance regeneration success and plantation growth rates following forest harvesting.

Several major field studies, as well as a hierarchical suite of lab to field studies focused on the effects of glyphosate on amphibian species, have been completed. Results of these studies provide a substantial empirical basis which taken as a whole demonstrates very low potential for significant direct deleterious effects of formulated glyphosate products on non-target organisms in forest ecosystems. One of the earliest of these studies was a long-term

investigation conducted in the Carnation Creek watershed of coastal British Columbia. This whole ecosystem experiment involved a fall aerial application of glyphosate (Roundup) in which the herbicide was applied at a rate of 2.0 kg a.i./ha to 41.7 ha of the watershed. General results were summarized by Reynolds and co-workers [53, 55] with more specific details provided in a series of published studies by several of the principal investigators involved. A key focus of the study was on comparative fate and effects of the herbicide in directly over-sprayed versus buffered stream channels. Feng *et al.* [43] documented maximum glyphosate residues of 162 μg/L in stream water, 6.8 μg/g dry mass in bottom sediments and <0.03 μg/L in suspended sediments of two intentionally over sprayed tributaries, dissipating to <1 μg/L within 96 h post application. Buffered streams were characterized by very low glyphosate residue levels <4 μg/L in stream water. Ratios of maximum stream water concentrations of glyphosate observed in buffered and over sprayed tributaries relative to literature toxicity values indicated a substantial margin of safety under either operational or worst case scenarios. Holtby and Baillie [56] examined the responses of coho salmon (*Oncorhynchus kisutch*) fingerlings and observed some stress and low mortality of 2.6% in caged fish located in the over-sprayed tributary. No similar stress or mortality were observed in other sites. Catch per unit effort in the over-sprayed tributary declined immediately after the application but recovered within 3 weeks. While this was taken to suggest that coho fingerlings had been stressed by some component of the herbicide spray, no treatment related changes in over-winter mortality, growth rates, probabilities of entering and leaving the tributary or timing of spring emigration were observed in the two years subsequent to herbicide treatment relative to results from 1 to 3 years of pre-spray monitoring. Kreutzweiser [57] also concluded that herbicide treatments did not unduly disturb stream invertebrates. While drift densities of most aquatic invertebrates did not increase in response to herbicide applications, the slight increase in drift response of *Gammarus* sp. and *Paraleptophlebia* sp. observed downstream of treated areas may have resulted from herbicide treatment. Feng *et al.* [42] also documented fate and persistence of glyphosate and its primary metabolite in terrestrial compartments. Residues in red alder and salmonberry foliage were 261.0 and 447.6 μg/g respectively and indicated good impingement on the target. Leaf litter residues, which averaged 12.5 μg/g for red alder and 19.2 μg/g for salmonberry initially, declined to less than 1 μg/g within 45 days post application (DT50< 14 days). In soils, glyphosate and AMPA residues were retained primarily in the upper organic layers of the profile, with >90% of total glyphosate residue in the 0 to 15cm layer. Distribution data for both glyphosate and AMPA suggested strong adsorption and a low propensity for leaching. Glyphosate soil residues dissipated with time resulting in estimated DT50 values ranging from 45 to 60 days. After 360 days, total soil residues of glyphosate were 6 to 18% of initial levels. Results of the Carnation Creek study were consistent with a similar study conducted by Newton *et al.* [41] in the Oregon Coast range. Additional work in the latter study examined the exposure of mammalian herbivores, carnivores, and omnivores. Results showed that retention of the herbicide varied with food preference; however, all species had visceral and body contents at or below observed levels in ground cover and litter, indicating that glyphosate did not accumulate appreciably in animal tissues.

The Fallingsnow ecosystem project conducted in the boreal forest of northwest Ontario is one of the few studies to comparatively examine the ecological consequences of herbicide treatments, including glyphosate, with other methods of vegetation management. In this experiment, treatments included aerial applications of triclopyr ester (Release) at 1.9 kg a.i./ha or glyphosate (Vision) at 1.5 kg a.i./ha with direct comparison to mechanical cutting using either brush saws or tractor-mounted cutting heads. Lautenschlager [58] concluded that herbicide treatments had relatively inconsequential effects on most ecological response parameters examined in this boreal forest site. As part of this multidisciplinary study, Simpson *et al.* [59] observed no substantial treatment-related differences in the movement of selected nutrients such as total organic N, NH^{4+}, NO^{3-}, K, Ca. Woodcock *et al.* [60] assessed the effects on songbird densities as determined by territory mapping, mist netting, and banding and observed 20 to 38 species breeding within various treatment blocks. First year post-treatment assessments revealed that mean densities of the 11 most common species increased by 0.35/ha on the control plots. In contrast, densities on treated plots decreased by 1.1/ha (brush saw), 1.6/ha (Silvana Selective), 0.14/ha (Release) and 0.72/ha (Vision). A point of emphasis here is that essentially any effective vegetation management technique will alter available habitat to some degree. In at least this one study, songbird densities were relatively less impacted by herbicide treatments as compared to mechanical treatments. Response to these habitat changes will vary with species, favouring certain species while resulting in out-migration of other species at least for some period of time. As a single species example, chestnut-sided warbler (*Dendroica pensylvanica*) had lower (p <0.05) mean densities on the brush saw-treated and Silvana Selective-treated plots than on the control plots and fewer (p <0.05) female birds were captured in the first post-treatment year.

A particular strength of the Fallingsnow ecosystem project was the detailed studies on plant communities where relative differences were tracked before and 1 to 5 years after treatments. Newmaster and Bell [61] showed that species richness and abundance of pteridophytes, bryophytes and lichens were reduced by all of the silvicultural treatments. Herbicide applications had the greatest initial effect on species richness, species abundance, and diversity indices. The authors noted that cryptogam diversity showed signs of recovery 5 years after treatment and that missed strips or untreated areas within a clearcut, provided a refuge for remnant communities and could play a key role in the rehabilitation of sites in terms of recovering the full suite of plant diversity. Bell and Newmaster [62] further reported that woody, herbaceous and graminaceous species showed transient declines in species richness, abundance or foliar cover, diversity indices, and rank abundance, as would be expected given the intent of the treatment. As a result, spruce trees proliferated in the regenerating plantations, but in no case did single layer monocultures occur. While herbicides had a relatively greater initial effect on plant community composition as compared to the two different cutting treatments, woody, herb, and grass layers showed substantial resilience to all treatments and recovered to pre-treatment levels within five years. Duchesne *et al.* [63] examined effects on total captures, species richness, diversity, and assemblages of adult carabids (Coleoptera: Carabidae) and found no effect on total capture rates but an increase in species richness and diversity in response to all treatments.

As noted by Guynn *et al.* [12] impacts of forest-use herbicides on amphibians is an area that has been historically understudied. In recognition of this general dearth of scientific knowledge and the potential for both aquatic and terrestrial life stages of amphibians to be directly exposed to formulated glyphosate products, Thompson and co-workers undertook a multi-tier, hierarchical project including both laboratory and field component studies [64]. Each tier of study provided unique and valuable data pertaining to overall risk assessment for amphibians. The authors also noted the need to consider potential multiple stress and multiple species interactions in ecotoxicological research. As the lead component study in this series, Edginton *et al.* [65] reported 96-h LC10 and LC50 estimates ranging from 0.85 to 3.5 mg a.i./L for early larval stages (Gosner 25) of *Rana clamitans* and *R. pipiens*. These endpoints remain among the lowest documented toxicity endpoints for amphibians exposed to formulated glyphosate products. The study confirmed that amphibian larvae were more sensitive than embryos and showed general equi-sensitivity among the four amphibian species tested. Results also demonstrated that larval amphibians are among the most sensitive of aquatic organisms when exposed to formulated products of glyphosate containing the POEA surfactant and thus the importance of testing end-use formulations. Surfactants are generally required to be used with glyphosate to allow effective uptake of this electronically charged molecule across plant cuticles. Inclusion of the surfactant also results in reduced losses from treated foliage *via* rain-wash [43, 66]. The inclusion of the POEA surfactant in many formulations is also very important from an ecotoxicological perspective. It is well recognized that POEA is the primary toxicant to aquatic species. POEA and other surfactants may affect membrane transport generally and often act as a general narcotic [32, 33]. As such POEA mediated toxicity is well established as a concern for aquatic organisms such as fish and amphibians for which transport of oxygen and other compounds across gill or skin membranes is a critical physiological function. Unfortunately, owing to the chemical complexity of the POEA surfactant and resultant difficulty in analysing for it in complex environmental matrices, the environmental behaviour of POEA in natural forest ecosystems has not been specifically studied. However, fate experiments conducted in the laboratory show that the surfactant is also readily degraded in soils with a half-life of less than 7 days, that desorption from soil surfaces is minimal, and that persistence in natural waters under laboratory conditions resulted in an estimated half-life of about 2 weeks. Results of these studies suggested that POEA would be lost from the water column following application by a combination of sorption to sediments and microbial metabolism [67]. The half-life of POEA in shallow waters (15 cm deep) in the presence of sediments has subsequently been reported as about 13 h [68], further supporting the concept that any potential direct effects of formulated products on organisms in natural waters are likely to occur very shortly post-treatment rather than as a result of chronic or delayed toxicity.

Tier II studies conducted by Chen *et al.* [69] confirmed the interaction of pH and Vision toxicity in *R. pipiens* larvae and showed parallel effects for zooplankton population response parameters, suggesting that the pH–Vision interaction is of general ecological significance. In addition, Tier II studies demonstrated that effects on zooplankton reproduction could also be exacerbated by food deprivation when presented as a concomitant stressor. *In situ* enclosure studies conducted by Wojtaszek *et al.* [70] in two different wetlands systems showed 96-h LC10 and LC50 values generally higher than those derived from laboratory studies. This result was attributed to reduced magnitude and duration of exposures resulting from natural degradation and dissipation mechanisms which are

active in real-world systems. Results clearly demonstrated the importance of including *in situ* manipulative studies in ecotoxicological risk assessments. Contrary to the results of the lab-based studies, the *in situ* enclosure experiment lead to the conclusion that typical silvicultural applications of Vision would not be likely to generate significant direct mortality in native amphibian larvae. This conclusion was strongly supported by both chemical and biological monitoring studies as reported by Thompson *et al.* [71] as the fourth and final tier of the research program. Results from these Tier IV studies showed no statistically significant differences in mean mortality among larvae of two different amphibian species (*R. clamitans* and *R. pipiens*) differentially exposed in over-sprayed, adjacent, and buffered wetlands. Results of the operational monitoring study were consistent with concentration-response relations from both Tier I and III studies since 99% confidence limits for real-world exposure concentrations in all wetland cases were below both estimated LC50 and LC10 values. As a general conclusion, results of this tiered research program indicate that aerial applications of the herbicide Vision, as typically conducted for conifer release in forestry, do not pose a significant risk of acute effects to the most sensitive aquatic life stages of native amphibians in forest wetland environments. The conclusion was consistent with specific risk assessments for formulated glyphosate products in aquatic systems [33]. Results of ongoing field studies consistently support this conclusion, thus allowing researchers to refocus their attention on more subtle but equally important potential effects on amphibian populations associated with possible indirect or multiple stressor interactions [72, 73].

Triclopyr

Triclopyr is the common name for ((3,5,6-trichloro-2-pyridinly)oxy)acetic acid, the active ingredient of formulated commercial products such as Garlon 3A and Garlon 4. These two products also represent two different chemical forms of triclopyr, that is the triethylamine salt and the butoxyethyl ester (BEE) respectively. Triclopyr mimics indole auxins as plant growth regulating hormones and causes plant mortality through induction of irregular cell growth, particularly in the stem tissues of vascular plants. Typical use rates for triclopyr are in the range of 4 kg a.i./ha, comparatively higher than those for glyphosate. Although triclopyr receives markedly less use in the international forest sector than glyphosate, it is a regionally important forestry herbicide in the southeastern USA and other areas where it is typically applied using ground-based techniques. The fate and effects of triclopyr in forest ecosystems have been previously reviewed [52]. In combination with data derived from several field studies conducted in a variety of forest ecosystems, it is well documented that triclopyr dissipates rapidly from foliage and soils. The primary degradation mechanism in soils is microbial and the principal metabolite is trichloropyridinol. Both laboratory and field study results suggest that triclopyr exhibits limited to moderate leaching or lateral mobility in soils [40, 74-76]. In aquatic compartments, BEE degrades *via* base-catalysed hydrolysis to yield triclopyr acid [77] which in turn may further degrade by either photolytic or biological means to yield the principal metabolite [52].

Wan [78], studied the comparative acute toxicity of Garlon 3A, Garlon 4, triclopyr, triclopyr ester, and their transformation products to juvenile Pacific salmonids, demonstrating that the ester was considerably more toxic than all other forms. The ester form of triclopyr is considered to be approximately 100 fold more toxic than the acid [79]. McCall [80], conducted simulations of the aquatic fate of triclopyr butoxyethyl ester emphasizing the importance of mechanisms converting the ester to less toxic forms as this is a critical determinant of potential toxic effects in fish such as coho salmon, as well as other aquatic organisms. Under low pH or cool temperature conditions, the transformation of ester to acid may be relatively slow and thus variations in these environmental parameters may strongly influence ecotoxicological outcomes. In this regard, toxicity of the ester form of triclopyr to fish, amphibians and aquatic invertebrates is the major concern in relation to potential ecological impacts and this aspect has received a substantial amount of scientific investigation.

Kreutzweiser and co-workers, conduced time-toxicity tests with rainbow trout (*Oncorhynchus mykiss*) under both laboratory and field studies. In flow-through toxicity tests [90] the effect of exposure time on the toxicity of triclopyr butoxyethyl ester (Garlon 4) to fish (rainbow trout, *Oncorhynchus mykiss*, and chinook salmon, *Oncorhynchus tshawytscha*) and stream insects (*Hydropsyche* sp. and *Isonychia* sp.). The toxicity of triclopyr ester to all species increased with increasing time of exposure to the ester. For example, median lethal concentrations for rainbow trout exposed for 1, 6, or 24 h were 22.5, 1.95, and 0.79 mg a.i./L of triclopyr ester. Results suggested that even under conditions where maximal predicted environmental concentrations (2.7 mg a.i./L) might occur, risk of acute toxicity would be very limited under typical exposure durations observed in flowing systems. In contrast, considerably higher risk of acute lethal effects could be predicted under conditions where the ester form might persist for more

than 6 h, even when initial concentrations were as low as 0.7 mg a.i./L. The authors noted the aquatic organisms in lentic systems (such as wetlands, ponds and lakes) are likely to be most at risk. These relations were subsequently confirmed in various field studies.

A major multidisciplinary study focused on the ecotoxicology of triclopyr ester (Garlon 4) following aerial application at a rate of 3.84 kg a.i./ha that was conducted in a typical boreal forest watershed of northern Ontario, Canada. A particular focus of this study was on the fate and effects of the more toxic form of triclopyr (BEE) in the stream under a worst case scenario of direct overspray [81]. Results showed an average deposit at the stream surface of 3.67 kg a.i./ha with BEE residues in stream water exhibiting instantaneous maxima of <0.35 mg a.i./L. A series of diminishing pulses were observed resulting from direct inputs during overspray of the stream channel upstream. Average concentrations of the BEE in stream water ranged from 0.05 to 0.11 mg/L during the first 12 to 14 h monitoring period and were below limits of detection within 72 h. Both the average concentrations and exposure durations observed in this field study were substantially below levels generating acute lethal responses for various aquatic organisms in either lab or field studies [e.g. 83-87]. Initial whole body tissue residues in samples taken from fathead minnow cages *in situ* at the downstream location (43 mg a.i./kg) were similar to those predicted from simulation models [80]. No statistically significant mortality was observed in three species of aquatic organisms (yellow perch, caddisflies or fathead minnows) caged *in situ* either in treated or control areas. The authors concluded that natural dissipation mechanisms including photolysis, hydrolysis and microbial action limited exposures to sublethal levels and that based on this study, significant impacts to aquatic organisms would not be anticipated under operational conditions where such streams would be protected by buffer zones of 60 to 100 m. Similarly, in a field experiment in which trclopyr BEE (Garlon 4) was directly injected directly into a small headwater forest stream, intensive sampling [82] showed maximal aqueous concentrations of 0.848 and 0.949 mg a.i./L at the monitoring stations nearest two discrete injection points. Average BEE concentrations ranged from 0.32 mg a.i./L at stations nearest injection points to 0.02 mg a.i./L approximately 225 m downstream. Results demonstrated rapid conversion of the BEE to triclopyr acid in this system, as well as significant sorption of the chemical to natural allochthonous (deciduous leaf pack) materials. Resultant short-term, pulse-type exposures of BEE were observed with magnitude decreasing and duration slightly increasing with downstream distance. Resultant exposure regimes failed to induce any mortality of resident brook trout, nor were there significant effects on the growth of 1 or 2 year old brook trout.

In contrast to the results of lotic system experiments, substantial toxicity to a variety of aquatic organisms has been observed in lentic studies characterized by longer duration of exposure to the more toxic BEE form of triclopyr. Kreutzweiser *et al.* [83] conducted a dose-response study on fish caged within *in situ* enclosures in a northern Ontario lake. Results showed median dissipation times for aqueous residues ranging from 4 to 8 days. All caged rainbow trout exposed to initial concentrations greater than 0.69 mg a.i./L died within 3 days and 43% mortality was observed at 0.45 mg a.i./L whereas no mortality was observed at the 0.25 mg a.i./L level. Using similar *in situ* enclosures in two different wetland ecosystems, Wojtasek *et al.* [84] studied the effects of triclopyr BEE (Release) on mortality, avoidance response, and growth of larval amphibians (*Rana clamitans*, *Rana pipiens*). A range of treatment concentrations were applied to yield nominal concentrations ranging from 0.26 to 7.68 mg a.i./L. Concentration-dependent mortality and abnormal avoidance response were observed but there were no significant effects on growth. Toxicity for the two test species (*R. clamitans* and *R. pipiens*) were less than those observed in prior laboratory studies [85-87], probably due to the rapid dissipation of BEE which showed a DT50 of less than 1 day in both of these shallow wetlands. The authors noted that LC10 and EC10 endpoints approximated aqueous concentrations of 0.59 mg a.i./L that is within the range for expected environmental concentrations in small wetland amphibian breeding habitats under direct aerial overspray scenarios, thus presenting a potential risk of impacts for a small proportion of native amphibian larvae. This conclusion was consistent with results of laboratory microcosm studies in which Chen *et al.* [88] showed that triclopyr BEE (Release) at environmentally relevant test concentrations (0.25 and 0.50 mg a.i./L) resulted in significant decreases in survival of both larval life stages of *R. pipiens* and a common wetland zooplankton species *Simocephalus vetulus*. Moreover results indicated that effects on amphibians and zooplankton may be amplified by other concomitant stressors such as low food availability or low pH.

Overall, risk assessments for triclopyr BEE based on early tier experiments identified a substantial risk of acute toxicity to fish, amphibians, zooplankton and aquatic invertebrates, particularly in lentic systems where dissipation of the ester form is limited in some way. The presumption of risk was confirmed by subsequent field studies in scenarios where longer term exposure to the more toxic ester form occurred, but not in lotic scenarios where the

duration of exposure to the ester was too short to attain toxic thresholds in aquatic organisms. Results emphasize the particular importance of understanding both the duration and magnitude of exposures that occur in real-world systems and the need for considering such natural exposure regimes when designing or interpreting research results and also when considering potential mitigative measures.

FOREST-USE INSECTICIDES

As compared to herbicides, fewer insecticides find widespread use in the forest sector internationally. Among those most commonly in use are the biological control agent *Bacillus thuringiensis* var. *kurastaki* (Btk) and the unique chitin-formation inhibiting chemical pesticide diflubenzuron (Dimlin®) (Table **1**). The use pattern for these products is highly sporadic with amounts applied varying dramatically in relation to the extent and severity of major insect pest outbreaks. Unlike herbicides, applications of insecticides are typically made to protect semi-mature or mature high value timber stands. Defoliating insect pests of significance historically in North America include the gypsy moth, spruce budworm, western spruce budworm, blackheaded budworm, jack pine budworm and Douglas fir tussock moth. Data provided by the USDA-Forest Service [16] indicates that of the total area treated for gypsy moth in the northeast region, 77% received applications involving Btk, while approximately 22% of the area was treated with Dimilin. In Canada, Btk is by far the most commonly used product accounting for approximately 86% of forest insecticide use [8], with the remainder being primarily tebufenozide (MIMIC). In the UK only four active ingredients were registered in 2004 as chemical insecticides for use in forestry [89]. Selected case studies for Btk and diflubenzuron are presented below to illustrate specific ecotoxicological issues of interest associated with forest uses of these active ingredients.

Increasingly, invasive insect species such as the mountain pine beetle, emerald ash borer, Asian long-horned beetle and brown spruce longhorn beetle are posing new and significant ecological and economic risks to the forest sector in North America [90, 91]. Similar invasive insect pest problems threaten forests in other countries, and often these are occurring in urban forest environments presenting several unique issues. For example, broadcast insecticide applications, as typically used against the major defoliating insect species, may be ineffective or publicly unacceptable as controls for invasive wood boring species. These issues have prompted the development and use of novel systemic injection techniques, as well as natural product insecticides such as azadirachtin [91] as alternative control techniques within broader integrated pest management strategies. A recent review [92] documents the environmental fate and effects information associated with several of these compounds which are purported to represent “reduced risk”.

Bacillus Thuringiensis Var. Kurstaki (Btk)

Among several strains of *Bacillus thuringiensis* with notable insecticidal activity, the proteinaceous crystalline toxin of Btk is known to be highly specific to larval Lepidoptera [93, 94]. The mechanism of action of Btk in Lepidoptera is the result of toxin induced rupture of the midgut followed by spore germination and septicemia in the body cavity that eventually results in death [95]. Several different formulations of Btk (see some examples in Table **1**), are used extensively in the USA and Canada, as well as for the control of Lepidopteran insect pests worldwide. One example of the latter is the use of Btk in an attempt to eradicate the invasive painted apple moth in New Zealand for which a comprehensive impact assessment has been published [96]. In North America, for major pests such as gypsy moth, spruce budworm, jack pine budworm and hemlock looper, applications are typically made by aircraft. Unlike conventional pesticides, the potency of Btk formulations is determined based on standardized bioassay response and reported in terms of Billions of International Units (BIUs). Typical application rates for Btk range from approximately 60 to 90 BIU/ha.

Results of published risk assessments [18, 96] indicate that given their highly specific mode of action, Bt products are unlikely to pose a significant hazard to vertebrates, fish, birds or insects other than macrolepidopteran larvae. Bt occurs naturally in soils throughout the world. The vegetative form of Btk does not generally persist in soil; however, endospores can survive in most types of soils for extended periods with half-lives of spores usually in the range of 100 to 200 days [97]. As noted in the New Zealand environmental impact assessment document [96], estimates on persistence of Bt toxins vary widely and there is some evidence to suggest that binding of Bt toxins to humic acids, organic supplements or onto soil particles protects the toxins from microbial degradation, without eliminating their

insecticidal activity. Leaf litter and soil samples collected following aerial spray Foray 48B for control of white-spotted tussock moth in Auckland, showed significantly enhanced levels of Btk-like isolates up to two years post-spray. Laboratory studies by Visser and other workers [98], had previously shown that formulated Btk products generally had no effect on functional parameters associated with soil microflora.

The New Zealand environmental impact assessment generally suggested that relative to all available options, Btk was likely to be the most acceptable approach for attempted eradication of painted apple moth in the urban area of Auckland, from a public, economic, efficacy and environmental perspective. The World Health Organization specifically concluded that "Bt products may be safely used for the control of insect pests of agricultural and horticultural crops as well as forests". While such comprehensive assessments typically support the use of Btk as environmentally acceptable, there are concerns associated with potential ecotoxicological impacts on non-target Lepidoptera and derivative indirect effects on insectivorous species, particularly birds, which may depend upon these organisms as a primary food source, as well as potential effects on non-target aquatic insects.

Several studies demonstrate that Btk causes immediate reductions in abundance and species richness of non-target larval Lepidoptera [99-102]. Butler and co-workers [103] conducted extensive studies on this aspect following applications of Btk for control of gypsy moth in oak forests of West Virginia, USA. During the treatment year, Btk produced significant decline of canopy-dwelling macrolepidopterous larvae. No differences in abundance of various caterpillar species were observed among treated and control plots during the weeks or months following treatment. Similarly, no difference between treated and control plots were observed in abundance of most species in 1992, the first post-treatment year. Non-lepidoptera species also appeared to be unaffected by the Btk treatment. The fact that abundance and richness of non-target lepidopteran larvae declined during each year of the three year study, even on non-treated plots, emphasizes the importance of using appropriate controls in field studies of this type. It also underscores the need to consider pesticide-induced perturbations in light of the natural variation in abundance that may occur due to both random and non-random factors which typically influence biological systems in natural environments.

Boulton *et al.* [99] assessed the impacts of Btk (50 BIU/ha as Foray 48B) on native, non-target Lepidoptera following treatments to 12,805 ha of Garry oak forests for control of gypsy moth in southeastern Vancouver Island, British Columbia, Canada. Significant variation in diversity among the Lepidoptera were not detected, but reduced richness and abundance on two different host plant species were observed. The authors noted potential concerns associated with such effects, particularly in highly fragmented forest stands such as those associated with urban or industrial areas. They also emphasized the importance of such effects on rare and endangered non-target lepidopteran species such as *Euchloe ausonides isulanus*, and *Euphydryas editha taylori* which are found only in oak meadows and rocky knolls. In a follow-up study, Boulton *et al.* [100] examined longer term recovery of non-target Lepidoptera noting that reductions were greatest one year post-treatment. Relative to the reference sites, each of 11 species that were initially reduced by the Btk applications showed an increase in the treatment sites within the next 3 years, by which time only four species remained significantly reduced in the treatment sites. The uncommon species were significantly reduced in the year of treatment but not one or three years post-treatment. Results of this study highlight the importance of long term monitoring following pesticide induced perturbations in relation to understanding the rate and process by which recovery in ecosystem structural or functional processes may occur. The study also emphasizes the important consideration of effects on rare and endangered species. In general, and somewhat surprisingly, this aspect has does not appear to have received sufficient scientific attention. In the case of Btk, such a concern has been raised for the Karner blue butterfly. Herms *et al.* [104] conducted a field survey and laboratory bioassay demonstrating significant dose-dependent mortality in response to Btk treatments and found that early and late instars were equally susceptible. The authors concluded that the Karner blue is both phenologically and physiologically susceptible to Btk as employed for gypsy moth suppression, even though the larval generation at risk and extent of phenological overlap may vary from year to year.

In cases where direct effects on species in one trophic level deleteriously affect those in other trophic levels, concerns over ecological implications are heightened. In the case of forest-use insecticides with highly specific modes of action, such as Btk, potential indirect effects of reduced prey or food availability on insectivorous predators are of particular interest. Two separate studies [105, 106] have examined such indirect effects on insectivorous birds and small mammals in Ontario, Canada. As would be anticipated, substantial reductions in

Lepidoptera larvae were observed in response to treatment. Many adult male shrews apparently emigrated and were replaced by young males and females. Effects on Nashville warbler and hermit thrush, chicks of spruce grouse, and adult male masked shrew were all attributed to indirect effects associated with reduction in the primary insect food source. Holmes *et al.* [105] further examined the hypothesis that food reductions caused by forest spraying with Lepidoptera-specific insecticides would affect songbird behaviour and reproduction. The comparative study of Tennessee warbler nests and parental behaviour involved spray blocks treated with Btk, tebufenozide (MIMIC) or left as an untreated control area. Nestling survival and growth were unaffected by the insecticide treatments. Nests in the treated blocks had smaller clutches, smaller broods and lower hatch rates than nests in the control block, but these differences were not statistically significant. Nestling diets were similar in the MIMIC and control blocks. There were slight differences in the behaviour patterns of female Tennessee warblers in the MIMIC and control blocks, with those from MIMIC treated spending less time at the nest and more time foraging. The authors concluded that the indirect effects of forest spraying with Lepidoptera-specific insecticides pose little risk to forest songbirds. Differential results may reflect species-specific behaviours, food preferences and ability to prey switch or broaden foraging ranges. The equivocal nature of these field study results, suggests that strategic species-specific biomonitoring and population modeling in conjunction with operational spray programs may be warranted to provide more conclusive evidence with regard to possible ecological consequences at broader spatial scales. Other studies have also documented potential indirect effects of Btk spraying associated with reductions in natural food for breeding black-throated blue warblers [107] and an endangered species – the Virginia big-eared bat [108].

Kreutzweiser *et al.* [109, 110] conducted a series of studies to examine the effects of Btk, as two different aqueous formulations of Dipel, on several aquatic invertebrate species (various species of Ephemeroptera, Plecoptera or Trichoptera). Results showed no significant mortality, drift response or consumption of treated leaf disks at levels well above label rates or expected environmental concentrations using either flow-through laboratory experiments or outdoor stream channel experiments. Although trends of reduced decomposition activity in treated outdoor stream channels were observed, there were no significant differences in mass loss of leaf material between treated and control channels. These results from laboratory and controlled field experiments indicated that contamination of watercourses with Btk is unlikely to result in significant adverse effects on aquatic invertebrates or microbial community function in terms of detrital decomposition. In a confirmatory field study, Kreutzweiser *et al.* [111] treated a section of a natural forest stream with Btk at 10 times the expected environmental concentration (200 BIU/mL) to determine effects on the aquatic macroinvertebrate community. Invertebrate drift density increased slightly, but only during the 0.5-h application and only at the site 10 m below the application point. There were no significant changes in taxonomic richness of benthic invertebrates after the application, but there were short-term alterations in community structure at the treated site after the application, as measured by a dissimilarity index. In 11 of 12 benthic taxa for which there were sufficient data, changes in abundance after the application were not significant compared with changes in abundance at the reference site. The stonefly *Leuctra tenuis* (Pictet) was reduced by ~70% at the treated site 4 days after the application, and abundance of this stonefly remained considerably lower, but not significantly different, from the reference site for at least 18 days. A follow-up study demonstrated that under laboratory conditions, Btk on leaf material was not toxic to *L. tenuis.* The Btk application had no significant effect on the growth or survival of caged caddisfly larvae, *Pycnopsyche guttifer*, in the treated stream.

Diflubenzuron

Diflubenzuron is a benzoyl-phenylurea insecticide that inhibits chitin deposition in arthropods. It is effective either as a stomach or contact insecticide. In forestry, diflubenzuron sees major use principally in the USA for suppression of gypsy moth. Two formulations (Dimilin 4L and Dimilin 25W) are registered in the USA and the active ingredient is also efficacious against tent caterpillar and several other forest insect species including pine false webworm [112] and eastern hemlock looper [113]. For suppression of gypsy moth, diflubenzuron may be applied *via* either ground or aerial methods at rates ranging from 9 to 75 g a.i./ha. Typical use scenarios under severe infestation conditions may involve multiple applications over several years.

In a synoptic review of potential environmental effects of diflubenzuron [114], adverse effects on crustacean growth, survival, reproduction, and behaviour have been observed at environmentally realistic levels ranging from 0.062 to 2 µg/L. Rebach and French [115] examined the effects of diflubenzuron on blue crabs and provide a review of potential effects in marine and estuarine environments. This review demonstrated substantial toxicity to these

species. Surprisingly, there appear to have been no field studies investigating potential effects on freshwater crayfish which are likely to be directly exposed during forest use. Mayflies, chironomids, caddisflies, and midges also have known sensitivity to diflubenzuron at similar aqueous concentrations, showing low emergence and survival as typical impacts. Fischer and Hall [116] reviewed the environmental fate and effects data on diflubenzuron with particular emphasis on aquatic systems. Organic matter and aquatic macrophytes are major factors influencing the adsorption and degradation of the compound. Reardon [117] presented an overview of field experiments examining the potential impacts of diflubenzuron (Dimilin 4L) on selected non-target organisms in an experimental broadleaf forest in West Virginia. Five non-target groups were monitored, including: fungi, bacteria, and invertebrates in leaf litter and soil; aquatic macroinvertebrates; canopy arthropods; pollinating insects and aquatic and terrestrial salamanders. Initial concentrations of diflubenzuron and degradation of residues on tree surfaces, in leaf litter, in soil, and in water were also determined. Except for aquatic macroinvertebrates, canopy arthropods, and native pollinating insects, there were no detectable effects of the treatment on the non-target groups. Diflubenzuron treatments were shown to decrease the densities and survival of several species of mayflies, stoneflies and a cranefly and reduce richness and abundance of non-target terrestrial arthropods, primarily macrolepidopterans and yellow jackets. Durkin [118] characterized the scientific database supporting the risk assessment of diflubenzuron (Dimlin) in forestry as large and somewhat complex, but concluded that direct effects of diflubenzuron on mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates were considered implausible, largely owing to the specific mode of action for this compound. Just as for Btk, the fact that diflubenzuron is an effective insecticide against Lepidoptera results in a substantial likelihood of effects on other non-target members of this group, as well as indirectly on insectivorous species such as birds, which may specifically rely on these insect populations as their primary food source. Potential effects on aquatic invertebrates were also considered possible depending upon site-specific conditions controlling deposition to surface waters and thus resultant exposure levels.

Numerous laboratory studies have demonstrated the sensitivity of aquatic invertebrate species to diflubenzuron. Hansen and Garton [119] showed that among complex stream faunal communities in the laboratory, mayflies and stoneflies were affected at 1.0 µg/L and that crustaceans were also particularly sensitive. These authors also noted that single species toxicity tests adequately predicted direct lethal effects, but not indirect effects resulting from altered interspecies interactions. Liber *et al.* [120] conducted an elegant field mesocosm experiment using a concentration-response design approach and derived field EC50 values for insect emergence inhibition ranging from 1.0 to 1.4 µg/L. Overall, they concluded that significant adverse effects on insect emergence could be expected at diflubenzuron concentrations of >1.0 µg/L with the time to recovery being concentration dependent. Boyle *et al.* [121] also employed outdoor mesocosms in a study exploiting the unique mode of action of diflubenzuron to examine the indirect responses following direct impacts at the primary consumer (*i.e.* invertebrate) trophic level. Direct reductions in invertebrate grazers caused indirect increases in algal biomass. Indirect effects including 50% reductions in biomass and in individual weight of juvenile bluegill occurred because of apparent decreases in invertebrate food resources. In contrast, no statistically significant impacts were observed on adult bluegill or largemouth bass for the duration of the experiment. Results indicated that diflubenzuron had both direct and indirect impacts on the experimental aquatic ecosystems under the conditions tested and although treatment regimes were not environmentally realistic in relation to forest use patterns, the study provides an excellent example of potential indirect secondary and tertiary effects in aquatic systems that would be difficult if not impossible to determine under laboratory conditions. In a semi-operational field study in mixed wood forests of central Ontario, Canada, Sundaram *et al.* [23] reported that the fate of diflubenzuron residues following aerial applications of Dimlin 25W at a rate of 0.07 kg a.i./ha. Results indicated that dissipation patterns differed among water, sediment and aquatic vegetation substrates, with reported DT50 values of <1.3 days in pond water and <14 days in all cases. Zooplankton and benthic invertebrate populations were monitored for up to 110-day post-spray in two over-sprayed ponds with comparison to control ponds. Significant mortality occurred in two groups of caged macroinvertebrates (amphipoda and immature corixidae) 1 to 6 days post-treatment. Three taxa of littoral insects (*Caenis*, *Celithemis* and *Coenagrion*) were also significantly reduced in abundance in the treated ponds 21 to 34 days post-treatment, but recovered to pre-treatment levels by the end of the season. Zooplankton (cladocerans and copepods) populations were reduced 3 days after treatment and remained suppressed for 2 to 3 months.

Harrahy *et al.* [122] noted that diflubenzuron may persist on hardwood leaves throughout the growing season up until the time of leaf fall. Non-target aquatic organisms that consume these fallen leaves may therefore be exposed to

the pesticide for a significant period of time. Several field studies have further investigated the potential effects of diflubenzuron on sensitive non-target aquatic invertebrates and confirmed effects under environmentally realistic scenarios. Griffith *et al.* [123] used Malaise traps to monitor emergence and flight distances of adult Plecoptera and Trichoptera from headwater streams in two different catchments of an experimental forest in West Virginia, USA before and after application of diflubenzuron. Stonefly, *Peltoperla arcuata* emergence was reduced in the first 4 months after treatment, as compared with the untreated catchments, however no differences in emergence of other species were observed. In a follow-up study [124], the flight of the stonefly *Leuctra ferruginea* was reduced in the treatment watersheds compared with the reference watersheds during the year following abscission of the treated leaves. Adult flight of other species did not decrease in the treatment watersheds during 1993. These results suggest that among aquatic invertebrates, stoneflies may be particularly sensitive to the effects of diflubenzuron even under scenarios of a single application. The authors noted that multiple applications of diflubenzuron over several years, which often occurs during gypsy moth suppression programs, may present a significant risk to these aquatic species. Similarly, Hurd *et al.* [125] observed significant reductions in the abundance of several taxa in treatment as compared to control watersheds following aerial application of diflubenzuron (Dimlin 4L) at a rate of 70 g a.i./ha. Most affected taxa included the stoneflies, *Leuctra* sp. and *Isoperla* sp., the mayfly, *Paraleptophlebia* sp., and the cranefly, *Hexatoma* sp. In a functional context, shredders were the dominant group affected, with reduced mean densities in treatment watersheds whereas densities of species such as Oligochaeta and Turbellaria increased in streams in treated watersheds. The authors again emphasized that since most aquatic insects oviposit in the watershed from which they emerge, repeated applications of diflubenzuron could have longer-term localized effects on invertebrate fauna in treated streams. In contrast to these studies, Boscor and Moore [126] studied the impacts of Dimilin at 70 g a.i./ha to a one-half mile stretch of White Deer Creek in central Pennsylvania. No spray-induced, adverse effects were detected on the organisms sampled, principally Ephemeroptera, Chironomidae, Trichoptera, and Plecoptera, for a period of up to 28 days after treatment.

The potential effects of diflubenzuron on non-target terrestrial insects have also been extensively examined. Sample [127] reported that the operational application of Dimilin [70, 75 g a.i/ha] resulted in greatest impacts on Lepidoptera which displayed reduced abundance and species richness at treated sites. No effects were observed among Coleoptera, Diptera, or Hymenoptera. Butler *et al.* [128] summarized results of a 6-year study conducted to evaluate the impact of diflubenzuron on the diversity and abundance of arthropods in West Virginia. Based on foliar sampling, overall arthropod family diversity and abundance, numbers of macrolepidoptera and beetles were significantly reduced in treated watersheds. Total arthropod abundance and macrolepidoptera abundance remained at significantly lower levels up to 27 months post-treatment. As noted by Durkin [126] some secondary effects resulting from reduced Lepidoptera prey may include increased foraging range, relocation and lower body fat content among foraging birds species. For example, Whitmore *et al.* [129] showed significantly lower fat reserves in seven of nine tested bird species following Dimlin applications to forests in the USA. Possible causal factors were listed as reduction in food availability and decreased biomass ingestion, increased energetic expenditures required in obtaining scarce food and reduced food quality in treated as compared to control sites. The latter study is an example of an investigation on functional (community energetics) rather than structural effects of pesticide use in forest ecosystems, an area which is generally under-studied. Another example is the study by Paulus *et al.* [130], who compared three methods for assessing the impacts of forest-use insecticides diflubenzuron and Btk on biological activity of soil organisms. While results were dependent on the monitoring technique employed, overall findings demonstrated transient effects on biological activity of soil organisms exposed to diflubenzuron but not Btk.

CONCLUSION

The cumulative wealth of scientific data available for modern forest-use herbicides and insecticides is extensive. Research spans multiple tiers of testing ranging from simple laboratory studies, through microcosm and *in situ* mesocosm studies and includes several comprehensive large scale field experiments. Higher tier field studies provide several unique benefits that are considered highly contributory to comprehensive ecotoxicological risk assessments. As many previous authors have suggested, it is impossible to replicate natural ecosystems, inclusive of all of their innate and interactive physical, chemical and biological components in the laboratory. In ecotoxicological risk estimation, direct use of data from any laboratory study carries the critical and highly questionable assumption of equivalence of the test system and the real world. As such, it is very prudent to continue the use of *in situ* mesocosm, manipulative field studies and long-term monitoring to confirm that extrapolative predictions based on

early tier laboratory studies are in fact valid. In higher tier field studies, experimentation should be focused on typical operational as well as worst case maximal use rates with common end-use products such that results incorporate any potential effects associated with surfactants or other formulants contained therein. In terms of response variables, these should be focused on population or community level response and recovery time, as these are typically most relevant to regulatory and policy decision making and involve levels of biological organization and interaction mechanisms (e.g. predation, competition, commensalism) that cannot be effectively simulated in laboratory experiments. Examination of the case studies presented here highlight all of these unique benefits as well as the overriding value of large scale field experiments in terms of negating or confirming risk. While these benefits and values are particularly important in forestry scenarios owing to the typically large scale of operations, similar advantages apply to field experimentation in other sectors as well.

Given the relatively specific mode of action of many modern pesticides, their general high water solubility and facile environmental degradation and metabolism, environmental concerns associated with modern forest-use pesticides differ significantly from historic issues associated with mass mortality, long-term persistence and bioaccumulation. Potential ecotoxicological impacts associated with modern day synthetic, natural product and biological pest control agents are likely to be much more subtle and are commonly associated with indirect or secondary effects associated with habitat alteration, reduced food resources or multiple stress interactions. For glyphosate and Btk, respectively the dominant herbicide and insecticide used in the forest sector internationally, case study evaluations reviewed here support the conclusions of several more comprehensive risk assessments. Based on the weight of scientific evidence currently available, these data and risk assessments suggest that the judicious use of these products, in accordance with product labels, pose little risk to forest environments or non-target wildlife species. In contrast, higher tier field studies conducted with triclopyr ester and diflubenzuron, confirm specific risks under environmentally realistic or operational conditions imposing a requirement for mitigative actions sufficient to negate the risk. In such cases it is considered prudent to use adaptive management strategies, including operational chemical and biological monitoring of small scale operational test programs to ensure that mitigative actions do in fact protect sensitive values and general ecological integrity of receiving environments. A highly positive sidelight of detailed operational monitoring studies is the ability to generate both exposure and effects data critical to effective probabilistic risk analysis. Overall, case study analyses presented here support the continued judicious use of pesticides as part of sustainable forest management. In cases where risks are identified, appropriate mitigative measures may still allow them to be employed where no other effective options exist.

With emphasis that the scientific knowledge base associated with potential ecotoxicological effects of major forest-use pesticides is both extensive and detailed, there are, as always, some areas where further research would be considered particularly valuable. These focus areas include: (a) evaluation of potential interactive effects of tank-mixed herbicides; (b) assessment of plausible multiple stressor interactions (e.g. chemical and concomitant drought stress); (c) investigations on impacts on key ecosystem functional processes; (d) development and application of cost-effective operational monitoring techniques applicable over broader spatial scales and longer time frames than typical empirical studies; (e) application of probabilistic analyses and; (f) risk assessments that include population modelling over larger spatial and temporal scales.

Relative to the available information base on forest-use pesticides, our scientific knowledge on potential impacts of alternative vegetation or insect pest control techniques is exceedingly weak. As noted by Scriber [131], all pest management programs carry some risk of negative environmental impacts; this includes the “do nothing” option. In general, it is inappropriate to assume that biological controls, natural pesticides or other non-chemical approaches pose no risks to ecological integrity of forest ecosystems. In fact there are several lines of evidence that clearly demonstrate this assumption to be invalid – see Thompson and Kreutzweiser [92] as but one example. It is imperative that all options with potential use in integrated pest management strategies be equally scrutinized against cost, effectiveness and environmental acceptability criteria. One, particularly valuable means of conducting such comparisons is through direct side-by-side multi-disciplinary field studies conducted at semi-operational or operational scales. Finally, from the ecological perspective alone, the potential deleterious effects associated with the “do nothing option” or with the use of ineffective options may in fact be greater than those associated with pesticide treatment or other pest control alternatives. As a generality there appears to be far too little scientific or policy attention paid to this aspect. Similarly, there may also be significant economic implications of weakly effective or non-intervention strategies. Multiple cases of exotic invasive plant or insect pests as currently extant in

the North American forest sector and elsewhere around the globe are unfortunately providing demonstrable evidence supporting the point that ineffective or non-intervention options are often not acceptable in either ecological or economic terms. Within an integrated management strategy, those options which best meet the three fundamental criteria of efficacy, economics and environmental acceptability should be made available and used by resource managers in optimizing the twin goals of sustainable resource use and protection of ecological integrity. All organisms, including humans, as integral components of forests and other global ecosystems are ultimately dependent on the successful achievement of those goals.

ACKNOWLEDGEMENTS

The author would like to acknowledge the financial contributions and support of the Canadian Forest Service, Department of Natural Resources Canada in making this chapter possible and to thank S. Holmes, D. Kreutzweiser, M. Coppens and two anonymous reviewers for their very helpful review and suggestions for improving the draft manuscript.

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CHAPTER 6

Impacts of Pesticides on Freshwater Ecosystems

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Abstract: Pesticides can enter surface waters via different routes, among which runoff driven by precipitation or irrigation is the most important in terms of peak concentrations. The exposure can cause direct effects on all levels of biological organisation, while the toxicant mode of action largely determines which group of organisms (primary producers, microorganisms, invertebrates or fish) is affected. Due to the interconnectedness of freshwater communities, direct effects can entail several indirect effects that are categorised and discussed. The duration of effects depends on the recovery potential of the affected organisms, which is determined by several key factors. Long-term effects of pesticides have been shown to occur in the field. However, the extent of the effects is currently uncertain, mainly because of a lack of large-scale data on pesticide peak concentrations. In the final section, we elucidate the different approaches to predict effects of pesticides on freshwater ecosystems. Various techniques and approaches from the individual level to the ecosystem level are available. When used complementary they allow for a relatively accurate prediction of effects on a broad scale, though the predictive strength is rather limited when it comes to the local scale. Further advances in the risk assessment of pesticides require the incorporation and extension of ecological knowledge.

INTRODUCTION

Modern agricultural practices rely on the usage of synthetic pesticides (mainly herbicides, fungicides and insecticides) in order to prevent losses by pests [1]. The global pesticide production reached significant levels after the Second World War and rose sharply from approximately 500,000 t/a in the 1950s to over 3 million t/a at the beginning of the 21st century [2]. This trend will probably continue over the next decades because of a demand for higher food production as the human population increases, monocultural production for biofuels and potentially introduction of new pests in many areas associated with climate change [2, 3], though introduction of pest-resistant plants and an increase in organic farming and integrated pest management may counter this trend. Given the large amounts of pesticides applied globally and given the fact that they are designed to harm biota, there is a high potential for adverse environmental effects also on non-target communities [4]. When pesticides enter freshwater ecosystems, they do interact with the biotic and abiotic components of the ecosystem. Abiotic factors can lead to degradation (photo-decomposition by sunlight or hydrolysis by water) or adsorption of the compounds on sediment or organic matter. The interaction with the biotic parts comprises uptake, metabolisation and accumulation in organisms, which in turn may lead to adverse affects on the freshwater biotic community. These adverse effects are the topic of this chapter and will be delineated in depth after a brief overview of the entry routes of pesticides in freshwater ecosystems.

Once released into the environment, pesticides can be subject to airborne and waterborne entry in aquatic ecosystems. Airborne processes encompass wind drift during pesticide spraying (spray drift) and volatilisation after application with subsequent atmospheric transport that may lead to the deposition of compounds in remote ecosystems (thousands of kilometres) from their initial application. For example, organochlorine insecticides such as dichloro-diphenyl-trichloroethane (DDT) and lindane (□-HCH), which exhibited high usage patterns from the 1950s to the 1970s have become ubiquitous in the environment due to their high environmental persistence and potential for long-range atmospheric transport [5, 6]. Organochlorines are at present even detected in the polar regions, although they were never applied there [6]. However, for the majority of currently used pesticides atmospheric transport is confined to regional translocations within a 300 km radius as they are less persistent and have a lower potential for long-range transport [7, 8]. The waterborne translocation of compounds is driven by precipitation events or irrigation. Precipitation and irrigation can wash compounds from the field into adjacent surface waters via runoff or subsurface flow or into the groundwater.

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The quantitative relevance of the exposure route (airborne or waterborne) varies depending on physicochemical properties of the compound as well as the geographical, geological, hydrological and climatic conditions and crop type. For Germany, a modelling study on the exposure routes estimated the input of 65%, 10% and 25% of diffuse pesticide load by field runoff, flow in drainage channels and spray drift, respectively, though this study did not include atmospheric transport [9]. Spray drift is greatest when the spraying is conducted aerially using aircraft and for crops such as vineyards or orchards where the spraying occurs in a horizontal direction [10]. However, several studies emphasised the relevance of the waterborne exposure route concerning pesticide concentrations as reviewed in [11]. A study in North Germany showed that concentrations in a small headwater stream were elevated by several orders of magnitude during heavy rainfall events in the pesticide spraying period [12]. For cotton in Australia, the endosulfan concentrations in a creek and a river were approximately 10-fold higher during runoff events than as a consequence of spray drift [13]. Similar observations were made in the Lourens River in South Africa with runoff-associated pesticide concentrations at the first strong rain event after pesticide application being approximately 50-fold higher for two pesticides compared to the spray drift concentrations [14]. In irrigation farming of rice in Japan, short-term peak concentrations of pesticides in adjacent water bodies occurred in association with heavy rain or irrigation events [15, 16]. Overall, for water bodies in agricultural areas, intensive rainfall (> 10 mm per day) or irrigation with consequent runoff and subsurface flows after pesticide applications is recognised as the most important route of entry, both resulting in episodic short-term peak pesticide concentrations. In rivers that are fed by agricultural tributaries, the exposure may be more continuous due to dilution and overlapping input from different tributaries, but is still seasonal.

The pattern of episodic peak concentrations has to be considered in investigations on the effects of pesticides on aquatic biota. Although a large number of studies has been conducted on the effects of pesticides in surface waters, the majority failed to clearly link effects to exposure, partly because the study design did not include a sufficient quantification of pesticide peak concentrations [11]. Hence, an exposure monitoring method should be adopted that captures runoff events in the spraying period. Automatic continuous water sampling or automatic event-triggered water sampling can be employed, but these methods are cost- and labour-intensive [12, 17, 18]. Therefore, alternative methods have been suggested such as a less costly version of an event-triggered water sampler [19], a suspended matter sampler for particle-associated hydrophobic pesticides [20, 21] or passive sampling using adsorbent membranes if continuous background exposure is absent or negligible [22]. To sum up, the determination of pesticide peak concentrations in the water bodies is crucial in studies on the effects of pesticides on freshwater organisms.

DIRECT EFFECTS OF PESTICIDES

The freshwater community consists of different groups of organisms such as fish, amphibians, invertebrates, plants or microorganisms. Pesticides can have direct and indirect effects on these organisms. Direct effects are caused by the physiological action of a pesticide within an organism. However, the biotic community is characterised by ecological interactions between species such as competition or predation and indirect effects refer to effects mediated via these interactions [23]. For example, mortality of water fleas as a direct result of exposure to a pesticide (direct effect) may lead to an increase of algae biomass due to a release from grazing pressure (indirect effect). We will firstly focus on direct effects as they are a prerequisite for understanding the indirect effects.

In general, direct effects of chemicals on an organism depend on the concentration; i.e. the dose determines the poison (Paracelsus). However, some further general factors influence the occurrence and magnitude of adverse effects:

- exposed life-stage: different life stages of organisms can be affected differently by the same exposure with younger life stages of fish, amphibians and invertebrates being in general more susceptible [24, 25];
- exposure duration: generally a longer exposure time leads to stronger effects [26, 27];
- biomagnification can imply a temporal delay in effects: for example, organochlorine pesticides can biomagnify along the food web, resulting in several orders of magnitude higher concentrations per lipid weight in organisms at the top of the food web [28];
- presence of additional stressors: the effect of a single compound can be enhanced in the presence of other pesticides [29, 30] or other stressors such as UV radiation [31], parasitism [32], predation [33] and food scarcity [34];

- population density: toxicant-induced effects on individuals in high density populations can reduce the negative intraspecific interaction and therefore be compensated [35], though the population structure may remain altered as the toxicant exhibits age-dependent mortality or delay in development [36, 37];
- history of the community: previous exposures to toxicants can modify the response of a community either by an acquired tolerance (pollution-induced community tolerance (PICT)) or a higher sensitivity [38-41].

These factors can, together with the biological and physicochemical characteristics of the exposed ecosystem, modify the potential direct effect of a pesticide entering a freshwater ecosystem. Therefore, the effects may differ between ecosystems with different modalities of these factors. For example, a certain concentration of a pesticide will most likely have stronger effects on a community of a forest stream that never received pesticide input in comparison to an agricultural stream that is subject to recurring pesticide exposure [42]. However, our knowledge about the comparative relevance and the differences in the modalities of these factors between natural ecosystems is very limited and does not allow for a generalisation of their influence on effects. Nevertheless, when studies are conducted within similar ecosystems (e.g. agricultural streams) or the factors vary only slightly between the sites, the pesticide concentration (or the derived toxicity) should be the most important predictor of the pesticide effect. Indeed, in field studies on the effects of pesticides on invertebrate communities in agricultural streams, most of the variability in community endpoints could be explained by pesticide concentration (or the derived toxicity) alone even across biogeographical regions [43-45]. The period and duration of exposure as well as the history of the communities were relatively similar in these studies. Nevertheless, the amplification of the effects of pesticides through these factors is one explanation why field effects have been reported at levels lower than expected based on laboratory or artificial stream experiments [42, 44]. Moreover, these factors are presumably most important when results from laboratory toxicity tests are extrapolated to the real world.

Pesticides can act on different endpoints that can be classified according to the level of organisation in biotic communities:

- suborganismal; e.g. changes in enzyme activity or chemical signaling [46], reduced respiration [47];
- organismic; e.g. increased mortality [48], change in morphology [49], delayed development [50], change in behaviour [51], increased susceptibility to infections [52] or reduced reproduction [53];
- population; e.g. changes in age structure [37], population growth rate [54] or mortality rate [55];
- community; e.g. changes in community composition [43, 56-59];
- ecosystem; e.g. changes in ecosystem processes [44, 60].

These levels are linked mechanistically in a hierarchical bottom-up order that can be described as a pyramid effect (Fig. **1**) [61, 62], and we outline this conceptual model as follows. A toxicant always acts on the physiological level of an organism first. Subsequently this may lead to individual-level effects such as delayed development or mortality. If the effect is strong and several individuals are impacted, the effect can propagate to the population level (e.g. the population growth rate may change). In cases of severe contamination, some or many populations or even whole groups of organisms (e.g. crustaceans) may go extinct and the niches may be occupied by other species. Thus, a change in community composition would be observed. For example, freshwater sediment microbial communities were exposed to a combination of fungicides, insecticides and herbicides, which lead to an alteration in bacterial community composition [59]. Finally, whether the specific functions of the affected species can be compensated for by other species (*i.e.* the degree of functional redundancy of the ecosystem [63]) ultimately determines the occurrence of an effect on an ecosystem process (e.g. breakdown of organic matter, primary production, nutrient recycling). In general, effects on all subjacent levels are a prerequisite for effects on higher levels of the pyramid (Fig. **1**). However, effects on ecosystem processes may occur without visible effects on the population or community level. For example, some studies in artificial ecosystems demonstrated that although the effects of herbicides on the rate of photosynthesis could be measured, no population effects could be detected among populations of primary producers [64, 65]. In most studies with artificial ecosystems, however, effects on ecosystem processes resulted from community and population level changes [66].

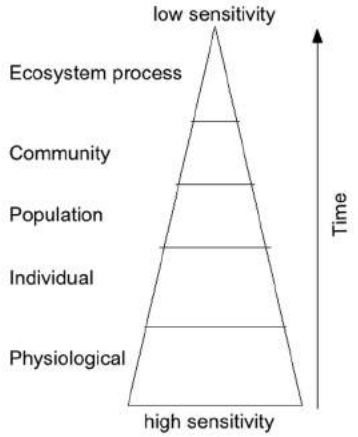

Figure 1: Effect pyramid. Effects on an upper level of biological organisation of the pyramid may occur with a lag phase and require effects on the subjacent levels. This is because higher levels of biological organisation have a lower sensitivity. See text for examples.

Conceptually, effects on lower levels of the pyramid (Fig. **1**) generally occur at lower concentrations than effects on higher levels since higher levels integrate several individuals or populations, of which some will be more tolerant. Thus, higher levels of biological organisation have a higher tolerance for pollution. Moreover, the effects on the different levels of organisation are not necessarily temporally synchronised as it may take from some hours to days for a suborganismic effect to affect individuals and from days to weeks for individual effects to affect populations, communities and ecosystems [61]. Studies on the effects of the pyrethroid fenvalerate demonstrated that population and community disturbance lagged behind and persisted while physiological or individual effects were not detectable anymore [36, 67].

Direct effects of pesticides have been reported on almost all biological endpoints for all groups of freshwater organisms. However, most studies were conducted in the laboratory whereas the focus of this book lies on effects in ecosystems [68]. Therefore, we mainly consider effects that have been observed under field or field-relevant conditions; *i.e.* either detected in field studies or in artificial ecosystems. In addition, we only include direct effects that were reported in the last two decades, so that the findings represent effects of currently used pesticides. Nevertheless, we briefly portray the effects on the aquatic wildlife that were reported since the 1950s and stimulated the writing of the historical book "Silent Spring" by Rachel Carson [69].

The significant agricultural use of organic pesticides, mainly organochlorine insecticides, started after the Second World War [70]. DDT, in particular, was used globally in large amounts for mosquito control and agricultural pest control (70-80% of total DDT used), reaching an annual use of approximately 400,000 t in the 1960s [71]. Reports of effects on wildlife and humans occurred soon after the widespread introduction of organochlorine insecticides [70, 72]. In the aquatic ecosystems, runoff of organochlorine insecticides following rain events in adjacent streams lead to severe fish kills and the eradication of the stream invertebrate fauna over stretches of several kilometres [70, 73]. Also aquatic and terrestrial birds in sprayed regions succumbed to lethal doses [72, 74]. More surprisingly, effects on the reproduction of several fish-eating birds were observed that comprised thinning of the egg shells resulting in the eggs being crushed during nesting, abandonment of nest and egg-eating by the parents [5]. The failure to reproduce affected bird colonies, resulting in population declines in species such as the brown pelican (*Pelicanus occidentalis*), great blue heron (*Ardea herodias*) and herring gull (*Larus argentatus*) [5]. Reproductive effects were even observed in regions with relatively low environmental exposure to DDT and occurred as a consequence of biomagnification of this highly persistent compound along the food chain, so that fish-eating birds at the top of the aquatic food web were finally exposed to biologically effective concentrations [5]. For example, after spraying of the Clear Lake in California for gnat-control, local populations of the western grebe (*Aechmophorus occidentalis*) declined, exhibiting fat concentrations of up to 100,000-fold the lake water concentrations, 400-fold the plankton concentrations and 200-fold the small fish concentrations in fat [75].

In the 1970s several persistent compounds (e.g. DDT, endrin, dieldrin) were banned in most countries due to their unacceptable effects on wildlife [76]. In agriculture, organochlorine insecticides were mainly substituted by the less persistent chemical families of pyrethroid and organophosphate insecticides. Although generally less widespread, effects

on non-target organisms were frequently reported in the 1980s. Between 1977 and 1984 approximately 56% of 128 fish kills in the United States were attributed to pesticide pollution, primarily to the organochlorine endosulfan and the organophosphate malathion [77]. Historically a trend can be observed from compounds with a broad mode of action affecting many non-target species to compounds with a specific mode of action that are less toxic for the majority of non-target organisms. Currently used insecticides such as pyrethroids are characterised by a 1000-fold lower toxicity for mammals compared to organochlorine pesticides [78, 79]. For example, an accidental input of the pyrethroid cypermethrin lead to the complete eradication of invertebrates over a stretch of 3 km but no fish kill was observed [80]. Nevertheless, given that there are usually some organisms in the freshwater community that are physiologically related to terrestrial pest species (e.g. insects), present and future pesticide use is likely to continue posing a threat to aquatic ecosystems. The effects of pesticides used in the last two decades on the different groups of organisms are summarised in Table **1**. Effects were reported for most groups and endpoints, though there are some notable differences. Field studies that show effects on macrophytes, phytoplankton and benthic algae as well as other microorganisms are scarce and are almost entirely limited to artificial ecosystem experiments. By contrast, several field studies have demonstrated effects in freshwater ecosystems on macroinvertebrates and zooplankton [45, 68, 81-85], fish [86] and amphibians [52, 87-90]. Here, the frequency of reported effects on macroinvertebrate and zooplankton assemblages is much higher than for fish and amphibians, for which only a few field studies reported effects at the population or community level. In the case of amphibians this is not surprising as they mainly appear in lentic (standing) surface waters, which generally receive less pesticide input than lotic (running water) habitats [91].

Table 1: Effects of pesticides on the different groups of organisms under field or field-relevant conditions reported in the last two decades: frequency of reported effects, field relevance and examples.

Effects level[a]	**Bacteria, protozoa and fungi**	**Phytoplankton and benthic algae**	**Macrophytes**	**Macroinvertebrates and zooplankton**	**Fish**	**Amphibians**
Suborganismal (S)	-	Genetic changes [222]	Increase Glutathione-S-transferase and chlorophyll ratio [223]	p-nitrophenylacetate esterase, Glutathione-S-transferase and Acetylcholin esterase inhibition [167]	Acetylcholine esterase inhibition [46]	Alteration of receptor binding and cell signalling [224, 225]
Individual (I)	Decrease in bacterial activity[226]	Decline in photosynthesis and mortality [227, 228]	Decline in frond area and weight, and mortality [229, 230]	Feeding depression and mortality [231]	Mortality [232]	Increase in parasite susceptibility and mortality [52, 233]
Population decline (P)	[234]	[235]	[236]	[11, 210]	[86]	[87]
Community: change in composition (C)	[59, 98]	[235, 237]	[238]	[42]	[86]	-
Associated Ecosystem processes (E)	Inhibition of microbial mineralisation [239]	Reduction in pH and O_2 [64]	Decrease in nutrient level, pH and carbonate cycle [238, 240]	Inhibition of organic matter decomposition and decrease of energy transfer [44, 241]	-	-
Frequency of reported effects[a]	I, P, C, E: low	S: low I,P,C,E: medium	E, C, S: Low P, I: medium	High for all levels, except E: low	S: high I: medium P,C:Low	P: medium S, I: high
Clear evidence from field studies	No field studies, only mesocosm (except one field study on C)	No field studies, only mesocosm	Not for E, C, S level	All levels	All levels	For S, P, I

[a] See first column for abbreviations: none: no studies; low: 1-5 studies; medium: 5 to 10 studies, high: > 10 studies

The dominance of reports on effects on macroinvertebrates and zooplankton followed by fish and amphibians compared to macrophytes, phytoplankton and benthic algae raises the question whether this is: a) an artifact of organism selection in biomonitoring; or b) observed impacts represent the real frequency of effects in these organism groups. There are several reasons that suggest an over-representation of effects on animals and specifically on macroinvertebrates and zooplankton:

- macroinvertebrates were much more frequently selected as biomonitoring organisms than other groups due to their well-described taxonomy, relatively high species richness, their sedentary nature and low expense of monitoring programs [92, 93];
- similarly, larger organisms such as fish and amphibians have received much more attention than plants and microorganisms because of their economic importance (fish) and due to individual preferences of researchers for vertebrates [94-96];
- the detection of effects on microorganisms was much more difficult because of the variability between sites and even adjacent micro-sites [97], and only recent advances in molecular techniques allow for a reliable detection of community changes in microbial assemblages in the field [98];
- macrophytes, phytoplankton and benthic algae as well as other microorganisms are in general more susceptible to herbicides than the other groups of organisms [65, 99] and since herbicides account for a major part of the applied pesticide mass [100], it is very likely that effects in the field have occurred but were not noticed;
- algae and microorganisms are known to have a fast recovery, hence effects may only be transient and not detectable after a few weeks [65].

We therefore conclude that the present picture concerning effects of pesticides on ecosystems is likely biased towards the fauna and especially towards invertebrates. In the next section we examine to what extent the biotic community is affected by different classes of pesticides.

Compound-Specific Effects on Different Groups of Organisms

The early days of ecotoxicology were driven by the myth of a "most sensitive species" that could be used as a standard test organism to predict the impacts of toxicants in ecosystems; *i.e.* no effects should occur in the ecosystem as long as no direct effects occur in the most sensitive species [101]. This quest for the "most sensitive species" relied on several assumptions, one being that the most sensitive test species for a set of compounds would be most sensitive to other compounds as well. This assumption has received extensive criticism and we will give a brief overview of studies with pesticides that contradict this assumption. Concerning herbicides, a study of van den Brink *et al.* [99] showed that the sensitivity of test species varies with the chemical class of the compound and that there is no single most sensitive primary producer. For example, the common duckweed *Lemna minor* was more sensitive to the herbicides diquat and linuron but less sensitive to diuron and metamitron than the green algae *Selenastrum capricornutum* [99]. Similarly, crustaceans are among the most sensitive species to organochlorine and pyrethroid insecticides, whereas the recently introduced neonicotinoid insecticides exhibit orders of magnitude higher toxicity to insects than to crustaceans [102]. In addition, the sensitivity of a species to a toxicant depends on the life stage and a sensitivity ranking of several species may therefore in some cases vary with the selected life stage [24].

However, this does not mean that patterns of sensitivity are completely stochastic, a broad classification into sensitive and tolerant taxa is possible when compounds are grouped according to their mode of action [103, 104]. In the case of pesticides, some substances have specific sites of action; e.g. photosynthesis or ergosterol synthesis that are only present in certain groups of organisms [105] (see Chapter 1). For example, pesticides that target the hormonal reproductive system of insects are unlikely to affect aquatic primary producers, which have a completely different reproduction system [106]. Other pesticides have a wider activity and target processes that are present in all or many organisms (e.g. cytochrome oxidase [105]), which complicates the prediction of effects.

There are some general rules of thumb on the sensitivity of the groups of aquatic organisms to herbicides, insecticides and fungicides. Herbicides are mainly targeting organisms that perform photosynthesis. A meta-study of artificial ecosystem studies on herbicides showed that for this class of compounds, primary producers are more

sensitive than aquatic animals [65]. Which group of primary producers (*i.e.* macrophytes, algae or microorganisms) is most sensitive depends on the mode of action of the herbicide [99].

A meta-study on insecticides showed that for this class of compounds, macroinvertebrates, zooplankton and fish are the most sensitive groups compared to other organisms [66]. Given that several currently used insecticides are designed to target invertebrates [1], macroinvertebrates and zooplankton are presumably more susceptible than fish. In fact, 16 currently used insecticides for which comparative toxicity data was available, exhibited highest toxicity to invertebrates and zooplankton [107]. In addition, several artificial ecosystem studies support this hypothesis since fish were less sensitive than invertebrates [108].

Fungicides are less extensively studied than herbicides and insecticides. Therefore sound generalisations about which groups are most sensitive to these compounds are problematic. Nevertheless, based on the mode of action, several fungicides should be most toxic to aquatic microorganisms, especially aquatic fungi [109]. An unpublished evaluation of experiments in artificial ecosystems did not indicate elevated toxicity by fungicides to the aquatic fauna and primary producers [109].

Recent studies have began to incorporate these differences in the modes of actions and proposed a new strategy for the search of sensitive species. A study of Wogram & Liess [103], extended and confirmed by von der Ohe & Liess [104], found that for macroinvertebrate species the variability of the sensitivity to organic chemicals is higher between taxonomic groups (primarily families and orders) than within groups and could be pooled in a relative toxicity ranking. For example, stoneflies were among the most sensitive taxa for organic chemicals, whereas gastropods are relatively tolerant to organic chemicals [104]. This study, however, did not distinguish toxicant mode of actions between organic chemicals and some studies demonstrated that such differences exist. Rubach *et al.* [110] showed that the relative sensitivity of macroinvertebrate taxa differed, albeit minor, between organophosphates, pyrethroids and carbamates. In addition, imidazole fungicides were more toxic to gastropods than many insect taxa [111, 112]. Furthermore, the differences in sensitivity ranking were even stronger for heavy metals and salinity [104, 113]. No relative sensitivity rankings have been established for other groups of organisms so far. Overall, we propose that with regard to toxicants with a similar mode of action a consistent, relative sensitivity hierarchy may be established at least for the different groups of organisms that can be used for risk assessment of pesticides, though no universal most sensitive species or group of species exists.

INDIRECT EFFECTS IN THE AQUATIC COMMUNITY

In ecosystems, species interact with other species and their abiotic environment. Direct effects of pesticides on a species can alter these interactions and therefore have an indirect (also termed secondary) effect on species that are otherwise not directly affected. The following ecological relationships may lead to indirect effects via propagation of direct effects:

- predation: comprises herbivore-plant, predator-prey and parasite-host relationships
- competition: inter- and intra-specific competition
- species-habitat: influence on habitat characteristics by some species
- mutualism or commensalisms

Indirect effects of pesticides have been reported frequently and have been summarised in different publications [65, 66, 114-116]. Here, we will give a brief overview on potential indirect effects of herbicides and insecticides. Fig. (**2)** sketches the direct and indirect effects of a herbicide and an insecticide in a freshwater ecosystem. As outlined in the last section, primary producers are generally at highest risk of being adversely impacted by herbicides. A reduction of the primary producers can lead to a decrease in the herbivore populations due to food limitation and/or habitat loss (Fig. **2**). For example, in a study on the effects of the herbicide atrazine on freshwater communities in artificial ponds, growth and reproduction of zooplankton (e.g. *Simocephalus serrulatus, Daphnia pulex*) decreased as a consequence of phytoplankton biomass reduction [117]. Similarly, amphibian tadpole biomass (e.g. *Rana catesbeiana*) decreased due to a reduction of food source (periphyton) and loss of macrophyte habitat (e.g. *Typha*

latifolia, Chara sp.) [116]. The indirect effect on herbivores as a consequence of a reduction in food may be less pronounced in nutrient-rich ecosystems in the field. Where a reduction in herbivores occurs, effects may subsequently propagate to higher trophic levels; e.g. predators that prey on the herbivores (Fig. **2**). For example, herbicide-induced reductions in zooplankton and macroinvertebrates (e.g. Chironomidae spp.) due to loss of primary producers as food and habitat resulted in a decreased total biomass of bluegill sunfish (*Lepomis macrochirus*) [116]. This ecological effect chain represents bottom-up indirect effects because the lowest trophic level (primary producers) determines the effects on higher trophic levels. While these indirect effects are due to predatory ecological relationships, competitive relationships between primary producers promote an increase of tolerant primary producers when sensitive competitors are eliminated by a pesticide (Fig. **2**). For example, algal blooms of *Chlamydomonas* sp. were observed after linuron strongly reduced macrophyte populations of *Elodea nuttallii* [118].

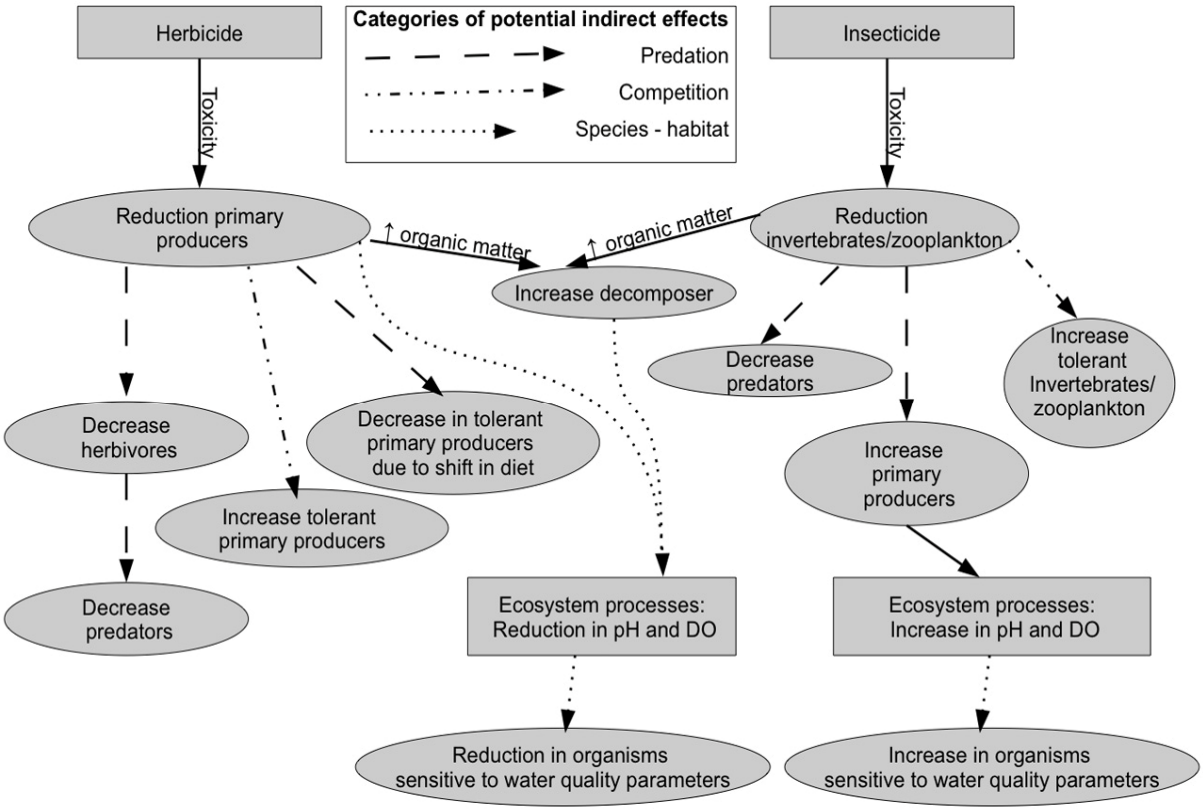

Figure 2: Schematic representation of direct (solid line) and indirect (dashed and dotted lines) potential effects of pesticides in freshwater ecosystems. See text for further explanation.

Photosynthesis is an important ecosystem process that influences water quality, and the inhibition of photosynthesis by herbicides results in a lower concentration of dissolved oxygen (DO) and lower pH values during daytime (Fig. **2**). In an indoor mesocosm study, the highest linuron treatments of 50 and 150 µg/L reduced DO and pH by up to 40% and 25%, respectively [119]. If a herbicide causes acute mortality of macrophytes, decomposition by decomposers may further enhance the reduction in pH and DO concentration [65]. This deterioration of water quality can then have detrimental impacts on sensitive invertebrate species and this represents a case of indirect effects resulting from species-habitat relationships (Fig. **2**). For example, a strong reduction of cladoceran and copepodan populations was partially attributed to a reduction in DO to only 20% compared to controls, following a 10 mg/L contamination with hexazinone in lake enclosures [120].

The schematic effects of the insecticide in Fig. (**2)** illustrates a combination of top-down and bottom-up indirect effect [121]. We present a scenario, demonstrated by many studies [66], where an insecticide adversely affected invertebrates and zooplankton species. The bottom-up indirect effect is represented by the decrease of fish population density due to

a reduction of invertebrate prey. For example, a significant reduction in macroinvertebrates such as ephemeropterans (mayflies) and dipterans as well as two zooplankton groups (Daphniidae and Cyclopidae) in outdoor ponds after treatment with methyl parathion led to decreased mean weights in rainbow trout (*Salmo gairdneri*) [122]. The top-down indirect effect commences with a release of primary producers from grazing pressure and may result in growth of their populations. In a mesocosm study on the effects of chlorpyrifos, the eradication (Insecta and Amphipoda) and reduction (Isopoda, Cladocera and Copepoda) of parts of the invertebrate community by the pesticide resulted in a two- to three-fold increase in periphyton chlorophyll-*a* accompanied by a bloom of *Oscillatoria* sp. [123]. Some tolerant invertebrate and zooplankton species may profit from the reduced competition with directly affected sensitive invertebrate species (Fig. **2**). In the aforementioned study on chlorpyrifos, Sphaeriidae molluscs and herbivorous rotifers (*Polyartha* sp.) increased as a consequence of reduced competition for food with more sensitive invertebrates [123]. Similar observations were made in a field study where, after pesticide exposure, sensitive species decreased and tolerant species increased [43]. The effects on ecosystem processes are ambiguous in this scenario. While the increase of primary producers increases the pH value and DO concentration, the decomposition of dead invertebrates/zooplankton by fungi or bacteria decreases these water quality parameters [66]. We assume that in larger freshwater systems and lotic systems the first mechanism would be more important. In the case of a strong reduction of the invertebrate fauna another important ecosystem process, leaf-litter decomposition can be inhibited [124]. A field study in 16 French streams demonstrated a three- to five-fold decrease in leaf-litter decomposition in streams with an insecticide-impaired invertebrate community [44]. Leaf-litter decomposition represents an important energy source in stream ecosystems and a reduction can even adversely impact river sections several kilometers downstream, since they rely on particulate organic matter input from upstream sections [125, 126]. Hence, indirect effects can occur a long way from the location of the direct effect.

Pesticides represent only one of the many disturbances (e.g. floods, droughts, land-use change, acidification, dredging *etc.*) that shape freshwater ecosystems [127] and other disturbances can also result in indirect effects [114]. The similarity of indirect effects of different disturbances depends on the disturbance type and selectivity of their effects on biota [128]. While some disturbances such as floods also occur in pulses, they presumably act less selectively on the trophic levels or groups of organisms in the biotic community; e.g. they are unlikely to only affect primary producers or invertebrates. By contrast, many of the currently used pesticides are relatively selective *i.e.* they act on a specific trophic level or group of organisms as outlined above. However, similar indirect effects have been reported for other contaminants such as pulses of heavy metals or accidental discharges of organic toxicants in freshwater ecosystems [114].

Indirect effects of pesticides have mainly been studied in artificial ecosystems because under field conditions a clear differentiation between direct and indirect effects is more difficult. In the field, the time and magnitude of pesticide input driven by precipitation events is unknown and the input usually comprises a mixture of pesticides that may directly affect several groups in the biotic community concurrently. Furthermore, the variation regarding environmental parameters, pesticide exposure and biotic community composition is usually rather high between sampling sites. Hence, the detection of indirect effects would require an extended Before-After-Control-Impact (BACI) sampling design [129] consisting of a spatially and temporally highly replicated monitoring of the biotic community in control and impacted sites before the pesticide input and immediately after the contamination to determine the direct effects and then a few times in weekly intervals to identify indirect effects. However, the efforts of such a study would be jeopardised by ignorance of the impact of the monitored runoff events. In the worst case scenario, any of the selected sites would be impacted (compare [44] where no impacts were detected in a field study in Finland). Even if direct and indirect effects could be detected using multivariate statistical techniques [130-133], causality could not be inferred and additional studies under standardised conditions would be needed [134]. The field studies conducted to date on the effects of pesticides did not aim to differentiate between direct and indirect effects. This may explain the lower effect thresholds that have been observed in field studies compared to artificial ecosystems [43, 44, 135], because the effects on invertebrates may not have resulted from direct toxicity of pesticides alone but as well may be a bottom-up indirect effect from depletion of primary producers or heterotrophic microorganisms. An alternative approach for assessing the direct and indirect effects in the field integrates ecological modelling [136]. The modelling is used to predict direct effects of an exposure event and the differences in the effects that are observed in the field are considered as indirect effects. However, currently used models do not allow for an integration of all the factors that can influence the strength of direct effects (see section “Direct effects of pesticides”) and therefore gives rise to high uncertainty.

EFFECT, DURATION AND RECOVERY DYNAMICS

Given the widespread application of pesticides, it is almost inevitable that some fraction enters freshwater ecosystems. Consequently, the authorisation for use of pesticides involves the passing of a value judgement on the question: "Which ecological effects are unacceptable?" [137]. For regulators in the European Union, long-term field effects on populations and communities are deemed unacceptable, though the operationalisation of "long-term" may vary on a case-by-case basis [138]. Similarly, the US EPA includes the assessment of recovery from pesticide stress in their risk assessment framework and regards potential irreversibility (*i.e.* permanent changes in the community structure or ecosystem processes) as an adverse effect [139]. From these perspectives follows that transient short-term effects are considered acceptable. The underlying hypothesis is that a toxicant can have only transient effects on an ecosystem and that the ecosystem may subsequently recover to an initial or reference state; *i.e.* the community recovery principle [140]. This hypothesis has been subject to criticism. Landis *et al.* [39] argue that because of the dynamic nature of ecosystems, any pesticide-induced effects are irreversible, rejecting the concept of recovery. Even if recovery can be observed on one level of biological organisation, changes may persist on other levels; e.g. the gene pool can be impoverished [141]. For example, fish populations of the brown bullhead (*Ameiurus nebulosus*) in the Great Lakes have been observed to have different genetic structures in populations that have been exposed to a mixture of organic toxicants and metals [142]. The US EPA acknowledges this criticism by defining recovery as "the return of a population or community to some aspect(s) of its previous condition" [139]. We agree with the criticism of Landis *et al.* [39], but would rather integrate it in the evaluation of studies on recovery; e.g. by studying effects on suborganismal endpoints in affected populations. Hence, we advocate the use of studies on the recovery of an affected artificial ecosystem or field ecosystem as a useful tool to deliver information on the toxicity of a pesticide that can be used to evaluate the acceptability of effects.

So far, almost all studies on the recovery of an ecosystem from the effects of a pesticide were conducted in artificial ecosystems and, in general, community composition was selected as the endpoint. Complete recovery was assumed when significant differences between treated and non-treated communities were not detected anymore. While controlling the type I error rate (reject the null hypothesis that recovery occurred when it is true), this entails the risk of a type II error (fail to reject the null hypothesis that recovery occurred when it is false) that may be more interesting in studies on recovery. Unfortunately, most studies on artificial ecosystems lack an analysis of the probability of a type II error of the selected test, which may be relatively high given that sample sizes in these studies are usually low (< 5 replicates per treatment). Therefore, the time to complete recovery is probably underestimated.

A meta-analysis of artificial ecosystem studies with various insecticides highlighted that the initial acute toxic effect of the substance is a critical factor for the time to recovery [143]. Methodologically, the toxicity of different compounds can be compared using the Toxic Unit (TU) approach [144], in which the results of laboratory toxicity experiments for a specific test organism (usually LC50) are used as a benchmark to compare the toxicity of concentrations of different compounds. The TU is given by

$$\mathrm{TU} = \frac{c_{\mathrm{i}}}{\mathrm{LC50}_{\mathrm{i,j}}}$$

where c is the concentration of compound i and j the benchmark organism. The benchmark organism should be selected according to its sensitivity for the study compounds. In the studies considered here, *Daphnia magna* or, in very few cases, a fish species (*Pimephales promelas, Oncorhynchus mykiss, Lepomis macrochirus*) was selected as standard test organism to compute the TU for insecticides. For herbicides, green algae (*Scenedesmus subspicatus, Selenastrum capricornutum, Chlorella vulgaris*) or macrophytes (*Lemna* spp.) were employed for TU calculation. For reasons of simplicity, throughout this chapter we use $TU_{Daphnia}$, TU_{fish} and $TU_{primprod}$ for the TUs based on *Daphnia magna*, fish and primary producers, respectively. Note that the TU approach assumes concentration addition *i.e.* the same concentration-response relationship for compounds, while this may differ in reality; e.g. one compound has no effects at a TU of 0.01 (1/100 of the LC50) while another compound may still have effects due to a flatter concentration-response curve [145].

In a meta-analysis of 26 artificial mesocosm studies with acetylcholinesterase-inhibiting insecticides and 18 studies with pyrethroid insecticides, no long-term community effects (> 8 weeks) were observed for a $TU_{Daphnia} < 1$ for fish,

microorganisms and primary producers [108]. Even with higher compound concentrations relating to a $TU_{Daphnia}$ between 1 and 100 only 4 of 15, 2 of 22 and 2 of 27 observations indicated long-term effects for fish, microorganisms and primary producers, respectively (observations with unknown recovery excluded). By contrast, freshwater insects, macrocrustaceans and microcrustaceans (Ostracoda, Cladocera and Copepoda) exhibited clear long-term effects above a $TU_{Daphnia}$ of 0.1 and even lower for pyrethroids, where concentrations between a $TU_{Daphnia}$ of 0.1 and 0.01 caused long-term effects in aquatic insects (1 of 10 observations) and macrocrustaceans (2 of 5 observations) [108].

A similar meta-analysis was performed for artificial ecosystem studies with photosynthesis-inhibiting herbicides, auxin-simulating herbicides and growth-inhibiting herbicides [65]. No long-term effects (> 8 weeks) were observed for molluscs over the whole range of tested concentrations (up to a $TU_{primprod}$ of 100). For zooplankton, long-term effects were reported in 3 of 16 cases with a $TU_{primprod} > 1$. Long-term effects on fish and amphibians occurred in 4 of 13 cases at concentrations relating to a $TU_{primprod} > 0.1$. For macrocrustaceans and insects no long-term effects were detected, except in 1 of 3 observations on auxin-inhibiting herbicides – but at a $TU_{primprod}$ of 0.01. All these effects were most likely indirect effects that resulted from the depletion of populations of primary producers or from the associated habitat degradation [65].

Phytoplankton and periphyton showed clear long-term effects for concentrations with a $TU_{primprod} > 1$, and in 1 of 8 cases with a $TU_{primprod}$ between 0.1 and 1, long-term effects were reported for phytoplankton. Macrophytes were more sensitive, with clear long-term effects in several studies above a $TU_{primprod}$ of 0.1, with 2 of 5 observations on auxin simulators indicating long-term effects between a $TU_{primprod}$ of 0.001 and 0.1.

To sum up, based on artificial ecosystem studies, long-term effects may occur when the concentrations exceed concentrations relating to a $TU_{primprod}$ and $TU_{Daphnia}$ of 0.01 for insecticides and herbicides. This seems to be in general agreement with two field studies on 20 streams in North Germany and 29 streams in Spain where the macroinvertebrate community exhibited long-term alteration at a $TU_{Daphnia}$ of similar magnitude [43, 135].

Only a few studies have scrutinised the duration of effects that are classified as long-term and they only focused on effects on macroinvertebrates. Recently, an artificial stream ecosystem study with the neonicotinoid insecticide thiacloprid reported the persistence of adverse effects on sensitive macroinvertebrate species half a year after a pulse exposure with a $TU_{Daphnia}$ of 0.014 [146]. An artificial pond ecosystem study demonstrated that at concentrations associated with a $TU_{Daphnia}$ of 20, the invertebrate communities of control ponds and treated ponds were still significantly different after 2 years whereas there was recovery at lower concentrations [67]. Very high concentrations of the insecticide methoxychlor ($TU_{Daphnia}$ of 10,000), which may occur from direct spraying of water bodies (e.g. mosquito control), implicated a different community in the treated stream compared to a reference stream over 5 years in a field study [84]. In the before mentioned study on 20 streams in North Germany, no full recovery of the community was observed within one year for a $TU_{Daphnia} > 0.001$ [43]. Relating these studies to generation times of invertebrates, which usually range from a few weeks to a year, illustrates that the recovery time is in the range of one to a few generations. Overall, these studies suggest that recovery in community endpoints can take over one year, and up to several years in cases of very high concentrations.

Factors Fostering Community Recovery Processes

The duration of community recovery from pesticide stress depends to some extent on the magnitude of the effect which in turn is determined by the concentration, exposure duration and toxicity of the pesticide and its transformation products [143, 147]. However, some other factors also influence the time to recovery:

- Ecological traits of species in the affected biotic community: In particular a short generation time, high reproduction rate, presence of resistant life stages and a high dispersal capacity of species augment recovery of populations [148]. For example, phytoplankton species generally recover faster from adverse effects of pesticides than macrophytes due to shorter generation times [65]. Similar observations were made for invertebrates with a short generation time [146, 149, 150] or high dispersal capacity [43, 151, 152]. Finally, microorganisms are presumably less vulnerable to pesticides due to short generation times and adaptability [41], though there may be exceptions; e.g. aquatic hyphomycetes [109].

- Timing and frequency of pesticide exposure: The timing of exposure is a crucial factor since the susceptibility of many species changes over the year because they may have terrestrial or resistant life-stages [43]. Concerning frequency of pesticide exposure, cyclical pulses of toxicant exposure can shape communities in the sense that they adapt [153] and recover until the next pulse occurs, while at the same time the exposure exerts a selection pressure on the communities [42]. Moreover, repeated pulses within a short period (weeks) are known to amplify effects in laboratory and mesocoms experiments [38, 108].
- Spatial dimension of effect: Large-scale contamination such as accidents require a longer time to recover since a higher magnitude of external recolonisation is needed to compensate for the effect [154]. Another aspect is that large-scale contamination often excludes the opportunity to avoid intoxication in refugia or by escaping, which again enhances subsequent recovery [155, 156].
- Position in the hydrological network: Several studies have demonstrated that the presence of undisturbed upstream sections foster recovery of the affected stream sections [43, 44, 157], presumably via recolonisation [158, 159] or energy provisioning [160].
- Regional species pool: The recovery of species that were locally exterminated from a disturbance such as pesticide pollution depends on, (1) the presence of these species in the regional species pool and, (2) that the ecological niche of this species is not occupied by a more competitive species [161].
- Climate: Several organisms develop faster and have more generations in regions with higher average temperature such as the tropics, which increases recovery of populations from disturbances [162, 163].
- Disturbance regime: the interaction with other disturbances and the type and frequency of other disturbances influence the recovery potential of the biotic community [128, 164].

To sum up, the recovery time of a freshwater community from pesticide stress is influenced by ecological, physicochemical, geographical and temporal factors.

How Frequent are Long-Term Effects Under Current Use Patterns?

In the previous sections we outlined the concentration levels that may cause long-term effects in the field. This raises the question, how frequently these exposure concentrations occur in the real world. For insecticides, Schulz [11] reviewed the concentrations given in field studies since 1982 and reported the maximum and minimum concentrations detected for each compound in each study. We calculated the respective $TU_{Daphnia}$ for the observed maximum concentrations for this data using the LC50 for *Daphnia magna* as given in the Pesticide Manual [165] in order to allow for a comparison with the long-term effect thresholds derived above. In the 64 studies, 162 of the 194 compounds measured, comprising 39 different insecticides, had concentrations above the limit of quantification. For these 162 observations, the $TU_{Daphnia}$ associated with the maximum concentrations exceeded 0.01 in 94 cases. Hence, in 58% of the observations in the respective field studies, the substances exhibited maximum concentrations that may cause long-term effects. To put this into the right context, one has to consider that: 1) some compounds without detections may not have been reported, hence the number of observations is limited to positive detections; 2) the study regions were not randomly selected but presumably based on some prior knowledge on pesticide pollution; 3) each of the 162 observations amalgamated up to 29 sampling sites and several sampling episodes; and 4) some regions with insecticide detections were sampled repeatedly [11]. However, a recent study on 83 pesticides in 17 agricultural streams over 4 years in the United States also reported that between 10% to 25% of the samples exceeded a $TU_{Daphnia}$ of 0.01 [166]. In addition, approximately 50% of the concentrations in the US streams exceeded a $TU_{primprod}$ of 0.01. By contrast, the TU_{fish} were rather low (most $TU_{fish} << 0.01$). Since no event-driven water sampler was employed in this study, the peak concentrations were most likely underestimated. Thus, the reported TUs represent a conservative estimate of the real exposure. Overall, the results confirm our conclusion that invertebrates and primary producers are at highest risk of being affected by pesticides and suggest that long-term effects of pesticides on both groups are not isolated cases.

RISK ASSESSMENT AND PREDICTION OF EFFECTS OF PESTICIDES

The beginning of widespread pesticide use in the middle of the 20th century was soon followed by reports of detrimental effects on ecosystems and human health [69]. Hence, today most countries require a pesticide risk

assessment for ecosystems and human health before authorisation of a substance is granted. The risk assessment procedure comprises a fate and an effects assessment. The fate side uses models and experimental data to assess the exposure in the environment (see chapter 2 in this book). In this chapter, we focus on methods to assess the effects; *i.e.* we give an overview of the different methods used to predict effects on aquatic ecosystems and describe their advantages and disadvantages.

Earlier, we mentioned that every effect of a pesticide has a physiological basis. However, the current science of ecotoxicology is very distant from a "grand unifying theory" that would mechanistically integrate all levels of biological organisation (Fig. **1**) and allows for the prediction of effects on the top levels from the lower levels. This holds true especially for the linking of suborganismal effects to higher levels. Currently, a clear link between responses at the suborganismal level and the fitness of individuals is still missing but would be a prerequisite for a sound suborganismal endpoint to be considered in risk assessment [167, 168]. Hence, although appealing from a precautionary principle point of view, suborganismal effects are at present no valid basis to predict effects on populations, communities or ecosystems. Currently, the approaches for ecological risk assessment of pesticides rely predominantly (1) on the individual (single-species laboratory tests) and ecosystem level and (2) on experiments. In the following we will sketch these as well as some alternative approaches.

Methods Relying on Toxicity on the Individual Level

The individual level has been the starting point of ecotoxicological research and still represents an important backbone supporting research on other levels of organisation. In fact, the vast majority of ecotoxicological data that has been produced to date (*i.e.* EC50 and LC50 data), originates from single-species toxicity tests. These tests allow for high replication and deliver relatively precise estimates of the toxicity endpoints (e.g. mortality or growth) under standardised conditions (temperature, water quality, age of test organisms *etc.*) [169]. For the first tier in pesticide risk assessment in a regulatory context, the acceptable concentration for a compound in the environment is derived by dividing the LC50 by a safety factor to account for uncertainties in the extrapolation from a single species in the laboratory to communities in the field. The uncertainties arise from abiotic and biotic factors that are not considered in single-species laboratory tests but may significantly modify the susceptibility of populations in the field such as ecological relationships within ecosystems [35, 170], recovery processes [36] and multiple stressors [32, 171]. In the European Union, a safety factor of 100 for acute toxicity tests for the invertebrate *Daphnia magna* and fish is applied to account for the above mentioned uncertainties [172]. The results from algal growth inhibition tests and chronic toxicity tests are divided by a safety factor of 10 to obtain the threshold concentration that should not be exceeded for the first tier in pesticide risk assessment. However, the use of this approach to predict effects in the field is relatively inefficient as it can be over- or underprotective [173, 174]. Underprotection of freshwater ecosystems can lead to losses of species and ecosystem services while overprotection may put unnecessary constraints on economic activities. Nevertheless, the single-species test is far less labour- and time-consuming than experiments on higher levels of biological organisation and are, therefore, an indispensable tool in the first tier risk assessment. Moreover, the results from these tests are critical for other research areas such as ecotoxicological modelling [175, 176], trait-based risk assessment [42, 94] or assessment of mixture toxicity [144, 177].

Alternative approaches to current single-species tests on the individual level can be categorised into: 1) those which use different methods to generate identical endpoints; and 2) those which generate different endpoints to assess the risk. Approaches of the first category include the use of different species or assays for testing and computational methods to predict toxicity data. Especially in the case of vertebrate testing, ethical concerns have promoted the development of alternatives such as the fish embryo test [178] or more recently the cell line test [179]. Another experimental development represents the rapid tolerance test that is a response to the scarcity of toxicity data for many compounds and species [113, 180]. Rapid testing involves simultaneous toxicity testing with field-collected taxa and sacrifices some precision in the determination of the toxicity endpoint in order to generate toxicity data on a wide array of species representative of natural communities [180].

A non-experimental method to obtain toxicity data is modelling. Quantitative structure activity relationship (QSAR) models represent a promising method to predict acute or chronic toxicity data [181, 182]. Here, the structure of compounds with known toxicity is used to predict the toxicity of unknown compounds. So far, QSARs have most successfully been applied to differentiate between narcotic and excess toxicity [183]. However, their value is

currently tenuous for compounds where the activity relies on exotic or unknown functional chemical groups. Moreover, several ecological models examine the influence of different test conditions on the determination of acute or chronic toxicity to allow for the adjustment to the respective field conditions [184]. For example, Ashauer *et al.* successfully (77% to 96% of explained variance) incorporated fluctuating and episodic concentrations of a pesticide using a threshold damage model to predict effects of realistic exposure conditions on the invertebrate *Gammarus pulex* [185], whereas standard toxicity tests utilise a constant or pulsed exposure. Finally, individual-level models can be used to analyse results from acute toxicity tests and explore mechanisms. For example, the energy budget model describes characteristics of individuals such as growth, metabolism or reproduction in terms of energy budgets [186] and the most commonly used energy budget model in ecotoxicology is DEBtox (see: http://www.bio.vu.nl/thb/deb/deblab/debtox/). However, most ecological models require toxicodynamic and toxicokinetic data that is available for a few species only and are therefore not widely applicable.

Species-sensitivity distributions (SSDs) present an alternative approach of the second category (generation of different endpoints) that was introduced to generate more accurate environmental quality targets [187]. SSDs integrate the results of single-species tests for a respective compound (or a mixture of compounds) to establish a statistical distribution of the sensitivity. The distribution function links the fraction of potentially affected species to the concentration of a compound. This allows for the derivation of a threshold concentration that is assumed to protect a defined percentage of taxa in the community (usually 95%). SSDs have received attention by regulators and are now used for the setting of environmental threshold concentrations in several countries including the US [188], the Netherlands, Australia and New Zealand [189, 190]. For herbicides and insecticides, two studies compared the thresholds derived with SSDs to effect concentrations observed in artificial ecosystem studies [99, 107]. For herbicides, SSDs based on chronic no-effect concentrations (NOEC) delivered threshold concentrations that were protective for artificial ecosystems, except for one out of nine compounds [99]. Similarly, the threshold concentrations derived from SSDs for 16 insecticides were protective for artificial ecosystems, though not in cases with repeated insecticide exposure where a safety factor of at least 5 was suggested [107]. However, species in artificial ecosystems are often more tolerant than natural communities [146] and therefore it remains to be demonstrated that SSDs are also protective in the field. Furthermore, the accuracy of SSDs for the prediction of effects in natural ecosystems has been questioned because they typically rely on the results of a few test species that are often not representative of natural communities and like single-species tests do not incorporate ecological relationships, multiple stressors or recovery processes [191]. In addition, their application range is limited due the scarcity of available toxicity data for many compounds [180]. In fact, toxicity data are often restricted to a few test species [135], while a minimum of 15 to 55 taxa have to be included in SSDs to arrive at thresholds with acceptable confidence limits [192], though as few as six taxa can be sufficient to derive protective concentrations [99]. The aforementioned rapid tests have been advocated as an experimental solution for the lack of species data [180]. Similarly, the use of, 1) expert judgement regarding the sensitivity of higher taxonomic groups (e.g., orders) combined with Bayesian statistical methods [193], and 2) statistical techniques such as interspecies correlation models [194, 195], allow for the construction of SSDs despite sparse data. Overall, SSDs represent a powerful tool to extrapolate individual level toxicity date to the community level, but do not consider ecological relationships so that the accuracy of the prediction is contentious.

Methods Relying on Toxicity on the Population level

Currently, impacts at the population level do not receive a lot attention in regulatory pesticide risk assessment [169]. However, since risk assessment is most interested in predicting effects on populations or communities, which are constituted of populations of many species, several authors have suggested that population level endpoints should inform risk assessment [196, 197]. Population level experiments can include ecological factors such as recovery, intraspecific competition and different life stages, and studies demonstrated that single species acute toxicity tests are a poor predictor for effects at this level [36, 198, 199]. The experiments can either be conducted for several populations in a community context such as artificial ecosystems that are discussed in the next section or for a single population. Yet, no consensus has been established on the experimental conditions in single population toxicity testing. More importantly, single population experiments are more labour and time-consuming and exhibit higher variability compared to single species tests [169], while not including ecological inter-species relationships and indirect effects. Therefore, advocates have focused on population level mathematical modelling for the prediction of effects [196]. The models can be broadly categorised into demographic models and individual-based models (for a

more thorough treatment of ecotoxicological population models see [196, 200]). Demographic models globally assign parameters such as fecundity or growth to age classes or the whole population and derive population level endpoints, which is most commonly the population growth rate. For example, in a study of the effects of diquat bromide on the bluegill (*Lepomis macrochirus*), the different age classes were parameterised with survival probabilities and fecundities and the model was used to assess the effects on the population growth rate [201]. Moreover, demographic models can be coupled with energy-budget models such as DEBtox to translate effects from the individual level to the population level [202]. By contrast, individual based models describe each individual in a population independently and the effects on the population level emerge out of the interaction and responses of the individuals when exposed to pesticides. These models can deliver new insights into mechanisms of toxic effects on populations and also allow for an explicit incorporation of the spatial dimension. For example, Van den Brink *et al.* [156] used an individual-based model to predict the effects of an insecticide on populations of the isopod crustacean *Asellus aquaticus* under different spatial scenarios. The results highlighted the relevance of habitat connectivity and the dispersal abilities, such as drift, of the species for its subsequent recovery in the impacted water body [156].

The major problem for ecotoxicological modelling on the population level remains the paucity of ecological data for other than the few better studied species, which hampers the parameterisation of models. This limits their value for prediction of pesticide effects. Nevertheless, they may be used to investigate effect mechanisms of pesticides in populations (and higher levels) to extrapolate empirical results from the individual level to the more meaningful population level. In addition, by modelling taxa based on generalised ecological traits such as generation time or dispersal capacity, population models can be valuable to select sensitive species or groups of species for the inclusion in artificial ecosystem experiments or identify indicator taxa in biomonitoring [149, 203].

Methods Relying on Toxicity on the Community and Ecosystem Level

The most frequently applied experimental method to predict effects of pesticides on the community level is ecotoxicological testing in replicated artificial ecosystems. Artificial ecosystem studies encompass different sizes and are accordingly differentiated into macrocosms, mesocoms and microcosms [204]. So far, most of the studies have been conducted in replicated mesocosms. Since mesocosms represent at least a part of an ecosystem, we do not draw a distinction here between the community level and the ecosystem level. In addition, mesocosm test systems possess all characteristics of real ecosystems such as ecological interactions, recovery processes, and depending on the experimental design, multiple stressors, though their configuration may be different.

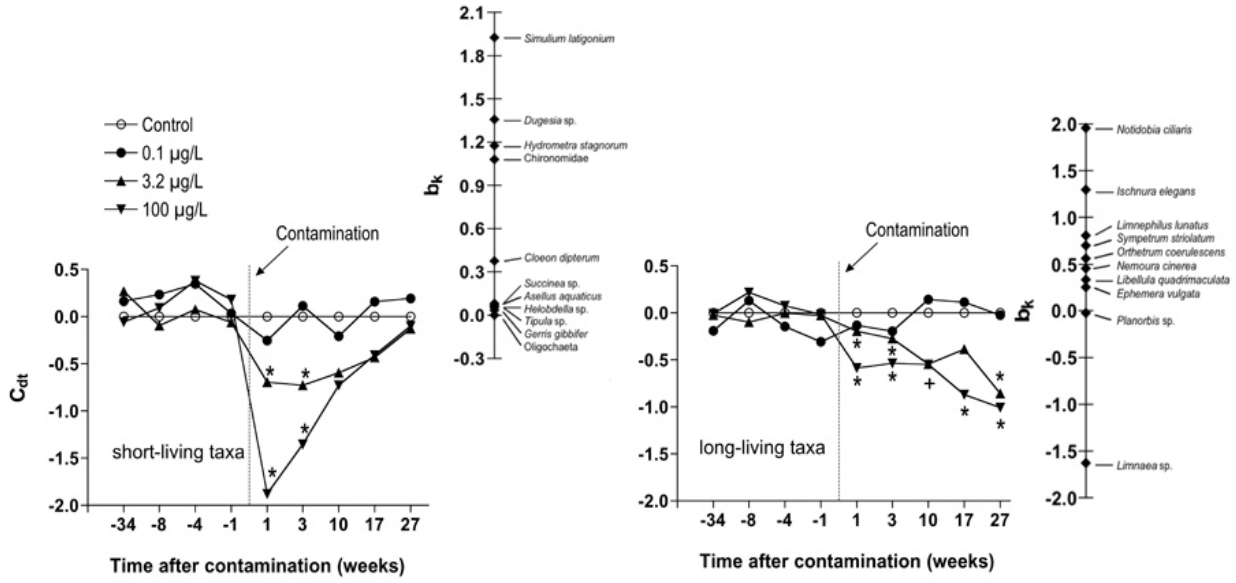

Figure 3: Effect duration of a neonicotinoid insecticide in mesocosms for short-living and long-living taxa. Asterisks indicate significant ($p < 0.05$, ANOVA, confirmed by both Games–Howell and Tamhane post-hoc tests) differences from the controls. C_{dt} = canonical coefficient for treatment d and week t. Modified and reprinted from [146] with permission from Elsevier.

Mesocosms can be constructed for both lentic (ponds) and lotic (stream) freshwater ecosystems. The construction of mesocosms starts with the physical containment structure, which is subsequently furnished with substrate and water. After allowing some time for stabilisation, sediments, plants and animals from natural ecosystems are introduced [204, 205]. The freshwater community should be established and replicate mesocosms should be similar in community composition before an experiment is run. Mesocoms can be constructed indoors or outdoors, with outdoor mesocosms being more realistic with exposure to field environmental conditions (rain, sun) but at the same time subject to higher variability due to seasonal variation and the risk of freezing during winter in temperate regions.

In general, experiments in mesocosm systems represent close-to-field conditions and include most biotic and abiotic factors that can influence the effects of a pesticide. Mesocosms have the advantage over field monitoring in that many factors can be controlled; the timing, duration and concentration(s) of exposure can be manipulated and the statistical power is higher since the abiotic factors between the replicated units are similar. Results from mesocosm experiments can be regarded as relatively accurate to predict effects in the field (effect thresholds) compared to other experimental methods and are therefore used as the highest tier in pesticide risk assessment. Nevertheless, there are some reasons why caution is warranted when predicting effects in the field from mesocosm experiments:

- Mesocosm communities do not necessarily mimic their natural counterparts under field conditions. The important trophic levels of vertebrates such as fish are often not included in artificial ecosystem experiments and the community composition for other trophic levels may be different compared to natural communities. Beketov *et al.* [146] surmised that long-term mesocosm studies had only 5-25% of long-living invertebrate taxa (uni- and semivoltine, life-cycle > 1 year) compared to 45-80% in natural streams.. Effects of the neonicotinoid thiacloprid on long-living invertebrate taxa persisted in a mesocosm study, while complete recovery of the short-living taxa occurred [146] (Fig. 3). Hence, potential long-term field-level effects would be underestimated when long-living taxa are underrepresented in the mesocosm system.
- Most studies were conducted in lentic systems and lentic communities contain different taxa and a lower fraction of sensitive taxa than lotic communities [206]. For example, 90 of 108 studies that were included in two meta-analyses on the effects of herbicides and insecticides were lentic [65, 108]. A lower fraction of sensitive taxa in the test system can lead to the underestimation of effects.
- Artificial systems are relatively variable over time because they are species-poor and lack redundancy [207]. At the same time they are poorly replicated and this raises concerns about the accuracy of the prediction. For example, mesocosm studies with esfenvalerate reported contradicting results concerning the development of primary production [208, 209], probably due to different ecological interactions between phytoplankton, zooplankton and fishes.
- Mesocosms have a low habitat complexity and only include a limited array of the various environmental conditions present in the field and therefore effects may still be over- or underestimated [196].
- It is uncertain to what extent the recovery dynamics of mesocosms are representative for the field situation. While in-stream recolonisation by drift from refugia or other, uncontaminated, sections of the freshwater system (mainly in lentic systems) is underestimated, external aerial recolonisation from other water bodies in the region may be stronger than in the field due to the presence of control streams in close vicinity.

The derivation of general concentration-response relationships that could be used in a predictive manner from field studies in freshwater ecosystems is difficult. This is because most field studies do not cover an exposure gradient as they are limited to a few streams or ponds (sample size < 10) and/or were not designed to deliver a regression. In addition, the causality between pesticide exposure and effects is often not clear [11]. In fact, field investigations on the effects of pesticides face two severe problems that hamper the establishment of a confident concentration-response relationship [42]. Firstly, the natural variation between communities at field sites is high, whereas reference sites often differ from pesticide-disturbed sites by more factors than only pesticide exposure [43]. Secondly, pesticide input during runoff events is associated with co-occurring changes in other environmental variables such as an increase in current velocity resulting in hydrological stress or increased turbidity that may confound effects of pesticides. We will outline three approaches to tackle natural variation and/or confounding factors. The first approach is to experimentally test the influence of potential confounding factors. Liess and Schulz

[210] constructed a bypass microcosm system connected to an agricultural stream and compared the effects of runoff with and without pesticide contamination on the dominant stream invertebrate populations. Only runoff events containing insecticides caused a significant decrease in the invertebrate populations [210]. In another study, the observed concentration range of environmental factors such as pesticide exposure and turbidity was investigated for effects on test species in laboratory experiments [211]. Since the test species showed only significant acute effects in response to pesticide contamination, the authors concluded that pesticides were the main cause of observed effects on invertebrate assemblages in the field [211, 212]. However, this approach is still confronted with natural variation when used to derive a concentration-response relationship.

A second approach to deal with natural variation and confounding factors represents the usage of ecological traits such as generation time or dispersal capacity to identify effects of pesticides [43]. The underlying ecological theory is that the sensitivity of taxa to a stressor and the occurrence of subsequent recovery patterns and indirect effects is dependent on their configuration of traits [161]. For example, species with a stream-lined body are more tolerant to hydrodynamic stress [213]. Hence, stressors can be regarded as a filter that selects taxa with a suitable trait configuration which results in an increase of these traits in the community [161]. Liess and von der Ohe [43] hypothesised that invertebrate taxa with a long generation time, low dispersal capacity, presence in water bodies during time of pesticide application and high physiological sensitivity would be most susceptible to pesticides and predicted a decrease of these "species at risk" (SPEAR) in the communities (see http://www.systemecology.eu/SPEAR/Start.html for online SPEAR calculator). In fact, they demonstrated a decrease of the fraction of sensitive taxa during the time of pesticide application in 20 streams in North Germany. A similar study was conducted in two regions of Finland and France and found a reduction in sensitive taxa with an increase in pesticide toxicity measured in terms of $TU_{Daphnia}$ [44]. Finally, an analysis of monitoring data comprising 28 tributaries in a Spanish river basin confirmed the relationship between pesticide input and decrease of sensitive taxa [135]. All studies established a significant relationship between pesticide toxicity and response of the sensitive taxa in the communities, or more technically speaking, between $TU_{Daphnia}$ and percentage of SPEAR species. Interestingly, the three concentration-response relationships were not significantly different and allowed for the derivation of an approximate effect threshold [42-44, 135]. According to these studies, slight effects occur already above a $TU_{Daphnia}$ of 0.001 and strong effects prevail above a $TU_{Daphnia}$ of 0.01 [44, 135]. This effect threshold lies approximately a factor of 10 below the effect threshold derived from mesocosm studies. The differences may be due to one of the following explanations:

1. The measured pesticide concentration (expressed as TU) in the field underestimates the peak exposure to which the ecosystem is exposed, resulting in a too low effect threshold.
2. The differences are due to different endpoints: the threshold for mesocosms refers to long-term changes in the communities, whereas the effect duration in the field studies is uncertain and the threshold may partly refer to observations of short-term effects.
3. The predictions from mesocosm studies underestimate the effects in the field due to differences in the factors outlined in the previous section (smaller fraction of sensitive taxa, different environmental conditions *etc.*)

Currently there is no consensus as to which explanation is most plausible. While some scientists argue that pesticide concentrations are usually underestimated in field monitoring [82] and consider the first explanation as the most likely, others emphasise the short-comings of mesocosm studies, which we have discussed earlier, and advocate explanation 2 or 3. However, if explanation 1 were true, this would mean that the different sampling methods (event-driven, grab sampling, passive sampling) employed in the three field studies (in some studies even in parallel) [22, 43, 44, 135] are subject to the same systematic error. More field studies would be needed to scrutinise this issue. Overall, the results from the abovementioned studies represent at least an accurate prediction of the order of magnitude at which effects may occur in the field. Nevertheless, the concentration-response relationship should be interpreted with care, when applying to, 1) areas with a different spectrum of applied pesticides, 2) lotic ecosystems, and 3) larger freshwater systems. In addition, the results can not be extrapolated to organisms other than invertebrates in the freshwater biotic community.

A third approach to tackle natural variation and confounding factors is the use of field-based sensitivity estimates. One method is similar to the SSD approach described before but uses sensitivity estimates from large field data sets to

predict thresholds. For example, a Norwegian dataset with 4200 sampling sites was used to assess the individual sensitivity of the frequently occurring and abundant taxa to toxicants and subsequently construct a field species sensitivity distribution (f-SSD)[214]. As with SSDs, the f-SSDs can be used to predict thresholds that should protect a certain fraction of the taxa in the community [214]. Recently, a new method was proposed by Kefford *et al.* [215] using dissimilarity indices to assess changes in the species pool across a contamination gradient. The method was applied to a larger data set on invertebrate data for 360 streams in North Germany and found a significant change in species composition with increasing modelled pesticide exposure [215, 216]. These methods are presumably more accurate in the prediction of effect thresholds than conventional SSDs relying on single species toxicity data. However, to apply this approach, large field monitoring data sets with concurrent pesticide measurements and biomonitoring data are required, which are very rare and presumably only available for governmental monitoring programs [135]. To date, governmental pesticide monitoring programs relied mainly on point water samples and were not adapted to detect episodic events such as pesticide runoff [82]. Hence, the predictions derived from these methods are likely less accurate than those from field studies using event-driven water samplers (see first section of this chapter).

Ecosystem Modelling

While many mechanistic models have been developed to predict the fate of contaminants in the environment, only a few mechanistic models target the effects of toxicants in freshwater ecosystems. These mechanistic effect models have only rarely been applied in ecotoxicology and are not thoroughly validated and compared to field data [217]. In one of the few published studies, Sourisseau *et al.* [217] calibrated and validated the ecosystem model Aquatox (http://www.epa.gov/waterscience/models/aquatox/) for control streams in a mesocosm experiment. The model was very sensitive to temperature parameters such as the optimal growth temperature for periphyton, filamentous algae and predatory invertebrates. Similarly, the optimal temperature for fish was a very sensitive parameter in another application of this model on the bioaccumulation of polychlorinated biphenyls [218]. Overall, these mechanistic models do not currently represent an alternative to other methods of prediction of effects and given the lack of ecological data for many species it is questionable if an adequate model can be used for the prediction of effects in the near future.

An alternative approach to mechanistic effect models is represented by statistical models that extrapolate observed concentration-response relationships from lower levels of biological organisation or from case studies to a larger scale. For example, Schriever *et al.* [219] combined a mechanistic fate model with a statistical model incorporating the concentration-response relationship observed in two central European regions to predict effects of pesticides on the European level [220]. However, due to data limitations and simplifications to allow for a wider prediction, such models do not provide accurate predictions of the effects, neither for specific pesticides nor on the small scale. Another example is the PERPEST model that predicts the magnitude and duration of effects of a certain concentration of a pesticide (and mixtures of pesticides) on various community endpoints simultaneously (e.g. community metabolism, phytoplankton and macro-invertebrates) [176, 221] and relies on a database containing results from freshwater mesocosm studies. A key advantage of PERPEST over single species/safety factor analyses is that it removes the need to extrapolate to the community level. However, the premise that the concentration-response relationships observed in a limited number of field case or mesocosm studies can be extrapolated to a wide range of freshwater ecosystems implicates uncertainties in the accuracy of the predictions. Nevertheless, due to the current limitations of mechanistic models and paucity of field studies, the statistical approach is certainly useful to identify potential hot spots of pesticide pollution and compounds of concern.

ACKNOWLEDGEMENTS

The authors like to thank Ben Kefford, Peter von der Ohe and three anonymous reviewers for valuable comments that helped to improve the quality of the manuscript. Special thanks to Mikhail Beketov for providing Fig. (3). RBS received financial support (SCHA 1580/1-1) from the German Science Foundation (DFG).

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CHAPTER 7

Ecological Impacts of Organic Chemicals on Freshwater Ecosystems

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Abstract: The ecological impacts of organic pollutants on freshwater ecosystems have attracted immense scientific, regulatory, and public attention over the past fifty years. In part, this reflects the significant role that freshwater ecosystems play as a repository for anthropogenic chemicals relative to other systems. Some of the most severe ecological impacts have been documented in freshwater ecosystems from persistent organic pollutants (POPs) such as polychlorinated biphenyls, polychlorinated dioxins and furans, and polycyclic aromatic hydrocarbons. Such chemicals can reside for long periods in freshwater sediments, which can then constitute a continual source to the environment even when direct inputs have ceased. Exposure of freshwater biota at lower trophic levels to persistent chemicals can result in transfer to, and ecological impacts at, higher trophic levels through bioaccumulation and biomagnification. In contrast to historically significant organic pollutants, the pervasive nature of new pollutant classes (e.g. pharmaceuticals, polybrominated diphenyl ethers, and perfluorinated surfactants) in global freshwater ecosystems is beginning to be recognized but the full spectrum of their ecological impacts is poorly understood. In this chapter we review documented and potential ecological impacts of organic chemicals in freshwater ecosystems. We focus predominantly on effects at the population, community, and ecosystem levels but, to the extent that our understanding of impacts at these higher levels is predominantly extrapolated from information derived at lower levels, we also include information at the organism and sub-organism level. In addressing each chemical class, impacts on microbial, plant, invertebrate, fish, and fish-eating bird populations are considered where data exists.

INTRODUCTION

The conceptual basis of the ecosystem, *i.e.* a system that includes living organisms interacting with each other and the inorganic components, was defined by Tansley [1]. The hierarchical and interconnected flow of energy and cycling of materials within an ecosystem lead to the concept of trophic structure [2] and subsequent bioenergetic studies of ecosystem development, including relationships between biota (food webs), diversity, and nutrient cycles [3]. From this pioneering work emerged the concept of hierarchical levels of organization within ecosystems (Fig. **1**). This hierarchical framework may be instructive in understanding the ecological impacts of contaminants; that effects at a given level of biological organization can propagate upward, or cascade downward, to other levels [4]; and that effects at one level can be understood mechanistically from information derived at lower levels in the hierarchy and interpreted ecologically from information derived from higher levels [5]. However, the greater the distance between any two levels, the more difficult it is to establish cause-effect relationships and, coupled with the non-linearity of many trophic relationships, clear examples of effects propagating from sub-individual levels to higher levels of organization are rare.

Current regulatory structures for protecting aquatic ecosystems typically rely on extrapolation of data derived from lower levels of biological organization (e.g. whole-organism toxicity tests) as these are often the only data available for criteria-setting. This situation stands in stark contrast to the protection goals of regulatory authorities, and the fundamental premise of ecological risk assessment (ERA), which is the protection of populations and communities. To bridge the gap between regulatory practice and the protection goals of ERA, much effort has been expended on understanding how ecosystems respond to contaminants at different levels of biological organization. Depending on the intensity and duration of exposure to organic contaminants, ecological impacts in the field may include avoidance, extirpation/extinction, loss of diversity and function, and, under severe situations, ecosystem collapse. Such large-scale impacts were observed in Lake Erie in the 1950s and in Great Lakes lake trout and fish-eating bird populations in the 1960s and 1970s [6]. Of course, ecosystems can recover when ameliorative action is taken as was witnessed in Lake Erie in the 1960s when phosphate inputs were reduced. Although examples of population collapse, community disruption, and ecosystem impacts in the field exist, unequivocal cause-effect relationships

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between individual contaminants and ecological effects at higher levels of biological organization are difficult to establish due to high biotic/abiotic complexity.

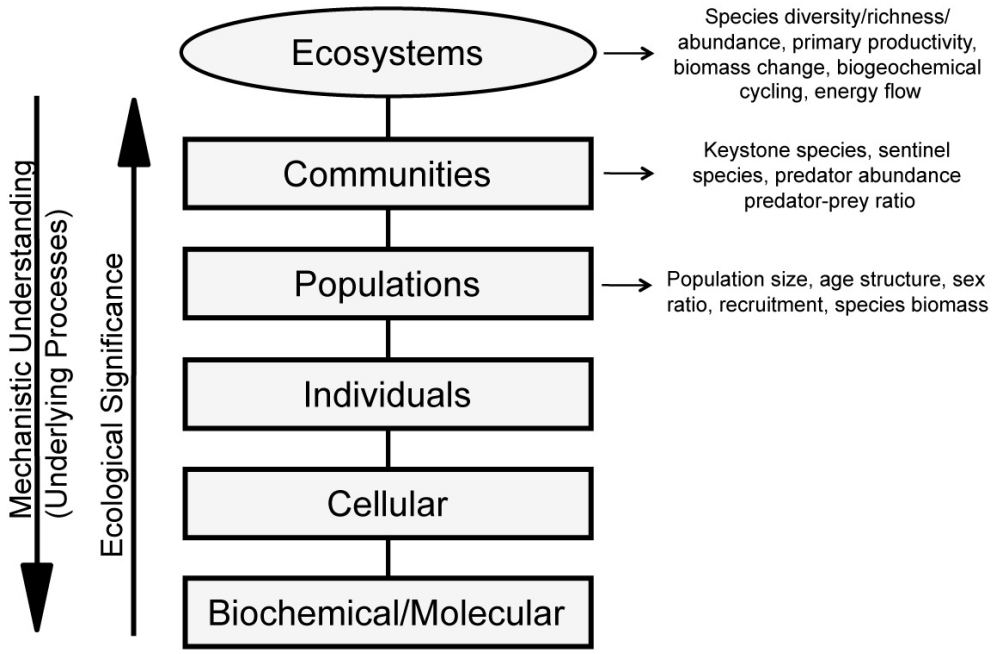

Figure 1: Schematic representation of the hierarchical organization of biological systems

Some understanding of the potential impacts of organic chemicals at higher levels of biological organization can be extrapolated from studies using non-chemical stressors to manipulate whole ecosystems [7, 8]. However, the practical, and arguably ethical, difficulty of manipulating whole ecosystems, communities or populations to understand the ecological impacts of contaminants limits public and scientific acceptance of such approaches. This may be partially overcome by conducting manipulative studies in model aquatic ecosystems (e.g. micro/mesocosms) and considerable knowledge about contaminant impacts at higher levels of biological organization have been derived through the use of such systems. Models can also help to understand potential ecological impacts of contaminants at higher levels of biological organization. Complex ecosystem simulation models have been developed (e.g. Comprehensive Aquatic Systems Model for understanding the impacts of pesticides) but have not been applied extensively because of the large number of explicit/implicit assumptions needed for parameterization, the large amount of data required about the fate and effects of the chemical(s) in an ecosystem, and difficulties related to model validation [4]. Better success has been met with population models and these are commonly applied in ERA [9, 10].

In this chapter, we review the potential ecological impacts of organic contaminants on freshwater ecosystems, focusing on microbes, plants, invertebrates and vertebrates. The chapter is organized by contaminant class, including those such as polychlorinated biphenyls, polychlorinated dioxins/furans, polycyclic aromatic hydrocarbons, and plasticizers (alkyphenol ethoxlylates, bisphenol A) with a long historical presence in the environment and those, such as polybrominated diphenyl ethers, fluorinated surfactants, and pharmaceuticals with a much shorter history. We exclude pesticides as these are covered in Chapter 6 of this book. The scope of this review is largely restricted to population, community, and ecosystem levels of biological organization, but we draw on information from lower levels of biological organization as needed.

HALOGENATED AROMATIC HYDROCARBONS

Halogenated aromatic hydrocarbons (HAHs) are a diverse class of organic chemicals, within which occur some of the most ubiquitous and toxicologically significant chemicals in aquatic ecosystems including: polychlorinated biphenyls (PCBs), polychlorinated dioxins and furans (PCDDs/PCDFs), and polybrominated diphenyl ethers (PBDEs). The unique

physicochemical properties of HAHs including hydrophobicity, low melting points, high octanol-water partition coefficients (Kow), and low volatility reflect the unique properties of halogens, which can comprise a significant percentage of the molecular weight of these compounds. Halogens have high electronegativity, a measure of how strongly atoms attract and hold electrons, and therefore the strength of covalent bonds. Fluorine, chlorine and bromine have electronegativity values of 4.0, 2.0, and 2.8, respectively, which are among the highest in the periodic table. The strong covalent bonds formed by halogens impart high molecular stability and hence a strong propensity to persist in the environment. The environmental persistence of these compounds increases the probability of exposure for environmental receptors while their hydrophobic nature can result in bioaccumulation/bioconcentration and subsequent biomagnification. For many HAHs, the combination of persistence and hydrophobicity has left an indelible imprint on many freshwater ecosystems and yielded a long history of scientific, regulatory, and public scrutiny.

Polychlorinated Biphenyls and Polychlorinated Dioxins/Furans

Background and Chemistry

Polychlorinated biphenyls were introduced in the 1920s as cooling and insulating fluids for industrial transformers and capacitors, fluorescent light ballasts, and as hydraulic fluids in the automotive and related industries [11]. The chemical properties of PCBs that made them ideal for these applications include low flammability and electrical conductance and high thermal and chemical stability. PCBs contain between 1 and 10 chlorine atoms attached in various configurations to biphenyl and were primarily marketed by the Monsanto Corporation between 1930 and 1977 under the trade name Aroclor. There are a total of 209 PCB congeners but only 100 to 150 occurred in formulations that were used and are now ubiquitously dispersed in the global environment [11]. PCBs were first reported in herring gulls and eagles in the mid-1960s [12], and have since been consistently identified in, among other matrices, human and animal adipose tissue, breast milk, and freshwater and marine sediments [13]. Evidence of chronic toxicity in humans and widespread effects in the environment led to implementation of the final PCB ban rule by the EPA in 1979, prohibiting the manufacture, processing, distribution and use of PCBs. PCBs have now been banned for 30 years, but it is estimated that approximately 70% of the PCBs manufactured remain in the environment [14]. In 2001 PCBs were listed as one of the "dirty dozen" POPs under the Stockholm Convention.

In contrast to PCBs, PCDDs/PCDFs have no commercial value and largely occur as historical by-products of the manufacture of organochlorine compounds (e.g. PCBs and pesticides such as 2,4,5-T and pentachlorophenol), the incineration of chlorine-containing substances such as polyvinyl chloride, and chlorine-based bleaching of wood pulp to make paper. Polychlorinated dioxins and furans are also created naturally as pyrolytic by-products of volcanic and forest fire activity. Polychlorinated dioxins and furans contain 1 to 8 chlorine atoms, yielding 75 and 135 possible congeners, respectively. Of these, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) congener is the most toxic and PCDD/PCDF congeners having chlorine atoms in the 2,3,7,8 positions appear to be most toxic and bioaccumulative. Once accumulated, clearance of the 2,3,7,8 congeners is extremely low and biomagnification occurs through food chains, at a rate of 3 to 10-fold for each trophic level [15].

Ecotoxicology

The environmental toxicology of PCBs and PCDDs/PCDFs has been well documented [12-14,16-18]. Animal studies indicate that PCBs and PCDDs/PCDFs cause teratogenic, mutagenic, carcinogenic, immunotoxic, and hepatoxic effects and both are known to disrupt endocrine and growth factor systems, including effects on the developing immune, nervous, and reproductive systems [18]. Mechanistically, the toxicity of PCBs and PCDDs/PCDFs is mediated through the aryl hydrocarbon receptor (AhR) which is present in jawed fish, mammals, reptiles, and birds but not primordial fish and invertebrates [19]. For this reason, invertebrates are generally insensitive to PCBs and PCDDs/PCDFs [20] and effects are most commonly observed only in biota of greater evolutionary complexity, although the degree of sensitivity varies considerably among species. Although much effort has been expended to identify cause-effect relationships between specific PCB and PCDD/PCDF congeners and ecological impacts at higher levels of biological organization, this has proven difficult because individual congeners exhibit varying degrees of toxicity and they are taken up and metabolized at different rates as they are passed up food chains [21] leading to different contaminant profiles in aquatic biota over time.

PCBs and PCDDs/PCDFs are hydrophobic. In aquatic ecosystems they may be accumulated through bioconcentration but given their hydrophobic nature and predominant association with sediments and lipids, bioaccumulation through

dietary sources is probably more significant. PCBs and PCDDs/PCDFs are generally metabolized and eliminated slowly from tissues so they not only accumulate but also increase in concentration as they are passed up food chains. Consequently, these chemicals are most commonly found in highest concentrations in predatory species at the top of food chains as documented in the Great Lakes and Arctic regions. In the Great Lakes, PCB concentrations have declined in water, sediments and biota by up to 95% from peak concentrations in the mid-1970s but they remain sufficiently high that they continue to be a major cause of fish consumption advisories [22].

PCBs and PCDDs/PCDFs appear to be relatively non-toxic to freshwater microbial communities at environmental concentrations. Salizzato *et al.* [23] found that the maximum concentration (0.90 □g/g) of PCBs extracted from contaminated sediment, a value well above those typically detected, was below the limit of sensitivity of the Microtox assay. Numerous studies have demonstrated that PCB and PCDD/PCDF congeners can be degraded by microbial communities in contaminated freshwater sediments [24-27]. Aerobic degradation typically involves attack of the carbon ring and subsequent metabolism of the molecule [24] while anaerobic degradation occurs through reductive dechlorination [25]. In general, the rate of dechlorination depends on the relative number and position of chlorine atoms on the molecule and generally decreases with an increase in the number of chlorine substituents. Although rates of metabolism attained in laboratory studies are often high, in situ removal rates are generally exceptionally slow due to poor bioavailability and mass transfer [28]. Nonetheless, it is common to observe population-specific increases in abundances and shifts in microbial community structure in PCB or PCDD/PCDF-contaminated sediments [27].

There is limited information on bioaccumulation and toxicity of PCBs and PCDDs/PCDFs for freshwater plants and algae [29, 30] and evidence of population and community-level effects is rare. Patterson *et al.* [31] evaluated the historical response of diatoms and chlorophytes in sediment cores from a PCB-contaminated freshwater lake. During the period of maximum contamination (estimated peak bioavailable sediment concentrations were 0.5 μg/L), minimal changes were observed in both diatom and chrysophyte assemblages. They hypothesized that the bioavailable fraction of PCBs in lake sediments was too low to cause detrimental effects in the limnetic phytoplankton communities. Kostel *et al.* [32] found that periphyton in a laboratory stream system accumulated up to one order of magnitude greater concentrations than sediment. Periphyton community structure shifted from a diverse diatom-based community to one co-dominated by fewer types of cyanobacteria. Yockim *et al.* [33] estimated bioconcentration factors up to 2083 for 2,3,7,8-TCDD for the freshwater alga *Oedegonium cardiacum* but did not indicate if effects on growth or population size occurred. Residues of up to 7000 ng/g in freshwater macrophytes (*Ceratophyllum* and *Elodea* spp.) were measured in a 30-day mesocosm study on 2,3,7,8-TCDD but no adverse effects were reported [34]. These limited data indicate that significant impacts of PCBs and PCDDs/PCDFs on plants and algae are unlikely at current environmental concentrations but they may serve as an important source of these compounds to higher trophic levels.

Information on the toxicity of PCBs and PCDDs/PCDFs in freshwater invertebrates is also relatively limited. Dillon and Burton [35] found that exposure to some PCB congeners killed 47 to 83% of freshwater fishes and invertebrates after 24 to 48 h at concentrations that were several orders of magnitude higher than those encountered under field conditions. However, most of the PCB congeners tested produced negligible mortality. In the freshwater cnidarian, Hydra aligactis, Adams and Haileselassie [36] estimated LC50s of 5 and 20 mg/L, and found bud regeneration was inhibited at 1 and 4 mg/L, for Aroclors 1016 and 1254, respectively. In these and other studies, effects occurred at concentrations much greater than those measured in freshwater sediments. West *et al.* [37] observed no toxicity in full life cycle exposures with the midge Chironomus tentans and the oligochaete Lumbriculus variegatus up to 9533 ng/g lipid of 2,3,7,8-TCDD. TCDD also had no effect on development and reproduction in the freshwater snail Physa sp. [38, 39], the water flea Daphnia magna [39, 40], the mosquito Aedes aegypti [38], and the aquatic oligocheate Paranais sp. [38]. In contrast, Ashley *et al.* [41] estimated LD50s between 0.03 and 1.5 ng/g body weight for 2,3,7,8-TCDD in a freshwater crayfish and toxicity was characterized by delayed mortality (15 to 40 days after treatment) and reduced activity.

Given the relative insensitivity of invertebrates to PCBs and PCDDs/PCDFs, evidence for population and community-level impacts is rare and often confounded by co-occurrence with other contaminants or non-contaminant factors. For example, De Lange *et al.* [42] found that sediment moderately contaminated by PCBs and PAHs affected the structure but not productivity of benthic macroinvertebrate communities, which they attributed to counteracting effects between contamination and an associated food surplus. Cooper *et al.* [43] compared two urbanized watersheds in Michigan USA, one whose sediments were heavily contaminated with PCBs, PAHs and metals compared to the other. They found fewer

insect taxa, reduced invertebrate index of biotic integrity scores, and higher sediment toxicity in the more industrialized watershed. Although PCBs exceeded the probable effects level at one site, their contribution to the benthic community impacts appeared to be low relative to other contaminants.

Collectively, the weight-of-evidence indicates that significant population or community-level effects from PCBs and PCDDs/PCDFs in invertebrates are improbable at environmentally relevant concentrations. This conclusion is supported by the hazard assessment of Loonen *et al.* [44] who concluded that invertebrates experience reduced hazard relative to fish, fish-eating birds, and mammals. Mechanistically, this has been attributed to the absence of the Ah receptor in invertebrates. However, although effects of PCBs and PCDDs/PCDFs might not be predicted for most invertebrates based on receptor-mediated toxicity, one area that has not been evaluated extensively is potential multi-generational effects resulting from long-term, low-level exposures and this may warrant some consideration in future assessments of these compounds.

Because of their relative insensitivity, association with sediments and occurrence at the base of most food chains, algae, macrophytes and macroinvertebrates play a key role in the bioaccumulation and transfer of PCBs and PCDDs/PCDFs to higher trophic levels. As such, some have been proposed as reliable indicators of PCB contamination in freshwater systems [45] and numerous studies have investigated uptake, metabolism, and trophic transfer by invertebrates of PCBs [46-54] and PCDDs/PCDFs [55-57] in aquatic invertebrates. Zebra mussels (Dreissena polymorpha) accumulated PCB 77 from sediments, diet and water at a rate 10 times more efficient than Lampsilis siloquoidea, the mussel to which they are often attached [58]. Accordingly, high densities of zebra mussels likely influence PCB contaminant dynamics in Great Lakes ecosystems [59]. The freshwater crustacean Mysis relicta played a key role in PCB transfer from sediments into the Lake Champlain food web [60], and Sallenave *et al.* [61] showed that accumulation of PCB 153 in spiked plant material by downstream collectors was enhanced by the presence of both scrapers and shredders in stream mesocosms. Kidd *et al.* [62] estimated that PCBs were accumulated in lower trophic level organisms between 1000 and 100,000 times over surrounding water and sediment concentrations and Rasmussen *et al.* [63] showed that each trophic level contributed a 3.5-fold biomagnification factor for PCBs in Great Lakes lake trout. For TCDDs/TCDFs, Muir *et al.* [55] determined biota-sediment accumulation factors (BSAFs) of 24.6 and 18.6 for crayfish and mussels exposed to TCDF in an experimental lake mesocosm study and BSAFs ranging from 0.31 to 1.62 for uncaged aquatic insects exposed to pulp mill effluent. In laboratory exposures, Loonen *et al.* [56] determined BSAFs of 1.6 and 0.07 for TCDD and octachlorodibenzo-p-dioxin and Pickard and Clarke [57] determined BSAFs ranging from 0.04 to 2.42 for eleven TCDD/TCDF congeners in L. variegatus.

In the Great Lakes region, declines in populations of both fish and fish-eating birds have been causally linked to the presence of PCBs and PCDDs/PCDFs [64, 65] with corresponding though often poorly understood impacts at the community and ecosystem level [66]. Lake trout populations in the lower Great Lakes provide an excellent case study on the role that contaminants likely played in regulating population levels. Lake trout population declines began in the 1930s in response to increased fishing pressure, advancements in fishing technology (e.g. improved fishing line materials), increases in invasive sea lamprey populations, and changes in food web structure caused by invasive fish species [67]. By 1960, a virtual collapse of lake trout populations in the lower Great Lakes had occurred. Extensive re-stocking of fingerlings began in the 1950s and continues to this day but has met with poor success. Hypotheses to explain the slow recovery of Great Lakes lake trout include poor survival to spawning age after stocking and hence insufficient numbers to ensure successful annual recruitment, failure to locate natural spawning grounds due to loss of olfactory acuity in hatchery-reared fish exposed to inappropriate spawning substrates, changes in reproductive performance due to complex changes in prey fish population densities, and exposure to contaminants [67].

There is strong evidence supporting the role of contaminants, particularly PCBs and PCDDs/PCDFs, in lake trout population declines [68]. Initial evidence for the role of contaminants was provided by Mac *et al.* [69] who found that up to 97% of lake trout fry reared in hatcheries between 1978 and 1981 died when exposed to water from the upper Great Lakes. Further, a higher than expected frequency of blue sac disease, which can lead to the death of eggs, was observed in lake trout from Lake Ontario in the 1970s suggesting that maternal transfer of dioxin and dioxin-like compounds to the eggs may have been responsible for the effects. Numerous studies have since established causal relationships between early life stage mortality in lake trout and exposure to PCDDs/PCDFs/PCBs [70]. Although the precise cascade of events from initial exposure to early life stage mortality is not fully understood, as Ah receptor agonists, PCBs and PCDDs/PCDFs are known to affect reproduction and development via disruption of endocrine function [71]. Ankley and Giesy [65], using a weight-of-evidence approach, outlined a series of laboratory and field studies conducted throughout

the 1990s on Lake Ontario lake trout which provide strong evidence to link PCDDs/PCDFs with lake trout population declines. These studies established that early life stage lake trout were exquisitely sensitive to PCDDs/PCDFs and other Ah receptor agonists, that these compounds were transferred maternally from adult fish to eggs, and that effects were consistent with observed pathologies, such as blue sac disease, in field-collected lake trout [70, 72-74]. Comparing the observed effects to residues of PCDDs/PCDFs and PCBs extracted from Lake Ontario sediment cores, Ankley *et al.* [48] concluded that the toxicity predictions are in excellent agreement with available historical data for lake trout population levels and suggest that evidence for recent improvement in natural reproduction is consistent with declining levels of persistent bioaccumulative chemicals in sediments and biota, a conclusion that is supported by Cook *et al.* [70].

With their position atop freshwater food chains, fish-eating birds often represent the most vulnerable group of organisms with respect to the effects of POPs. Population declines of fish-eating birds such as herring gulls, cormorants, and Caspian terns in the Great Lakes have been linked to contaminant-induced reproductive effects [75]. Keith [76] found that reproductive success in herring gulls in Lake Michigan was approximately one third of the normal rate and Gilbertson [77] found that reproductive success was about 10% of expected rates in nesting sites in Lake Ontario. Initially, reproductive failure in fish-eating bird populations was attributed to egg shell thinning resulting from exposure to the pesticide DDT but high rates (up to 30%) of embryo mortality among gull populations [66] and continued observation of developmental and reproductive abnormalities after the ban of DDT in the early 1970s indicated other chemicals were also contributing to population declines. Experiments in which eggs were transferred from clean to contaminated sites for herring gulls [78] and Foster's terns [79] found lower hatching success and behavioral anomalies in adults, thus supporting the contaminant-based theory of observed declines. Many studies documented numerous consistent symptoms including increased embryo and chick mortality, growth retardation, congenital deformities (*i.e.* cross-bill syndrome), feminization of embryos, and abnormal parenting behavior. These effects became known as the Great Lakes Embryo Mortality, Edema, and Deformities Syndrome (GLEMEDS; [80]), a syndrome previously documented in animal studies on known Ah receptor agonists.

Causal evidence that PCBs and PCDDs/PCDFs contributed to population declines in Great Lakes fish-eating birds was developed in the 1990s. Ludwig *et al.* [81] evaluated reproductive success in a population of Caspian terns in Saginaw Bay, Michigan following a one in a hundred-year flood in 1986 that released sediment-bound PCBs. PCBs accumulated rapidly in tern eggs, accounting for 98% of the toxic equivalents (TEQs), and concentrations in the eggs approached the lethal dose required to kill 95% of chicken embryos. The percent of chicks that hatched from first and second clutches the following year was 28% and 0%, respectively, compared to corresponding 5-year rates of 60% and 43% and the 3-year average hatchling deformity rate increased 163 times over the historic rate. Using a weight-of-evidence approach, Ludwig *et al.* [82] reviewed available data from studies on cormorant and Caspian tern populations around the Laurentian Great Lakes to test the hypothesis that deformities in embryos and chicks of these species were caused by contaminants measured as TEQs. Hatching deformities and abnormalities were comparable to those observed in chickens exposed to PCBs and dioxins and were correlated with concentrations of PCBs and TEQs, which were present at concentrations sufficient to cause the effects. Overall, they rejected the null hypothesis and concluded that there was a relationship between the incidence of deformities in both bird species and exposure to planar halogenated compounds measured as TEQs or total PCBs. Giesy *et al.* [83], also using a weight-of-evidence approach, concluded that lethality and deformities in embryos of colonial fish-eating Great Lakes birds were caused by multiple planar dioxin-like compounds which expressed their effects through a common mechanism of action.

With strong evidence for the role of contaminants in causing population declines in Great Lakes fish and fish-eating birds, effects at the community and ecosystem levels might be expected. For example, the primary route of exposure for affected bird populations is contaminated fish, so loss of predatory bird species might be expected to cause changes in fish, and possibly other organism, populations [80] *via* a cascade of trophic interactions. However, understanding trophodynamic changes and shifts in food web structure in the context of contaminants is difficult as non-contaminant drivers of change in ecosystem structure (e.g. introduction of exotic species) must also be considered. Some studies have attempted to address this complexity by examining expected shifts in contaminant profiles in food webs experimentally [49, 84] and *via* modeling (e.g. [51]) but empirical evidence of shifts in community structure and ecosystem function resulting specifically from POPs remains elusive [66].

Numerous studies have measured PCB and PCDD/PCDF residues in adult amphibian and reptile tissues but evidence for effects of PCBs and PCDDs/PCDFs at the population and community level is scant [85, 86]. A study at

a PCB-contaminated site in Peducah, Kentucky found that tissue-borne PCBs were significantly higher in larvae than adults of various anuran species but found no evidence of adverse effects at the population level [87]. PCB tissue levels in frogs in five PCB-contaminated southwestern Michigan wetlands were lower than those in sediments and suggested that the apparent lack of effects on frog populations could be explained by limited contaminant accumulation [88]. Jung and Walker [89] estimated that embryos and tadpoles of green frogs (*Rana clamitans*), leopard frogs (*R. pipiens*), and American toads (*Bufo americanus*) are 100 to 1000-fold less sensitive to TCDD-induced lethality than most fish species. Reeder *et al.* [90] observed a significant shift in sex ratios favoring males, and increased prevalence of intersexuality, in field populations of cricket frogs (*Acris crepitans*) and concluded that the evidence suggested a strong association between population declines and TEQ body burdens. They suggested that amphibian populations could be affected at environmentally relevant concentrations; however, in most studies effects occur at concentrations significantly higher than those typically measured in water and sediments. Based on their work with *X. leavis*, Levine *et al.* [91], suggest that the apparent insensitivity of anurans to dioxins reflects low affinity binding by the Ah receptor. Bishop *et al.* [92] suggested that high mortality in snapping turtles (*Chelydra s. serpentina*) in 1984 and 1985 in Hamilton Harbor, Ontario was strongly correlated with tissue PCB concentrations. Bishop *et al.* [93] observed a significant increase in abnormal development with increasing HAH exposure in snapping turtles eggs at various sites in the lower Great Lakes; the strongest correlations were associated with PCDD/PCDF concentrations. Eisenreich *et al.* [94] found no evidence of immediate effects on embryonic development and hatching success of maternally-exposed snapping turtle eggs collected from the Hudson River, USA relative to those from a reference site; however, high mortality and lower growth rates, correlated with PCB concentrations in the eggs, were observed eight months after hatching.

Polybrominated Diphenyl Ethers

Background and Chemistry

Polybrominated diphenyl ethers (PBDEs) are a diverse group of chemicals that are structurally similar to PCBs, and like PCBs have 209 congeners. Produced as octa-, penta-, or deca-BDE formulations, PBDEs are used primarily as flame retardants in commercial products, including building materials, electronics, furnishings, motor vehicles, plastics, polyurethane foams, and textiles [95, 96]. Commercial PBDE products are typically composed of a complex mixture of congeners, although most mixtures are dominated by one or two specific congeners [97]. The three dominant forms used in industrial manufacturing (deca-, octa- and penta-BDEs) each have specific uses, *i.e.*, penta-BDE (polyurethane foams), octa-BDE (rigid plastics e.g. ABS) deca-BDE (textiles, resins, and rigid plastics). By 1990, worldwide production of PBDEs had surpassed peak production of PCBs; coupled with the fact that PBDEs are not chemically bonded to the materials with which they are associated, and are thus readily released during product use and disposal, they now occur widely in the global environment [98].

PBDEs have low water solubility (typically in the low μg/L range) and log K_{ow} values ranging from 5.9-6.2, 8.4-8.9, and 10 for the penta- octa- and deca-BDE, respectively [99]. The penta-, octa-, and deca-BDEs have thus been detected globally in a wide variety of matrices, both biotic and abiotic [97, 100]. In a recent study of marine water and sediments in Japan, concentrations in water, sediment, and fish and invertebrates were in the low pg/L range, ng/kg range, and ng/g range, respectively [101]. In sediments, deca-BDEs generally dominate the total PBDEs, while in biota tetra-BDE (BDE-47) tends to dominate, an observation consistent with other studies [100]. Not all PBDEs appear to biomagnify and those that do, do not appear to biomagnify to the same degree as PCBs [101, 102]. Further, metabolism, specifically debromination, occurs in a number of species, including fish and microbes [98, 103]. Regional comparisons of total and specific PBDE congener concentrations in the environment indicate that PBDE concentrations in Europe are approximately 10-fold below those in North America [100] and concentrations in arctic marine mammals are currently 10 to 100-fold lower than in temperate species [100, 104]. Currently, the penta-BDE mixture is banned in Europe, the main manufacturer of the penta- and octa-BDEs has begun a phase-out of these congeners, and the deca-BDE, despite industry arguments, is now being banned in jurisdictions across the globe [98]. A series of recent reviews have examined their history, chemistry and toxicology in marine and freshwater ecosystems [95-100, 104, 105].

Ecotoxicology

Due to the chemical nature of PBDEs, and their similarities to PCBs, toxicological research has predominantly focused on freshwater and marine organisms. In mammals, PBDEs may act as hormone mimics affecting thyroid

function, sexual development and behavior; be cytotoxic (increased apoptosis and necrosis); and may be linked to tumor formation and cancer [98]. The mode of action of PBDEs is uncertain; like PCBs and dioxins, PBDEs were originally thought to act *via* the Ah receptor but recent evidence suggests that this may not be the case as the biomarkers associated with AhR activation are not up-regulated following PBDE exposure [97, 99]. PDBEs have been implicated in disruption of thyroid hormone homeostasis in rodents [97, 99], though it is unclear if this mode is shared with other vertebrates and invertebrates, especially those with no analogous hormonal system. Recent work has found evidence for disruption of oxidative phosphorylation and inhibition of complex II of the mitochondrial electron transport chain in fish, especially for hydroxylated forms of PBDEs [106].

The limited toxicological research on PBDEs has generally focused on whole organism or molecular responses and studies investigating potential effects of PBDEs at the population/community level are rare. Indeed, the majority of work has focused on characterizing concentrations of PBDEs in fish, birds, marine mammals and invertebrates [102, 107-111]. When examining microbial populations for their ability to metabolize various PBDEs, a strain of bacteria (*i.e.*, *Burkholderia xenovorans* LB400) was shown to exhibit some toxicity when exposed to mono-BDE. The toxicity was attributed to a metabolite formed during the biotransformation process, and this strain also produced hydroxylated PBDEs which, based on work with vertebrates, are thought to be more toxic than the parent compounds [103, 106]. Several recent reviews [103, 106] reveal the paucity of acute toxicity information for aquatic organisms. However, the limited data that are available indicate that acute toxicity from PBDEs or their metabolites is unlikely. In embryonic zebra fish, the 72-h EC50 for developmental effects (e.g. developmental arrest, edema) was 14.5 μg/L (25 nM) and the adult LC50 after 96 h was between 174 and 232 μg/L (300 and 400 nM) for the hydroxylated BDE-47 [106]. Based on current environmental concentrations and appropriate safety factors, the authors concluded that concerns for wildlife for this PBDE metabolite are unwarranted. The 24-h LC50 to *D. magna* for PBDE congener 153 was >210 μg/L with some chronic effects on reproduction at concentrations >12.5 μg/L [112]. Due to their physicochemical properties (e.g. hydrophobicity), PBDEs are not ideal candidates for microcosm/mesocosm-based toxicity studies and because of their similarities to PCBs (chemical, toxicological, and in terms of co-occurrence) it would be difficult to attribute any observed effects at the population or ecosystem level in the field to PBDEs alone. This issue is highlighted by Jaspers *et al.* [110] who measured a variety of POPs (PCBs, PBDEs, HCHs and DDT) in predatory aquatic and terrestrial bird tissues. Of the seven species examined, six showed no relationship between tissue burden and condition index. The lone exception was the barn owl, which showed negative correlations with PCBs and DDT, which were 100 and 10-fold higher in concentration, respectively, than the PBDEs. The same relative difference between total PCB and PBDE burden in salmonid tissue concentrations have been reported (43,100 ng/g lipid and 2440 ng/g lipid, respectively) in Lake Michigan [102], meaning the attribution of ecological effects solely to PBDEs in the field is unlikely or not occurring at this time.

POLYCYCLIC AROMATIC HYDROCARBONS

Background and Chemistry

Polycyclic aromatic hydrocarbons (PAHs) are a class of POPs comprised of thousands of individual substances that contain two or more fused aromatic rings composed of carbon and hydrogen atoms. PAHs are formed through pyrogenic, petrogenic, diagenetic, and possibly biogenic sources [113]. Pyrogenic sources may be natural, such as forest fires and volcanoes, or industrial, such as the incomplete combustion of fossil fuels, fugitive losses during petroleum extraction, transport, and industrial emissions [114]. Petrogenic sources result from diagenetic processes – low temperature, high-pressure reactions of biogenic materials that occur over geological time scales that lead to the formation of petroleum and other fossil fuels. PAHs are generally hydrophobic and many interact strongly with sedimentary organic carbon [113] and bioaccumulate in aquatic biota, particularly those at lower trophic levels [115, 116]. As such, PAHs are commonly associated with sediments and particulate matter and ecotoxicological concerns have therefore focused on toxicity to aquatic benthic communities and impacts on associated food chains. Historically, only 16 PAHs have been prioritized as environmentally significant and thus received the focus of research; however, it is now recognized that aquatic communities may be exposed to, and potentially affected by, hundreds of PAHs [116] and information on their potential risks are poorly understood.

Ecotoxicology

The toxicology of PAHs in aquatic environments has been well documented and numerous reviews/books are available in relation to bioavailability [113, 115], bioaccumulation [115, 117] and toxicity [114, 118, 119]. PAHs

can adversely affect aquatic organisms physically (e.g. smothering, attenuation of light, habitat modification, and reduced food availability) and directly, *via* toxicity from parent or photosensitized PAHs [114]. The former is most commonly associated with accidental releases of petroleum while the latter results from exposure to PAHs associated with oil or derived from natural or industrial sources. The toxicological effects of PAHs are numerous (see Table **14**.1 in [114]). The primary mode of action of PAHs is narcosis [120]. However, some PAHs act as pro-carcinogens through metabolic formation of DNA adducts, a potentially critical initial step in carcinogenesis [121]. DNA adduct formation has been used as a biomarker of PAH exposure in aquatic organisms [119]. PAHs can also induce immunosuppression [122] as indicated by increased incidences of disease in Japanese medaka exposed to benzo[a]pyrene [123]. PAHs and their derivatives may also affect estrogenic activity. Rainbow trout hepatocytes exposed to anthracene exhibited anti-estrogenic activity, possibly mediated through binding to the Ah receptor [124]. Villeneuve *et al.* [125] found that several PAHs and hydroxylated or methylated PAH derivatives induced estrogenic responses in three separate cell lines.

A unique property of some PAHs is the ability to absorb energy from the ultraviolet spectrum of sunlight, resulting in excited state molecules that, through the subsequent loss of energy, can be several orders of magnitude more toxic than the parent molecules [126-128]. This phenomenon, referred to as phototoxicity, has been demonstrated in freshwater invertebrates [129-132], fish [133-134], and amphibians [135-138]. While most of these studies were conducted under laboratory conditions, PAH-UV interactions in the field have been observed [126, 138, 139] and it has been speculated that synergistic interactions between UV light and PAHs in aquatic habitats may be a contributing factor in amphibian population declines [140]. Others have argued that phototoxicity in the field is ecologically irrelevant because abiotic factors (e.g. dissolved organic carbon), physiological mechanisms (e.g. metabolism/excretion) and physical structures (e.g. integument, burrowing, larval cases) mitigate exposure to UV radiation [141].

Evidence for impacts of PAHs at higher levels of biological organization in the field is scant. Unlike many of the classic POPs, PAHs do not biomagnify [115]. Greatest PAH tissue residues appear to be associated with primary consumers and detritivores in sediments, and tissue concentrations generally decrease with increasing trophic level due to species-specific differences in toxicokinetics and increased biotransformation, especially in vertebrates [115, 142]. Thus, effects on populations and communities are more likely to result from direct exposure to PAH or indirect ecological effects than to food chain transfer and subsequent direct effects at higher trophic levels.

Freshwater microbial communities are both affected by and adaptable to PAHs. In a field-based microcosm study, Baker and Morita [143] found that glucose mineralization and phosphatase levels declined significantly but methane and CO_2 production rates significantly increased in sediment bacterial communities after a 4-week exposure to crude oil designed to mimic a spill. Nitrogen fixation was not affected by 0.1% (v/v) oil, but was reduced after 8 weeks by 1.0% oil. In contrast, Nyman [144] found that exposure of wetland sediment microbial communities to two types of crude oil stimulated bacterial metabolic activity as indicated by measurements of redox potential and respiration. PAHs occur naturally, so it is not surprising that microbial communities have evolved the capacity to degrade them [145], and PAH-degrading capacity is much greater in contaminated soil where selection has favored bacteria capable of withstanding exposure [146]. However, in situations of heavy contamination (e.g. oil spill), ecosystem integrity and function may be affected due to lower microbial diversity as this reduction disrupts the tight coupling and interdependence among consortia, and between consortia and grazers. For example, Nyman [144] observed an increase in metabolic activity and oil degrading activity in their wetland sediment study, but this came at the expense of microbial diversity, with tolerant species becoming dominant as sensitive species declined in abundance.

The toxicity of PAHs to freshwater algae and macrophytes has been evaluated in laboratory and field studies. Bott and Rogenmuser [147] exposed algal communities to three oil extracts in stream microcosms for several weeks. No. 2 fuel oil extracts depressed algal biomass (measured as chlorophyll *a*), decreased diatom occurrence, and resulted in dominance by blue-green algae. Used crankcase oil extracts also depressed biomass, but Nigerian crude extracts did not, and both of these extracts had less effect on algal community composition than did the No. 2 extracts. Marwood *et al.* [148] observed effects of PAHs at environmentally relevant concentrations on photosynthesis in natural algal assemblages and attributed this to phototoxicity. Burk *et al.* [149] found that total plant cover, total and mean number of species, and Shannon diversity declined progressively for two years after an accidental oil spill in a marsh and eighteen species found before the spill were absent the following season. However, the vegetation of the marsh showed substantial recovery by the third and fourth years. McGlynn and Livingston [150] modeled

adsorption/desorption and potential effects of sediment PAHs at low concentrations by rooted aquatic plants in field and laboratory experiments. The macrophytes' roots assimilated PAHs and the assimilation exhibited saturation. Growth of the macrophytes was inhibited by PAHs but at concentrations several orders of magnitude greater than threshold effects levels for aquatic animals.

Bestari *et al.* [151] and Sibley *et al.* [152, 153] exposed freshwater plankton communities to creosote in microcosms for 83 days at concentrations ranging from 0.06 to 109 mg/L. Creosote had no direct toxic effect on phytoplankton whose population densities and diversity in all treatments exceeded those in the controls and exhibited a parabolic relationship relative to both time and total PAH [152]. In contrast, zooplankton abundance and diversity was significantly reduced by creosote, with a 7-day community-level no-effect concentration of 5.6 μg/L [153]. The zooplankton community was dominated by rotifers, which proliferated at the expense of more sensitive cladocerans and copepods. Recovery to pre-treatment abundance levels occurred in all concentrations by the end of the 83-day exposure. The growth of phytoplankton populations appeared to be stimulated by both indirect (lower grazing pressure from zooplankton) and direct (hormetic stimulation by PAHs) effects.

Several studies have examined the response of freshwater benthic macroinvertebrate communities to PAHs. Crunkilton *et al.* [154] monitored the response of benthic macro-invertebrates in a small Missouri, USA stream into which 1.5 million liters of domestic crude oil had been spilled. Sensitive members of the benthic community (aquatic insects, mussels, snails) declined to <0.1% of expected abundance 25 days after the spill and species diversity indices and the abundance of mayfly and stonefly genera were below water quality criteria for Missouri streams up to 11 months after the spill. The impacts were attributed to physical obstruction of both substrate and organisms, and PAH toxicity. West *et al.* [155] evaluated the effectiveness of a carbonaceous resin to reduce the bioavailability of PAHs in field-contaminated sediments as a basis for potential remediation using laboratory toxicity tests and field colonization studies. The resin significantly reduced pore water concentrations of eight measured PAHs in both laboratory and field sediments. In laboratory tests, bioaccumulation and phototoxicity in L. variegatus were significantly reduced; in the field-deployed sediments, the resin amendment also decreased pore water PAH concentrations but did not improve benthic invertebrate colonization. Den Besten *et al.* [115] investigated impacts of PAH-contaminated sediments on benthic macroinvertebrates in the Rhine-Muese Delta in The Netherlands. Highly contaminated sediments contained significantly fewer taxa, had lower species diversity compared to reference sites, and produced significant toxicity in sediment bioassays with the invertebrates C. riparius and D. magna. De Lange *et al.* [156] evaluated seasonal variation and bioavailability of PAH in contaminated floodplain lake sediments in relation to benthic invertebrate community structure. While sediment-associated PAH concentrations occurred at levels at which effects were predicted, biovailability was low and the PAHs were not associated with observed impacts on benthic community structure.

Cooper *et al.* [43] compared benthic community and fish population structure in two sub-watershed wetlands of a western Michigan lake, one of which is highly contaminated with PAHs and metals as a result of a long history of industrial activity. Significantly fewer insect taxa, reduced fish species richness and catch per unit effort, and lower invertebrate and fish index of biotic integrity scores were found in the industrialized watershed. Cormier *et al.* [157] used a formal strength-of-evidence methodology [158] to infer causes of impairments at two sites in the Little Scioto River, Ohio, USA, which is heavily contaminated by sediment PAH. At the upstream site, they concluded that impairment of the benthic community and fish populations was due to altered habitat substrate (predominance of fine-textured sediment) and low dissolved oxygen. At the downstream site, impacts included lower diversity and dominance by pollution-tolerant invertebrates and reduced fish growth, elevated PAH tissue concentrations and increased incidences of abnormalities in fish, all of which could be causatively explained by concentrations of sediment PAHs. Lesko *et al.* [159] assessed the effects of contaminated sediments on reproductive potential of female brown bullhead (*Ameiurus nebulosus*) collected from the Black and Cuyahoga Rivers, Ohio, both contaminated with metals, PAHs and PCBs. Females from the most contaminated (Cuyahoga) river had higher fecundity and the population size was larger compared to the reference river, which they attributed to an enhanced food supply due to reduced competition from predators. However, fish diversity in the Cuyohoga River was lower and incidences of tumors higher relative to the reference river [160]. Evidence that PAHs can act as endocrine disrupters has largely been developed for fish [71, 161, 162]. While studies to date have not linked endocrine effects directly to population or community-level effects, evidence that PAHs may impair reproduction in fish, either through altered sex steroid metabolism or biosynthesis (see [71] for examples), reduced growth or abnormalities in larval fish [163] suggest that endocrine-induced population effects are possible.

Studies investigating the effects of PAHs on amphibians and reptiles have largely focused on organism and physiological responses, either through direct exposure to PAHs [136, 164, 165] or synergistic exposure to UV light as described above. Few studies have examined the effects of PAHs at the population and higher levels of biological organization in amphibians. Physiologically, amphibian responses to PAHs are similar to other vertebrates [118]. Lefcort *et al.* [166] studied the effects of oil and silt on the growth and metamorphosis of larval mole salamanders, *Ambystoma opacum* and *A. tigrinum tigrinum* in oil-contaminated ponds and outdoor microcosms treated with used motor oil. In both test systems, both species had reduced size and weight compared to controls that was attributed to an indirect effect of reduced algal growth (salamander food) and direct toxic effects.

FLUORINATED SURFACTANTS

Background and Chemistry

Surfactants are surface-active materials that, at low concentrations, are capable of reducing the surface tension of a liquid *via* selective adsorption at the interface [167]. Surfactant molecules are amphiphilic, characterized by a hydrophilic (water-soluble) 'head' group attached to a hydrophobic (water-insoluble) 'tail' portion. In conventional, hydrocarbon-based surfactants, the hydrophobe is typically an oleophilic (lipid soluble) hydrocarbon. In perfluorinated surfactants (PFSs) fluorine atoms replace hydrogen atoms on the hydrophobe. The replacement of hydrogen with highly electronegative fluorine atoms on the hydrophobe renders PFSs both hydrophobic and oleophobic, capable of repelling both water and oils. Increasing the number of fluorine atoms in the hydrophobe increases chemical stability as bond strength generally increases with an increase in the number of fluorine constituents [168]. The exceptional persistence rendered by the high molecular stability has led to the detection of PFSs in a variety of biotic and abiotic matrices on a global scale [169-172].

The global pervasiveness of PFSs reflects both a long history of manufacture (since the 1950s) and widespread use as surface treatments for carpets, fabrics, and paper products to repel soil, oil, and water and applications such as fire fighting foams, adhesives, electronic insulators, cosmetics, cleaners, among others [167, 170]. Recently, concerns over the occurrence of perfluorooctane sulfonic acid (PFOS) in the environment, especially in sensitive Arctic regions, resulted in a cessation of production in 2000 by 3M Corporation and the recent inclusion of PFOS under Annex B of the Stockholm Convention on POPs, indicating that its use should be restricted.

Ecotoxicology

Environmental concerns about PFSs have predominantly focused on two compounds: perfluorooctanoic acid (PFOA) and PFOS. Over the past decade, the toxicity of PFOS and PFOA to environmental receptors has been well studied as reviewed in [170, 171, 172]. However, with the exception of the microcosm studies described below, most of this work has been conducted at the organism level.

Several studies have assessed the toxicity of PFSs in freshwater macrophytes and algae. Boudreau *et al.* [174] and Boudreau [175] assessed the toxicity of PFOS and PFOA in the algae *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*, and the aquatic plant *Lemna gibba*, under laboratory conditions at chain lengths of 4 to 7 carbons. In tests with PFOS, 96-h growth inhibition NOEC values were 5.3 and 8.2 mg/L for *P. subcapitata* and *C. vulgaris*, respectively, and 6.6 mg/L for *L. gibba* (wet weight). In tests with PFOA, laboratory EC10 values for growth ranged from 5.7 to 59.4 mg/L for *C. vulgaris* (96-h) and *L. gibba* (7 d), respectively [175]. Colombo *et al.* [176] calculated a NOEC value of 12.5 mg/L for growth inhibition in *P. subcapitata* exposed to the ammonium perfluorooctanoate. Liu *et al.* [177] assessed four perfluorocarboxylates and two sulfonates to the alga *Scenedesmus obliquus* and found that toxicity based on cell density ranged from none (PFOA) to 21.6 mg/L (perfluorotetradecanoic acid). Latal *et al.* [178] showed that perfluoro-hexanoic, -heptanoic, -octonoic, and -nonanoic acid were more toxic than PFOS and PFOA to three species of algae (LC50s range: 6.0-24.3 mg/L). Blue-green and diatom species were comparable in sensitivity but both were more sensitive than green algal species. In an outdoor microcosm study, Boudreau *et al.* [174] determined a 42-day NOEC (frond number) for PFOS of 0.2 mg/L for a population of *L. gibba*. Hanson *et al.* [179, 180] estimated NOEC values in excess of 0.3 mg/L and 23.9 mg/L for PFOS and PFOA, respectively, for two species of *Myriophyllum* in outdoor microcosm studies.

With one exception, freshwater invertebrates appear to be relatively insensitive to PFSs. Boudreau *et al.* [174] estimated NOEC values for immobility in 48-h exposures of 0.8 and 13.6 mg/L for *D. magna* and *D. pulicaria*.

NOEC values in tests with PFOA for both *Daphnia* species indicated reduced toxicity relative to PFOS [175]. In both cases, daphnids were only sensitive to carbon chain lengths ≥ 8. Ji *et al.* [181] estimated LC50s of 17.95 mg/L for PFOS and 199.51 mg/L for PFOA for the daphnid *Moina macrocopa*, which is approximately twice the LC50 determined for *D. magna*. In a 7-day chronic test, *M. macrocopa* experienced significantly reduced reproduction at 0.31 mg/L for PFOS, which was approximately seven times lower than the effect concentrations observed over the 21-day exposure in *D. magna*. The greatest toxicity observed for PFOS in any aquatic species is the 20-day LC50 of 9.2 μg/L reported for *C. tentans* [182]. In the same test, *C. tentans* did not respond to PFOA in 10-day exposures at concentrations up to 100 mg/L. In a series of indoor microcosm studies, PFOS caused a significant reduction in zooplankton abundance and altered community structure at concentrations ≥10 mg/L [183]. In a similar test with PFOA, Sanderson *et al.* [184] determined a lowest observed effect concentration (LOEC) of between 10 and 70 mg/L depending on taxonomic group. In these studies, zooplankton communities became dominated by rotifers with simultaneous declines in cladoceran and copepod species at the highest concentrations. In a 35-day outdoor microcosm study, Boudreau *et al.* [174] estimated a community-level NOEC of 3.0 mg/L for zooplankton, with significant declines in zooplankton abundance at 30 mg/L. Kannan *et al.* [185] estimated a bioconcentration factor of approximately 1000 for PFOS in Great Lakes benthic invertebrates and Higgins *et al.* [186] estimated lipid-normalized BSAF values of 33 and 42 for PFOA and PFOS indicating that both compounds may be accumulated from sediments and thus available for trophic transfer in freshwater food chains.

In fish, studies indicate that toxicity thresholds of PFSs are typically much higher than environmental concentrations. Du *et al.* [187] observed no mortality in zebra fish exposed to PFOS in a 70-day exposure but did observe significant declines in growth and various biochemical and genetic endpoints at concentrations as low as 50 μg/L. Hagenaars *et al.* [188] also found no mortality in carp fry exposed to PFOS concentrations up to 1 mg/L but did observe significantly reduced condition factor and liver indices as low 0.1 mg/L. Colombo *et al.* [176] estimated a 96-h LC50 for the ammonium salt of PFOA of 400 mg/L. PFOS and PFOA are hepatotoxic, affecting hepatocyte membranes indicative of necrosis and interfere with fatty acid metabolism [189]. PFOS and PFOA exposures can also decrease circulating sex steroids in fish depending on species, age, and sex [189, 190]. Contrary to mammalian studies, PFOS and PFOA appear to be relatively weak peroxisome proliferators in fish [189]. In freshwater fish, PFOS and PFOA bind tightly to serum proteins [191] and bioaccumulate (from highest to lowest) in fish in the blood, kidney, liver, and gall bladder [192]. Kannan *et al.* [185] found that PFOS concentrations in Chinook salmon were 20 times greater than in their prey species and Furdui *et al.* [193] estimated log bioconcentration factors of 4.1 and 3.8 for PFOS and PFOA, respectively, in Great Lakes lake trout. These data provide evidence of food chain transfer of PFAs. Interestingly, Kannan *et al.* [195] found notable concentrations of PFOS in fish eggs suggesting oviparous transfer.

Numerous studies have measured residues of PFSs in aquatic fish-eating birds [169, 185] but few have assessed toxicity. Newsted *et al.* [194] determined a 5-day LD50 of 150 mg/kg body weight in young (2-day old) mallard ducks exposed to food-borne PFOS. The concentration of PFOS in mallard livers associated with mortality was at least 50-fold greater than the single maximum concentration that has been measured in livers of avian wildlife indicating low risk. Adult mallards fed PFOS up to 150 mg/kg feed showed no treatment-related effects [195]. Based on this work, they estimated an avian toxicity reference value of 0.021 mg/kg body weight per day. Kannan *et al.* [185] recorded the highest concentrations of PFOS from bald eagles in the Great Lakes and estimated a biomagnification factor of 10 to 20. Excretion of PFOS in bald eagles appears to be more rapid than classical POPs [185], but the potential for binding of PFOS with serum proteins and production of metabolites whose toxicity is poorly understood, warrants further investigation with respect to potential risks to fish-eating bird populations.

The occurrence and toxicity of PFSs in amphibians and reptiles is limited to only a few studies. PFS concentrations ranging from 137 to 250 ng/g (wet weight) have been measured in green frogs, yellow-blotched map turtles, and snapping turtles from the Great Lakes [185]. Ankley *et al.* [196] observed reduced growth and delayed metamorphosis, which can impact population stability, in northern leopard frogs at 3 mg/L PFOS and hypothesized that this may have been the result of impaired thyroid function.

The weight of evidence indicates that PFSs pose limited risks to freshwater organisms as toxicity thresholds are typically well above concentrations of PFAs measured in the field. Beach *et al.* [171] derived protective screening-level concentrations for PFOS of 2.3 mg/L for freshwater plants and algae and 1.2 μg/L for aquatic invertebrates, the latter value reflecting the sensitivity of *C. tentans* [182]. A tissue-based threshold value of 87 mg/kg wet weight was

determined to be protective of fish. Collectively, the evidence does not support a causal link between current PFS contamination and population or community-level impacts in aquatic systems.

PHARMACEUTICALS AND PERSONAL CARE PRODUCTS

Background and Chemistry

Pharmaceuticals and personal care products (PPCPs) encompass a wide variety of chemicals and applications, including the cure and prevention of disease in humans and livestock, diagnostic treatment (e.g. x-ray contrast media), growth promotion in livestock, and chemical additives (e.g. musk fragrances, antibacterial agents) in personal care products [197]. Thousands of PPCPs are produced and used daily around the world, in quantities that now approach those typical of agrochemicals [198]. Sources of PPCPs to freshwater environments include wastewater treatment effluents, run-off from agricultural fields amended with manure and sewage, direct addition *via* livestock excretion, and leaching from landfill sites.

In contrast to many of the legacy chemicals addressed in this chapter, knowledge of pharmaceuticals in the environment, and the attendant concerns for human and environmental health, emerged only in the late 1990s. While evidence of hormonally active pharmaceuticals date back to the 1960s [199], the pervasive nature of PPCPs has only recently been brought to light because of advancements in analytical technology that made it possible to detect PPCPs at the low concentrations at which they typically occur. In North America, the widespread occurrence of PPCPs in surface waters was documented by Kolpin *et al.* [200] who identified 82 compounds from surface waters of 139 streams with many compounds co-occurring. Many studies have since added to the list of PPCPs known to be present in the environment [201]. These studies show that PPCPs occur predominantly at sub-µg/L concentrations. However, although the majority of PPCPs occur at low concentrations and degrade rapidly under most environmental conditions, continual addition to the environment renders them effectively "pseudo-persistent" [202]. Moreover, pharmaceuticals are designed to elicit biological effects, which is the basis of their therapeutic activity [203]. The combination of pseudo-persistence and biological activity has lead to uncertainty about how PPCPs will behave toxicologically in the environment and legitimate questions about potential risks to environmental receptors.

Ecotoxicology

The fate and effects of PPCPs in aquatic systems has been summarized in several reviews [203-209]. There is general consensus that acute exposure of aquatic organisms to PPCPs carries negligible risk [210, 211]. In a review of over 360 acute toxicity endpoints in freshwater organisms for 107 human PPCPs, Webb [212] found that <10% were toxic at concentrations <1 mg/L, a value approximately 3 to 4 orders of magnitude above concentrations typically measured in freshwater ecosystems. Thus, interest in PPCPs from an ecological impacts perspective is presently focused on potential impacts from chronic exposures. Here, ecological impacts, if any, will likely depend on the type of PPCP. For example, Fent *et al.* [210] found that chronic LOECs in laboratory test species are about two orders of magnitude greater than maximum concentrations in sewage treatment plant effluents. However, their assessment did not include antibiotics or hormones, both of which warrant additional detailed investigation regarding potential risks to aquatic biota [205, 206].

One of the best studied PPCPs is ethynylestradiol (EE2), a synthetic compound widely used in birth control formulations and commonly detected in sewage treatment plant effluents and biosolids. As a hormone mimic, EE2 is a potent endocrine disrupting agent in freshwater vertebrates and has been implicated in a number of cases of sexual disruptions reported in freshwater fish exposed to sewage treatment effluent [213-214]. EE2 induces synthesis of the egg yolk precursor vitellogenin in male and juvenile fish, can cause increased incidences of intersex (gonads possess features of both sexes), feminization of male fish and reduced fertilization success [215, 216]. However, few studies have linked these changes to actual changes at higher levels of biological organization. One exception is the study of Kidd *et al.* [217] who showed that chronic exposure to environmentally relevant concentrations of EE2 over several years led to the collapse of a population of fathead minnows in a whole lake exposure. In that study, Palace *et al.* [216] found evidence of intersex and inhibited development of testicular tissue in males of pearl dace (*Margariscus margarita*) and suggested a trend toward reduced population abundance and smaller young-of-the-year size classes in the EE2-treated lake. Interestingly, and reflective of the transient nature of PPCP contamination, fish populations were observed to recover after exposure was halted. Watts *et al.* [218] and Dussault *et al.* [219] found no evidence for effects of EE2 in *C. riparius* and *C. tentans*, respectively, in life cycle tests. However, Dussault *et al.* [220]

showed that *C. tentans* and *H. azteca* accumulated EE2 (bioaccumulation factors of 31 and 142, respectively) and could therefore serve as a source of this compound to vertebrate receptors at higher trophic levels. Jensen *et al.* [221] estimated an EC50 of 0.011 μg/L for 17-α-trenbolone, an endocrine-active growth additive used in livestock, in fathead minnows; this concentration is comparable to those measured in beef cattle feedlot runoff [222].

Evidence for effects of non-endocrine PPCPs at higher levels of biological organization is rare but may occur in microbial populations exposed to antibiotics and veterinary medicines. In a recent review of the occurrence and toxicity of antibiotics in aquatic ecosystems, Kümmerer [207] suggests that bacteria and microalgae are 2 to 3 orders of magnitude more sensitive than organisms at higher trophic levels so the prospect for effects on microbial communities cannot be discounted. Indeed, Backhaus and Grimme [223], in a bioluminescence inhibition test with *Vibrio fischeri*, found toxic effect values (EC10) for two antibiotics in the range of concentrations expected in surface waters. Tetracycline was found to disrupt nitrification at concentrations found in some freshwater sediments [224]. Of particular concern with this class of pharmaceuticals is the potential for the development of resistance in freshwater bacteria and this has been demonstrated in natural bacterial populations in sediments associated with aquaculture [207, 225, 226].

Richards *et al.* [227] evaluated the effects of a PPCP mixture composed of ibuprofen, fluoxetine, and ciprofloxacin at individual concentrations of 10, 100, and 1000 μg/L on populations of macrophytes (*L. gibba* and *Myriophyllum sibiricum*), plankton, and bacterioplankton in a 35-day microcosm study. Significant decreases in growth of the plant species at intermediate and high concentrations and eventual loss of plant populations in the high treatment were observed. A significant increase in overall abundance and a significant decrease in diversity of phytoplankton occurred at the high concentration. The higher abundance reflected a large increase in one species that dominated the phytoplankton community; other phytoplankton species were unaffected or were eliminated, explaining the lower diversity. Similarly, zooplankton increased in abundance and had reduced diversity at the highest concentration. The mixture had no effect on bacterioplankton abundance. The authors concluded that the individual risks posed by these compounds in freshwater ecosystems were negligible.

In a 49-day microcosm study (34 days exposure and 14 days recovery), to a four-tetracycline mixture at individual concentrations of 10, 30, 100, and 300 μg/L, Wilson *et al.* [228] measured biomass production, community respiration, and primary productivity, as well as phytoplankton and zooplankton community responses. Phytoplankton abundance and community respiration decreased significantly at the two largest concentrations but primary productivity was unaffected. Community metabolism (ratio of productivity to respiration) decreased significantly at the two greatest concentrations due to significant increases in respiration. Zooplankton were not affected by the tetracycline mixture. The effects observed in this study are approximately 2 orders of magnitude greater than concentrations expected for tetracyclines in freshwater systems. Hillis *et al.* [229] evaluated the effect of monensin, an antibiotic commonly used in beef and poultry, on zooplankton communities in a 50-day microcosm study at concentrations ranging from 0.5 to 500 μg/L. The community-level NOEC (50 μg/L) was approximately 50 times greater than environmental concentrations. McGregor *et al.* [230] observed no impacts of monensin on macrophyte populations up to 100 μg/L in a microcosm study. Sanderson *et al.* [231] evaluated ivermectin, a commonly applied anti-helmenthic drug that has been shown to be highly toxic to aquatic invertebrates, in a long-term (250 day) microcosm study. They demonstrated that ivermectin could pose risks to aquatic organisms at or below the predicted environmental concentrations.

Overall, while there are some exceptions for classes of PPCPs such as estrogens and antibiotics, the weight of evidence indicates that the probability of acute or chronic effects at higher levels of biological organization in aquatic ecosystems is small. Indeed, based on a simple hazard approach comparing the ratio of the predicted effects concentration (PEC) and the predicted no effects concentration (PNEC), Tarazona *et al.* [232] suggest that the likelihood of observing ecosystem-level effects would be expected at ratios of approximately 10 or higher. Such high PEC/PNEC ratios for PPCPs are rarely observed in freshwater environments.

COMPOUNDS IN PLASTICS

Background and Chemistry

The production of plastic yields a variety of potential environmental contaminants. The two most common, which are addressed here, are bisphenol A (BPA, 4,4'-dihydroxy-2,2-diphenylpropane) and nonylphenol (NP), a member of

the alkylphenol ethoxylates (APEs). BPA is a key building block used to produce polycarbonate plastics and epoxy resins [233]. Polycarbonates are incorporated into sheeting, glazing, bottles and storage containers and epoxy resins are used as protective coatings on buildings, boats and vehicles; collectively, this usage accounts for 95% of BPA in the plastics industry [234]. NP is the basis for non-ionic surfactants commonly used in the manufacture of industrial and domestic detergents, pesticide formulations, emulsifier and dispersing formulations, cosmetics, and paints.

BPA is not especially stable in the end product and has been observed to migrate into surrounding environmental matrices. Although BPA is relatively short-lived in the environment (the half-life in water ranges from several hours to a few days depending on initial conditions [235]), continuous inputs and the ubiquity of plastics in the environment, has resulted in routine detection of this compound [233, 236]. BPA is most commonly detected in waters downstream of wastewater treatment plants with concentrations in the effluent typically an order of magnitude greater than in receiving waters [235, 237]. In a review of BPA concentrations in rivers and groundwater, Sharma *et al.* [235] found BPA occurred predominantly at low ng/L and low μg/L range, respectively. In a recent exposure analysis for North America and Europe, median BPA concentrations in freshwater systems were 0.081 and 0.01 μg/L and 0.6 and 3.4 ng/g in sediments, respectively, [238]. Despite low persistence and generally reduced global presence relative to other organic contaminants, BPA has attracted attention from an ecotoxicological perspective because it can bioaccumulate and has been shown to act as an estrogen mimic. For example, BPA is structurally similar to the potent estrogen diethylstilbestrol and has been shown in the yeast estrogen assay to bind and activate the estrogen receptor in vertebrates [233, 239, 240].

NP enters the environment *via* industrial and commercial sources [241, 242]. Due to their occurrence in cleaning agents, high concentrations of NP ethoxylates enter wastewater treatment plants. Here, metabolic degradation leads to the production of NP, which is released into receiving waters [241]. Not surprisingly, NP has been widely detected in systems with wastewater inputs, with the resulting distribution between environmental compartments driven primarily by its physicochemical properties. Due to low water solubility and a K_{ow} >4, fugacity modeling has shown that NP partitions preferentially into sediment, with concentrations downstream of inputs reaching the mg/Kg range compared to low μg/L for water [241, 243]. NP undergoes significant degradation in the water column, with a half-life of a few days [244], but in sediments, half-lives >60 years have been reported [241]. The bioaccumulation potential of NP is generally low to moderate [242], although recent work with zebra fish estimated BCFs >1000 [243]. As an endocrine disruptor, NP can impair reproduction and sexual development as has been shown in fish [241, 245-247]. These estrogenic effects are more pronounced in NP relative to the parent ethoxylate forms and current environmental concentrations may result in population-level effects *via* effects on reproductive fitness [236, 246].

Ecotoxicology

There is a reasonable body of literature examining higher-level effects of NP in aquatic ecosystems. The most extensive is a series of papers that describe the impacts of NP on sediment dwelling nematode, plankton and microbial communities in 230 L aquatic microcosms over an 8-week application phase and a 6-week dissipation phase. The nematode community initially had high abundance and low Shannon diversity, with dominance by one species, *Eumonhystera filiformis* [248]. At week 7, abundances declined and diversity increased but did not correspond to the NP concentrations. The maturity index was the only response that showed a treatment-related response; being significantly lower at the highest concentration (3.4 mg/kg sediment) relative to controls and other treatments. The relative insensitivity of nematodes was attributed to decreased bioavailability due to binding of the NP to the cationic groups in the sediment as a result of the pH of this particular test system. Changes in phytoplankton and periphyton species richness and diversity were not correlated with NP concentrations as the measured NP concentrations were approximately 10-fold lower than those known to cause direct toxicity [249]. However, changes in community composition of phytoplankton were noted, with Conjugatophyceae, which were dominant in all microcosms during the pre-treatment period, being dominant only in the controls and lowest NP concentration post-treatment. In contrast, Cyanophyceae came to dominate at intermediate and higher test concentrations. The authors interpreted this trend as evidence for differential grazing by zooplankton, specifically decreased grazing pressure at higher NP exposures due to direct toxicity on the zooplankton through estrogenic effects [250]. Abundances of copepod larvae were the most severely affected, with declines up to 95% at 200 mg/L NP, with no recovery observed during the 6-week post-treatment period in the three greatest concentrations. Cladocerans were less sensitive, recovering in all but the highest NP exposure, a response that may have been

facilitated by the shift in phytoplankton to smaller species [250]. Time-dependent NOEC values for the community ranged from 19 to 44 mg/L. Interestingly, these NOEC values are lower than those of many single species laboratory invertebrate tests [242], though comparable to that estimated for *D. magna* (EC50 of 16.5 mg/L for population growth (*r*) in a 21-day test) [251]. In a subsequent study, using the same microcosms, Hense *et al.* [252] concluded that decreases in zooplankton reproduction, attributed to the endocrine effects of NP, resulted in a delayed shift in phytoplankton community structure and increases in rotifera, due to reduced grazing and competitive pressures. Microbial communities, specifically bacteria and microfungi in sediments, tended to increase in abundance with elevated NP concentrations in these test systems, with only a slight change in the overall microbial community [253]. This could be attributed to increased food resources due to zooplankton mortality at higher NP exposures, as has been observed elsewhere with mass zooplankton and benthos mortality [254]. In microbial microcosm test systems lacking sediment, zooplankton and benthos, water borne microbial communities showed no significant differences in diversity up to 5 mg/L NP [244].

In fish, NP has been shown to affect behavior and survival and, through estrogenic effects, reproduction [245]. Indeed, field populations of freshwater fish exposed to NP *via* wastewater effluents consistently show endocrine modulated effects such as vitellogenin expression, gonadal abnormalities, reductions in circulating testosterone and reproductive dysfunction, and reductions in the gonadosomatic index, all of which can result in population-level effects through impaired fecundity [241]. However, while some studies show a correlation between NP and these effects downstream of wastewater effluents [255], assigning direct causality to NP when many other contaminants that share a mode of action and input source co-occur is difficult. A modeling exercise using data from laboratory and field studies was conducted to examine the potential impacts on populations of brook trout (*Salvelinus fontinalis*) and fathead minnows exposed to NP for three years at 1 and 30 µg/L [245]. Depending on model parameterization, they predicted an increase in population size of 17% or a decline by 28% at 30 µg/L but no significant change at 1 µg/L NP. Fathead minnows showed a similar response, with population reductions up to 21% and a shortened spawning season at 30 µg/L, but full recovery was anticipated within two years after exposure.

There is a robust body of knowledge on the acute and chronic effects of BPA to a suite of aquatic organisms at the individual level under laboratory conditions (see review by Mihaich *et al.* [256]). In acute exposures, EC50 and LC50 values (24 to 96 h) are typically in the mg/L range for invertebrates (1.1 to 16 mg/L), while chronic testing for invertebrates found NOEC values ranging from 0.25 mg/L in the snail *Marisa cornuarietis* for female growth to >3 mg/L for *D. magna* reproduction [256]. Primary producers appear to be slightly less sensitive than invertebrates. For example, the EC50 for growth for the diatom *Skeletonema costatum* was 2.5 mg/L, while the EC50 for growth for *L. gibba* was 32 mg/L [256]. Based on currently measured environmental concentrations, BPA is unlikely to induce acute effects in these organisms. However, Oehlmann *et al.* [233] suggest that effects at higher levels of biological organization may occur through subtle impacts on reproduction and development in vertebrates and invertebrates. They summarized the toxicological literature for types of responses in organisms exposed to BPA and concluded that invertebrates were generally more sensitive than vertebrates such as fish. In snails significant increases in reproductive effects and super-feminization have been observed, which is consistent with the proposed mode of action of BPA as an estrogen mimic. For example, in a 180-day study with *M. cornuarietis*, the EC10 for egg production (increase) was 13.9 ng/L, which is within the range of some environmental concentrations. Other invertebrates appear to be less sensitive. A NOEC of 1 mg/L was determined for reproduction in *D. magna*, and conflicting results have been reported in marine copepods, with some showing inhibition of larval development and others showing accelerated growth, including increased egg production at 20 µg/L. In *C. riparius*, emergence of second-generation individuals was delayed at concentrations as low as 78 ng/L. In fish, the majority of papers report feminization effects *in vivo* and expression of the vitellogenin protein, but at concentrations in the high µg/L and well above what is typically observed in aquatic environments. However, some studies have reported changes in circulating concentrations of some hormones at more environmentally relevant concentrations. Oehlmann *et al.* [233] cite one study on brown trout that reports impacts on sperm quality, a delay in ovulation, and inhibition of ovulation in the low µg/L BPA range, with the interpretation that this could lead to delayed breeding in less favorable periods, with potential impacts at the population-level for these fish. Based on available data, Oehlmann *et al.* [233] felt that BPA could be contributing to adverse reproductive outcomes in populations of exposed fish, but to date, no studies have shown this causally in the field. Staples *et al.* [257] summarized the chronic laboratory data (growth, reproduction and mortality) for this compound, developing species sensitivity distributions and estimating chronic predicted no effects concentrations (PNEC or the 5th centile of the distributions), which are considered

protective of populations, communities and ecosystems. They determined PNEC values of 11 to 71 µg/L BPA and concluded, based on current environmental concentrations, that higher-level effects are not anticipated.

CONCLUSION

Global freshwater ecosystems have a long history of contamination from organic pollutants. While an enormous amount of research has been conducted to assess the impacts of organic contaminants on freshwater systems, much of this has been generated at lower levels of biological organization (organism and lower) and clear, cause-effect examples of contaminant-associated impacts at the population, community, and ecosystem level are generally rare (Table **1**).

Table 1: Relative state of current understanding, including cause-effect relationships, of the ecological impacts of the organic chemicals addressed in this review in relation to levels of biological organization. Relative rating based on evidence from laboratory, field, and cosm studies. XXX: clear evidence of impacts with some causal relationships established; XX: evidence of impacts but causal relationships not established or uncertain; X: possible evidence of impacts but causality not established; ___ no evidence of impacts.

Chemical	Sub-organism	Organism	Population	Community	Ecosystem
PCBs	XXX	XXX	XX	X	X
TCDDs/TCDEs	XXX	XXX	XX	X	X
PBDEs	XXX	XX	X	___	___
PAHs	XXX	XXX	XX	X	X
Pharmaceuticals	XX	XXX	X	___[1]	___
Bisphenol A	XXX	XXX	X	___	___
Nonylphenol	XXX	XXX	XX	X	X

[1] There is some evidence that community-level effects could occur in microbial communities

Our present understanding of how freshwater ecosystems respond to contaminants is largely based on work with persistent, bioaccumulative legacy chemicals (e.g. PCBs, dioxins, and PAHs) and the information derived from this work has proved essential in developing protective regulatory criteria and implementing ecosystem-based management strategies to mitigate effects. However, there is much research that remains to be done. For example, future research should focus on the potential impacts of more recent chemical classes (e.g. PBDEs, PFSs, and pharmaceuticals), whose physicochemical properties, environmental behavior, and potential impacts on aquatic ecosystems, has not been fully elucidated. In addition, since contaminants rarely occur individually in the environment, there is a need to better evaluate the impacts of chemical mixtures in aquatic systems and potential risks that result from exposure to them. Like individual chemicals, the historical focus for chemical mixtures assessment has been at lower levels of biological organization and there is little information about their effects at the population or community level. The potential effects of mixtures should be considered in the context of cumulative impacts, with emphasis on interactions between both chemical and non-chemical (e.g. nutrients, sedimentation, *etc.*) stressors. In terms of population and community-levels assessments, one of the ideal tools to undertake such studies is model aquatic ecosystems such as microcosms or mesocosms as these facilitate evaluation under "close-to-field" conditions, including direct and indirect effects, both of which may be critical aspects to quantify the fate and effects of organic chemicals in freshwater systems. Finally, greater resources are needed for chemical and biological monitoring of aquatic systems for the purpose of assessing trends in exposure to, and impacts from, chemicals to provide a stronger foundation on which to support regulatory and research initiatives.

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CHAPTER 8

Impact of Pollutants on Coastal and Benthic Marine Communities

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Abstract: In recent years, sources, types and levels of contaminants in the marine environment have increased as a consequence of anthropogenic activities worldwide. Chemical substances are usually present in the marine environment at different concentrations. They are accumulated in the tissues of marine organisms exerting damaging effects at different levels of organization, from organisms to communities and ecosystems. The understanding of their effects and distribution has increased substantially since the early reports on the biological effects of marine pollution and associated monitoring problems. This Chapter has been divided into five main sections: (i) molecular, cellular and tissue level biomarkers in assessing effects, reviewing the use of biomarkers in monitoring effects; (ii) biological effects at organism and population level; (iii) bioassay studies at organism level, focusing on ecotoxicology and discussing toxicity estimation and bioassay limitations; (iv) ecological effects at community level, including structural parameters, such as richness, diversity, *etc.*, but also the proportion of opportunistic and sensitive species, discussing the multiple pressure interactions; and (v) measuring pollutant effects in integrative assessments, both in evaluating risk and assessing the ecological status of the ecosystems. The main objective of the Chapter is to bring together the knowledge on the biological effects of pollution, developed in recent years, at different biological levels of organization.

INTRODUCTION

In recent years, sources, types and levels of contaminants in the marine environment have increased as a consequence of anthropogenic activities worldwide [1, 2] (Table **1**). Chemical substances are present usually in the marine environment in different concentrations; they are not necessarily accumulated in the tissues of marine organisms, but they still can cause toxic effects (e.g. herbicides) even at very low concentrations, exerting damaging effects [3]. The understanding of their effects and distribution has increased substantially since the early reports on the biological effects of marine pollution and associated monitoring problems [4] (Table **1**). However, there is a need to develop methods for the identification, estimation, comparative assessment and management of the potential risks posed by chemical pollutants to aquatic living resources and marine ecosystems [5, 6]. Hence, the relationships between pollutants and direct and indirect effects on the marine environment, across different levels of organization, together with the operational framework for establishing causal-effect relationships are presented in Fig. (**1**).

Assessment of environmental pollution cannot be based solely upon chemical analyses, because this approach does not provide clear indication of the deleterious effects of contaminants [5, 7]. In addition, the increasing number and types of potential pollutants (*i.e.* polybrominated diphenyl ethers, endocrine disruptors, pharmaceuticals, *etc.*) entering the marine environment requires novel strategies for pollution assessment. Consequently, there is general agreement that the most appropriate approach for the assessment of environmental pollution is by integrating a suite of chemical and biological measurements [5, 7-9].

In this respect coastal organisms are of high importance in environmental toxicology as sentinel species, because they are can be used in the assessment of the effects of pollution through biological effect measurements [10]. Bivalve molluscs have been one of the most widely-used indicators to determine the existence and toxicity of chemical substances. Therefore, due to their sessile nature, wide geographical distribution and high bioaccumulation capacity, they have been considered as ideal for the detection of the biological effects of pollutants [11].

In order to analyse the extent of disturbances of a biological system and to quantify the state of health, the integration of several biological effects at different levels of biological organisation has been suggested by several authors [6, 12]. Biological effect measurements incorporate three approaches: (i) biomarker studies at molecular, cellular and tissue level; (ii) bioassay studies at whole organism level; and (iii) ecological surveys at community and population level [13].

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Table 1: Effects on marine life, from different types and sources of pollution. Source: adapted from WorldWatch Institute (http://www.gdrc.org/oceans/marine-pollution.html).

Type	Primary Source / Cause	Effect
Nutrients	Runoff - approximately 50% sewage, 50% from agriculture, cattle, and other land use. Also airborne nitrogen oxides	Feed micro- and macroalgal blooms in coastal waters. Decomposing algae depletes water of oxygen, and releasing toxins can kill marine life
Sediments	Erosion from mining, forestry, farming, and other land-use; coastal dredging	Water turbidity; impede photosynthesis. Clog gills of fish. Smother and bury coastal ecosystems. Toxins
Pathogens	Sewage, livestock	Contaminate coastal swimming areas and seafood, spreading cholera, typhoid and other diseases
Alien Species	Transported in ballast water, aquaculture, *etc.*	Outcompete native species, reduce biodiversity, introduce diseases, increased incidence of red tides
Persistent Toxins (PCBs, metals, DDT *etc.*)	Industrial discharge; wastewater discharge from cities; pesticides from farms, forests, home use, *etc.*; seepage from landfills	Poison or disease in marine life, especially near major cities or industry. Contaminate food-webs and seafood. Fat-soluble toxins that bio-accumulate in predators can cause disease and reproductive failure
Oil	46% from cars, heavy machinery, industry, other land-based sources; 32% from oil tanker operations and other shipping; 13% from accidents at sea; also offshore oil drilling and natural seepage	Low level of pollution can kill larvae and cause disease in marine life. Oil slicks kill marine life, especially in coastal habitats. Tar balls from coagulated oil litter beaches and coastal habitat. Oil pollution is down 60% from 1981
Plastics	Fishing nets; cargo and cruise ships; beach litter; wastes from plastics industry and landfills	Discarded fishing gear continues to catch fish and crustaceans. Other plastic debris entangles marine life or is mistaken for food. Plastics litter beaches and coasts and may persist for 200 to 400 years
Radioactive substances	Discarded nuclear submarine and military waste; atmospheric fallout; also industrial wastes	Hot spots of radio activity. Can enter food web and cause disease in marine life. Concentrate in top predators and shellfish, which are eaten by people
Thermal	Cooling water from power plants and industrial sites	Kill off corals and other temperature sensitive sedentary species. Displace other marine life
Noise	Supertankers, other large vessels, military exercises, and machinery	Can be heard thousands of kilometres away under water. May stress and disrupt marine life.

This Chapter has been divided into five main sections: (i) molecular, cellular and tissue level biomarkers in assessing effects; (ii) biological effects at organism and population level; (iii) bioassay studies at organism level (as a part of the previous section, focusing on ecotoxicology); (iv) ecological effects at community level; and (v) measuring pollutant effects in integrative assessments. The main objective of the Chapter is to bring together knowledge on the biological effects of pollution developed recently at different biological levels of organization.

MOLECULAR, CELLULAR AND TISSUE LEVEL BIOMARKERS IN ASSESSING EFFECTS

Recent studies have applied the "biomarker approach" to assess deleterious effects in biological systems [8, 16]. However, there is still some debate relating to their definition and, especially, their use in environmental risk assessment [17, 18]. According to McCarthy and Shugart [19], biomarkers are defined as measurements of body fluids, cells, or tissues, that indicate in biochemical or cellular terms, the presence of contaminants (exposure biomarkers) or the magnitude of the host response (effect biomarkers). Owing to the short time of response, biomarkers are used as early warning signals of biological effects caused by environmental pollutants in order to

predict changes at higher levels of biological organisation; *i.e.* populations, communities or ecosystems [5]. In general, responses at lower biological organisation levels are more specific, sensitive, reproducible and easier to determine, but more difficult to relate with ecological changes. Conversely, responses at higher biological organisation levels are indicative directly of ecosystem health; hence, more relevant to environmental management. However, they are more difficult to determine, less specific and only manifest at a late stage, when environmental damages have already occurred [20, 21].

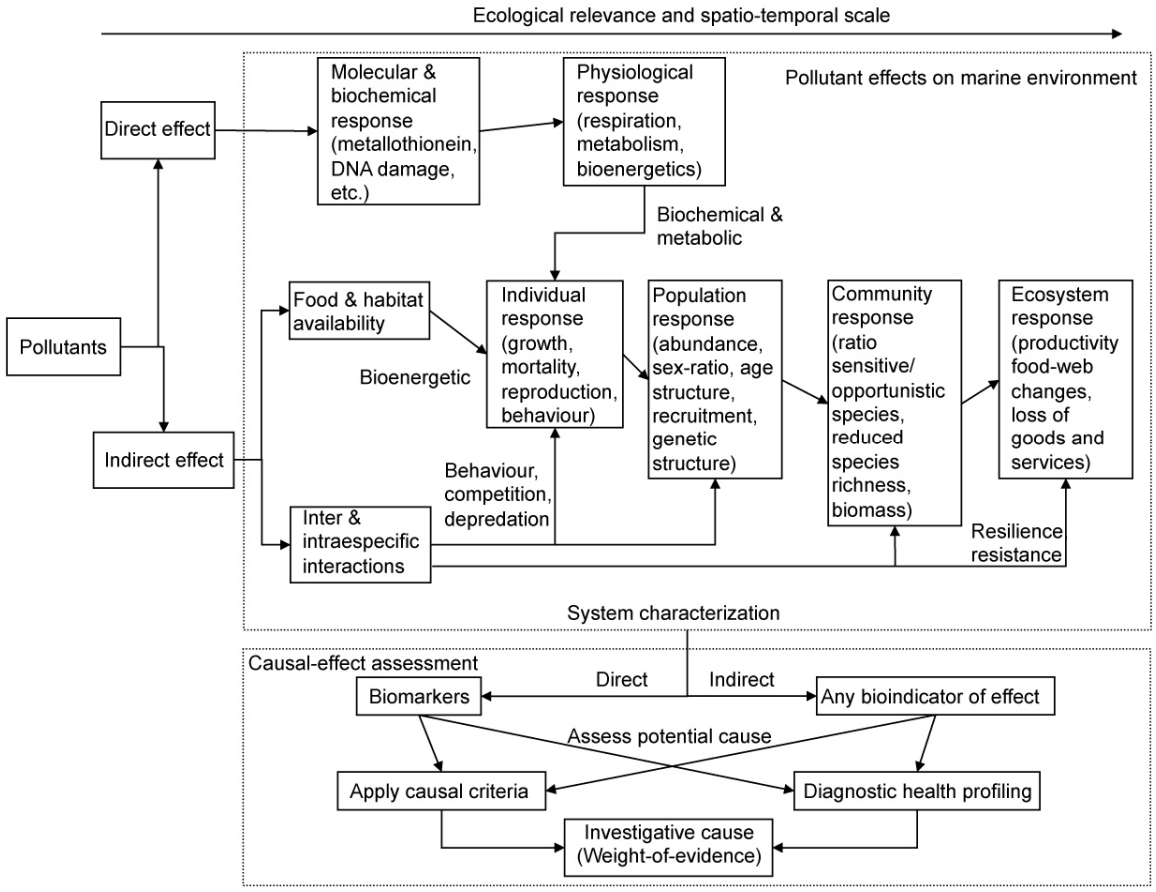

Figure 1: Relationships between pollutants and their direct and indirect effects on the marine environment along a gradient of ecological relevance and spatiotemporal scale, together with the operational framework for establishing causal-effect relationships using multiple lines of evidence (adapted and modified from Clements [14] and Adams [15]).

The biomarker approach should be applied as a 'multi-marker' approach, since the use of a single biomarker in environmental studies does not reflect the integrated response of the organism [5, 6]. Furthermore, for each biomarker, specificity, temporal relevance, inter- and intra- individual variability, baseline values and the existence of confounding biotic and abiotic factors should be defined before its application [8].

Despite the above mentioned shortcomings, biomarkers could be a very valuable tool to determine cause-effect relationships, in the assessment of environmental pollution in the operational and investigative monitorings defined in the European Water Framework Directive (WFD) [22]. Thus, against the background of the development of the European Marine Strategy and of relevant Directives, the biomarker approach has been recommended and introduced in monitoring programmes for the surveillance of the marine environment, as an approach that complements the use of diagnostic chemical analysis with the assessment of biological effects [7, 23].

Over the last 20 years, an increasing number of ecotoxicological papers or research dealing with pollutants have focused on the study of causality between pressure of xenobiotics and responses at the molecular, cellular and tissue level. The possible effects of various contaminants on the health status of several coastal organisms are reviewed herein.

Metal toxicity occurs when the rate of metal uptake into the body exceeds the combined rate of excretion and detoxification of available metal [24]. The first measurable effect of metals is on the inducibility of metallothionein (MT) genes and MT protein synthesis [25, 26]. In mussels, two families of MT isoforms have been characterised recently [27]; it has been suggested that these isoforms may accomplish different functions in presence of different metals and stressors. Whereas the monomeric MT10 isoforms have been reported to be synthesised constitutively and involved in essential-metal regulation, particularly of Cu and Zn, the dimeric MT20 isoforms are inducible and are involved mainly in Cd and hydroxyl radical detoxification [26, 28].

Organic pollutants are taken up readily into the tissues of aquatic organisms; here, biotransformation via Phase I (functionalisation) and Phase II (conjugation) metabolism can in part, determine the fate and toxicity of the contaminants [29]. Of central importance to Phase I metabolism is the mixed function oxygenase system, whose terminal component cytochrome P450 (CYP) exists as a superfamily of proteins, capable of oxidising a wide variety of substrates in numerous aquatic species [30]. One specific form, CYP1A1, can be estimated in fish liver by the measurement of 7-ethoxyresorufin-O-deetilase (EROD) activity and has been used as a biomarker of exposure to organic aromatic xenobiotics [23, 31]. In contrast with fish, marine invertebrates, especially mussels, have a low ability to metabolise organic pollutants, due to the lack of efficient isoforms of P450, which are only found in terrestrial organisms. Thus, one of the most important sentinel species (mussels) does not possess this important biotransformation system, discouraging the utilisation of EROD induction in pollution biomonitoring programmes, using mussels as sentinels. However, recent studies have suggested that the assessment of peroxisome proliferation and multixenobiotic resistance (MXR) may be more appropriate, as biomarkers of exposure to organic xenobiotic compounds in molluscs [32]. In this respect, both laboratory and field studies have shown that organic xenobiotics (such as phthalate ester plasticizers, Polycyclic Aromatic Hydrocarbons (PAHs) and oil derivatives, Polychlorinated Biphenyls (PCBs), certain pesticides, bleached Kraft pulp and paper mill effluents, alkylphenols and estrogens) all provoke peroxisome proliferation in aquatic organisms [33, 34]. Accordingly, MXR-related gene expression has been detected, either in terms of induction or inhibition, in molluscs exposed to anthropogenic organic pollutants [35, 36].

It is important to note that biomarkers can be affected by mixtures of different chemicals present in the field, giving rise to additive, synergistic and/or antagonistic effects. Several studies have demonstrated decreases in the activities of several enzyme markers of organic compounds in molluscs from polluted sites, probably due to complex interactions occurring in the marine environment between mixtures of pollutants [16, 37]. Similarly, acetylcholinesterase activities, inhibited by some pesticides (in particular carbamates and organophosphorus compounds), can be inhibited also by metals [38].

At a cellular level, tolerance to metals is based upon sequestration by a range of cellular ligands, such as MTs, followed by compartmentalisation within lysosomes [25]. Thus, metals bound to metal-binding proteins may enter lysosomes, and follow the catabolic pathway as would any other cellular protein. However, excessive concentrations of metals can cause alterations of structure, permeability and integrity of the lysosomal membrane when storage capacity of the lysosomes is overloaded [39, 40]. Impairment of lysosomal functions and, thereby, of food assimilation, can result in severe alterations in the nutritional status of the cells and whole organisms, and could be indicative of disturbed health. Previous studies have reported lysosomal enlargement, increased intralysosomal accumulation of metals and enhanced production of lipofuscins in metal exposed mussels, as a detoxification mechanism to minimise toxic effects of excess metals [28, 41, 42]. Additionally, a decrease in lysosomal membrane stability has been documented upon exposure to a progressively higher Cu concentration in laboratory experiments [43, 44] and in a transplant study in a copper mine [45].

Nevertheless, lysosomal responses are not metal-specific and, thus, are considered as a biomarker of general stress [46]. For instance, destabilised lysosomal membranes have been found also in marine organisms exposed to organic compounds, or collected from sites with mixture of contaminants [16, 47, 48]. Likewise, enhanced lipofuscin deposition has been observed in the digestive cells of mussels exposed to PAHs under both laboratory and field conditions [47, 49]. Intralysosomal neutral lipid accumulation in aquatic organisms (*i.e. Mytilus edulis*) has been reported upon exposure to organic chemicals in laboratory experiments [50, 51] and in sites polluted with oil derivatives [41, 52].

There is evidence [48] that reactive oxygen species (ROS) are formed in the presence of a wide range of contaminants, such as metals (e.g. Cu, Fe), PCBs and some pesticides, provoking alterations in proteins, DNA and

membrane structures/functions. ROS are detoxified by antioxidant enzymes and scavenger molecules. In marine mussels caged for 4 weeks, in an industrialised harbour of north-west Italy, a biphasic trend for single antioxidants (catalase, glutathione *S*-transferases, glutathione reductase, total glutathione) and the total oxyradical scavenging capacity was shown. There was no variation or increase during the first 2 weeks of exposure to the polluted site, followed by a progressive decrease up to a severe depletion of ROS in the final part of the experiment [48]. The decreased capacity to neutralise specific ROS has been shown to correlate with the occurrence of alterations at various sub-cellular targets, including lysosomal membranes and DNA [53, 54]. Oxidative DNA damage, revealed as high levels of the mutagen 8-oxo-7,8-dihydro-2'-deoxyguanosine and lipid peroxidation, measured in terms of high malondialdehyde levels, has been found in mussels from polluted areas [55, 56].

Genotoxic compounds, such as persistent organic pollutants (POPs), can alter the integrity of DNA structure, either directly or through their metabolites [57], causing mutagenesis [58]. Biomarkers of genotoxicity include DNA damage, which is based upon potentially pre-mutagenic lesions (such as DNA adducts, base modifications, DNA-DNA and DNA-proteins cross-linking and DNA strand breaks) and chromosomal damage [59]. The presence of micronuclei is an indicator of chromosome breakage or chromosome loss [60]; this technique has been used extensively in invertebrates [61, 62]. Caged mussels exposed to seawater polluted by aromatic hydrocarbons, displayed a continuous increase of micronuclei frequency in gill cells, reaching a plateau after a month of caging [63]. The incidence of micronuclei has also been linked to the induction of leukaemia cells in the clam *Mya arenaria*, suggesting that the micronucleus test is a very good indicator of the potentially life-threatening consequences of genotoxic exposure [64].

The effects of environmental pollution have been identified also at tissue level and, therefore, histopathological changes in target tissues have been proved to be sensitive markers of health status in aquatic organisms [65, 66]. In bivalve molluscs, highly significant correlations between tissue pathologies and contaminants (Pb, Hg and PCBs) have been observed in mussels from the east coast of the USA [67]. In the digestive gland of mussels exposure to Cu, Cd and the water-accommodated fraction of different oils induced alterations in cell type ratios of digestive tubules, with basophilic cells increasing in number, in relation to digestive cells [28, 68]. This morphological change of digestive tubules has been observed, accompanied by atrophy of the digestive epithelium in molluscs [45, 69], apparently involving augmented autophagic processes [70]. Following the *Prestige* oil spill [69] that occurred in November 2002 in the Atlantic, north-west coast of Spain, digestive gland atrophy was demonstrated in mussels, indicating disturbed health, due partly to disturbances in digestion and metabolism [71]. Another severe tissue alteration, the incidence of granulocytomas, has been observed in molluscs from highly polluted areas [72].

There is a growing concern that chemicals in the environment, either natural or synthetic, can interact with the endocrine system, causing reproductive disturbances in aquatic organisms that may affect recruitment and lead eventually to deleterious population effects [73, 74]. Endocrine–disrupting chemicals such as phytoestrogens, alkylphenols, synthetic estrogenic hormones and bisphenol A mimic estrogenic hormones and thus cause estrogenic or feminising effects, whereas other chemicals such as tri-butyltin (TBT), used in antifouling paints for ships, and synthetic androgenic hormones cause androgenic effects [73, 75]. In invertebrates, the endocrine regulation of reproduction and development is not as clear as in vertebrates [76]. Vitellogenin (VTG)-like proteins, yolk proteins produced only in sexually mature females, have been observed in male molluscs exposed to different xenoestrogens such as alkylphenols [34, 77, 78]. In contrast, reduced VTG-like protein levels have been described in female molluscs inhabiting PAH contaminated sites or, after exposure to North Sea oil, indicating a possible anti-estrogenic effect of PAHs [34, 79]. Moreover, exposure of females to certain metals, such as Cu, provoked an increase in VTG-like protein levels and accelerated spawning, maybe as a result of possible acute toxic effects, or as an effect on hormone regulation of gamete development [45, 80]. These controversial results in females highlight the need for more basic research to understand biomarker responses, before their implementation in monitoring studies. On the other hand, it should be noted that the development of more advanced techniques for biomarker quantification could also be helpful in the interpretation of the multi-marker approach.

Overall, the utility of stress indices, based upon molecular, cellular and tissue responses in sentinel species, could provide a comprehensive indication of the impact of chemical pollutants in coastal marine environments. However, the need to increase the knowledge on biomarker baseline values and confounding factors is also highlighted.

BIOLOGICAL EFFECTS AT ORGANISM AND POPULATION LEVEL

The impacts of metallic and organic pollutants on marine biota cover a plethora of direct and indirect effects on organisms and populations. These effects have been reported in a wide variety of biota such as bacteria, fungi, plants and animals.

Among the inorganic metallic pollutants, Cd, Cu, Cr, Pb, Hg, Ni and Zn are some of the best studied in terms of speciation, toxicity, bioavailability or bioaccumulation in marine ecosystems. Other metals with toxic effects in marine communities are Al, Sb, As, Se or Ag. Unlike other contaminants, all of these metals occur naturally in the environment; some of them (e.g. Cu and Zn) have essential functions in several biota at low concentrations. The degree of increase in levels of these metals in the environment, with respect to the background levels, and the degree to which they have toxic effects on biota, depends upon a wide number of factors such as: (i) their geochemical behaviour; the physiology and condition of the target biota; (ii) chemical speciation and; (iii) the presence of other toxicants, or environmental conditions [81]. In order to assess the lethal responses of marine biota to the metallic pollution, several approaches have been carried out since the 20th Century. Laboratory bioassays have permitted comparisons of toxicity between different pollutants and among different species. Moreover, these bioassays have shown that the first developmental stages of several species of invertebrates are highly sensitive to several toxicants [82, 83]. Research has also been carried out in order to evaluate sublethal responses in marine biota to inorganic metals; this has included effects on growth, sexual maturity, diseases, luminescence, metabolism, *etc.*

The research undertaken on the biological effects of the organometallic pollutants has shown that some of these compounds are substantially more toxic than the inorganic metal to marine organisms. As an example, methylmercury is substantially more toxic to several biota than inorganic mercury, because it is more efficiently transported across the gut [84] and because of its ability to diffuse through lypophilic media and cross cell membranes. Another highly researched pollutant is TBT, which has toxic effects in a wide variety of biota, whereas inorganic tin is less toxic. TBT effects include lethal toxicity and effects on growth, reproduction, physiology, and behaviour [85]. Several of the negative effects are due to interferences with the endocrine function, and manifested as the imposex phenomenon. In dioecious gastropods, imposex presents as the superimposition of male organs in females [86]. In some species, such as the dogwhelk *Nucella lapillus,* this masculinisation has provoked the local extinction of populations in several polluted areas, during the 1970s and 1980s [87]. Because TBT has negative effects at a very low concentration (e.g. ~ 1 ng Sn/L [87]), it has been considered as one of the most toxic xenobiotics ever produced and introduced deliberately into the environment [88]. At those sites where measures were taken to reduce the input of TBT into marine ecosystems, the previously affected species have shown a recovery [89].

PAHs are relatively ubiquitous organic molecules, within marine ecosystems. There exists more than 100 different PAHs, with both natural (e.g., forest fires) and anthropogenic (e.g., burning processes or oil spills) inputs into the ecosystems. The fact that PAHs occur generally as complex mixtures in the environment makes the evaluation of toxicity more difficult [90, 91]. This difficulty is increased by the fact that some have shown higher toxicity as a consequence of photoactivation [92]. Although there is a need for research to clarify the biological effects of PAHs in marine ecosystems [90], toxic (carcinogenic) effects have been found in phytoplankton, zooplankton, invertebrates and vertebrates because they form DNA adducts. Most of these toxic effects are related to a reaction with macromolecules (like nucleic acids or proteins), or an interaction with lipids in cell membranes or other cellular constituents [93, 94]. These processes can cause diseases (including tumours) and negative effects on immunosystems, growth, reproduction and behaviour.

Chlorinated pollutants (*i.e.* PCBs, organochlorine pesticides, herbicides and fungicides) have effects on the physiology and behaviour of marine biota. Almost all of these compounds are not known to occur naturally in the environment and some are very persistent. Negative effects were found on growth, reproduction, luminescence, metabolism, *etc.* Some of these effects are due to endocrine disruption. Although some of these effects in marine organisms are relatively well known, others are still poorly understood [95].

Pollution effects on organisms can imply consequences at the population level. These can occur in different degrees, from changes in the population dynamics or genetic diversity, to the local extinction (as the above-mentioned case

of some gastropod extinctions due to TBT pollution). The linkage between pollution and population can be more complex to assess than is the case for individual organisms. Nevertheless, the recent development of genetic techniques presents some assessments of this issue [96]. Populations might respond with increased genetic variation (e.g. resulting from new mutations), or decreased genetic variation (e.g. resulting from population bottlenecks) [97]. The loss of genetic diversity can imply a reduced adaptive potential of populations to changes in environmental characteristics or to the presence of new pollutants [96, 98, 99]. Because of the complexity of the processes involved, the loss of genetic diversity is not predictable based solely upon knowledge of the mechanism of toxicity of the chemical contaminants and the life cycle of the biota [97].

Several studies of the effects of contaminants in populations have been carried out with meiobenthic species. This approach is related to the fact that several meiobenthic species have short generation times; this permits a study for the duration of a full life cycle. Studies carried out near offshore platforms, combined with laboratory experiments, found genetic diversity in meiobenthic copepods (*Nitocra lacustris, Cletodes sp., Enhydrosoma pericoense, Normanella* sp*., Robertsonia* sp*., and Tachidiella* sp.) correlated inversely with the degree of sediment contaminants (hydrocarbons); however, other causes could be attributed [100, 101]. Laboratory assays have found that the exposure to polybrominated diphenyl ether (BDE-47) and copper can reduce genetic diversity and alter genotype composition without affecting population abundance of some meiobenthic species [102, 103].

Genetic studies have also been carried out on macrobenthic taxa. Research undertaken on mussel, barnacle, prawn and isopod species suggests that pollution may reduce genetic diversity, but other causes cannot be discarded. Moreover, long-term exposure to metal pollution does not necessarily result in decreased genetic diversity [104, 105].

The exposure of the populations to pollutants can select rapid genetic changes or small-scale evolutionary processes associated with a genetically-inherited increase in tolerance to the pollutants (a process called microevolution [106]). This microevolution can buffer, partially, the effects of pollution in populations. As an example, an estuarine oligochaete species (*Limnodrilus hoffmeisteri*) was found to be more resistant to cadmium and nickel pollution following two generations of selection in laboratory assays [107].

BIOASSAY STUDIES AT ORGANISM LEVEL

An important component of pollutants arriving in the marine environment is retained in the bottom sediments, where they can reach concentrations several orders of magnitude higher than in the overlying waters [108]. Hence, in coastal systems, bottom sediments need to be characterized in environmental studies. Historically, such characterization has been limited to physicochemical analysis [109]. However, the chemical analyses by themselves do not provide evidence of biological effects on organisms; therefore, they do not assist in confirming the effect that they induce on ecosystems [110, 111]. Thus, toxicity tests on marine systems, among other biological methods of ecotoxicological evaluation, are necessary as a complement to physicochemical analyses to assess the potential effects of pollutants on organisms and biological communities [109].

Ecotoxicology, as the science that studies all of the adverse biochemically-mediated effects of all chemicals on all living organisms, including all their interactions within organisms and among species in the environment [112], is applied to the evaluation of the effects caused by pollution on the marine environment by means of bioassays.

Bioassays

Bioassays are used to evaluate the environmental quality through the measures of toxicity in natural samples, and to predict the ecological risk of contamination. These tests show numerous advantages: (i) the test organisms only respond to the bioavailable fraction of a pollutant; (ii) also, as opposed to chemical analyses that detect only previously well-known compounds, bioassays can help identify new toxic elements whose noxious effects had not been described previously [113]. In addition, (iii) they offer quantitative information on sediment toxicity, which provides a basis for discriminating between impacted and unimpacted sites. The results from these tests are also relevant ecologically because they commonly use resident species. As such, the tests undertaken provide a way to compare the sensitivities of different organisms.

The toxicity tests under laboratory conditions are carried out with the purpose of establishing any relationship between exposure of pollutants and the effects caused on individual organisms. By means of these tests, dose-response relationships are established, determining the relationship between the concentration or dose of the toxin and the noxious effect on an organism. Following the proposal of the use of toxicity tests as an appropriate tool for the valuation of marine pollution [83], they have become a fundamental part of the evaluation of environmental risk; they provide a direct measure of toxic adverse effects, complementing the traditional physicochemical measures [114].

At present, the list of bioassays to assess toxic effects of exposure to marine bottom sediments, as a measure of risk to populations is very extensive [115-118], given the multiple combinations that exist between the elements and the conditions to be selected. These bioassays, carried out in highly defined, controlled and reproducible conditions, can be applied to total sediment, suspended sediment, elutriate, pore water and/or sediment extract. The response variables to be measured include long-term toxicity, acute toxicity, bioaccumulation, endocrine effects, effects on reproduction, carcinogenesis and mutagenesis. Different marine organisms belonging to different trophic levels (bacteria, algae, molluscs, echinoderms, annelids, fishes, *etc.*) and in different development phases can be used in bioassays. This approach offers a wide range of biological possibilities for investigation [119, 120].

The organisms used in these tests are selected on the basis of: (i) their sensitivity; (ii) their ecological, commercial or recreational relevance; (iii) their high availability and abundance; (iv) their ease of culture or maintenance in laboratory; (v) and the simplicity of the analysis of results [121-123]. Finally, the responses obtained in test species can be qualitative or quantitative, but should be unequivocal, easily observable, describable, measurable, biologically significant and reproducible [124].

In a typical bioassay, the response of an aquatic organism to a toxic substance is related to the toxic concentration in water/sediment, together with the time of exposure. A commonly used technique to measure the dose-response relationship requires exposure time to be held constant over a series of different concentrations in order to record the proportion of individuals that present a specific biological response; e.g. mortality-survival, fertilization-non fertilization, or mobility-non mobility. The final objective is to obtain a toxicity curve of a substance or compound for an organism that defines the relationship between concentration (dose) and response (see Chapter 1).

Toxicity Estimation

To estimate the toxicity of a substance or sample, the EC50 is used extensively since it is a statistically-reliable measure. The EC50 is defined as the Effective Concentration that produces a specific effect on 50% of a population based upon experimental laboratory tests. It is used as a standard measure of toxicity and it permits a comparison of the toxicity of different compounds on an organism, or the toxicity of the same compound on different organisms. However, on the basis that the final objective of toxicological studies is to protect ecosystems, the EC50 alone is not sufficient. Therefore, it is necessary to obtain a second parameter that defines the toxicity threshold; *i.e.* the concentration above which they begin to show adverse effects. In this sense the NOEC (the highest experimental concentration in which the response does not present statistically-significant differences, with regard to the control) and LOEC (the lowest experimental concentration in which the observed response is significantly different from that of the control) are defined. These last reference parameters present a higher potential for utilisation from the point of view of their ecological application. Nevertheless, some debate exists presently on the suitability of NOEC and LOEC as estimates of the toxicity threshold because of their strong dependence on the experimental design, and an alternative frequently used is the concentration causing a lower level of effect, such as the EC10 [125, 126]. Moreover, the reason for not using LOEC and NOEC as endpoints in many instances (e.g. regulatory, water quality guidelines) is the statistical unreliability of the calculated values for such endpoints [125, 126].

Bioassays Limitations

The laboratory bioassays have some limitations since they do not necessarily reproduce the range of potentially relevant environmental factors present in nature [127, 128]: (i) the species used are not necessarily part of the communities that inhabit the studied sediments and, as such, they may not be representative of the species found in the area of interest [129]; (ii) the laboratory conditions are controlled, whilst the factors (i.e. abiotic:. climate, temperature, hydrodynamics, quality of water and/or sediment; and biotic: development stage, reproductive state, health, presence of other individuals and/or species, etc.) are changing in the environment [125, 130]; (iii) biomagnification through trophic webs, effects of

nutrients, habitat alteration, inter- and intraspecific predation or competition relationships are not taken into account [131]; and (iv) laboratory bioassays do not predict the indirect effects that often characterize the responses of the ecosystems to stress [132]. Therefore, although these bioassays have improved our understanding of the effects of pollutants, their results are difficult to extrapolate to the environment, because they lack ecological "realism" [133], since too many components exist in an ecosystem that make it impossible to predict accurately the effect that a toxic substance can exert. For example, the effect of a toxicant varies not only between species within an ecosystem, but also in the same species in different ecosystems. On the other hand, in natural environments, a substance may not produce adverse effects on a particular species, but does so in its predators or its food source, which influences finally the survival of the organism. Thus, in the absence of a thorough understanding of natural systems and their processes there is always a high degree of uncertainty [134]. Hence, it is necessary to obtain the highest amount of data on ecosystem dynamics, abiotic compartments and xenobiotics' toxic effect on the species. The analysis of these data permits the prediction, with a higher reliability, of the ecological risk associated with an episode of contamination (Environmental Risk Evaluation). Similarly, all this knowledge needs to be applied together for the restoration of ecological health in contaminated systems [135, 136]. This is the reason why ecotoxicological information is important when establishing environmental monitoring programmes [137].

Because of the necessity of obtaining more complete and useful information from an ecological perspective, testing on the basis of a single species has not been considered sufficient in recent years. When evaluating potentially contaminated samples from the environment, the use of a test battery, including species belonging to different habitats, development stages, multiple trophic and evolutionary levels, and (even) different times of exposure, is recommended as a more appropriate strategy [138, 139].

For the purpose of chemical registration, regulatory authorities require bioassays with individual chemicals.. These bioassays need to be conducted under standard conditions and there is no interest in reproducing the broad variability of natural ecosystems. On the other hand, bioassays with environmental samples are normally conducted in order to detect the presence of chemicals a priori unknown. In that case, the bioassay is a tool in the 'tool-box'. Once the hot-spots are identified, subsequent more expensive techniques may be applied to study the ecological effects.

EFFECTS ON COMMUNITIES AND ECOSYSTEMS

There is general agreement amongst marine investigators that measuring a suite of indicators across levels of biological organization is often necessary to assess ecological integrity [14]. As the effects of pollutants on the marine environment may be identified at all levels of biological organization, these indicators should include biochemical, population, community, and ecosystem responses (Fig **1**). In previous sections, the most common approaches in assessing those effects at low organizational levels have been presented. In turn, warnings of pollutant effects at community and ecosystem level are scarcer. However, as ecological integrity requires the protection of a good structure and functional processes at those high levels [140, 141], demonstrating biochemical and physiological responses to pollutants may not be sufficient.

Following Clements [14], the key to predicting the effects of contaminants on communities and ecosystems, is to understand the underlying mechanisms. Thus, establishing a cause and effect relationship between stressors and responses at higher levels of organization is problematic. The structure and functioning of these systems may be altered for many reasons other than contaminant exposure. Hence, Clements [14] suggests that one of the major goals of ecotoxicology is to develop an improved mechanistic understanding of ecologically-significant responses, to contaminants.

A review of the effects of pollutants on aquatic ecosystems in different parts of the world can be seen in Islam and Tanaka [142]. These authors describe a decrease in species diversity, changes in community structure, degradation of habitats, decline in abundance and biomass, diminution in yield of marine resources, *etc.* Hence, Wolfe [143] and other authors (see also Fig. **1**) have systematized the bioindicators of pollution for marine monitoring programmes at community and ecosystem levels. These approaches include measurements of abundance, biomass, richness, dominance, similarity, ratio opportunistic:sensitive species, age-size spectra, trophic interactions, energy flow, productivity, and the loss of goods and services. The response to pollutants (metals, organic compounds), of some of these indicators, is examined below.

Richness, Diversity and Evenness

In a recent review, Johnston and Roberts [144] make a meta-analysis of 216 papers in which the most frequently used measures of diversity and evenness were species richness (number of species per unit area), the Shannon–Wiener index and Pielou evenness (Margalef's richness and Simpson's diversity were used occasionally). The vast majority of the contributions concluded that there were significant negative effects of pollution upon species richness, with occasional increases in species richness and diversity associated with nutrient enrichment. Only 20% of the papers did not detect the effects of contamination upon diversity. When an effect was detected, its response ratio based upon species richness and Shannon–Wiener diversity tended to be greater than reductions in the Pielou evenness. Hence, a 30–50% reduction in species richness and diversity were identified in all habitats exposed to all contaminant types. In turn, Dauvin [145] does not consider species richness a good indicator of disturbance in estuaries, due to marked changes linked to salinity gradients.

There is also good evidence that offshore discharges of oil-based drilling fluids by the oil and gas industry have caused reductions in benthic species diversity or other changes in community structure at distances of <1–3 km from drilling locations [146, 147].

Although there is a large degree of variability when comparing laboratory toxicity values and benthic measures, Long *et al.* [148, 149] have demonstrated that (from almost 1500 samples) in 92% of the samples classified as toxic, at least one measure of benthic diversity or abundance was less than 50% of the average reference value. These findings have been used in the derivation of sediment quality guidelines (SQGs), as commented below.

Pollution of marine habitats has been associated with a reduction in biodiversity, either as a result of reduced species richness, increased dominance of tolerant species (i.e. decreased evenness), or a combination of both factors following the Pearson and Rosenberg paradigm [150]. However, the abovementioned meta-analysis [144] indicates a remarkable similarity in the response ratios across habitat and contaminant types. Hence, pollution was never associated with the complete exclusion of life from a particular location (commonly 50–70% of species were able to tolerate the contaminant load). In some cases, estuarine communities showed very high abundance and biomass values together with very high levels of contaminants [145, 151]. This paradox is explained by the delay between the period with the maximum runoff and the maximum contaminant input (at the end of autumn and during the winter) and the recruitment period for the dominant species (throughout the spring and summer), together with the absence of anoxic conditions.

Ratio Opportunistic/Sensitive Species

From the previous Section, it is clear that the identity of pollution-tolerant and –intolerant species is of great interest. Pollution-tolerant and opportunistic species have long been recognized as potential bioindicators of impacted systems [130, 152, 153]. Macrobenthic communities respond to pollution by means of different adaptive strategies [130]: (i) r-selected species, with short life-span, fast growth, early sexual maturation and larvae throughout the year; (ii) k-selected species, with relatively long life, slow growth and high biomass; and (iii) T, stress-tolerant species, not affected by alterations.

These strategies have been used in developing different indices that can be used to assess the environmental quality status in estuarine and coastal systems (see reviews in [154, 155]). From the high number of indices based upon sensitive/opportunistic ratio of species, probably the most successful are AZTI Marine Biotic Index (AMBI) [156] and the Benthic Quality Index (BQI) [157], which are on the basis of many other methodologies, most of them used within the WFD [158].

From the extensive publications using these indices (and especially from that of AMBI), it can be observed that increases in metals [159-161], organic compounds and TBTs [146, 162, 163] produce a decrease in benthic community quality detected by this index. Hence, a primary mechanism driving these changes, as a result of exposure to contaminants, is the elimination of sensitive species and the subsequent monopolization of resources by tolerant and opportunistic species [144].

In some cases such as at Restronguet Creek in the Fal estuary system [147], copper and zinc pollution from mining is strongly suspected to have caused the exclusion or restriction of several species of bivalves, including

Cerastoderma edule, Macoma balthica, Mytilus edulis and *Scrobicularia plana*, as well as the changes in nematode metal tolerance. However, some species (such as *Nereis diversicolor*) are able to adapt to pollution. Normally, in these extreme situations of metal pollution, infaunal communities are dominated typically by metal-tolerant opportunistic deposit-feeding polychaetes [144]. In fact, sediment metal chemistry and benthic infauna surveys undertaken over 33 years with sampling before, within and after tailings deposition from a metal (Pb, Zn) mine in Greenland [161] have shown dramatic changes of benthic fauna composition. Faunal recolonisation 15 years after closure of the mine was slow. Of the metals, Pb had the greatest impact, with deterioration of benthic communities above a threshold of 200 mg/kg, decreasing diversity and dominance of sensitive species, and increasing tolerant and opportunistic species; *i.e.* long-lasting effects on the biological system.

In turn, from the relatively few data on hard-bottom substrata communities, it has been suggested that macroalgal communities are relatively resilient to pollution [144]. However, some research has shown metal- and nutrient-impacted rocky shores to contain degraded communities of macroalgae. Opportunistic algal species with rapid growth rates, including *Ulva* and *Enteromorpha* dominated, replacing relatively diverse communities of large perennial algae and sessile filter feeders seen in more pristine areas [164-168].

Trophic and Other Interactions

Pollution may affect diverse components of the ecosystem, through primary species structuring the community. Hence, Roberts *et al.* [169] review the ways in which the contamination of biogenic habitats may affect other compartments (*i.e.* epifauna), describing four pathways: (i) colonisation by mobile fauna; (ii) inhibition of larval settlement; (iii) feeding by herbivores and predators; and (iv) post-ingestive effects on fauna.

The effects of habitat-bound contaminants, on the abundance of epifauna, may be driven by the behavioural responses of dispersing organisms [169]. For instance, recruitment of epifauna is reduced to macroalgae experimentally-spiked with copper as a result of behavioural preferences for uncontaminated algal hosts. Thus, exposure to habitat-bound contaminants is likely to be spatially complex. Hence, small-scale variation in contaminant concentrations interacts with variation between organisms in their ability to disperse among alternate habitats.

In addition, the accumulation of metals by macroalgae and seagrasses represents a potentially important pathway of contaminant exposure to grazing organisms (herbivores and detritivores), which are responsible for much of the transference of metals to higher trophic levels [169]. In fact, the algae *Ulva lactuca* and *Enteromorpha intestinalis*, collected from contaminated sites and used to feed herbivorous gastropods, produced complete mortality of the latter organisms within 1–4 weeks of continuous dietary exposure [170]. Similar effects have been described at other trophic levels [169].

Interactions of Pressures within the Ecosystems

The potential for interactions to occur between chemical contaminants and habitat factors (e.g., food and habitat availability) has been identified as being important for understanding the ecological effects of pollutants [171]. Thrush *et al.* [171] demonstrated a way of determining likely interactions and also that multiplicative effects, such as stressors, frequently interact across environmental gradients. This pattern suggests a strong role for regression-based analysis of field gradients in the determination of contaminant effects. This study highlights the potential variation in response to metal contaminants across ecological landscapes; it provides an insight into fitting ecotoxicological responses into ecosystems. The complex community effects, mediated by impacts on foundation or key species and ecosystem engineers, have been assessed by Thrush *et al.* [171], highlighting the need for improved integration of ecological patterns with contaminant-stress responses.

Moreover, Crain *et al.* [172] in an analysis of 171 studies that manipulated two or more stressors in marine and coastal systems, found that cumulative effects in individual studies were additive (26%), synergistic (36%), and antagonistic (38%), which is very close to what would be expected from a random distribution (33-33-33%). The overall interaction effect across all of the studies was synergistic, but interaction type varied in relation to response level (antagonistic for community, synergistic for population), trophic level (antagonistic for autotrophs, synergistic for heterotrophs), and specific stressor pair (seven pairs additive, three pairs each synergistic and antagonistic). Addition of a third stressor changed the interaction effects significantly in two-thirds of all of the cases; it doubled

the number of synergistic interactions. Hence, although pollutants can affect communities and ecosystems, their effects can be reinforced when other pressures or stressors (*i.e.* nutrient inputs, habitat loss, hypoxia, *etc.*) are present. Generally, organisms living under conditions close to their environmental tolerance limits appeared to be more vulnerable to additional chemical stress [173].

MEASURING POLLUTANT EFFECTS IN AN INTEGRATIVE ASSESSMENT

Much discussion has taken place about the lack of a coherent terminology to differentiate the various assessment types and the diverse nature of aquatic environmental integrative tools and methods in assessing ecological integrity [141, 174]. Following Borja *et al.* [141], these approaches can be divided into two categories: (i) those evaluating risk and state of a particular system (*sensu* the Drivers-Pressures-State-Impacts-Response (DPSIR) approach); and (ii) those assessing the ecological integrity status of the whole ecosystem under an ecosystem-based approach.

Evaluating the Risk and State of a System

The ecotoxicological effect measurements must be used, within the context of ecological risk assessment (ERA), as a tool to assess the likelihood of harm being caused to ecosystems, or their components through exposure to a specific concentration of a chemical.

Among the approaches used to overcome the limitations shown above, some authors propose the use of multispecies tests, or using different compartments of the system (*i.e.* chemical analysis, bioassays, impacts on benthic communities) in the assessment. This approach is developed within the context of an integrative assessment, considering several lines of evidence (LOE); *i.e.* sediment contamination, toxicity and benthic fauna.

Another relatively recent approach is the weight of evidence (WOE) approach, which is the result of combining different measures of environmental quality to establish an overall assessment of environmental health. The philosophy behind WOE is a preponderance/burden of evidence approach, where the conclusions drawn from individual components are considered not as a sum of these components, but relative to one another [175]. WOE determination incorporates judgements concerning the quality, extent, and congruence of the data contained in the different LOE. It includes also observational (e.g. ecology) and investigative or manipulative (e.g., toxicology used to determine cause-and-effect) components. Ideally, any WOE framework will be easily understandable by lay personnel or decision-makers; it will also appropriately differentiate between hazard (the possibility of impact) and risk (the probability of impact) [176].

One of the first sediment quality WOE frameworks was the sediment quality triad (SQT). The triad concept was conceived more than 20 years ago by Long and Chapman [177] to provide a sediment quality evaluation based upon three components: (i) chemistry, to determine chemical contamination; (ii) bioassays to evaluate toxicity; and (iii) benthic community structure to determine the status of resident fauna exposed to the sediment contaminants. These three original components provide the basis for the SQT, or contaminated sediment risk assessment [178]. However, the traditional SQT is based on correlation, not causation; it can provide definitive conclusions regarding the pollution status of contaminated sediments, but cannot provide definitive conclusions in all cases, and cannot derive causation without further studies. Hence, the traditional SQT can be considered as a screening-level ERA, with causation examined at a higher tier [179]. Hence, the SQT needs to include additional LOE to address all aspects of ERA.

Individual LOE involved in contaminated sediments evaluation should include [175]: (i) measures of sediment chemistry to determine the level and extent of pollution and modifying factors (e.g., grain size, total organic carbon) compared to SQGs, and to answer the question “are contaminants present at levels of concern?”; (ii) measures of resident benthic community structure to determine whether community structure has been altered, possibly due to pollution; and (iii) measures of toxicity to determine whether the contaminated sediments are affecting the biota.

Additional LOE can include: (i) measures of biomagnification, usually involving measurements of body burdens in sediment-dwelling invertebrates, and food chain modelling to answer the question “are any contaminant of concern capable of biomagnifying and likely to do so?”; (ii) measures of exposure such as biomarkers or body burdens (bioaccumulation) to determine which sediment contaminants, if any, are bioavailable and to try to determine

causation; (iii) toxicity identification evaluations (TIE) to attempt to assign causation; and (iv) determinations of sediment stability to determine whether only surficial sediments should be evaluated or whether deeper sediments, which may be exposed during storm or other events, need to be evaluated by answering the question "is the sediment stable or is it prone to erosion resulting in exposure of deeper, more contaminated sediments and/or contamination down-current?"

In summary, a battery of different LOE selected for specific purposes should be developed, maximizing flexibility in the use of WOE within a wide variety of situations and locations which exist in the environment.

For sediment risk assessment, the recommended WOE is the tabular decision matrix (TDM); this is the most effective and logical basis for presenting WOE in a manner readily understandable. TDM was used under the SQT first proposed by Chapman [125] and improved by other authors [176, 180, 181]. Such a matrix must be based on a strong quantitative, statistical evaluation / summarisation prior to merging into more qualitative matrix tables. Each LOE is established on the basis of a graduation (a scoring system) to rate each measurement endpoint as indicative, moderate, or negligible/low ecological risk. These LOE are summarized in SQGs, toxicity test results and biotic indices. The classification of the toxicity tests to use in the ordinal ranking scheme is based upon comparison with sediment toxicity guidelines and/or standards established previously in national ring, or intercalibration tests using the same species of organisms [182]. The integration of data-reducing techniques is very useful to incorporate into a tabular matrix, as emphasised by Chapman [183]. Some steps in the SQT are assigned more weight than others based upon expert knowledge of the sediment assessment, system behaviour and factors interpretation computed from Best Professional Judgement. As mentioned previously, to develop a tabular matrix, a ranking scheme must be applied for categorisation. An example of this application is shown in Tables **2** and **3** [184].

Table 2: Ordinal ranking scheme applied for 'weight of evidence' categorisation. PIAE = Potential Impact for Adverse Effects.

Rank	**Pollutant**	**Classification**		
Contamination[1]	Metals and metalloid	average < AL1 (low PIAE)	AL11<average<AL2 (moderate PIAE)	average > AL2 (high PIAE)
	PAHs	values< AL1 (low PIAE)	AL1< values < AL2 (moderate PIAE)	AL2 > 1 (high PIAE)
	PCBs	values< AL1 (low PIAE)	AL1< values < AL2 (moderate PIAE)	AL2 > 1 (high PIAE)
Toxicity[2]	Amphipods mortality	< 25% (not toxic)	25-50% (moderate toxicity)	> 50% (high toxicity)
	Microtox	EC50>1000 (not toxic)	1000>EC50>500 (moderate toxicity)	EC<500 (high toxicity)
Benthos[2]	Biotic index	0–3.3 (no alteration)	3.3–5 (moderate alteration)	5–7 (high alteration)
Overall Risk Assessment		No significant adverse effects (0-1 positive)	Potential significant adverse effects (1-3 positive)	Highly significant adverse effects (more than 3 positives)

[1] For sediment pollution, two guideline values, obtained from previous studies undertaken in Spanish Ports [185], are used: Action Level 1 (AL1) and Action Level 2 (AL2).

[2] Toxic thresholds for amphipods from DelValls *et al.* [182], whilst benthic reference values have been derived for Basque Country ecosystems based on Borja *et al.* [156].

In WOE assessments, there is no single correct way to relate sets of variables and other approaches are considered, such as multivariate analyses (e.g., Principal Component Analysis) which are used typically in WOE determinations [183, 186].

Some authors [183, 187] have also detailed a tiered scheme for WOE components. The tier testing approaches are recommended for regulatory purposes as it permits keeping risk assessment cost-effective and feasible. It allows an assessor to undertake only as much sampling and analysis as are needed to come to a reasonable decision. Moving through the tiers (steps), one moves from a broad to a more focused scope and from general benchmarks to more detailed, directed tests [188]. Although WOE assessments (such as the SQT) have improved since their initial development, future WOE applications should consider specific LOE in terms of risk assessment, ensuring that both exposure and effect assessment are addressed adequately, as are both causation and ecological relevance. As emphasised by Chapman *et al.* [180], WOE assessment (such as the SQT) should not be used to develop a single numerical index; any simplification of WOE should be site- or region-specific, not generic.

Table 3: Example of tabular matrix with the Sediment Quality Triad (Lines of Evidence, for management), using a port from the Basque Country (northern Spain).

Stations	**Chemistry**			**Toxicity**		***In situ* alteration[1]**	**Overall risk assessment[2]**	**Explanation / contamination of concern**
	Metals and metalloids	PAHs	PCBs	Amphipods mortality	Microtox	AMBI		
1	-	+/-	+	+	+	+	+	Sediments polluted. Biological effects associated with PAHs and PCBs.
2	-	-	-	-	-	+/-	-	Clean sediments. Not polluted. Moderated *in-situ* alteration not associated with chemicals in sediments and no effect in laboratory.
3	-	-	-	-	+	+	+/-	Moderate toxicity and alteration. Either biological effects are associated with other contaminants or with confounding factors such as organic matter, ammonia and/or sulfides.
4	-	-	+/-	+/-	+/-	+/-	+/-	Moderated degradation by organics. These sediments could become polluted.
5	-	+	-	-	-	+	+	Degradation associated with PAHs. Sediment toxicity tests must be cross-checked.

Note: use of symbols provides for a convenient and rapid visual assessment of all endpoint results, as well as assessment of the concordance among endpoints, for a given site. Symbols indicate: +, contamination, effect or alteration are observed; +/-, moderate contamination, effect or alteration; -, no contamination, effect or alteration.

PAH = Polycyclic Aromatic Hydrocarbons; PCB = Polychlorinated Biphenyls

[1] Benthic community *in-situ* alteration using the AZTI Marine Biotic Index (AMBI) [156].

[2] For overall risk assessment (see Table **2**): two moderate (+/-) equal to one positive.

However, the components of WOE assessments will change as new, more ecologically-relevant measurement endpoints are discovered and applied. In particular, it is expected that: (i) particular WOE "tools" will be refined and validated; (ii) interpretative guidelines will be more fully developed; and (iii) chronic toxicity tests and community responses will be further incorporated into WOE assessments.

In summary, environmental quality, assuming the persistence of a suitable habitat, can be determined only by the responses, or condition of multiple (never single) measures undertaken as part of integrative assessments.

The uncertainty and high variability, inherent in both ecosystems and methods of measurement, require a burden of evidence approach. The WOE approaches are, and will continue to be, most useful where they are flexible and responsive to study goals, ecological realities, and social concerns [175].

Assessing the Ecological Integrity of an Ecosystem

One criticism of the sole use of diversity indices, as measures of ecological impacts is that such measures do not consider alterations to the structure of communities; therefore, they may mask more effects than they elucidate [144]. Following these authors, indices which consider taxonomic relatedness and multivariate analyses of community structure are more sensitive and powerful means of detecting ecological impacts than studies that consider diversity and species richness (or other indices) alone. These criticisms, together with the interactions and synergistic effects described above, have led to the use of multiple indices and integrative methods to assess ecological integrity in marine waters [189]. These methods take into account the concept of environmental or ecological status, which includes the structure, function and processes of marine ecosystems, bringing together natural physical, chemical, physiographic, geographic and climatic factors; subsequently, integrating these conditions within the anthropogenic impacts and human activities in the assessment.

Hence, the environmental status concept defines quality in an integrative way, using several biological parameters (*i.e.* macroalgae, macroinvertebrates, *etc.*) together with physico-chemical and pollution elements, including ecosystem attributes (such as food web dynamics, species diversity, and the distribution of life histories) that are not direct biological properties but functions of the entire ecosystem. They are important because they provide information about the functioning and status of the ecosystem; they have been perceived widely as additional and potentially useful indicators of environmental status [141]. This approach is intended to permit an assessment of the ecological status at the ecosystem level ('ecosystem-based approach' or 'holistic approach' methodologies), more effectively than can be carried out at a species or chemical level (*i.e.* quality objectives). However, there are few examples of pollutant impacts at the whole marine ecosystem level [142, 190], in an integrative way, because they are masked by other human pressures as commented upon above.

An overview of these kinds of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems world-wide can be seen in Borja *et al.* [189]. Overall, the legislative measures world-wide tend to converge in defining environmental water quality in an integrative way. However, the degree of convergence is variable, based generally upon studies carried out in single systems, which do not permit generalisation.

In practical terms, managers and decision-makers need simple, but scientifically well-established methodologies capable of demonstrating to the general public the evolution of a zone (estuary, coastal area, *etc.*), taking into account pollution and other human pressures or recovery processes [155, 189]; likewise, capable of guiding the implementation of successful management. Within this context, there is a major scientific challenge to develop tools to define adequately the scale and present condition of marine ecosystems in terms of biological performance, as well as to monitor changes through time, and similarly, to identify and address, through management, the causes of observed impairments [189].

Some of these challenges have been addresses in assessing ecological status in large ecosystems in the USA and Europe [166, 191]. However, with the success of these tools come additional challenges. The proliferation of indices to assess the status adds an element of confusion back into what they had been intended to simplify [140]. Some of the confusion arises because of the different processes used for developing, calibrating and validating methods in different regions; this leads to inconsistencies in assessment across regions. Additional confusion results from

indices developed for multiple types of biota, providing managers with multiple, often conflicting, answers for a single water body [140]. Whereas the last decade was characterized by an explosion of methods, the next decade should be one of consolidation and agreement [140].

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CHAPTER 9

Chemical Pollution on Coral Reefs: Exposure and Ecological Effects

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Abstract: In this chapter we review the effects of anthropogenically derived chemical pollutants on tropical coral reef ecosystems. A wide range of compounds, including pesticides, trace metals and petroleum hydrocarbons enter reef systems through various pathways and affect different reef species and/or life history stages. Tools for evaluation of chemical stress on coral reefs consist of molecular, biochemical, physiological and ecological bioindicators, providing information at organismal or community levels. This chapter collates and assesses available information on different chemical stressors in the marine environment and the effects on reef-building corals. Ecological effects from chemical stressors are strongly dependent on exposure characteristics. Three probable pollution scenarios are discussed and their individual properties evaluated. Short-term, pulse-like pollution events including oil spills or antifoulant deposition through ship groundings often have a direct and severe impact upon multiple trophic levels of the system. However, these events are typically localised and possibly irrelevant on an ecosystem-wide scale. In contrast, recurring pollution events such as input from river floods or chronic pollution from land runoff (e.g. sewage treatment effluent or herbicides), may exert subtle effects on lower trophic levels of the system, affecting species fitness and driving adaptation. Effects from recurring or chronic pollution are more likely to combine and interact with other environmental factors, but remain poorly understood. Over time, chronic sub-lethal stress may decrease resilience of reef organisms to other forms of environmental stress like elevated sea surface temperatures and ocean acidification.

CORAL REEF ECOSYSTEMS AND SYMBIOTIC PRODUCTION

Coral reefs are biogenic structures that often contribute significantly to the seaward section of tropical shorelines, buffering the coast from wave action and erosion [1]. Coral reefs are among the most biologically diverse and productive systems in the world and many coastal communities depend upon these economically and culturally important ecosystems as a source of income or resources. Coral reefs are crucial to tropical fisheries and tourism and provide many island populations with primary building materials. Reef-related tourism alone generates vast revenues in some parts of the world. For example, in the 1990s, an estimated $140 billion was generated by Caribbean reefs annually [2]. The ecological value of coral reefs is also enormous. There are over 600 species of calcifying corals, and these contribute directly to the habitat of thousands of species of tropical fish, algae and invertebrates. The physical protection offered by coral reefs enables formation and persistence of associated ecosystems such as seagrass beds and mangrove forests, allowing for the existence of essential habitats, hatching grounds and fisheries [3]. These ecosystems are in turn crucial for the reefs, as they play an important part in the sustenance of the marine foodweb by providing detrital matter that helps maintain the lower trophic levels of the reef food web, by functioning as a sediment trap limiting particulate matter reaching reefs and by acting as nursery grounds for many juvenile fish that find their way out to the reef in adulthood [1].

Reef-like structures have existed on earth for over 500 million years, with modern reefs developing around 250 million years ago [4]. Most coral reefs are located in tropical oceans within 30° of the equator with water temperatures ranging between 18 and 30 °C (Fig. **1**). Coral reefs are primarily formed by calcification processes of scleractinian (hard) corals and coralline algae, providing a structural basis for reef-dwelling organisms. The extraordinary productivity of coral reefs that may seem remarkable within a marine environment harbouring low nutrient concentrations can be explained by the photosynthetic contribution of intracellular microalgae to host tissues [5]. Reef building corals all host endosymbiotic dinoflagellates (zooxanthellae) of the genus *Symbiodinium*. Coral hosts profit from this mutualistic relationship by obtaining high-energy photosynthetic products in the form of sugars, amino acids, carbohydrates and small peptides from the algae, while the symbiotic algae receive inorganic plant nutrients, refuge and protection within the polyp tissues [5, 6]. The symbiosis serves the main purpose of

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restricting nutrient outflow into the surrounding oligotrophic water column, and as a result the host is endowed with substantially more energy than would otherwise be available to heterotrophs, enabling corals to extract calcium carbonate from surrounding waters and secrete it as a skeleton [6, 7]. As coral symbiosis based upon algal primary production is the engine driving coral reef ecosystems, stressors that interfere with photosynthetic processes could undermine the basis of this biologically and economically important marine habitat with serious consequences [8].

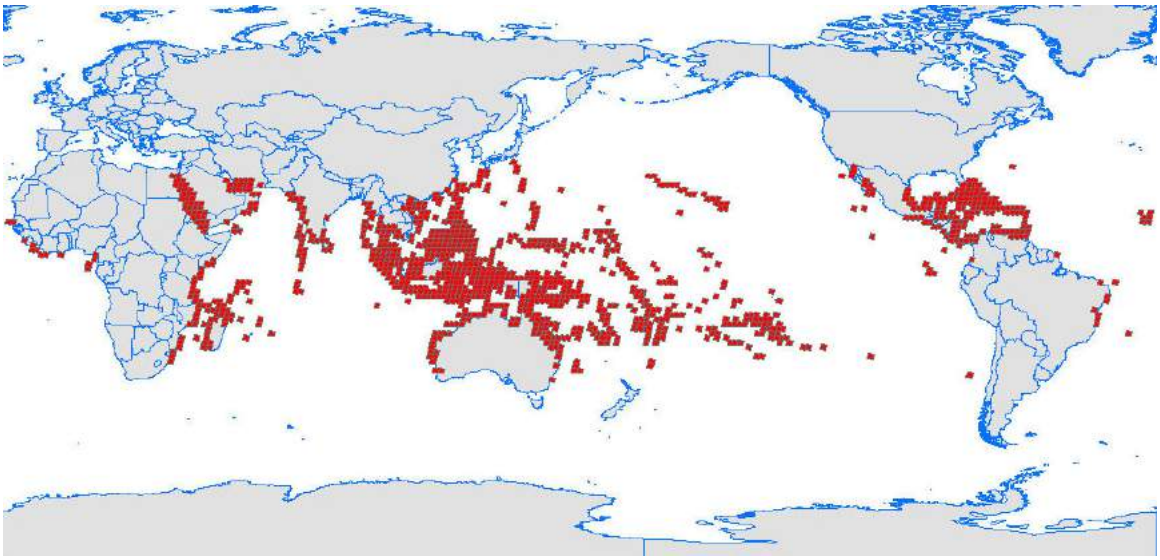

Figure 1: Distribution of coral reefs around the world (source: http://www.nasa.gov/).

Most scleractinian corals reproduce by broadcast spawning, with many species releasing eggs and sperm into the water column simultaneously at annual spawning events [9]. Typically fertilisation of the highly buoyant eggs is external and mobile planula larvae develop over 48–72 h [10]. The swimming planulae are usually competent to undergo settlement on the substratum followed by metamorphosis into a juvenile coral polyp after 96 h. Larvae of many coral species are known to require a biochemical inducer from their preferred settlement substratum, crustose coralline algae, to trigger metamorphosis [11]. Juvenile coral polyps are generally less than 2 mm long and are susceptible to predation as well as smothering by sediments and other stressors [12]. It takes between five and ten years for many coral species to become reproductive and these early life histories, including fertilisation, larval development, larval metamorphosis and the juvenile stage, are all critical for the long term resilience and health of coral reefs and their vulnerability to anthropogenic stress needs to be considered [13, 14].

Over the last century and a half in which intensive agriculture, fishing practises and industries evolved, an estimated 30% of coral reefs worldwide have been severely depleted. Between 50 and 70% of coral reefs are thought to be under direct and immediate threat from climate change and human activities [15, 16]. Coral reef cover in the Caribbean has been reported to have declined over 80% in the last 30 years [17]. Climate related changes in ocean acidity and temperature, nutrients and chemical pollution are the main proposed reasons for coral reef declines and cause for concern [12, 16, 18-21]. In this chapter we aim to critically review and collate information about the possible ecological effects of chemical pollution on coral reefs. We assess potential pollutants, sources and the likely exposure of reef-building corals and their zooxanthellate symbionts. Furthermore, we evaluate what trophic level is most likely to be affected by various pollutants and in what particular way an organism will be impacted. Finally, we aim to assess evidence that links exposure to effect and identifies gaps in our knowledge concerning the effects of anthropogenic pollutants on coral reefs.

SOURCES OF MARINE POLLUTANTS

Waterborne chemicals affecting tropical marine communities can have both point and non-point sources and may be transported to reefs from distant origins. Different classes of contaminants are often associated with a particular environmental compartment because of their physicochemical characteristics. Fig. (**2**) presents a conceptual model for

potential contaminant routes reaching reef ecosystems. Transport, dispersion, and ultimately biological effects of pollutants in marine systems depend on the persistence of these chemicals under tropical conditions and their bioaccumulation and biodegradation rates. Typically pollutants with a higher solubility in surrounding waters will find their way further offshore. Association of pollutants with particulate matter may increase environmental persistence. Because of the rapid sorption of many contaminants to sediments, it is not surprising the largest reservoirs of chemical stressors will be found in estuaries, wetlands or nearby urban centres. Nevertheless, suspended sediments transported in monsoonal flood-plumes have the potential of contaminating sites further offshore. Additionally, volatilisation from surface waters, transport through the atmosphere and redeposition elsewhere can deliver residues far from their original sites of application [22]. Biota carrying accumulated loads of persistent chemicals in their tissues can also transport pollutants between ecosystems and far from their application or deposition sites.

Terrestrial runoff from rivers and streams contaminated by agricultural, industrial or urban activities is usually the most important route for chemicals to enter marine waters (Table **1**). The array of potential contaminants is very wide from organics such as pesticide residues, pharmaceuticals and hydrocarbons to industrial waste products, metals and organometallic compounds. In highly protected areas such as Australia's Great Barrier Reef (GBR), chemicals of concern are contemporary pesticides; most specifically herbicides originating from farming activities [23]. In more highly populated regions such as south-east Asia, a much wider range of urban and industrial contaminants also threaten coral reefs.

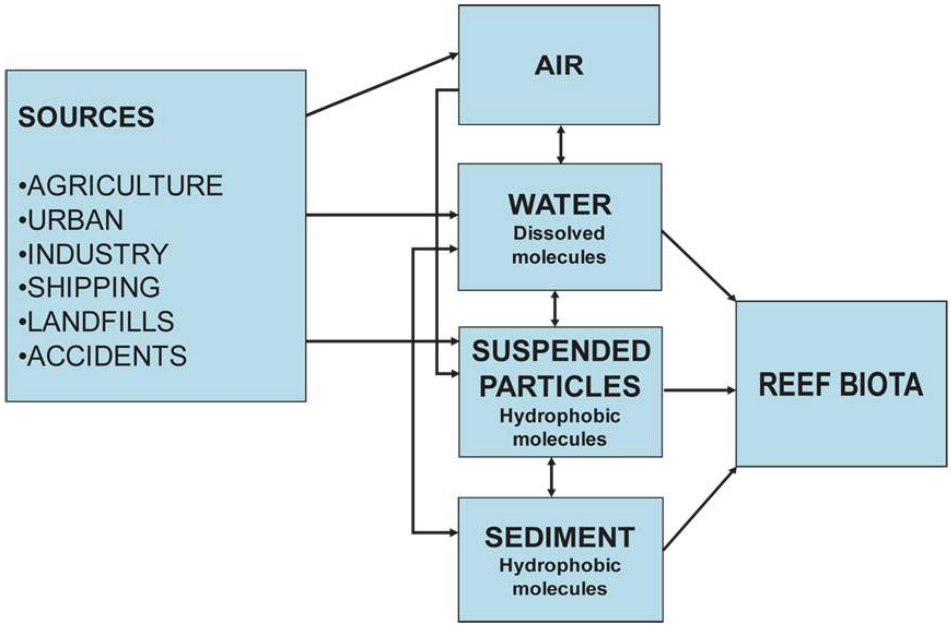

Figure 2: Conceptual model for pollutant pathways in marine systems.

Industrial activities including mining and smelting operations are sources of metals and dioxin-like compounds while shipping operations, refineries and oil extraction and explorative processes introduce hydrocarbons and trace metals to the marine environment [24, 25]. In addition, urban sources such as sewage outfalls, desalination plants and landfills can contribute considerably to contaminant loads [1]. The potential off-site movement of chemical residues depends on both physicochemical properties of compounds (e.g. partition properties) and environmentally specific factors such as hydrology, sediment composition, temperature and biological degradation parameters [26]. While the breakdown of organic compounds is likely to be more rapid in the tropics than in temperate regions, the warmer conditions may increase sorption of organics to particulates thereby increasing their persistence in some environmental compartments. Direct contamination of marine waters can result from inputs associated with boating (hydrocarbons and antifouling paint applications) or chemical-based fishing (e.g. cyanide, [27]). Oceanic waste disposal, ship groundings and incidental spills are additional routes of chemical pollution. Coral reefs are often in close proximity to shipping lanes where contaminated bilge water is disposed of or cargo is spilled. Dredging of channels can resuspend metals and other buried organic pollutants [28].

Table 1: Main contaminants, sources and concerns in regards to tropical coral reefs

Contaminant group	Representatives	Sources	Main concerns
Insecticides	DDT	Agricultural & urban runoff	Survival, reproduction, early life transitions & genetic effects. (Bioaccumulation for persistent OC pesticides)
	Dieldrin		
	Chlorpyrifos		
	Carbaryl		
	Permethrin		
Herbicides	Diuron	Agricultural & urban runoff, antifouling applications, ballast water discharge	Photosynthesis & calcification
	Atrazine		
	Hexazinone		
	Glyphosate		
Antifouling agents	Irgarol-1051	Shipping activities & marine structures	Photosynthesis & calcification Survival, reproduction, early life transitions & genetic effects
	Zn-pyrithione		
	TBT		
Industrial OCs	Dioxins	Thermal processes (atmospheric deposition) & terrestrial runoff	Bioaccumulation, reproduction in birds & mammals, metabolism & genetic effects
	PCBs		
	Furans		
Oil products & PAHs	Often unspecified mixtures	Shipping operations, industrial discharge, oil exploration, mining activities & spills	Bioaccumulation, survival, reproduction, metabolism, growth & genetic effects
Metals	Copper	Agricultural runoff, various urban and industrial sources, oil explorative activities & antifouling applications	Bioaccumulation, survival, reproduction, growth & behaviour
	Zinc		
	Mercury		
	Cadmium		

STRESS EVALUATION

Coral reef ecosystems are composed of a diverse community ranging from algae to mammals interacting in a variety of ways on multiple hierarchical levels. All reef organisms are likely to vary substantially in their sensitivity and response to individual pollutants or combinations thereof. Furthermore, susceptibility of species and possible stressor interactions may differ considerably depending on the life stage of exposed organisms. Even though reef-building corals are only one component of the ecosystem, they are considered fundamental for its existence and hence in this review we will concentrate on available knowledge and key questions related to potential effects of pollutants on reef-building corals and their endosymbionts.

The delicate interaction between coral host and *Symbiodinium* sp. is particularly vulnerable to water quality declines and stress from high sea surface temperatures and high light intensities [3, 12]. From a toxicological point of view, chemicals can affect the host animal, the algal symbionts, or both. While contact of host with contaminants is often direct, multiple membranes need to be crossed before a chemical can reach the intracellular algae. Studies in the past have suggested physiological differences between reef building organisms may determine their susceptibility to chemically induced stress. For example, branching coral species seem more vulnerable to some chemical contaminants than massive corals [29] and small-polyped species seem more susceptible to pollution stress than large-polyped corals [30]. Orientation, growth form, life stage, reproduction strategy, mucus production and lipid content are all factors that will determine how easily survival of a coral species is influenced by chemical stress [1]. A wide range of laboratory assays have been used to evaluate effects of chemical pollutants on corals over the past four decades. Table **2** provides an overview of assays used to evaluate stress in multiple life history stages of hard corals.

Prior to 2000 most studies focussed on the effects of metals, PAHs and older organochlorine pesticides on corals (for reviews see [1, 53]). Due to increased application of modern pesticides and altered pollution profiles, up to date information is now required on the distribution and impact of contemporary pollutants on coral reefs.

Table 2: Examples of bioassays and biomarkers used to assess the effects of contamination on scleractinian corals.

Life stage	Host or symbiont	Assay description	Reference(s)
Adult	Host & symbiont	Quantification of bleaching	[13, 31, 32]
	Host	Calcification rate	[33, 34]
		Lipid content	[31]
		Reproductive output	[31]
		Photopigment composition	[31]
Adult	Symbiont	Respirometry	[35, 36]
		Primary production	[35]
Adult and isolated symbionts	Symbiont	^{14}C fixation	[34]
Adult and juvenile	Symbiont	PAM fluorometry to estimate electron transport in photosystem II	[14, 31, 37-39]
Gametes	Host	Fertilization success	[13, 14, 40, 41]
Larvae	Host	Larval settlement and metamorphosis	[13, 14, 42-44]
Adult	Host & symbiont	Gene expression (various genes)	[45-49]
		Stress protein analysis: e.g. MnSOD, GPx, GST, HSPs, ubiquitin	[50-52]

The purposes of toxicity testing are to: (I) determine cause and effect relationships; (II) determine thresholds for lethal and sub-lethal effects to enable the derivation of water quality guidelines; and (III) to use this information in combination with exposure concentrations to evaluate and compare hazards and ideally determine the magnitude of risk potential and mitigation options. While the majority of toxicological data for most chemical contaminants originates from temperate studies and species, in the past decade there has been an increase in the number of studies examining the effects of relevant contaminants on tropical marine species, including corals. A number of assays have been developed to evaluate sublethal effects of chemical pollution on scleractinian corals. These are molecular, cellular and physiological diagnostic indicators that provide a means of assessing both qualitative and quantitative responses to a variety of pressures, individually and collectively. Organisms respond to environmental changes by regulating metabolic pathways to prevent physiological damage. These expressions precede population-level changes and are useful indicators if linked to specific physiological or ecological events [54].

Often a combination of pressures can result in mortality or impaired biological function. Thus, the evaluation of environmental effects on biological systems associated with anthropogenic pressures such as pollutants must begin with understanding causal linkages between stressor and effect [45]. For adult corals, biomarkers of effect include molecular (e.g. gene regulation), biochemical (e.g. lipid content), physiological (e.g. reproduction, calcification) and ecological (e.g. bleaching due to loss of symbionts, distribution) endpoints. The effects of contaminants on zooxanthellate photosynthesis have been assessed using respirometry, ^{14}C fixation and pulse amplitude modulated (PAM) fluorometry [55]. A technique is based on fluorescence quantum yield measurements as a function of photochemical efficiency of photosystem II (PSII) in phototrophic organisms, which can be applied *in situ* or in the laboratory (for a technical review, see [56]). The degree of quantum yield inhibition may be directly correlated to the stressor concentration the evaluated organism is exposed to [57].

Previous studies have demonstrated that different stressors may trigger similar responses through alternating pathways. For example, exposure to metals [32, 58], cyanide [27], herbicides [14], elevated temperature [59, 60] and ocean acidification [61] can all induce bleaching. It is also possible that a single stressor induces a variety of responses that can only be detected using an assortment of different biomarkers [62]. Molecular analysis of gene expression can evaluate the relative impact of stressors, by identifying specific responses to individual stressors [49, 54, 63]. The field of transcriptomics identifies transcription of certain genes at a given time and allows monitoring

of gene expression which in turn can identify gene function and mechanisms behind a particular biological response [47, 64], although establishment of a causal link between genomic and physiological responses has often proved difficult. In effect, while physiological or ecological endpoints indicate stress effects at organismal, population or even ecosystem levels, genetic tools may reveal subtle and specific effects at a genomic level, preceding physiological responses such as coral bleaching or impaired photosynthesis.

CHEMICAL STRESSORS ON CORAL REEFS

In this section we separately investigate organic biocides and industrial pollutants, metals and oil hydrocarbons. We will discuss pathways of exposure and summarise known effects on scleractinian corals. Finally, we will describe ecological impacts associated with these contaminants and evaluate risk posed to coral reef communities.

Pesticides and Antifouling Agents

The second half of the 20th century has seen a massive increase in population growth and a corresponding expansion of world food production. The acceleration of agricultural output can partly be ascribed to the introduction of organochlorine (OC) pesticides such as DDT, which eventually allowed for the shift from classical small-scale farming to industrialised agriculture. The use of pesticides in combination with fertilisers has been growing steadily and has become an essential element of modern agriculture, by preventing the spread of pests and exotic species and by enhancing growth potential, resulting in dramatic increases in crop yields. By the late 1960s environmental drawbacks arising from the use of DDT for agricultural purposes, or as a vector of disease control, became apparent and usage of selected persistent OC pesticides (e.g. DDT, HCB, dieldrin and chlordane) was slowly phased out in most developed countries. Other classes of pesticides such as organophosphates, carbamates, organotins and pyrethroids soon filled these roles. Today a wide variety of pesticides are used in specialised farming globally, with enormous annual application rates (see Chapter 4).

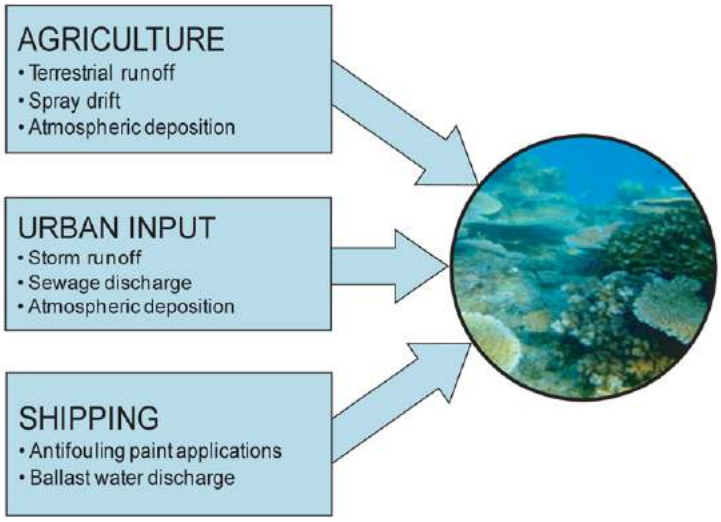

Figure 3: Sources of pesticides reaching coral reef sites.

Modern pesticides generally exhibit shorter half-lives in the environment when compared with their predecessors. Improved usage patterns and progressive application techniques, alongside highly specialised modes of action, make unintended side-effects to non-target species considerably less of a concern. However, care must be observed. The economies of countries in tropical areas are often based on agriculture, and depend on intensive use of pesticides to maintain and improve production [65]. As agricultural activities are primarily concentrated in river valleys and coastal plains, it is not surprising that agrochemical residues originating from terrestrial applications are ubiquitous in streams and rivers, eventually draining into estuaries and coastal seas [23, 66]. Multiple studies have shown that the main source of pesticides in nearshore coastal areas is agricultural application, transported via terrestrial runoff (especially during the monsoon season) [23, 67, 68]. The effects of pesticide residues are of great concern as many of these compounds and their breakdown products have been reported to influence both human and environmental health. It is

therefore vital to characterise transport and fate of pesticides and their toxicity to non-target organisms to confidently assess risk associated with application, especially in tropical areas where pesticide usage patterns are generally much higher than in temperate zones [65]. Fig. (**3)** provides an overview of sources of biocides reaching reef sites.

In addition to terrestrial sources, biocides are intentionally released into the marine environment by incorporation into antifouling paint formulations where they function to prevent unwanted growth of a wide range of algae and invertebrates on boats or other marine structures. The organometallic biocide tributyltin (TBT) proved the most effective antifouling ingredient in the latter half of the 20th century and is a common contaminant in harbours globally. Although normally uncommon on coral reefs, TBT as well as metal biocide 'boosters' have been identified in extreme concentrations on reef rubble following ship groundings [69, 70]. The use of TBT has been regulated internationally since 1990 due to recognition of its severe impact on aquatic ecosystems. In 2003 the International Maritime Organization (IMO) and their Marine Environment Protection Committee (MEPC) banned application of TBT as an antifouling agent on ships completely. As of 2008, TBT-based antifouling paints must be removed or covered with a sealer-coat [71]. Since the phasing out of TBT in antifouling paints alternative coatings have been developed often using copper or zinc in combination with an organic booster biocide. These are typically herbicidal in nature, as primary colonisation of hull surfaces by microalgae allows for a convenient base for subsequent attachment and growth of seaweeds and invertebrates. This may result in elevated environmental concentrations in areas of high yachting activity, particularly in and around harbours [72]. Studies have shown that biocide dissipation from hulls of vessels is a function of leaching and degradation rates, water movement, sorption and hull treatment amongst other parameters [73]. The same studies have suggested two of the most popular booster biocides in use, diuron and Irgarol 1051, to persist in the water column while other booster biocides disappear more rapidly [73]. Although some data are available on biodynamics of antifouling agents in temperate regions, there is little comparable information on bioavailability in tropical waters.

Until 2000, most studies on pesticides in tropical marine ecosystems focussed on OC compounds, as these persistent chemicals have dominated the pesticide market for decades in tropical areas. Studies also focused on estuaries, as this is likely to be where most terrestrially derived sediments settle and therefore where most concentrated reservoirs of persistent chemicals will be detected. Furthermore, studies focused on commercial species such as fish that may affect human health. Apart from a few studies performed in the Florida Keys [74-76], Bermuda [34] and the Philippines [77], most available data on pesticide dynamics on coral reefs are associated with the Great Barrier Reef. The limited studies assessing banned persistent organochlorine substances on coral reefs found concentrations to be generally low [74-76, 78] or declining [53, 79]. While organochlorine residues (e.g. endosulfan) have been measured in recent times [78-80], it is the current generation of pesticides that are of greatest concern to inshore marine ecosystems. Organophosphorous (OP) insecticides such as chlorpyrifos, or herbicides such as glyphosate (Round-up®); triazine herbicides such as atrazine, simazine, ametryn and Irgarol 1051; and urea herbicides such as diuron and tebuthiuron are some of the key pesticides that may affect coral reef biota [23, 67, 81].

Recent studies have found contemporary herbicides to be ubiquitous in waters nearby coral reefs [23, 34, 68, 81, 82]. Systemic herbicides are of particular ecological concern to coral reef systems, as these compounds are developed for quick environmental uptake through the root system of plants and are therefore relatively water soluble. A chemical's solubility will determine whether it mainly exists in the water column or is associated with suspended particulate matter, and therefore more likely to sink to the bottom. In contrast to the latter fraction that precipitates nearshore and becomes incorporated in the sediment, chemicals dissolved in the water column can travel greater distances and exert adverse effects far from their application sites. Thus, apart from a greater potential to reach reef sites, herbicide pollution can have severe consequences for ecosystems dependent on primary production. Herbicides target a wide range of physiological processes; however the herbicides most commonly detected on coral reefs are the photosystem II (PSII) herbicides (Table **3**). This class of herbicide acts by inhibiting electron transport through the photosystem in chloroplasts by reversibly binding to a specific electron-acceptor protein (D1-enzyme in PSII). These herbicides outcompete the normal ligand for binding sites on this highly conserved protein vital for plant photosynthesis [83]. The D1-enzyme also forms part of the photosystem in symbiotic zooxanthellae of corals and is likewise affected by herbicide exposure. As far as the holobiont (host combined with symbiont) is concerned, direct effects of herbicide exposure are a decrease in algal photosynthetic efficiency, limiting energy flow from symbiont to host [84]. Secondary effects of restricted electron flow in PSII include a build-up in reactive oxygen leading to oxidative stress [85], a process intensified by high illumination [86]. Further effects include disruption of

membrane structure and chlorosis, as well as aberrations in energy dynamics due to the reduced availability of photosynthetic products, ATP, NADPH and ferredoxin [87]. As inhibition of photosynthesis can lead to decreased algal production and eventually expulsion of symbionts (bleaching), herbicide contamination may disturb the fragile keystone algal-coral relationship so important for all ecological processes on coral reefs.

Impact

Table **3** provides an overview of studies on pesticide effects on hard corals. Little is known regarding the effects of OC pesticides on reef building corals. Suspected adverse effects caused by OCs range from carcinogenesis, interruption of neurological function, changes in cell metabolism and gene expression, to endocrine disruption and interference with reproduction [78].

In an early study on organochlorine pollution, McCloskey & Chesher [89] observed photosynthetic depression in a number of scleractinian corals after *in situ* exposure with DDT, dieldrin and a PCB with concentrations in the mg/L range. However, even these extremely high concentrations did not result in alterations of feeding behaviour, polyp expansion, sediment clearing or skeletal crystal formation. Olafson [90] explored bioaccumulative potential of OCs in reef biota and found DDT, chlordane and lindane able to accumulate in coral tissues. In a recent study assessing effects of short-term exposure (up to 96 h) to low insecticide concentrations on different life history stages of the branching coral *Acropora millepora*, endosulfan (OC), chlorpyrifos and profenofos (OPs) were found to affect photosynthetic performance and/or density of zooxanthellae within adult branches at relatively high concentrations [13]. In addition, profenofos-exposed branches expressed permanent tissue retraction. These neurotoxic insecticides did not inhibit fertilisation of gametes as may be expected from the absence of neurons in oocytes and sperm, yet larval metamorphosis was heavily impacted, with 50% effect concentrations (EC50) for inhibition of metamorphosis as low as 0.3-1.0 µg/L [13]. This study found the swimming behaviour of the larvae was not affected by the insecticides, an observation confirmed by Acevedo [91], who demonstrated that far greater concentrations of chlorpyrifos and carbaryl were required (mg/L) in order to cause mortality amongst larvae of the brooding coral *Pocillopora damicornis*. These findings suggest that the mode of impact of investigated insecticides in coral larvae involves specialised pathways instead of general neurotoxicity. In contrast, another study on adult colonies of *P. damicornis* demonstrated 50% mortality (LC50) to occur after 96-h exposure to 6 µg/L chlorpyrifos [92]. While most insecticides tested appear to negatively affect corals or their larvae, a recent study indicates that the "eco-friendly" larvicidal agent, *Bacillus thuringiensis* ssp *israelensis* (Bti), used extensively to control mosquitoes in the tropics, is harmless to coral larvae [93].

Herbicides readily penetrate coral tissues and rapidly (within minutes) reduce the photosynthetic efficiency of the endosymbiotic zooxanthellae. No apparent acute effects have been observed on host animals, fertilisation of gametes or metamorphosis of larvae after short-term exposures to diuron [14]. Nonetheless, bleaching of established recruits or adult coral branches is a common reaction to high concentrations or chronic exposures of PSII herbicides [14, 32, 94]. The dissociation of symbiosis is considered a sub-lethal stress response and a secondary effect most probably caused by oxidative stress in zooxanthellae as a result of chronic photoinhibition. Although the mechanism is still not entirely understood, the main hypothesis is that by expelling symbionts, host corals can reduce the number of damaged symbionts within their tissue, while at the same time limit their exposure to reactive singlet oxygen [86, 94].

In a study on the effects of the antifouling herbicide Irgarol 1051, Owen and co-workers [34] found isolated *in vitro* symbionts of *Madracis mirabilis* to cease incorporation of $H^{14}CO_3^-$ after 4-8 h exposures to concentrations as low as 63 ng/L. The effects of PSII herbicides on coral symbionts has flow-on effects to the host, which suffers a proportional decrease in the energy translocated as sugars to the animal tissue [84] and this can lead in the long term to reduced reproductive output [31]. Jones [86] reviewed the toxicological effects of various PSII herbicides on a range of coral life history stages and isolated symbiotic algae and argued the most sensitive endpoint to be inhibition of photosynthesis in algal symbionts. Overall, in directly comparable exposure experiments using adult branches of multiple coral species, concentrations of tested herbicides reducing effective quantum photosynthetic yield (10-h EC50) of symbiotic algae *in hospite* ranged over three orders of magnitude (Fig. **4**), while significant reductions in photosynthetic yields were observed at concentrations as low as 50 ng/L (Irgarol 1051), 200 ng/L (diuron) or 300 ng/L (ametryn) [38, 39, 86]. These outcomes were supported by tests on isolated symbionts [88] and can be directly compared with toxicological results on other aquatic primary producers, including tropical estuarine microalgae [95], seagrass [96], crustose coralline algae [97], marine diatoms [98] and freshwater algae [99]. Conversely, recovery of normal photosynthetic rates occurred quickly after placement in clean exposure medium [38, 39]. This would suggest short-term exposure to PSII herbicides inflicts no permanent damage.

Table 3: Toxicological studies on the effects of selected pesticides on different life history stages of scleractinian corals

Contaminant	Life stage	Toxicological endpoint[1]	Toxicological data (References)[2]
Herbicides			
Diuron	Adult	Bleaching	[39, 86]
		Symbiont density	[31]
		PSII inhibition	[14, 31, 38, 39, 77]
		Photopigment composition	[31]
		Lipid content	[31]
		Respiration	[77]
		Fecundity	[77]
	Juvenile	Bleaching	[14]
		Tissue retraction	[14]
		PSII inhibition	[14, 84]
		Energy acquisition	[84]
	Isolated symbionts	PSII inhibition	[39]
		$H^{14}CO_3^-$ incorporation	[88]
	Larvae	Metamorphosis	[14]
	Gametes	Fertilisation	[14]
Atrazine	Adult	PSII inhibition	[39]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[88]
Irgarol 1051	Adult	Bleaching	[39]
		PSII inhibition	[39]
		$H^{14}CO_3^-$ incorporation	[34, 88]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[88]
Simazine	Adult	PSII inhibition	[38]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[88]
2,4-D	Adult	Tissue damage	[74]
		PSII inhibition	[77]
		Respiration	[77]
	Isolated symbionts	$H^{14}CO_3^-$ incorporation	[115]
Ametryn, hexazinone, Tebuthiuron, Ioxynil	Adult	PSII inhibition	[38]
Insecticides			
Endosulfan, chlorpyrifos, profenofos, carbaryl, permethrin	Adult	Symbiont density	[13]
		PSII inhibition	[13]
	Larvae	Metamorphosis	[13]
	Gametes	Fertilisation	[13]

1. For methodology and exposure durations please refer to text or original publications

2. For quantitative toxicological data please refer to text or original publications

However, chronic exposure experiments of coral branches to low (1-10 µg/L) diuron concentrations showed, apart from decreased photosynthetic rates, severe visible bleaching, partial colony mortality, two to five fold reductions in tissue lipid content and significant reductions in fecundity [31]. In laboratory exposures, the widely used non-PSII herbicide 2,4-D (a growth inhibitor) failed to exert any effect on colour, tissue extension, photosynthesis and metabolism of adult corals except in extremely high concentrations (100 mg/L) [74, 77]. When exposed to a formula containing a 2,4-D amine salt including a 'wetting agent' (dispersant) though, severe toxic effects were observed at 100 µg/L [74].

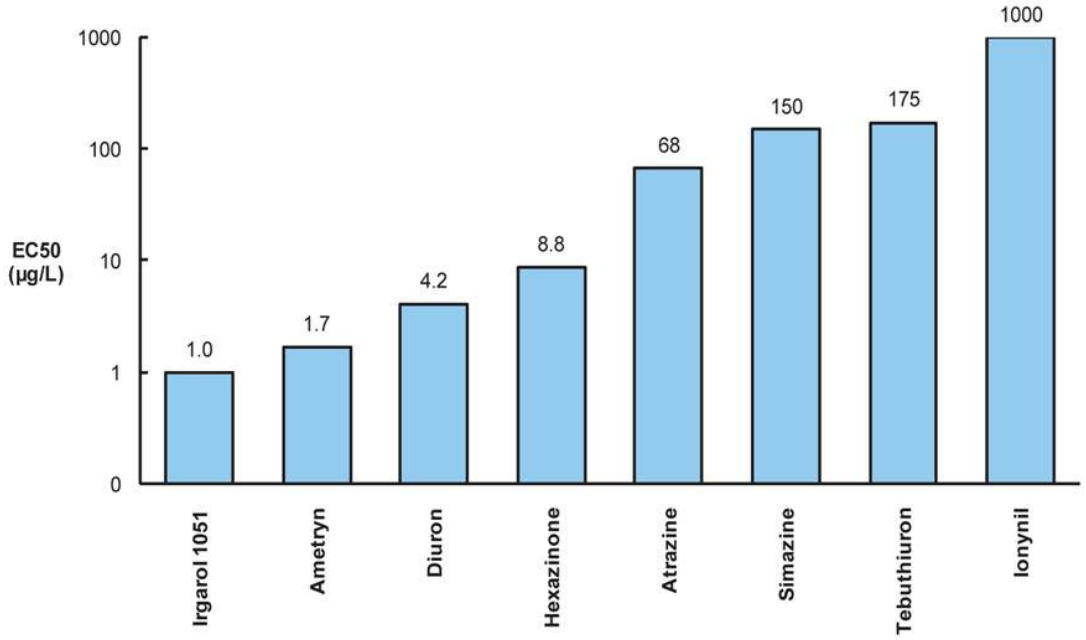

Figure 4: EC50 for several herbicides impacting effective quantum PSII yield of zooxanthellae symbionts in tissues of different coral species. Lower values indicate greater toxicity with susceptibility ranging over three orders of magnitude. Data after [38, 39, 86].

The historic focus on persistent pesticides has found that the key concern is associated with accumulation in the food chain and potential effects in air-breathing top predators. A key finding in the last decade relates to PSII herbicides. There is increasing evidence of widespread relatively low level exposure of inshore reefs (e.g. on Australia's GBR) to these chemicals. Concentrations are typically below levels at which adverse effects may occur on photosynthesis (e.g. diuron <20 ng/L). However, during major flow events concentrations have been determined in flood plumes near inshore reefs that are sufficiently high for effects to be detectable (diuron 0.1-1 µg/L) [23]. Furthermore, as flood plumes often carry elevated concentrations of several pollutants simultaneously, it is likely that nearshore reef systems are exposed to combinations of chemical stressors. Herbicides are commonly detected in complex mixtures within fresh and seawater systems. As multiple factors potentially interfere with the same physiological mechanism (e.g. PSII electron flow), additive or even synergistic toxic effects may occur [100]. A recent study has confirmed that the phytotoxicity of PSII herbicides commonly detected in GBR waters towards benthic microalgae is additive [101]. Overall, the margin of safety between observed concentrations and measurable effects is relatively small and the potential risks from chronic exposure remain unclear. Herbicide-induced interference with primary producers may exert a bottom-up pressure on the system, potentially decreasing reef resilience to other environmental stressors as elevated temperatures and ocean acidification.

Industrial Organochlorines: PCBs, Dioxins and Furans

Polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are ubiquitous organic contaminants. This group of chemicals is extremely persistent, has a tendency to bioaccumulate in biotic tissues and includes some of the most toxicologically potent compounds known (see Chapter 7). Although a global treaty aimed at the reduction and possible elimination of PCBs and dioxin-like chemicals has been established in 2001, significant concentrations are still found in marine environments worldwide. Evidence exists that distribution of PCBs, PCDD/Fs into the aquatic environment is mainly due to atmospheric deposition of volatilised molecules [102]. Despite many studies in temperate and arctic regions and extensive reporting on the concentrations and some effects of PCBs and PCDD/Fs in tropical marine species including mammals [103-105], fish [106, 107] and invertebrates [79, 107], to our knowledge no studies have been performed assessing concentrations in tropical waters or on coral reefs and effects on reef-building corals remain speculative. Dioxin-like substances bind to the aryl hydrocarbon receptor in vertebrates and invertebrates, resulting in interference with a broad range of cellular processes. Concentrations of these compounds in water are presumably too low to cause direct effects on primary producers and environmental impact is mostly

associated with bioaccumulation, affecting air-breathing organisms at the top of the food chain. Therefore, likely ecological impacts of dioxin-like pollutants on coral reefs will consist of top-down imbalance of the system.

Oil Hydrocarbons and PAHs

Crude oil, refined petroleum and their combustion products all contain aromatic hydrocarbons, including some polycyclic aromatic hydrocarbons (PAHs). These compounds enter the marine environment through anthropogenic processes or have naturally occurring sources (Table **1**) [53]. A study by Capone and Bauer [108] suggested that in the late 1980s an estimated average of 6 million metric tons of petroleum products were released into our oceans annually. As most petroleum products are hydrophobic in nature, the majority of aromatic hydrocarbons introduced into the marine environment will associate themselves with particulate matter and be deposited in the sediment [108], where these compounds tend to persist. Benthic filter feeders or sessile organisms are at risk through direct contact or ingestion of oil compounds. Straughan [109] argued the biological consequences of oil spills should be determined by the nature and interaction of a multitude of factors, including type of oil, dosage, remedial action, prior exposure, presence of other stressors, differences between biota and many physical environmental, climatic and seasonal factors. Generally, a mixed product containing a broad spectrum of hydrocarbons is released to the marine environmental where it may affect a variety of biological processes [108].

Table 4: Studies on the effects of selected oil products on different life history stages of scleractinian corals.

Contaminant	Life stage	Toxicological endpoint[1]	Toxicological data (References)[2]
Crude oil	Adult	$H^{14}CO_3^-$ incorporation	[110]
	Larvae	Metamorphosis	[42]
	Gametes	Fertilisation	[42]
Dispersed crude oil	Larvae	Metamorphosis	[42]
	Gametes	Fertilisation	[42]
Fuel oil 467	Gametes	Fertilisation	[120]
Dispersed fuel oil	Adults	Tissue damage	[121]
	Gametes	Fertilisation	[120]
2-stroke oil (vegetable)	Adult	Bleaching	[122]
		PSII inhibition	[122]
	Gametes	Fertilisation	[122]
2-stroke oil (mineral)	Adult	Bleaching	[122]
		PSII inhibition	[[122]
	Gametes	Fertilisation	[122]
Product formation water (PFW)[3]	Larvae	Metamorphosis	[42]
	Gametes	Fertilisation	[42]
	Symbionts	PSII inhibition	[123]
Drilling mud[3]	Adult	Mortality	[124]

1. For methodology and exposure durations please refer to text or original publications

2. For quantitative toxicological data please refer to text or original publications

3. PFW and drilling mud often contain toxic concentrations of both metals and hydrocarbons

The potential of oil contamination to coral reefs is high given their often intense commercial activity and proximity to shipping lanes [1]. Acute exposure of reef ecosystems to oil spills can occur through accidental discharge from ships or as a result of terrestrial runoff. Production platforms in the vicinity of coral reefs have the potential to contaminate surrounding waters through discharge of production formation water (PFW) [42], a complex mixture that may contain petroleum hydrocarbons, suspended solids, metals, naturally occurring radioactive materials, organic acids and inorganic ions amongst other substances [110]. Likewise, the drilling of oil-wells may introduce mud heavily contaminated with petroleum hydrocarbons and metals to the marine system, while refineries and pipelines are additional sources with potential for chronic pollution by oil products. Floating oil can be deposited on reef flats or to interfere with reproductive processes in buoyant gametes or larvae [111]. Large oil spills are often moderated by application of surface dispersants that dissolve slicks into smaller droplets. However, application of

dispersants is likely to increase hydrocarbon concentrations in the water column and thus increase exposure to benthic reef organisms [42, 112]. Several studies have demonstrated that a combination of dispersal and oil products expressed higher toxicity than either component separately [42, 113, 114], yet it has been suggested modern dispersants may be less toxic to marine biota [1]. Once in the water column petroleum hydrocarbons rapidly become associated with organic matter and suspended particles. Volatile components evaporate while non-volatile components are deposited into the sediment. This deposited fraction is unlikely to absorb, evaporate, dissolve or be biologically degraded [53, 108]. Oil products may also occur in globulised form dispersed through the water column and can settle onto the reef [115]. Aromatic hydrocarbons, either in the form of dispersed oil or as water soluble components, can be absorbed by coral tissues while oil globules can adhere to coral surfaces [116]. The high lipophilicity of aromatic hydrocarbons stimulates rapid passive uptake in the coral tissue, while detoxification can be slow [114, 117]. Residues of aromatic hydrocarbons have been reported to remain present in coral tissues months after exposure occurred [118], yet evidence suggests hydrocarbon deposits in sediments and coral tissues to be substantially reduced after two years at high-energy reef sites [76, 119].

Impact

Adult coral colonies can be killed or injured by direct contact with oil or drilling mud [116, 124, 125]. Filter feeders and benthic organisms that cannot escape the oil or contaminated sediments will typically see bioaccumulation of toxic compounds, genetic mutations and metabolic disorder in their tissues. Corals exposed to hydrocarbons have been shown to exhibit loss of zooxanthellae (bleaching), impaired reproduction and tissue damage [116]. Field studies on mud discharges during oil well drilling found decreased coral growth rates in *Montastraea annularis* exposed to the fluids, while several years of exposure found 70 to 90% reduction in coral cover within one hundred meters from the drilling site [126]. These findings were supported by laboratory studies predicting decreased growth, metabolic aberrations and nutritional abnormalities [127, 128]. More recently, Raimondi and colleagues [124] observed tissue mortality in adult cup corals after exposure to drilling muds. Bak [129] observed decreased coral cover, diversity and local recruitment after chronic exposure to refinery petroleum on a Caribbean reef and argued chronic pollution to have more severe effects than single spills, yet comparable detrimental effects were observed after an acute major oil spill in Panama [130]. Guzmán and co-workers [130] also observed a strong correlation between injured corals and oil residues in sediment during five years of monitoring coral health after this spill. Aromatic hydrocarbons have been shown to decrease photosynthetic performance of dinoflagellate symbionts in some corals [113, 131]. Jones and Heyward [123] observed decreased photochemical efficiency and subsequent expulsion of zooxanthellae by host corals exposed to PFW as a result of photoinhibition in the symbionts. Freshly isolated symbionts were affected at slightly lower concentrations [123]. Tissue retraction as an environmental stress response has been observed after exposure to low concentrations of oil or dispersed oil, but normal tentacular expansion recovered within a week [114]. It has also be hypothesized that aromatic hydrocarbons may cause a reduction in coral tissue lipid contents, thereby limiting fat reserves necessary for increased mucus production or proliferation of mucus secretory cells [116, 118, 121].

Decreased reproductive success of both brooding and broadcasting corals after oil exposure has been observed in a number of studies. Two histological studies showed impaired gonadal development [121, 132], while in field studies infertility and decreased egg size as a result of increased injury occurrence were observed five years after exposure to a major oil spill [132]. Loya and Rinkevich [111] noted oil products could induce premature expulsion of larvae in a brooding coral. More recently, Negri and Heyward [42] suggested that early life stages may be more sensitive to pollution than established adult colonies. In annual mass spawning events, as experienced in the Indo-West Pacific or Australian waters, gametes of broadcast spawning coral species are released in the water during brief synchronised events. The highly buoyant eggs float to the surface where fertilisation occurs [133]. If the spawning event coincides with an oil spill, an entire year of reproductive effort is threatened while adverse environmental conditions endure with implications for larval settlement and parent colonies. A number of laboratory studies have reported a dose-dependent inhibition of fertilisation, metamorphosis and settlement of broadcast spawning scleractinian corals after exposures to oil and/or dispersant [42, 120, 134]. For example, crude oil inhibited larval metamorphosis at 82 μg/L and this was reduced to 33 μg/L in the presence of a non-ionic dispersant [42]. Despite these findings, effects of oil (whether or not dispersed) on gamete fertilization, embryogenesis, larval metamorphosis and settlement are unknown for the majority of both broadcast spawning and brooding species.

Marine pollution from PAHs and oil hydrocarbons is associated with localized events as runoff from urban centres, oil exploration and extraction activities and accidental spills. These chemicals are highly hydrophobic and therefore

contamination typically remains relatively confined, although spillage from offshore drilling operations can travel for great distances. Likely effects on coral reefs will consist of overall disturbance of biological homeostasis by exerting effects over multiple trophic levels. The greatest impacts on coral reefs are likely to occur if hydrocarbons come into direct contact with coral spawn during mass reproduction or at low tide on shallow reefs. Interactions with other environmental stressors are improbable, as effects from spills and shipping incidents often cause severe mortality and will overwhelm subtle adverse effects from other factors.

Trace Metals and Metalloids

Metals are a physical component of rocks and soils and enter the environment through natural weathering and erosion processes. Many metals are biologically essential, yet most have the potential to become toxic above certain threshold concentrations [135]. Industrial activities such as mining and smelting as well as agricultural applications (*i.e.* organometallic pesticides and fertilisers) and urban waste have substantially contributed to the release of elevated quantities of trace metals into the environment [53]. Even though recognition of toxic potential and legislation in the past have seen a great reduction in metal output, environmental contamination continues. Terrestrial runoff and sediment-bound transport through freshwater streams and rivers eventually delivers these contaminants to estuaries and inshore seas (Fig. **1**). Metals are strongly associated with particulate matter and therefore not usually directly available to aquatic biota. However, particulate metals in sediments can be solubilised by acidic juices in the gut of sediment-feeding organisms, and thus become available for accumulation in biotic matrices through passive uptake across permeable surfaces such as gills or the digestive tract [136, 137]. Biological availability and solubilisation rates of trace metals from particulate matter are dependent upon a variety of environmental variables, including sediment cation exchange capacity and organic content, dissolved oxygen concentrations, pH, salinity, temperature and redox potential amongst other factors [53, 137, 138]. Furthermore, remobilisation and resuspension of sediments may return metals to the water column [28]. High environmental metal concentrations are generally restricted to locations adjacent urban centres, industrialised areas or sites draining areas of intensive agriculture [139]. Trace metals and metalloids have a multitude of applications and sources, yet the most abundant metals entering the environment in elevated quantities as a result of agricultural activities are copper (Cu) and zinc (Zn), used as constituents of fertilisers or biocides; arsenic (As), cadmium (Cd) and mercury (Hg) as components of some fungicides. Lead (Pb), nickel (Ni), aluminium (Al), manganese (Mn) and iron (Fe) often enter marine waters as the results of mining activities (as do As and Hg), industrial or urban waste discharges and runoff. Tin (Sn) has generally been introduced into the environment as a biocide, principally as constituent of antifouling paint formulations, e.g. tributyltin (TBT) [139, 140].

Numerous studies exist on metal contamination and effect on corals [141-144]. In adult corals, metals might be absorbed and occur in various capacities. As early as 1971, Livingstone and Thompson [145] found trace metals to be incorporated into the aragonite (a carbonate mineral) of coral skeletons. Quantification of these built-in skeletal metals is currently used as a biomarker that reflects environmental conditions during the coral's lifetime [146-148]. Trace metals can also be found in skeletal cavities [149], integrated within the organic matrix of coral skeletons [150], or absorbed onto exposed surfaces of the skeleton [151]. Besides skeletal inclusion, several studies have demonstrated trace metals present in coral tissue [33, 152, 153]. Pathways through which corals absorb metals may vary. Brown and colleagues [151] found that corals retract their tissue in response to environmental stress, and thus may be more susceptible to direct uptake of metals by exposed skeletal spines. Another responsive action to physical or chemical stress is the excretion of high quantities of mucus with a high affinity to bind metals that may actively reduce metal uptake [127]. Additionally, it has been suggested corals are able to regulate internal metal concentrations through the physiology of their zooxanthellae endosymbionts. Studies with related symbiotic organisms like sea anemones indicated zooxanthellae to be responsible for the majority of metal uptake and accumulation [154]. In response to elevated metal concentrations, zooxanthellae can enhance calcification rates. Furthermore, zooxanthellae may be involved in the active uptake of trace metals, accumulating higher concentrations of metals than do host coral tissues [58, 143, 145, 155]. Subsequent stress-induced expulsion of symbionts by the coral host may act as a regulatory response mechanism in reaction to high metal concentrations [32].

Impact

Bioavailability, physiological effects and fate of trace metals are highly dependent on the chemical form and oxidation state in which metals exist, as reflected by their toxicity [164]. Thus, it is of clear importance to distinguish between

individual metal species present in a particular biological compartment [165]. Once introduced in a biotic matrix, trace metals have the potential to affect nutrient cycling, cell growth and regeneration, as well as reproductive cycles and photosynthetic potential [1, 53]. Table 5 summarises a range of effects on corals caused by exposures to metals and organometallic compounds. Elevated levels of copper, zinc and tin in the effluent of a tin smelter in Thailand caused reduced growth and calcification rates in branching corals [33]. In a study considering corals in a Hong Kong estuary exposed to elevated concentrations of metals, pesticides, nutrients, sewage effluents and suspended sediments over a prolonged period of time, it was argued metals were mainly responsible for declines in coral cover, diversity, abundance and growth rates [30]. Laboratory exposure of the massive coral Porites lutea to elevated iron concentrations resulted in bleaching. It was noted that corals that had been pre-exposed to an iron-enriched environment responded in a less drastic way, suggesting development of some form of iron tolerance [58]. Jones [32, 94] found elevated copper concentrations to induce rapid bleaching in the branching corals Acropora formosa and Seriatopora hystrix, while no inhibition of photosynthetic efficiency of zooxanthellate endosymbionts was observed. The author suggested copper-induced bleaching to occur without affecting the algal photosynthesis but may be related to effects on the host coral. However, in a longer term exposure experiment on Plesiastrea versipora, low concentrations of copper were observed to reduce symbiont response to a host signalling factor regulating photosynthesis, while stress responses as inhibition of photosynthetic efficiency or bleaching were not detected [166]. To further emphasise the toxic effects of copper, two separate studies showed how copper has a detrimental effect on the metabolism of both the branching coral Pocillopora damicornis and the massive coral Porites lutea [35, 167].

Table 5: Toxicological studies on the effects of selected trace metals and metalloids on different life history stages of scleractinian corals

Contaminant	Life stage	Toxicological endpoint[1]	Toxicological data (References)[2]
Trace metals			
Copper	Adult	Mortality	[32, 156]
		Bleaching	[32, 157, 158]
		PSII inhibition	[86]
	Larvae	Mortality	[159]
		Metamorphosis	[44, 160]
	Gametes	Mortality	[144]
		Fertilisation	[40, 144, 160, 161]
Zinc	Gametes	Fertilisation	[40, 161]
Cadmium	Gametes	Fertilisation	[40]
Nickel	Larvae	Mortality	[162]
		Settlement	[162]
Iron	Adult	Bleaching	[58]
Organometallic substances			
TBT	Adult	Mortality	[156, 163]
		Bleaching	[163]
		PSII inhibition	[163]
	Juvenile	Mortality	[163]
	Larvae	Metamorphosis	[43, 160, 163]
		Settlement	[43]
MEMC	Adult	Symbiont density	[13]
		PSII inhibition	[13]
	Larvae	Metamorphosis	[13]
	Gametes	Fertilisation	[13]

1. For methodology and exposure durations please refer to text or original publications

2. For quantitative toxicological data please refer to text or original publications

In a recent study on the effects of an organometallic fungicide containing mercury on different life history stages in *Acropora millepora*, 2-methoxyethylmercuric chloride (MEMC) severely affected adult branches exposed to 10 µg/L MEMC. Branches bleached and some host tissue died at this concentration, while at a lower concentration (1 µg/L) polyps retracted and photosynthetic efficiency decreased [13]. Early life history stages proved very sensitive to MEMC exposure. Lowest observed effect concentrations (LOECs) inhibiting fertilisation of gametes and larval metamorphosis were established at 1 µg/L MEMC (EC50 values of 1.7 µg/L and 2.5 µg/L, respectively) [13]. In a series of experiments assessing effect of trace metals on fertilisation and settlement success of selected coral species, copper was found to be a highly effective inhibitor of fertilisation in all species tested, with fertilisation rates dropping proportionally with increasing copper exposure concentrations (EC50 = 15-40 µg/L Cu). Zinc, lead and cadmium were much less potent. However, high interspecific variety in sensitivity was observed [40, 41]. In contrast to the high sensitivity of hard-coral gametes to copper exposure, gametes of the soft coral *Lobophytum compactum* exhibited a surprising resistance to copper toxicity (EC50 = 261 µg/L) [168]. Settlement of *Acropora tenuis* larvae was significantly reduced at concentrations of 42 µg/L Cu (48-h EC50 = 35 µg/L) [44]. Negri and Heyward [160] found fertilisation of gametes and larval metamorphosis of *Acropora millepora* reduced when exposed to low concentrations of copper and TBT. Copper proved the most potent inhibitor of fertilisation in this study (4-h EC50 = 17.4 µg/L), while TBT proved more toxic towards larval metamorphosis (24-h EC50 = 2 µg/L). The same study also showed that surfaces coated with antifouling paints containing copper or TBT to inhibit both fertilisation and metamorphosis [160]. These findings were confirmed by Victor and Richmond [144] who exposed *Acropora* gametes in Guam to low copper concentrations and calculated 50% inhibition of gamete fertilisation after 12-h exposure to 11.4 µg/L Cu. TBT is known to inhibit protein synthesis [169] and some cnidarians such as the sea anemone *Aiptasia palliida* have been shown to exhibit reduced resistance to infection and decreased zooxanthellae densities after chronic exposure to very low concentrations (0.05 µg/L) of TBT [170]. In another study involving antifouling paint contamination, sediment polluted with a mixture of TBT, copper and zinc, all components of commercial antifouling formulations used until recently and acquired at a ship grounding site in Australia, demonstrated potential to interfere with normal larval behaviour [43]. Modern antifouling formulations that contain organometallic components used as replacements for TBT are often not exempt from environmental impact; e.g. the biocide zinc-pyrithione (Zpt) is detrimental to embryonic development of both sea urchins and mussels [171]; however, no toxicological studies of this compound have been performed on corals. In a review by Reichelt-Brushett and McOrist [143] on trace metal contamination within corals from around the world it was made evident that, although high interspecific, regional and temporal variance was observed, environmental concentrations of selected metals (especially copper) were often near or exceeding concentrations proven to exert detrimental ecological effects on scleractinian corals.

Trace metal pollution is often limited to areas adjacent to urban and industrial centres or near river deltas. As metals are relatively immobile in the marine environment, adverse effects are likely to be exerted on a localised scale. Copper and organometallic substances containing tin or mercury are significantly more potent than other trace metals and affect a broad range of variables in a variety of species. Consequences for the system will consist of both bottom-up and top-down effects. Chronic, low-level metal contamination may decrease resilience of marine organisms to other environmental stressors such as elevated temperatures, ocean acidification and other chemical pollutants.

Interactions of Multiple Stressors

Nearshore marine pollution often occurs in combination with other natural and anthropogenic sources of stress for resident biota. A globally changing climate results in increasing sea surface temperatures and ocean acidification, arguably the most important factors when considering stress on coral reefs [3, 19, 35]. Moreover, in the tropics monsoonal rainfall delivers vast amounts of fresh water, nutrients and suspended sediments to estuaries and inshore reef systems [172]. At this time of the year seawater temperatures approach thermal tolerance limits for many coral species [6, 60]. Thus, during flooding events, inshore coral reefs can potentially face combinations of low salinity, high turbidity, nutrient and pesticide exposures during episodes of thermal stress. It is assumed that nearshore corals and other sensitive organisms may be at extreme risk to combinations of stressors but little research has been performed to test this hypothesis.

Of the studies performed on stressor interactions, most have dealt with temperature in combination with an additional stress factor. Temperature affects physicochemical properties of membranes, including permeability, fluidity and diffusion rates. This will have a likely effect on a chemical's toxicity that is dependent on target site delivery [38]. Simultaneously, temperature can affect toxin solubility, speciation and (bio)degradation rates but also an organism's sensitivity to a

particular chemical [164]. The PSII herbicide diuron was observed to take longer at 20 °C than at 30 °C to reach a similar response in *Seriatopora hystrix*, while a decreasing sensitivity to the herbicide was observed at higher temperatures [38]. Another good illustrative example has recently been provided in a study where high dissolved inorganic nitrogen (DIN) concentrations were correlated with a decreased resilience of corals to high temperature [173]. Wooldridge and Done [174] subsequently proposed how combinations of high temperature and high DIN concentrations work synergistically and may be a causative mechanism for large-scale coral bleaching. However, the multiple sources of stress (often simultaneous events), coupled with the great complexity of marine ecosystems and their high variability obscures the establishment of simple causal relationships between stressors and observed effects, which greatly complicates assessment of tolerance, resilience and ecological implications of stress [175].

SYNTHESIS

Tropical coral reefs are among the most biologically diverse and productive ecosystems in the world but their continued existence is threatened by a number of factors. Despite their extended geological subsistence, coral reefs appear very sensitive to changes in environmental conditions. Elevated ocean acidity and surface temperature, high turbidity and nutrient concentrations through terrestrial runoff and chemical pollution from various sources are the main pressures exerted on modern reef systems and among the proposed reasons for global coral reef declines [16, 19]. Community alterations or a shift from autotrophy to heterotrophy will eventually affect the entire reef community, and could possibly change the dominant ecological process from calcium carbonate deposition to erosion [176]. Pressures on coral reef ecosystems are likely to increase further as a result of expanding coastal agricultural practises and industrialisation, population growth and climate change. While limiting the effects of climate change is a global challenge, management approaches to minimise the effects of pollution pressures on nearshore coral reefs can contribute towards sustainable exploitation of our marine resources; curbed inflow of suspended sediments, nutrients and chemical stressors are potential means to protect our reef systems in a shifting environment [12, 177, 178]. We have shown substantial differences exist in sensitivity and response of scleractinian corals and their zooxanthellae symbionts to various types of chemical pollutants. Furthermore, susceptibility may differ considerably depending on its life history stage. Evaluation of environmental stress on coral reefs caused by chemical pollution is constrained by the limited number of assessment endpoints, implying further research is required on a broad range of species and life history stages.

Chemical pollutants can enter and affect a reef ecosystem in a number of ways. The type of exposure often determines the severity and scale of effects. Coral reef ecosystems may be chronically exposed to combinations of stressors at low concentrations, regular pulses of chemical stressors and/or be subject to acute exposures of specific chemicals at relatively high concentrations during an accidental pollution event (Table **6**). While accidental pollution events (e.g. oil spills) will most likely overshadow effects from other potential stressors and have profound effects at all trophic levels, they are often localised, not harming the overall structure and function of large ecosystems. In contrast, regularly recurring pollution events such as input from river floods or chronic pollution from land runoff (e.g. sewage treatment effluent or herbicides) is more likely to affect a larger area and exert subtle effects, driving the system towards genetic and ecological adaptability. In these scenarios, the combined effects of chemical mixtures may significantly increase total toxicity and associated risk.

Low-level chronic pollution and recurring pollution events may interfere with the resilience of lower trophic levels instead of directly impacting the ecosystem. Because adverse conditions persist for prolonged periods of time, fitness of species is affected in the long run and this may lead to an increased vulnerability to various other stressors, including those related to climate change.

Ecological risk assessment requires knowledge of the spatial and temporal distributions of key stressors in relation to the most vulnerable tropical species. Exposure is a function of the intensity (magnitude), timing, frequency and duration of adverse conditions. Thus, ecological exposure scenarios may become very complex, especially when multiple stressors are involved and exert pressures on interconnected biological compartments. With all relevant information taken into consideration, it is not likely that the chemicals discussed in this chapter will significantly impact reefs single-handedly as concentrations are generally low or localised. For example, a number of chemical pollutants have been identified at low concentrations on the GBR, including the herbicide diuron and the insecticide chlorpyrifos (both primarily used in coastal agriculture), TBT and copper (antifoulants) and PAHs from oil spills and boating/shipping activities. The toxic effect concentrations of these pollutants and their observed annual mean

(background) and peak event (e.g. oil spill, ship grounding, flood plume) concentrations can be combined as risk quotients (effect concentration divided by environmental concentration), providing semi-quantitative estimates of risk and safety margins for GBR species (Table **7**).

Table 6: Profiles and likely effect for three types of pollution scenarios on coral reefs.

	Scenario I: Chronic pollution	**Scenario II: Recurring pollution events**	**Scenario III: Accidental pollution events**
Description	Non-pulse	Regular pulse	Random pulse
Input pathways	Terrestrial runoff Atmospheric deposition	Flooding events	Accidents
Area of concern	Large	Medium-Large	Localised
Timeframe	Chronic long-term disturbance	Moderate transient disturbance	Major short-term disturbance
Recovery phase	No	Limited	Yes
Stressor interactions	Very important	Very important	Less important as event overshadows environmental factors (although recovery may be affected)
Likely effects	Subtle and broad effects driving adaptation Bottom-up consequences on a large scale	Localised mortality Subtle and broad effects driving adaptation Bottom-up consequences on a large scale	Localised mortality Whole foodchain affected, limited large scale effects
Pollutant type	Agrochemicals Metals Antifouling agents Pharmaceuticals	Agrochemicals Metals	Oil hydrocarbons PAHs Antifouling agents

Table 7: Concentrations of environmentally relevant chemical pollutants on the Great Barrier Reef (GBR) linked to effect

Chemical (most sensitive endpoint)	**LOEC (µg/L)**	**Background inshore GBR (µg/L)**	**Event inshore GBR (µg/L)**	**Risk quotient LOEC/ background[1]**	**Risk quotient LOEC/ event[1]**	**Exposure scenario[2]**
diuron (PSII inhibition)	0.3-1	0.001-0.020	0.1-1	>15	>0.3	I - II
chlorpyrifos (metamorphosis)	0.4	0.0004	0.0007	>1000	>570	I - II
TBT (settlement)	0.4-0.9		<3.6 µg/g (sediment)[3]			I - III
copper (fertilisation)	10-100	0.2[4]		>50		I - II
PAHs (fertilisation)	2-30		0.1-0.65		>3	I - III
Total oil hydrocarbons (fertilisation)	2-30		10-2000		>0.001	III

LOEC=Lowest observed effect concentration.

[1] Higher numbers indicate a greater safety margin.

[2] Exposure scenario is indicative of which scenario is more likely for a given type of chemical to be relevant in terms of risk: (I) chronic exposure, (II) recurring event-style exposure, (III) random event-style exposure (Table **6**)

[3] Concentrations of TBT are usually specified in µg/g sediment; to our knowledge no information is available for water concentrations found in Australia.

[4] Concentrations found offshore in northern Australia[179].

Few chronic pollutants on the GBR approach concentrations that may cause harmful effects to corals as indicated by high risk quotients (>10 for background concentrations) for diuron, chlorpyrifos and copper. Only during short term events, such as in river plumes, does the concentration of diuron become great enough to affect corals (risk quotient = 0.3). However, risks posed by mixtures of pollutants may well exceed those presented by individual chemicals.

Despite the relatively low risk associated with chemical pollution on the GBR, evidence is emerging that pollution reduces the resilience of corals and other organisms to global climate change [174] and more research is required to document the increased sensitivity of corals to pollutants at elevated temperatures or under more acidic conditions. Even though the GBR is one of the most highly monitored regions in the tropics, risk quotients can only be calculated for a small number of pollutant types as indicated by data gaps in Table **7**. This general lack of data is more extreme in other tropical regions where other pollutant types may be more relevant and stronger links between regional water quality monitoring and relevant toxicological testing is required. In spite of our growing understanding of the effects of chemical stressors on reef species, there are still gaps in our knowledge which complicate assessment of ecological significance. To characterise a risk to an ecosystem, all relevant information concerning exposure and effect needs to be evaluated, including concentrations and distribution patterns of chemicals. Extremely limited information is available for concentrations of chemical pollutants on coral reefs worldwide. Obtaining these data is difficult as the majority of tropical coral reefs are situated in developing areas and most available data originates from more developed areas such as Florida or the GBR, where usage patterns of pesticides and discharge of industrial and urban waste is likely to differ from pollution patterns in South-east Asia, the Pacific islands or Africa.

Research in the last two decades has identified that coral reefs are threatened by a variety of stressors including elevated ocean temperatures, ocean acidification, overfishing, nutrient input and turbidity and that these pressures vary considerably between regions. While overfishing and nutrification are currently considered as the most detrimental local stressors to coral reefs, current knowledge on the distribution and effects of chemical pollutants is too limited to assess how these compare with other stressors. While laboratory studies have identified toxic thresholds of corals and other tropical organisms to a widening range of pollutants, further monitoring and research needs to be undertaken in several key areas:

- Stronger efforts need to be made linking pollution monitoring and ecotoxicological studies to better assess risk.
- More studies are required on the subtle effects of chronic pollution (Scenario I) and how low levels of pollution may inhibit the recovery of coral reefs from disturbance.
- Further experimental work needs to be undertaken to better understand the potential interactive effects between combinations of pollutants in flood plumes during tropical monsoons.
- Identification of which pollutants in a region may be reducing the resilience of corals and other organisms to climate change is essential to effectively target and develop management policies that may improve the survival prospects of our tropical coral reefs.

ACKNOWLEDGEMENTS

This work was supported by the Australian Government's Marine and Tropical Sciences Research Facility, implemented in North Queensland by the Reef and Rainforest Research Centre Ltd. JvD received financial support from the University of Queensland. Entox is a partnership between Queensland Health and The University of Queensland.

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CHAPTER 10

Impact of Contaminants on Pelagic Ecosystems

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Abstract: Most of the primary production of the world's oceans takes place in the water column, thereby fuelling not only marine pelagic food-webs, but also most benthic communities. In addition, nearly all marine organisms depend on the pelagic zone for some part of their life-cycle. Although most contaminants have physico-chemical properties that cause them to associate with organic material particles and eventually be transported to sediments, direct contaminant inputs are predominantly to pelagic ecosystems. Taking both the ecological importance and the contaminant load into account, there is a surprising lack of scientific knowledge concerning the effects of contaminants in pelagic systems. The main reasons are presumably the difficulty in linking exposure with processes at a scale relevant for environmental management, and challenges involved in using pelagic fish and zooplankton species for experimental studies (excluding the 2-3 copepod species used for regulatory toxicity testing). Contaminants have been shown to affect primary producers as well as secondary producers-consumers, but there is very limited knowledge about ecological impacts. Top predators in marine ecosystems (piscivorous fish species, marine mammals, seabirds) will be particularly at risk from persistent organic contaminants since they will biomagnify. Although there is evidence of effects caused by such substances in the past, there is a need for continuous updates including "new" contaminants. Most relevant for lower trophic levels, micro- and mesocosm studies under controlled conditions are critical for increased understanding of processes and putative effects of contaminants in the pelagic zone. Some field-based strategies have been suggested and implemented to varying degrees for environmental management of contaminants in the water column, including risk-based modelling, bioassay-analyses of environmental samples or extracts (e.g., through the use of passive samplers), caging of organisms and, finally, collection and analyses of native organisms.

INTRODUCTION

The pelagic zone of the oceans constitutes the single largest ecosystem of the world and contains the organisms that form the basis for most marine food chains and all fisheries resources. The characteristics of the marine pelagic ecosystem have been extensively reviewed [1]. Verity *et al.* [1] clearly indicate that the various forms of anthropogenic impacts on the seas, may result in, *i.e.* overexploitation, habitat changes, extinctions, increased disease, species replacements, and how an integrated understanding of resource availability and predation pressure is required for effective environmental management. As will become apparent later in this chapter, increased concentrations of contaminants may affect both bottom-up and top-down processes. Although causing less obvious effects than, for example, overfishing or habitat modification, contaminants are nevertheless important for our understanding and proper management of human interactions with marine pelagic ecosystems.

There are of course spatial and temporal variation of physical and chemical parameters in the pelagic zone, both vertically and horizontally, but it is comparatively stable compared to habitats in most terrestrial or freshwater ecosystems (e.g., Kaiser *et al.* [2]). However, in terms of productivity there are large differences between areas. Whereas coastal areas and shallow seas are among the most productive per area of any ecosystem on the planet, oceanic areas generally have low biomass and productivity [2]. Sunlight-driven primary production needs to take place in the upper reaches of the oceans, sometimes limited to the upper ten or twenty meters. The part of the pelagic zone with the highest primary production will in most cases also be the area that receives contaminant inputs and will have the highest concentrations of such substances. Although there is an extensive literature on oceanographic trace metals, including non-essential metals such as mercury, cadmium and lead, and their behaviour in relation to hydrographic processes and nutrients [3], there is limited data for organic contaminants (see [4]). Organic contaminants are generally thought to be associated with dissolved or particulate organic material, to some extent inorganic particles, and will thus be gradually removed from the water column through sedimentation. Contaminant exposure to pelagic organisms will therefore be from low concentrations in water, through ingestion of particles with

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somewhat higher concentrations, through uptake of organic material with associated contaminants or through trophic transfer (which would lead all the way from bacteria and protists to marine mammals, seabirds and humans). Although it should theoretically be simple to quantify the relative distribution and bioavailability of a given substance in pelagic waters by knowledge of its lipid-solubility (and hence affinity for organic material), complex biotic and abiotic processes results in concentrations of contaminants in water, particles or organisms that are difficult to predict (e.g., Ruus *et al.* [5], Vethaak *et al.* [6]). The available data support some general observations; for example, bioaccumulation and possible biomagnification of polycyclic aromatic hydrocarbons in invertebrate food chains, but not in vertebrates [7, 8, 9] (but see Berrojalbiz *et al.* [10]), trophic transfer of persistent organic contaminants [11, 12, 13, 14] and mercury [15, 16], and finally, more or less species- and exposure-dependent accumulation of other trace metals [17, 18].

Major sources of contaminant inputs to the pelagic zone are atmospheric deposition, riverine inputs, shipping activities, land run-off and point discharges. A small proportion of marine contaminants will be directly deposited on the seafloor through activities such as dredging or drilling operations. Sediment-associated contaminants may eventually be a source of input to the pelagic zone through diffusion, resuspension or trophic transfer, but there is limited knowledge about links between contaminants in benthic or demersal species and their predators in the pelagic zone. An emerging problem is the presence of plastic debris and associated contaminants. Contaminants can interact with both floating microplastics and plankton, and thus potentially enter food chains that may ultimately affect humans [19]. Preliminary data show that chemicals in plastic microparticles (<1 mm) are being taken up by marine organisms, including mussels [20]. There is as yet limited knowledge of any effects.

A distinction needs to be made between coastal and oceanic areas. Coastal areas are for natural reasons the waters of the world's oceans with the highest inputs and levels of contaminants, but at the same time areas with a high variability in environmental factors such as particle load, primary production, salinity and temperature. About 30% of oceanic primary production occurs in shelf and coastal environments, constituting less than 10% of the total area of the ocean [21]. The factors discussed above will affect the behaviour of contaminants and how they may impact marine ecosystems [22]. Oceanic areas are less variable than coastal areas and sources of contaminants are limited to atmospheric deposition, offshore oil and gas activities, shipping discharges and, to a lesser extent, the presence of plastic debris.

Over the last decade there has been an increasing number of studies reporting the concentrations of contaminants in surface and microlayer water [23, 24], associated with plastic resin pellets [25], passive samplers [26, 27], particulate material [24, 28] and caged or pelagic organisms [5, 24, 29]. As will be discussed in greater detail below, there are obvious problems in trying to assess the effective concentration of contaminants in water-masses, both due to the variable solubility, speciation, association with particles and bioavailability of contaminants and because water-masses move and mix.

There is even less data for contaminant-related effects in pelagic ecosystems. Nearly all marine model organisms for laboratory- or field-based studies on contaminant effects are benthic species, including blue mussel (Mytilus edulis; [30, 31]), dab (Limanda limanda; [32, 33]), eelpout (Zoarces viviparus; [34]) and flounder (Platichthys flesus; [35, 36], [37]). There are however, some studies that have targeted pelagic species or used caged species. The BECPELAG (Biological Effects of Contaminants in Marine Pelagic Ecosystems; [23]) workshop investigated effects and levels of contaminants in pelagic systems through field-collected organisms [38], caged organisms [39] and bioassays of water and passive sampler extracts [40]. Organisms studied ranged from invertebrates to fish. The results from the workshop clearly showed that levels and effects of contaminants in field-collected organisms were less clear than in organisms caged in the same area. Other studies have focused on species at the top of food chains such as swordfish, for which there are indications of relationships between contaminant levels and sublethal endocrine disrupting effects [41].

The aims of this chapter are to review the current understanding of how contaminants affect pelagic ecosystems, outline approaches and to suggest research directions.

CHALLENGES

There are reasons why benthic organisms and systems have been preferred to pelagic systems in contaminant research. As hinted to above, ecological importance is certainly not the reason and many pelagic fish species are as

economically important as benthic species. One reason for the preference of benthic species for research in general is accessibility – intertidal or shoreline species require less infrastructure for their collection and study than organisms in the water column. Secondly, benthic species are generally more amenable to being kept in the laboratory and there is hence much more general knowledge about their biology. Thirdly, and possibly most important, concentrations of contaminants are orders of magnitude higher in sediment than in the water column, at least in theory resulting in higher exposure levels for sediment-dwelling than for pelagic organisms. However, exposure levels in the two habitats will vary considerably for different groups of contaminants. Pelagic organisms will generally be exposed to higher levels of the more easily degradable substances than their benthic counterparts. Finally, there is a difference between benthic and pelagic organisms in our knowledge of their exposure history (or at least perceived knowledge). Whereas many benthic species, for example blue mussel, are sedentary and stationary, pelagic species move continuously. Although contaminants may enter marine ecosystems through pelagic waters, there is a feeling that it is easier to quantify exposure for benthic than for pelagic species. In enclosed water bodies such as fjords or estuaries this may be true, but in the open sea it is not obviously a clearer relationship between contaminants in abiotic matrices such as sediment and epibenthic organisms than between concentrations in water and pelagic organisms. Even for benthic organisms there are not obvious quantitative relationships between contaminants in sediment and the tissues of sediment-dwelling organisms [42, 43], and sediment-related factors such as black carbon strongly affects bioavailability even of organic contaminants [8, 44].

One major challenge for understanding contaminant exposure and effects for pelagic organisms concerns their presence in and exposure to different water masses. For planktonic organisms this is not necessarily the case as they will remain associated with a water mass for periods of time, but nekton such as fish will clearly be exposed to different levels of contaminants as they move through more or less contaminated water masses.

A relevant question here is how contaminant exposure in marine ecosystems can be most precisely estimated. For species with low metabolising capacity, accumulated concentrations of many organic contaminants and non-essential metals will be a reasonable estimate for long-term exposure. Other species, and particularly vertebrates, will to a larger extent regulate their intake and accumulation of non-essential metals and metabolise and excrete a variable fraction of absorbed organic contaminants. Although some organic contaminants have half-lives in the range of years in most organisms [45, 46], most are metabolised at least to some extent and some, such as alkylphenols and polycyclic aromatic hydrocarbons, to the extent that tissue residue analyses are less useful than analyses of metabolites in bile or other excretory fluids [47, 48, 49]. As mentioned above, there is a complex relationship between contaminant concentrations in abiotic matrices (sediment, water) and concentrations in tissues, particularly for mobile species. Contaminant exposure may therefore be most accurately determined from tissue concentrations for persistent substances and metabolite levels for others. There are however some other alternatives and we will focus particularly on the pelagic organisms here. There is limited knowledge about the ecotoxicology of this group of organisms, but zooplankton does not appear to metabolise organic substances efficiently [50] (but see Magnusson *et al.* [51]), and they accumulate a range of metals [52] as well organic contaminants [11, 53] and would therefore be a useful matrix by which to estimate exposure in any given water-mass. Using zooplankton for this purpose would however need to be part of a carefully designed experiment to ensure spatial representivity, and vertical migration patterns would need to be taken into account. In the photic zone phytoplankton could be used for the same purpose, although any vertical movement would have to be considered for the species used. A second alternative is to use passive samplers: a range of different materials have been used, including membranes with a lipid inside [54], silicone sheets [26, 55], various plastics [56], coated membranes [57] or polyurethane foam [58]. Common to most passive samplers as they have been deployed until now is the need for a mooring system. Passive samplers are generally deployed for a period of three to six weeks prior to extraction and chemical analyses.

PRIMARY PRODUCERS

Phytoplankton forms the basis of marine food webs and embodies the carrying capacity of marine ecosystems. In the classical view, the main route for organic carbon was through zooplankton feeding on phytoplankton, but it is now well established that microzooplankton, bacteria and probably viruses play crucial roles in affecting the trophic dynamics and composition of plankton communities [59, 60]. Our knowledge of how and whether contaminants affect these organisms and interactions between them is limited.

The increase in primary production in coastal waters since the 1970s, at least to some extent due to increased nutrient inputs, has received much attention from the scientific community as well as from environmental managers. In many coastal systems, phytoplankton blooms are common events and a significant amount of this phytoplankton biomass will sediment through the water column, settle on the bottom and the nutrients be remineralised in surface sediments [61]. Increases in the occurrence of algal blooms have been linked to phenomena such as oxygen deficiency and mass kills of benthic fauna and fish as well as the formation of foam on beaches (produced by algae species such as Phaeocystis) and toxic shellfish.

To what extent will chemical stressors affect primary producers? Given the large amount of new, industrially produced substances, this is an important and relevant issue for the coming decades. Results from experimental studies indicate that certain chemicals may have a direct impact on plankton communities and food chains, and may thus potentially affect the carrying capacity of estuarine and coastal ecosystems. The most important compounds for causing toxic effects upon phytoplankton are pesticides and biocides, especially those with a herbicidal mode of action. The antifouling agent TBT has been shown to affect phytoplankton communities at concentrations that are present in coastal waters [62]. Effects include reductions in population development rate and shifts in species composition – *i.e.*, towards species that are more tolerant to TBT pollution. Worldwide measures to restrict TBT in antifouling paints (with a total ban by 2008) has lead to the development of alternative antifouling compounds such as zinc pyrithione (ZPT), copper pyrithione (CPT), Irgarol 1051 and diuron [63, 64, 65]. Residues of these novel antifouling agents are currently found worldwide, especially in estuarine and coastal waters near and in contaminated marinas. Irgarol 1051, like other triazine herbicides, is a strong inhibitor of photosystem II and reduces growth and productivity of sensitive phytoplankton species [66]. Some phytoplankton species appear to be more sensitive to Irgarol 1051 than others. For example, a 23-h exposure to Irgarol (112 ng/L) decreased the abundance of some eukaryotic species to less than half of the controls [67]. Zamora-Ley *et al.* [63] found in a marine harbour that Irgarol 1051 caused changes in several phytoplankton species with increasing herbicide concentrations.

Maraldo and Dahllöf [64] found that the acute toxicity of the antifouling agents ZPT and CPT among natural phytoplankton communities was similar to that of TBT [62], which in turn was higher than those reported for Zn and Cu alone [64]. The sensitivity towards ZPT and CPT was dependent on the phytoplankton community structure and the density of algae and suggested an enhanced effect of ZPT and CPT under phosphate-limiting conditions.

The effects of the herbicide atrazine on marine phytoplankton typical of the German Bight (North Sea) were demonstrated in mesocosm experiments [68]. The authors reported reduced photosynthesis accompanied by lower chlorophyll concentrations and reduced primary production. Other recent experimental work have demonstrated that the pharmaceutical clotrimazole can affect marine microalgal communities at picomolar concentrations, but the true potential for impact on marine primary producers has not been established [69].

The development of plankton communities in estuarine and coastal waters is governed by highly dynamic physical and chemical processes. This makes it hard to predict or establish the effect and ecological significance of chemical compounds on these communities. The potential impact of chemicals on phytoplankton and phytobenthos communities in coastal waters is known to depend on environmental factors such as salinity, temperature, nutrients, and exposure to UV-A and UV-B radiation and contaminants. Although contaminants may affect phytoplankton, any effects might be masked by other factors and interactions. To tackle this problem field studies complemented with mesocosm experiments should be conducted to improve control over factors and to improve the ecological relevance of the findings.

Another aspect of chemical stress on plankton and other organisms higher in the food chain are natural toxins produced by marine algae. As a consequence of changes in the coastal zone, the frequency and intensity of toxic algal blooms might increase, resulting in increased levels of natural toxins. The risk of toxic algal blooms can also increase as a result of unintended introductions of new invasive species, for example by ballast water releases. However, it remains difficult to quantify ecological impacts of such natural toxins because available toxicity data are limited. The relative contribution of anthropogenic chemical compounds and natural toxins on the total chemical pressure under field conditions is therefore unknown, and we lack insight into any interactions between these groups of chemicals.

SECONDARY PRODUCERS AND TERTIARY CONSUMERS

Secondary production includes the consumption of primary producers and biomass generated by heterotrophs. Tertiary consumers include predatory fish and fish-eating mammals and birds. Long-term changes of offshore

zooplankton appear to be mainly associated with climatic and hydrographic phenomena [70]. Any direct or indirect effects of contaminants on marine zooplankton are not well understood. Bioaccumulation of metals and organic contaminants in marine zooplankton including jellyfish has been reported, [71, 72]. An obvious challenge in this context is the identification and separation of different species in a sample. In a comprehensive study, Hoekstra and co-authors concluded that concentrations of organic contaminants in zooplankton predominantly reflected chemical partitioning and that there was limited biotransformation by the *Calanus* species investigated [71]. Although organochlorine contaminants do not appear to be metabolised extensively by zooplankton, there is some evidence that polycyclic aromatic hydrocarbons may be [10].

Toxicity information for zooplankton is limited, except for the few species used in toxicity testing (mainly *Acartia, Nitocra*, *Tisbe* and mysids, [73, 74, 75]), although there is some indication that, e.g. insecticides affect coastal zooplankton [76]. Toxic effects have been shown for TBT at concentrations present in coastal waters [77]. The observed effects included reduced population development rate and shifts in species composition.

A high potential for bioaccumulation of endocrine disrupting compounds (*i.e.*, organotins, flame retardants) and indications of endocrine disrupting effects have been demonstrated for the estuarine mysid *Neomysis integer* [72, 78]. This species plays a key role in the transfer of energy between phytoplankton and fish production in estuaries and along shallow coastal waters in northern Europe, and between benthic and pelagic food webs. Furthermore, some studies have investigated effects of contaminants on population-level effects in the ecologically very important copepod genus *Calanus* [79, 80]. A limited number of studies have evaluated the application of sublethal effect protocols and biomarkers, in phyto- and/or zooplankton species [78, 81]. However, there have been some recent studies using transcriptomic approaches for ecologically important *Calanus* species [82, 83, 84].

A number of studies indicate that eggs and larvae of pelagic and demersal fish that float in surface and subsurface layers may be particularly sensitive to diffuse contaminant exposure (including PAHs from oil pollution) and sublethal effects [85, 86, 87]. Unfortunately, the full impact of contaminants on critical life stages of fish and other nekton is still largely unknown.

Several studies have demonstrated effects of contaminants on sublethal responses in selected pelagic fish species. In studies with saithe (*Pollachius virens*) as part of the BECPELAG workshop, tissue-level effects were observed in fish collected close to a production platform in the North Sea [88]. A North Sea monitoring study using a predominantly demersal feeding species, haddock (*Melanogrammus aeglefinus*), reported a range of effects in this species linked to the presence of populations in or near areas with offshore activity [89]. There were substantially increased levels of DNA damage and changes in the lipid composition of membranes in haddock collected in areas with high offshore activity. The effects were corroborated by other biomarkers and showed a total picture of a population with increased DNA damage mainly due to PAH exposure (indicated through elevated PAH metabolite concentrations), but also increased oxidative stress resulting in changed lipid composition [89]). However, the ecological significance of the observed effects remains unresolved.

Fossi and co-workers [41] showed that large pelagic predators, bluefin tuna (*Thunnus thynnus*), swordfish (*Xiphias gladius*) and Mediterranean spearfish (*Tetrapturus belone*), contained increased levels of vitellogenin (VTG), a yolk precursor protein only expected to be present at appreciable quantities in female fish. Such levels are most likely caused by accumulation of endocrine-disrupting substances through their diet. Another study by De Metrio *et al.* [90] supported these findings and showed that close to a quarter of caught male Mediterranean swordfish (*Xiphias gladius*) displayed ovotestis (intersexuality), again possibly caused by endocrine-disrupting compounds (EDCs). Furthermore, elevated VTG levels were found in liver tissue. The causes of these phenomena are not yet known, but bioaccumulation of endocrinologically active substances is a possible explanation. The evidence of wide-spread EDC exposure in the marine environment is supported by studies of Scott and co-workers [91, 92], who observed offshore male cod (*Gadus morhua*) and male dab (*Limanda limanda*) with elevated levels of VTG.

Because of bioaccumulation and biomagnification processes in food webs, globally distributed persistent organic pollutants (POPs), including EDCs, may attain high concentrations, in pelagic top predators. Such substances may reach levels that result in effects on reproductive and/or immune systems. This has been well illustrated in field studies on Baltic grey and ringed seals, and semi-field studies with Wadden Sea harbour seals. Those studies have shown that

reproduction and immune functions can be impaired in top predators following biomagnification of PCBs in the food chain (see review by Vos *et al.* [93]). Reproduction effects have resulted in population declines and may also have contributed to the mass mortalities observed in some European seal populations due to virus infections.

Numerous other cases refer to mass mortalities by infectious diseases, poor reproductive performance, immuno-suppression, thyroid abnormalities and other non-reproductive disorders in marine mammals and fish-eating birds (for reviews, see Vos *et al.* [93] and Law *et al.* [94]). Such effects have to some extent been associated with the presence of POPs (e.g., organochlorine compounds, brominated flame retardants and metabolites) and other endocrine disrupting and/or immunotoxic compounds in the body fat [95]. Bennett *et al.* [96] found an association between chronic exposure to mercury and infectious disease in harbour porpoises. An increase in disease susceptibility in contaminant-exposed whale and dolphin populations has further fed speculation about a possible negative influence of contaminants on the immune system [97]. Accumulation of persistent and lipophilic contaminants, including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (coplanar PCBs), were found in several albatross species feeding in the open oceans, specially the North Pacific Ocean. Possible adverse effects of these compounds to these birds may be expected from toxic equivalent (TEQ) levels [98]. However, in most of these cases, it was not possible to confirm a cause-effect relationship between a specific chemical or group of chemicals and individual or population level effects. Studies over the last decade have shown high concentrations of a range of substances of concern in marine top predators, including TBT [99, 100], toxaphenes [101], polybrominated diphenyl ethers [101, 102, 103, 104], perfluorooctane sulfonates PFOS and perfluorooctanoic acid PFOA [105, 106]), nonyl- and octylphenol [107] and phthalate esters [108]. Single and combined impacts of food-chain accumulation of these contaminants and subsequent high concentrations in marine pelagic secondary producers and tertiary consumers has yet to be elucidated. In addition to the above, increasing levels of human pharmaceuticals, personal care products and aquaculture veterinary pharmaceuticals in coastal pelagic ecosystems is an area of concern with limited knowledge of any ecological impacts [109].

INTERACTIONS BETWEEN ENVIRONMENTAL FACTORS

Three of the most obvious pressures from human activity in marine waters are eutrophication, oil and contaminant inputs. For eutrophication there is extensive data on nutrient and bloom dynamics in coastal areas [110]. There are large amounts of data on the environmental physiology of many algal species. There is also a substantial body of knowledge on how oil and offshore-related discharges affect marine ecosystems, not least from monitoring following accidental spills from, for example, Exxon Valdez [111] or Prestige [112]. Aspects of the consequences of offshore-related effluents were evaluated recently through the BECPELAG workshop [23]. Finally, there is a large literature on the presence and effects of contaminants in coastal ecosystems even at low exposure levels [113]. Although there is limited evidence of large-scale effects of contaminants in marine ecosystems, possibly with the exception of Puget Sound, USA [114] and the North Sea and Baltic in the 1970-80s [86, 115], there is reason to believe that chronic exposure to low levels of contaminants will affect pelagic organisms.

Eutrophication, oil and contaminant inputs are co-occurring features of most estuaries and harbours in industrialised countries. Organic enrichment, the presence of oil, contaminants and variable oxygen availability would be expected to interact in their effects on marine biota, but there are surprisingly few studies on whether and to what extent this is the case (but see Gunnarsson *et al.* [22] and Herman *et al.* [116]). Natural waters contain both dissolved (DOM) and particulate organic material (POM), both of which may act as “sponges” to mop up organic and many inorganic contaminants in the water column. Increased levels of organic material could therefore be expected to modulate effects of contaminants through decreased bioavailability in water or increased sedimentation and “co-precipitation” of contaminants. For filter-feeding organisms in the water column, association of contaminants with particles may actually increase exposure as both food and water will contain contaminants. For predators this process would decrease water-borne exposure, but increase exposure through the food chain. Water-soluble components of oil would behave as other contaminants in this context, whereas dispersed oil would be expected to behave like DOM. It is not clear how algal, bacterial and protist interactions may be affected, although specific effects from contaminants on any one group would be expected to affect energy and nutrient flows in the network. Association of contaminants with particles will generally decrease residence time in the water column and thus shift exposure from pelagic to sediment ecosystems.

Despite existing knowledge about eutrophication effects in pelagic systems, there is a need for further knowledge about how natural systems behave under conditions of varying nutrient or carbon availability and there is limited understanding about how oil or contaminants may interact in such systems. Small-sized organisms could be thought to be at greater risk since they would be expected to accumulate higher concentrations of contaminants, but organisms that accumulate non-limiting substrates may also have a high uptake [117]. The question remains whether organisms that accumulate high concentrations of contaminants are most sensitive to the effects of the contaminants. In addition to ecological consequences of modulating the systems themselves, changes in both small and medium scale pelagic processes could strongly affect fluxes and effects of contaminants in coastal ecosystems through affecting sedimentation and transfer to higher trophic levels.

Combined effects between UV radiation and contaminants on plankton community structure in coastal zones have been observed in several recent studies. Major coastal and marine contaminants that still often exceed environmental risk limits in estuarine and coastal waters, such as TBT, PAH, Irgarol or atrazine have phototoxic capacity and proven or suspected impact on planktonic species composition and communities. Microphytobenthos and phytoplankton might be especially sensitive to such phototoxic effects. What appeared to be a synergistic interaction between TBT exposure and UV-B radiation effects on a natural planktonic assemblage was found by Sargian [118] and Pelletier *et al.* [119] using a microcosm approach. Deleterious effects of TBT exposure were significantly more pronounced when cells were co-exposed to enhanced UVB levels. The same author also found a reduced bacterial production in the presence of TBT. Hjorth and co-workers [120] observed effects of the polycyclic aromatic hydrocarbon pyrene on a natural marine plankton community using a food-web approach in a mesocosm. Direct and indirect effects on the function and structure of bacteria, phytoplankton and to a lesser degree on zooplankton communities were found. The change in system function suggested that PAHs might be an important stress factor for pelagic systems, as a one-time exposure of a single compound changes the development of a pelagic community.

An important finding was recently reported by Echeveste *et al.* [121]. These authors performed *in situ* experiments on board of a research vessel in the NE Atlantic Ocean that determined the influence of complex mixtures of organic pollutants on oceanic phytoplankton populations. The results of these experiments suggest that current levels of POPs are only 20 times below the levels at which significant influence on ecosystem function (primary productivity) would be found.

Table 1: Alternative strategies for pelagic environmental assessment.

Approach	Advantages	Disadvantages	References
Exposure and/or effect modelling	Reproducible; Direct link to risk assessment.	No direct link to environmental impact.	[27, 122]
In situ extracts	Identify specific mechanisms and substances; Sensitive and reproducible; Possible to test systems not otherwise included (e.g., early life stages in fish).	Limited volume/area; Laboratory testing for effects.	[40, 123 - 125]
Caging	Reflects local exposure over deployment period; Can use organisms with desirable characteristics.	"Semi-natural" exposure situation; Food availability unknown; Exposure at one point.	[26, 29, 39, 126]
Mesocosm studies	Can control vital parameters. Some ecological relevance; Improves scope for interpretation.	Reduced biological and physical complexity relative to field situation.	[6, 68, 77, 118, 120]
Field sampling	High ecological relevance.	Difficult to assess area integrated over; High natural variability.	[38, 89]

APPROACHES

There are substantial logistical challenges involved in the study of how contaminants may affect pelagic systems or species. Micro- or mesocosm studies are required for detailed studies of specific effects or interactions between

factors. For lower trophic levels, mesocosm studies are generally required to assume any kind of ecological relevance. In the field, four approaches have been used:

I. modelling of contaminant distribution and subsequent effects by comparing with lab-data;

II. estimating exposure through whole-water extraction or passive samplers and either model effect – as for (i) – or measure using a battery of bioassays, e.g., *in vitro* techniques;

III. cage organisms in the area of interest;

IV. mesocosm studies; and

V. field-collection of organisms.

The five approaches all have weak and strong characteristics, outlined in Table **1**.

RESEARCH NEEDS

As will be apparent from the above, there are large blank areas in our understanding of how and whether contaminants impact pelagic ecosystems. On the other hand, knowledge of the pelagic zone is clearly vital in the management of our oceans. In this context it is important not to view the pelagic zone in isolation, but remember that pelagic processes are important to both the surface layer and benthic ecosystems. Future research should be directed towards integrating and not dividing our understanding of different environmental compartments.

As for all other fields in ecotoxicology, we face a major challenge in developing methods to assess the effects of contaminant mixtures. For pelagic systems this may be particularly relevant since even the less persistent contaminants will be present in the water column near the source. In addition to contaminant mixtures, there is a scarcity of knowledge on how other factors modulate contaminant impacts or combination effects. Micro- and mesocosm model systems (see below) should be useful tools in this context.

It will be clear that there is a need for an improved understanding of how contaminants affect both primary producers and microbial loop components. Current knowledge is limited to effects on single algal species and there is virtually no knowledge of impacts in more complex systems that include bacteria and protists.

There is some understanding of how some contaminants affect a limited number of zooplankton species (e.g., calanoid copepods), but little is known about the wide range of mesozooplankton species, including metamorphosing stages and effects on their sensory systems [127].

It is inherently challenging to keep pelagic fish species and their early life stages for experimental studies due to the need for specialised sampling techniques and large volume aquarium systems. In contrast to primary producers and zooplankton, there is a substantial knowledge of general physiology and biochemistry that can be applied for fish, even though there may be species-dependent contaminant-associated effects. There are even larger obstacles involved in experimental studies of pelagic top predators.

In addition to experimental micro- or mesocosms, four approaches have been used for the assessment of contaminant effects in marine pelagic ecosystems: modelling, *in situ* extracts/passive samplers, caging and field collection. Both laboratory- and field-based methodologies are needed and they complement each other.

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CHAPTER 11

The Role of Aquatic Ecosystems in the Elimination of Pollutants

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Abstract: Contamination of aquatic ecosystems is always of concern to environmental scientists; however, these systems also possess unique capabilities allowing them to eliminate or remediate certain levels of pollutants. Primarily through the presence of vegetation, aquatic ecosystems are known to be capable of removing or at least decreasing pollutant loads travelling through the aqueous phase. In addition to vegetation, soil/sediment and microbes play a significant role in transferring or transforming pollutants to acceptable levels in aquatic ecosystems. This chapter focuses on some of the primary literature describing phytoremediation of organic pollutants (e.g. hydrocarbons and pesticides) and inorganic pollutants (e.g. metals and nutrients). Research indicates the popularity and success of phytoremediation techniques used to clean up both organic and inorganic pollutants from the water column. While certain caution should always be exercised, phytoremediation continues to serve as a successful means of pollutant remediation in aquatic ecosystems.

INTRODUCTION

Aquatic ecosystems are often receptacles of point- and non-point source pollutants from spills, sprays, or runoff events. While much emphasis is placed on aquatic ecosystem damage from pollutants, research has demonstrated these unique systems have resilience and assimilative capacity in the mitigation of such pollutants. This chapter will focus on aquatic ecosystem responses to metal, nutrient, and pesticide inputs, primarily discussing the concept of pollutant remediation *via* plants (phytoremediation) and microbes. Because various review articles have been published regarding specific phytoremediation techniques [1, 2], this chapter is not meant as an exhaustive literature review. Instead, it provides a broad understanding of some of the principle concepts involved in aquatic system remediation (through plants) of common pollutants.

PHYTOREMEDIATION

Phytoremediation is generally defined as the use of plants and associated microbes to remove, contain or render harmless environmental pollutants [2, 3]. The nature of pollutants will affect their ability to successfully undergo phytoremediation. For example, while organic pollutants can be degraded, inorganic pollutants such as nutrients are unable to be degraded. Instead, through processes of phytoremediation, inorganic pollutants can be stabilized or sequestered. According to Susarla *et al.* [4], three general factors affect pollutant uptake and distribution within plants used in phytoremediation efforts: physicochemical properties of the pollutant (e.g. octanol water partition coefficient, vapor pressure, water solubility); environmental conditions (e.g. pH, temperature, soil moisture, organic matter); and plant characteristics (e.g. available enzymes and root systems). In addition to the factors affecting pollutant uptake, phytoremediation itself has five major mechanisms by which the process may operate [2, 4, 5, 6].

1. Phytoextraction/Phytoaccumulation: The pollutant is taken up by the plants, but not completely or quickly degraded, resulting in accumulation within the plant.
2. Phytovolatilization: The pollutant is converted by plants into a volatile form and released.
3. Phytostabilization: Typically observed with metals, plant root exudates alter the soil environment allowing the pollutant to precipitate.
4. Phytotransformation/Phytodegradation: The pollutant is eliminated *via* plant enzymes or enzyme co-factors.
5. Rhizodegradation: The pollutant is treated *via* enhanced activity of bacteria or fungi associated with plant roots in the rhizosphere.

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Not all mechanisms are equally effective for remediation of all pollutants. Phytoextraction, phytoaccumulation, and phytostabilization are efficient mechanisms for remediation of many metals, including cadmium, chromium, lead, nickel, and zinc. Mercury, selenium, and various chlorinated solvents are effectively remediated through phytovolatilization. Pollutants such as munitions, chlorinated solvents, and certain pesticides are best remediated through phytotransformation and phytodegradation. Rhizodegradation is an effective mechanism for remediation of radionuclides, certain organic chemicals, and metals.

AQUATIC SYSTEM REMEDIATION OF ORGANIC POLLUTANTS

Studies of organic pollutant remediation in aquatic systems tend to focus on structures such as oxbow lakes, detention ponds, riparian buffer zones, vegetated drainage ditches and constructed wetlands. As with inorganic pollutants, remediation occurs not only in and around vegetation, but also within sediment and aqueous phases *via* chemical and microbial processes. Polarity and lipophilicity of pollutants give reliable indications on their ability to be remediated *via* vegetation. Limited plant-pollutant uptake will be achieved with chemicals which are extremely polar due to difficulty in crossing biomembranes [7]. On the other hand, extremely lipophilic pollutants quickly penetrate biomembranes, only to be sorbed to root material. It is the pollutants with intermediate lipophilicity which are best remediated by vegetation. These pollutants can be translocated to upper plant parts, rather than become concentrated in root material [7]. According to Chaudhry *et al.* [8], by reducing plant wax viscosity, uptake of non-polar compounds will be enhanced. Additionally, factors which increase leaf cuticle hydration increase the permeability of hydrophilic compounds.

Once pesticides are absorbed in plant material, three main reactions are responsible for pollutant transformation [8]:

1. Degradative (e.g. hydrolysis and oxidation)
2. Synthetic (e.g. conjugation)
3. Rearrangement (e.g. epoxide formation)

Specific Examples of Organic Pollutant Phytoremediation

Euliss *et al.* [9] compared reduction of petroleum hydrocarbons found in sediments with sedge (*Carex stricta*), switchgrass (*Panicum virgatum*), and gamagrass (*Tripsacum dactyloides*) versus sediments under willow (*Salix exigua*), poplar (*Populus* spp.) or no vegetation. Significantly fewer residues of petroleum hydrocarbons (70%) were in sediments with sedge or grass; whereas only 20% fewer residual hydrocarbons were noted in the sediments containing trees or no vegetation. Two aquatic plants, *Juncus fontanesii* and *Lemna minor* have reportedly removed phenol concentrations ranging from 8 to 48 mg/L [10]. Polychlorinated biphenyls, another common organic pollutant, has been shown capable of being transferred from an aqueous spiked solution into plant material from the common reed (*Phragmites australis*) and rice (*Oryza sativa*) [11].

A great deal of phytoremediation literature addresses the ability to reduce pesticides. Many studies examine remediation capabilities within stream mesocosms, constructed wetlands, or vegetated drainage ditches. Several studies have examined the influence of vegetation on the reduction of pyrethroid insecticide concentrations in aqueous solution. In a mesocosm experiment, Moore *et al.* [12] reported *cis*-permethrin reduction ranging from 67 ± 6% in common cattails (*Typha latifolia*) to 71 ± 2% in cutgrass (*Leersia oryzoides*). Another study conducted by Moore *et al.* [13] examined permethrin mitigation in constructed ditches in Yolo County, California. The ditch distance needed to reduce permethrin concentrations to half of their original inflow concentration ($D_{1/2}$) in non-vegetated ditches (50-55 m) was basically twice that of vegetated ditches (21-22 m). Bennett *et al.* [14] determined that in order for initial bifenthrin and lambda-cyhalothrin aqueous inflow concentrations to be reduced to 0.1% of their initial value, a vegetated ditch 280 m would be necessary. Other vegetated ditch studies have reported 87% of the mean measured lambda-cyhalothrin was associated with plant material [15]. In a constructed wetland experiment, 49% of measured lambda-cyhalothrin was associated with vegetation, while 76% of cyfluthrin was found in vegetation [16].

Various studies have also examined the remediation of organophosphate insecticides and different herbicides with vegetation. After dosing a field-scale constructed wetland in the Mississippi Delta, USA, Moore *et al.* [17] reported

43% of the measured mass of the insecticide diazinon was associated with wetland plant material. In a California study, diazinon was amended into two constructed ditches, one vegetated and one non-vegetated. Ditch half-distances ($D_{1/2}$) were calculated (see previous paragraph for description) and results indicated a non-vegetated ditch would need three times the distance (158 m) of a vegetated ditch (55 m) to remediate the same diazinon concentration [13]. Comparing methyl parathion transport in vegetated versus non-vegetated constructed wetlands, Moore *et al.* [18] reported pesticide concentrations were detected in outflow samples of the non-vegetated wetland 30 min after initial dosing. During the same time sequence, methyl parathion concentrations in the vegetated wetland were only measured at 20 m (slightly less than half way through the system). Semi-permeable membrane devices deployed in both wetlands confirmed that, although methyl parathion concentrations reached the non-vegetated wetland outflow, no pesticide was detected in the vegetated wetland outflow [18]. Experimental constructed wetland mesocosms at the University of Mississippi Field Station were utilized for specific pesticide phytoremediation studies in the late 1990s. Results from those studies indicated that 25% and 10% of measured chlorpyrifos and metolachlor (herbicide), respectively, were associated with wetland plant material [19, 20]. A study examining atrazine mitigation in a vegetated drainage ditch populated with *Polygonum* spp., *Leersia oryzoides*, and *Sporobolus* spp. reported 61% of measured herbicide concentrations were associated with plant material [15]. Rice *et al.* [21] examined radiolabelled pesticide concentrations in aqueous solution in vegetated versus non-vegetated systems. In different systems vegetated with *Ceratophyllum demersum*, *Elodea canadensis,*and *Lemna minor*, 1%, 4%, and 23% of ^{14}C-metolachlor, respectively, remained in aqueous solution, while 61% of the pesticide was present in non-vegetated system aqueous solutions. Likewise, ^{14}C-atrazine was amended into identical systems. Percentage of pesticide remaining in aqueous solution was 41%, 63%, and 85% for *C. demersum*, *E. canadensis*, and *L. minor*, respectively. In non-vegetated systems, 85% of ^{14}C-atrazine remained in aqueous solution [21]. Rose *et al.* [22] monitored reduction of the herbicide fluometuron in open and vegetated ponds for consecutive growing seasons. Significant differences (58% reduction in vegetated pond versus 41% reduction in open pond) was noted during the second incubation of the second season.

Microbial Remediation of Organic Pollutants

Organic pollutants represent a vast range of chemicals with diverse properties and varying degrees of toxicity and recalcitrance to microbial remediation. Common pollutants include petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), nitroaromatics, polychlorinated biphenyls, industrial solvents and various pesticides [23]. The biodegradation of these materials has received a substantial amount of interest over the last two decades and interactions between various organic pollutants and microorganisms have been examined from physiological, molecular, and even evolutionary perspectives [24, 25, 26, 27]. At a basic level organic materials can be separated into those that are biodegradable (*i.e.* are transformed by microorganisms into more innocuous products, ultimately into carbon dioxide and water), those that are persistent (materials which are not biodegradable in certain environments), and those that are recalcitrant (materials which are resistant to biodegradation in most situations). While individual microorganisms are capable of degrading simple organics, typically the complete biodegradation of organic pollutants may involve the metabolic activity of several microbial populations acting as a consortium [28].

As well as the fundamental capability of natural bacterial populations to degrade an organic pollutant, a number of other considerations are important and may impact the effectiveness of microbial remediation [28]. Organic pollutants typically occur as mixtures of different groups of chemicals and even within specific groups (e.g. PAHs) there is a great deal of variability on degradability [28, 29]. Different organic substrates (or their degradation byproducts) may interfere with the microbial pathways used to degrade other substrates, impairing effective remediation. As well as substrate variability, certain microorganisms might also require electron acceptors other than oxygen; for example, sulfate reducing bacteria may be particularly effective in the reductive dehalogenation of highly substituted materials such as organochlorine compounds [30]. Even if metabolically suitable microbial populations and electron acceptors are present, interactions between the microorganisms and pollutant may be limited. Organic compounds can become associated with polymers in the soil matrix limiting their accessibility to microbial populations [31], and the same could occur in aquatic sediments. At a more fundamental level, many organics are also insoluble in water, and this is likely to be a limiting factor in the degradation of materials such as polychlorinated biphenyls in aquatic ecosystems, although the microbial production of surfactants may overcome this to some extent [23]. Limited accessibility of microbial populations to organic pollutants also means that the concentration of pollutant that the microorganisms are exposed to may be substantially lower than the actual

concentration in the system, and these lower concentrations may be below the threshold needed for induction of degradative enzyme systems even if the natural microbial populations contain them [32, 33]. The movement of microorganisms towards increasing concentrations of pollutants (*i.e.* positive chemotaxis) may be just as important as actual degradative ability in the microbial remediation of some organic contaminants [29].

Populations of the bacterium *Pseudomonas putida* can show both chemotaxis towards and the ability to degrade the two-ring PAH naphthalene if they possess the appropriate plasmid [34], and natural microbial populations are likely to show the same capabilities. Naphthalene is a common organic micropollutant in water and many bacteria capable of degrading naphthalene have been isolated [29]. Similarly a large number of bacteria appear to be capable of degrading three-rings PAHs such as phenanthrene, and as with naphthalene degraders, these bacteria represent a diverse range of bacterial taxa [35, 36, 37]. The capability of microorganisms to degrade higher molecular weight PAHs such as benzo[*a*]pyrene, a five-ring carcinogenic compound that is a commonly formed from combustion of organic material, is much more limited [37]. Those bacteria that can oxidize benzo[*a*]pyrene generally do so through cometabolism, requiring the presence of other organic substrates either for metabolism or to stimulate PAH degradation [38, 39]. Various fungi have been shown to be potential degraders of benzo[*a*]pyrene and other high molecular weight PAHs in terrestrial environments [37, 40], but the importance of fungi as degraders of PAHs in aquatic ecosystems is not known.

Nitroaromatic organic compounds released from incomplete fossil fuel combustion and as feedstock in the manufacture of materials such as pesticides are generally regarded as being fairly recalcitrant to bioremediation, especially through oxidative reactions [27, 41]. Few microorganisms are capable of using nitroaromatics substrates as their sole source of carbon and/or nitrogen, although many more appear to be capable of reducing nitroaromatics to corresponding aminoaromatics through the action of various nitroreductases [27]. This typically occurs under anaerobic conditions, and may be the major method by which poly-nitroaromatic compounds can be degraded [42]. Intermediate products, however, may be more toxic than the original pollutant, and effective mineralization in aquatic sediments is likely to require consortia of many different interacting microbial populations. Simpler mono- and di-nitroaromatics are mineralized aerobically by some bacteria that potentially use them as a source of carbon, energy, and nitrogen [27]. Various actinomycetes and pseudomonads can hydroxylate the nitro groups in 2-nitrophenol and 4-nitrophenol, releasing nitrite and forming dihydroxybenzene which is subsequently mineralized [43, 44, 45]. These reactions are important in the microbial degradation of the pesticides parathion and methyl parathion which are first hydrolyzed to yield 4-nitrophenol, which is subsequently hydroxylated [46]. Monoxygenases and dioxygenases are involved in the hydroxylation of mono- and di-nitroaromatics, respectively, but other aerobic degradation mechanisms exist in some bacteria [27]. However, compared to many non-nitrogen containing organic pollutants, nitroaromatics are more resistant to microbial mineralization and the majority of studies have been at the bench- or laboratory-scale rather than in natural environments.

As with nitroaromatics, most organic molecules that contain substituted groups are more recalcitrant to microbial remediation than simpler hydrocarbons. This is especially true of halogenated organic pollutants, even those with relatively simple modifications of aromatic hydrocarbons such as chloro- and fluoro-benzene. However, while most bacteria in natural environments have no ability to degrade these compounds, continued exposure to simple halogenated aromatics encourages genetic exchange between bacterial populations and has been shown to result in the evolution of new degradative pathways [47, 48]. Organics with more extensive substitutions such as the pesticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) or polychlorinated biphenyls (PCBs) are more difficult to degrade. Microorganisms that degrade these materials do so through either an oxidative process, in which they use the pollutant as the main substrate for metabolism, or through reductive dehalogenation, in which the halogen groups are replaced with hydrogen [42]. Reductive dechlorination of PCBs appears to be common in contaminated aquatic sediments and typically involves populations of anaerobic microorganisms such as the sulfate reducing bacteria [30, 42]. However, while microbial consortia may process chlorinated organic compounds completely, most of the isolated bacteria that are capable of reductive dehalogenation do not do so completely so that end products of remediation may still contain chlorine groups. A notable exception is *Dehalococcoides ethenogenes* which can reductively dechlorinate the solvent tetrachloroethene to ethene, using perchloroethene as an electron acceptor for metabolism [49, 50]. *D. ethenogenes* is also interesting in that while most bacteria that have been studied from the perspective of pollutant remediation belong to well studied microbial groups such as the Proteobacteria or Firmicutes, *D. ethenogenes* is related to the Chloroflexi [51], a poorly studied group of unusual

photosynthetic organisms. This illustrates the importance of considering the possible role of all microorganisms in natural ecosystems for bioremediation, not just those that have been previously studied. It suggests that other poorly studied groups of bacteria may have novel metabolic pathways for pollutant removal that are as yet undiscovered.

AQUATIC SYSTEM REMEDIATION OF INORGANIC POLLUTANTS: METALS

Aquatic systems ranging from lotic (*i.e.*, rivers, streams) to lentic ecosystems (*i.e.*, wetlands, lakes, oxbows) have the ability to transform heavy metal pollutants from the water column through various ecological processes. Plants, sediments and microbes actively participate through biological and chemical processes to transform, remediate, and stabilize toxic metal pollutants. Marshes or constructed treatment wetlands are most often used for phytoremediation of metals [52]. However, the remediation capabilities of the system are not limited to plants. Sediments actively participate in forming metal complexes, reducing certain metal forms, and binding elements to particulate matter. Lentic conditions create ideal circumstances for decreases in soil redox, habitat for aquatic plants, and reducing toxic, soluble valence forms of metals to insoluble, reduced non-toxic forms.

Phytostabilization is the most common form of phytoremediation whereby assimilation and transformation of elements are restricted to the roots and there is no translocation of elements to the shoot. Often these plants are called root accumulators [53]. Plants with a higher concentration of element within the plant tissue than in the surrounding substrate (*i.e.*, water or sediment) are often considered hyperaccumulators and often exhibit luxury uptake. Luxury uptake is the ability to increase elemental concentrations within the plant tissue beyond the needs of the plant for normal metabolic functions [54]. The optimal plant for phytoextraction should not only be able to tolerate and accumulate high levels of heavy metals in its harvestable parts, but also have a rapid growth rate and the potential to produce a high biomass in the field. An ideal plant for rhizodegradation should have rapidly growing roots with the ability to remove toxic metals from solution over extended periods of time [5].

Studies in plant response to heavy metals have suggested that plants have evolved two different physiological mechanisms which enable them to tolerate metal toxicity: accumulators and excluders [55]. Accumulators concentrate sequestered metals in plant parts at low to high concentrations above background concentrations. Excluders have differential uptake and transport between root and shoot which result in constant low shoot/root levels over a wide range of external concentrations. In accumulators, root uptake and transport are more or less in balance, but metals can still accumulate in the roots. Excluders do not generally regulate metal uptake, with restriction of transport from root to shoot as the likely mechanism reducing metal toxicity. Studies have suggested that plants growing on metalliferous soils cannot prevent metal uptake, but can only restrict it and hence accumulate metals in root and shoot tissues at varying concentrations. Different plant species globally have shown considerable differences in their uptake ability for various metal species. Baker [55] highlighted 12 different wetland and upland species that had 18-fold variation for zinc, 240-fold difference for lead, and 273-fold for cadmium.

Phytoremediation of metals has several advantages:

- Metals can be selectively removed at low concentrations (*i.e.* a polishing step)
- It can occur on site with through flow, or biomass can be transported to a specific site
- There is an initial low capital investment and low operating cost as compared to traditional methods of remediation

Specific Examples of Metal Phytoremediation

Maine *et al.* [56] identified two strategies of metal remediation depending on the plant species used. Submerged non-rooted *Eichornia crassipes* retained the majority (97%) of metals in macrophytic biomass, while a community co-habitated or completely dominated with *Typha domingensis* had the majority of metals associated with the sediments. This example illustrates the varying degrees of assimilatory capacity between aquatic plants. Water hyacinth (*E. crassipes*) has also been used to phytoremediate iron-rich wastewaters in constructed wetlands [57]. Iron removal by water hyacinth was largely due to the process of rhizofiltration and phytostabilization, since chemical precipitation of iron oxides was followed by flocculation and sedimentation. In this study, phytoremediation seemed to not be very

substantial in iron accumulation in comparison to chemical precipitation. Rhizofiltration was the predominant mechanism of remediation of iron since a substantial portion was localized in the roots. Iron phytoextraction was possibly negligible due to the physiological barriers to iron transport to aerial tissues. Caution must be exercised with the use of *E. crassipes,* since it is considered a noxious weed in many countries.

Sharma and Gaur [58] examined the ability of *Lemna polyrhiza* to remediate zinc, lead, and nickel. It was noted that the plant had a rapid increase of metal assimilation within 12 hours, with subsequent assimilation reaching a plateau. It is hypothesized that within the initial 12 hours, rapid, passive uptake of metals occur, while thereafter the assimilation occurs at a slower rate due to metabolic control. A consequence of too great a concentration of heavy metals is the decline and inhibition of chlorophyll synthesis. Thus, most plants have an evolutionary and metabolic constraint to assimilation of certain elements.

Zazo *et al.* [59] examined two species, *Typha latifolia* and *Carex lurida* for their phytoremediation ability in reducing hexavalent chromium. Irrespective of the plant species, as there were no significant differences between species; hexavalent chromium removal was enhanced by plants, with a decrease in soil redox promoted by organic root exudates released by the plants. In low redox conditions, iron and sulfate reduction is increased. Additionally, concentrations of ferrous iron and sulfides increase in the sediment pore water which in turn reduces hexavalent chromium to Cr_{III}. Soils high in organic humic substances will also possess the ability to transform and sequester toxic metals ions. Humic acids constitute a large organic carbon fraction and represent a significant electron donor reservoir for metal reduction and amelioration [59].

Often plants will significantly phytostablize contaminants whereby metals are reduced in and around the roots [60, 61]. The aquatic plant rhizosphere provides a particularly effective, locally oxidized/reduced environment for metal precipitation and adsorption outside the root. *Phragmites australis* roots have been shown to accumulate Fe, Cu, Zn, Pb and Cd, with little to no translocation of metals within the plant to rhizomes and shoots. Iron plaque formation of Fe-oxyhydroxides formed by oxygen evolution by the roots and microbial metabolism is believe to be a mechanism of avoiding toxicity of reduced forms of Fe and Mn to roots under flooded conditions. Vesk *et al.* [62] identified where various element species occurred within the roots of aquatic plants. Iron was often present at highest concentrations at the root surface and decreased within the cell, while trace metals (Cu, Zn, Pb) had highest concentrations occurring within the plant cell, and decreasing towards the root surface. Meyers *et al.* [63] examined the uptake and distribution of lead sequestered by hydroponically grown *Brassica juncea.* The study showed lead uptake was restricted to the root tissue suggested rhizofiltration, where the concentration of lead was always two to three orders of magnitude greater in roots than in shoots. Electron microscopy work revealed substantial and predominantly intracellular uptake at the root tip, while endocytosis of lead within the plasma membrane was not observed. Further experiments demonstrated uptake of lead increase as concentration of lead in solution increased.

In some instances an interaction occurs between metals. Studies have shown [64] that manganese absorption by plant tissue will be suppressed or depressed by high levels of iron precipitate or assimilation. For example *Juncus effusus* showed reduced concentrations of manganese in shoots as a result of high iron concentrations. Thus, phytostabilization of one element could result in deficiencies in other elements important to metabolic functions such as growth.

Plants can accelerate and promote bioremediation of metals and other contaminants by stimulating the growth and metabolism of microorganisms through the release of nutrients and oxygen. There is a significant amount of information concerning the influence of aquatic plants on metal fluxes at larger scales. There is also a substantial amount of information concerning small scale laboratory research addressing kinetics of metal uptake in aquatic plants. There is still a very relevant need for research understanding processes of metal accumulation and transformation in the field and how it affects larger scales.

Microbial Remediation of Metals and Metalloids

The microbial remediation of metals differs from that of organic pollutants as metals are not degraded into what are ultimately innocuous products [65]. Rather, interactions between microorganisms and metals may change the redox state of the metal or alter its mobility in the environment. At a basic level, interactions between microorganisms and metal contaminants in aquatic ecosystems can be separated into four broad types: (1) microbial redox transformations that

change the metals mobility; (2) volatilization or precipitation from the water column; (3) absorption of metals to microbial cells or cellular products (biosorption); and (4) microbial transformations of other chemicals that indirectly influence metal behavior [66]. Commonly, a number of these processes will be involved in the microbial remediation of metals and metalloids; for example, dissimilatory metal reduction as part of anaerobic respiration (a redox transformation) can result in a metals precipitation or biosorption. Aquatic ecosystems harbor appreciable numbers and diversity of bacteria that metabolize or are resistant to toxic metals and many of these organisms are capable of biotransforming elements into forms of different mobility and toxicity. Fig. (1) illustrates some of the microbial processes that can be involved in transforming metals in oxic and anoxic layers of aquatic environments.

Microbial interactions with arsenic are an example of naturally occurring metal-microbe processes that may have remediation potential. Studies suggest that arsenic resistant bacteria are a common component of both aquatic and terrestrial ecosystems, even those not suffering from arsenic pollution [67, 68]. Bacteria possess a number of genetic and physiological systems for dealing with arsenic toxicity including redox transformations and its incorporation into organic forms [69]. The metabolic process of arsenite (As_{III}) oxidation converts arsenic to the less toxic arsenate (As_V) and has been shown to occur in a number of bacteria, either as a resistance mechanism or as a form of energy generating metabolism [70, 71, 72, 73]. The microbial oxidation of arsenite has been proposed as a bioremediation strategy for aquatic environments [70], as the resulting arsenate is much less soluble and can be more easily removed through steps such as alkaline precipitation with lime [74].

While inorganic arsenic becomes less mobile and toxic following oxidation, the opposite is true for other metals. The oxidized forms of chromium and the radioactive metals uranium and technetium are much more water soluble than their reduced forms [65, 75] so that, in contrast to arsenic, it is the microbial reduction of these metals which may be more beneficial to the remediation of aquatic environments. Differences in the mobility of various metals in different redox states also highlight a fundamental difference in remediation strategies for aquatic and terrestrial environments. In solid phase systems (soils) it is typically beneficial to increase metal mobility so they are removed from the system; however, in aquatic phases the opposite approach (to encourage microbial redox processes that decrease metals mobility and increase their precipitation or adsorption) is often more desirable [75].

The reduction of chromium from the toxic and highly soluble Cr_{VI} to relatively insoluble Cr_{III} has been demonstrated in a wide range of microorganisms including bacteria, fungi, and algae [76, 77]. Chromate reducing enzyme systems vary greatly between bacteria and include both soluble enzymes, those associated with the cell membrane, and enzymes capable of reducing Cr_{VI} either aerobically or anaerobically [78, 79]. Such diversity suggests that bacterial chromate reductases may be useful in the bioremediation of chromium contaminated sites in a wide range of environments [80]. As is the case with arsenic, chromium resistant bacteria appear to be ubiquitous, having been recovered from both chromium impacted and non-polluted environments [81].

Toxic metals can also be removed from aquatic environments *via* their precipitation with the products of other microbial redox processes. Dissimilatory sulfate reducing bacteria, a common group of bacteria in aquatic sediments that utilize sulfate as the terminal electron acceptor in anaerobic respiration, produce hydrogen sulfide as a waste product. The sulfide produced can precipitate and immobilize arsenic [82] and abiotically reduce Cr_{VI} to Cr_{III} [83]. Sulfate reducing bacteria have also been shown to reduce aqueous concentrations of cadmium, copper, iron, nickel and zinc through the formation of insoluble metal sulfides in model systems [84, 85] and similar processes are possible in natural environments. Dissimilatory iron-reducing bacteria convert Fe^{3+} to Fe^{2+} during their anaerobic respiration, and Fe^{2+} can also abiotically reduce and immobilize toxic metals such as chromium [83]. Some iron-reducing bacteria such as species of *Geobacter* and *Shewanella* are also capable of directly reducing other metals as part of their metabolism, and may be useful in the reductive remediation of technetium, cobalt, and uranium [86, 87, 88].

While sulfate and iron-reducing bacteria are typically found in anoxic aquatic sediments, they can also be important components of aquatic biofilms [89, 90]. Biofilms are naturally occurring communities of attached microorganisms found on any submerged surface, and they are characterized by a complex architectural structure often with both aerobic and anaerobic layers [91, 92]. They often support a diverse range of microbial populations in close proximity to each other, which can be important in bioremediation [93]. Furthermore, the cells within biofilms are enclosed within a matrix of extracellular polysaccharides or slime, which itself can remove metal contaminants from the surrounding water through the process of biosorption [94, 95, 96]. The combination of sulfate reduction and

potential biosorption/precipitation of metal sulfides within the biofilm structure can be a particular effective method of remediation and has been shown to be effective for metals such as chromium, copper, and lead [97, 98, 99].

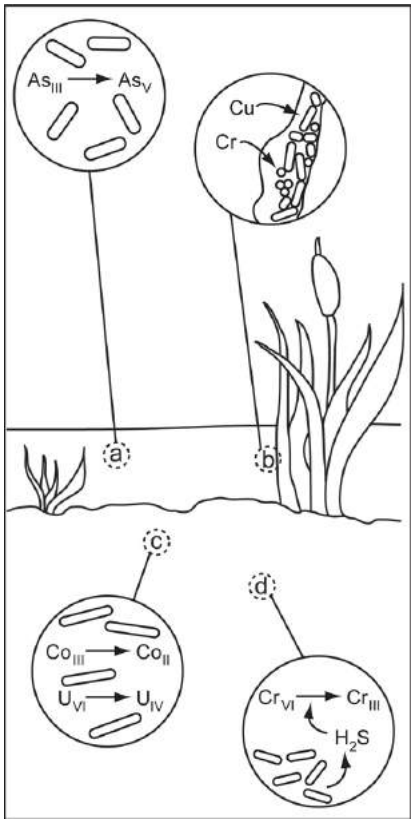

Figure 1. Microbial processes that can remediate metal pollution in aquatic ecosystems include: (a) the aerobic oxidation of metals such as arsenic which can reduce their mobility and toxicity; (b) the absorption of metals such as chromium and copper to biofilms associated with sediments and aquatic plants; (c) the microbial reduction of metals such as cobalt and uranium to less toxic forms by iron-reducing bacteria such as *Shewanella*; (d) indirect transformations resulting from microbial metabolism that result in metal precipitation, such as the reduction of chromium following the production of hydrogen sulfide by sulfate-reducing bacteria in anoxic sediments.

Sulfate-reducing bacteria in anoxic sediments and biofilms are important mediators of mercury methylation [100, 101, 102], which can have negative impacts on human activities because methylmercury is highly toxic and subject to biomagnification through aquatic food webs [103]. However, sulfate-bacteria may also play some role in the removal of aqueous mercury (Hg^{2+}) *via* the production of hydrogen sulfide as a waste product which can react with Hg^{2+} to form the much less soluble mercuric sulfide [104]. Of more importance from a remediation aspect is the enzymatic reduction of Hg^{2+} to elemental mercury (Hg^0) which is common and widespread throughout bacteria [105, 106]. Elemental mercury is insoluble and much less toxic than other forms. It is also volatile so that the microbial reduction of Hg^{2+} to Hg^0 is a significant mechanism that can contribute to the removal of mercury from natural waters to the atmosphere [106]. Mercury contaminated environments select for microorganisms capable of carrying out this transformation [107], which is encoded for by a number of mercury resistance (*mer*) genes [105, 106].

Bacterial mercury resistance genes are usually located on plasmids and are often components of transposons [105, 106, 108, 109]. These mobile genetic elements can be passed between bacterial species *via* the process of horizontal gene transfer and the evolutionary history of *mer* genes suggest that this has been a relatively frequent occurrence in the past [110]. The same phenomenon has been shown for arsenic resistance genes, which are also often borne on plasmids [111].

Mobile resistance genes demonstrate the capability of natural microbial communities to respond and adapt to environmental pollution both from metals and organic pollutants [112]. From an applied perspective they present an

excellent opportunity to incorporate biotechnology into bioremediation in that the genes can be transferred into specific bacterial species that may be suitable for a particular environment. Such an approach is likely to be particularly beneficial in environments where there are multiple contaminants, in that traits such as metal resistance may be passed onto to organic-degrading bacterial populations [23].

AQUATIC SYSTEM REMEDIATION OF INORGANIC POLLUTANTS: NUTRIENTS

Unlike previously described organic pollutants and metals, nutrients are a vital component in aquatic systems. Productivity and trophic status of aquatic systems impart information on not only the stability, but also the relative ecological health of aquatic systems. Problems arise however, when nutrients in aquatic systems reach levels in excess of the natural system's capacity to utilize them. Nutrient concentrations must strike a fine balance between the needs of the aquatic system and excessive levels which will lead to ecological problems such as hypoxia or harmful algal blooms.

Several studies have examined abilities of aquatic plants to remediate excessive nutrient concentrations. Cronk and Fennessy [113] warn that nitrogen and phosphorus removal from water by vegetation is not the major pathway for nutrient remediation where concentrations are high. More success can be achieved with plants in a nutrient phytoremediation scenario when overall nitrogen and phosphorus concentrations have lower loads. Nutrient uptake by plants is also dependent on several factors including season, plant growth rate, plant biomass, and latitude [113]. In a two year study in northwest Mississippi, USA, Kröger *et al.* [114, 115] reported that vegetated drainage ditches reduced 53% and 43% of the dissolved inorganic nitrogen and maximum inorganic effluent phosphorus loads, respectively. Mesocosm scale studies reported $83 \pm 3\%$ and $40 \pm 8\%$ decrease in aqueous ammonia and nitrate concentrations, respectively, in systems vegetated with *Ludwigia peploides* [116]. Although it was least effective in decreasing ammonia and nitrate concentrations, the aquatic plant *Leersia oryzoides* was more effective than *L. peploides* at removing organophosphorus ($29 \pm 7\%$) [116]. Two separate studies examined the use of *Eichhornia crassipes* in remediating excessive nutrients from water. In a system with a 21 day hydraulic retention time, 100% removal of total nitrogen and phosphorus was achieved after nine weeks of treatment through *E. crassipes* [117]. Using a 31-day batch growth experiment with *E. crassipes*, reductions of total Kjeldahl nitrogen, ammonium, and total phosphorus were 92%, 99%, and 99%, respectively [118].

CONCLUSIONS

Aquatic systems are resilient habitats which receive many point and non-point-source pollutants. Rather than focus on their contamination, this chapter was devoted to the abundance of literature demonstrating remediation capabilities – primarily phytoremediation – of these aquatic systems. Although the literature presented within this chapter is not an exhaustive review of all the research conducted in these specific areas, it provides a solid foundation for those interested in the ability of vegetation to clean waters receiving pollutants. Cautions exist, of course, when using phytoremediation for any pollutant. While benefits certainly exist, there are also drawbacks to using phytoremediation tools. For example, harvested biomass from metal phytoextraction may be a hazardous waste. Improper initial planning may lead to a potential food chain effect due to consumption of contaminated plants. Just as a mechanic cannot fix every problem with a wrench, phytoremediation should be considered as a valuable tool in practitioners' environmental toolbox. Remediation of aquatic systems is equivalent to an "ecological tag-team" of physical, chemical, and biological processes conducted in plants, sediment, and water.

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Concluding Remarks

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THE EXTENT OF CONTAMINATION

The new millennium started with a legacy of unprecedented contamination of the world ecosystems left in the wake of the various activities of humankind. Chemical pollutants have become so diverse (see Chapter 1) and widespread that there is hardly any region of the world that is not currently affected by their impacts. With the exception, perhaps, of the desert wilderness areas (for which information on pollution is still lacking), every other ecosystem on earth, from the polar regions to the tropics, whether on land or in the oceans, has been shown to contain residues or traces of organic and inorganic pollutants of anthropogenic origin.

Even if most of the contaminants originate in industrial, developed countries located mainly in temperate regions, it has become obvious that natural transport processes have carried many pollutants far from their sources to places like mountain tops, polar caps and remote islands in the oceans. Those transport routes have been thoroughly examined in Chapter 2, which shows the enormous progress achieved to date in this field of environmental research. As a consequence, current models on fate and transport of contaminants are becoming more accurate, and no doubt will minimise in future the expensive analytical monitoring that was once required to understand the movement and accumulation of chemical pollutants in ecosystems. However, monitoring will still remain necessary to determine whether the remedial actions taken as a result of risk assessments and regulation are effective. This is particularly true of recent contaminant classes (e.g., fluorinated surfactants, pharmaceuticals) as little is known about these in terms of potential ecosystem level effects (see Chapter 7). Therefore, the use and development of monitoring devices such as passive samplers will be crucial in future.

As our knowledge of pollution increases, so also do the efforts to eliminate the most toxic pollutants. Encouraging examples of bioremediation by aquatic plants and micro-organisms are already available for some pesticides and metallic pollutants, as explained in Chapter 11. The natural capacity of aquatic ecosystems to eliminate most kinds of chemical pollutants is already being fostered by natural selection, because of the increasing pressure that such ecosystems experience in our heavily contaminated world. Indeed, nature always moves towards a point of equilibrium, thereby mitigating the impacts caused by pollution. Phytoremediation systems harness the adaptivity of natural systems and are already being used to sequester metal contaminants in many places, and in future could become essential management tools to eliminate pesticide residues in agricultural regions once long-term maintenance issues are resolved adequately. However, before they become widely accepted we need first to investigate the microbe-plant interactions in order to enhance the capabilities of the system, and also to explore the possibility of phytoremediation of 'emerging' organic contaminants such as new pesticides and even persistent organic compounds like dioxins, perfluorinated compounds, *etc.* The recalcitrant nature of the latter chemicals is certainly a problem that may require the combined action of both natural bioremediating systems and artificial remediation initiatives (e.g. incineration at high temperatures).

Impacts on Terrestrial Ecosystems

Throughout human history, metals and metalloids have accumulated within soils and surface waters of the earth, sometimes as a consequence of extraction from otherwise stable ore-bodies, redistribution of materials rich in metals (e.g., guano) or through combustion of fossil fuels. Our understanding of the ecological risks associated with increased environmental deposition of metals has improved as a consequence of the study of heavily polluted environments, where various elements such as Cd, Cu, Zn, Se, As and Hg have been deposited in high concentrations with severe and obvious detrimental effects on flora and fauna. However, we are only now beginning to appreciate the subtle and slow shifts in ecosystem function and dynamics that may occur through the movement of some elements through the food chains. Those flora and fauna that have been detrimentally affected through the bioaccumulation and biomagnification

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of elements such as Cd or MeHg, can only be detected by careful examination of their trophic movement through ecosystems, and by experimental manipulations. While the evidence to date has been summarised in Chapter 3, it is clear that more carefully planned studies in a wider selection of taxa (e.g., pelagic and coastal fish and invertebrates, insectivorous and predatory birds, and marine and terrestrial reptiles) are needed if we want to predict the overall consequences of increasing pollution by metallic and metalloid elements in ecosystems.

Among the most common organic pollutants are pesticides, which are routinely applied to the majority of the vast agricultural landscapes of the world as well as to many forested areas. Within the immense literature available on the subject, only effects at the community and ecosystem levels have been considered in this book. The known ecosystem impacts of pesticides have been studied mostly within the agricultural fields and pastureland with livestock, a summary of which is presented in Chapter 4. Our knowledge of pesticide impacts outside of those fields where pesticides are applied (the so-called off-crop areas) is scarce and in some cases anecdotal, which explains why the assessment of other terrestrial ecosystems surrounding the agricultural land, such as scrub or shrub landscapes and deserts, could not be considered in this book. This is precisely one of the areas where future studies on this topic should focus: the comparative assessment of impacts either of individual pesticides or their mixtures between the crop areas and the surrounding ecosystems (e.g., wetlands, scrubland, adjacent forests, etc). To a certain extent this has already been accomplished for bird communities in England and the USA, but much more work needs to be done in other countries (e.g., tropical ecosystems) to obtain a full picture of the effects at ecosystem level.

In parallel to scenarios in the agricultural field, Chapter 5 examines in detail four case studies of pesticides currently in use for forest management - two insecticides and two herbicides. Based on that evidence we are beginning to understand now the complex issues involved in these practices, but we are still unable to identify critical thresholds at which the system becomes unsustainable. Obviously, the damage that one single pest can cause in a monospecific boreal or temperate forest should not be measured only in economic terms, as it really affects entire animal and plant communities that depend on that forest. Here is where a balance must be struck between the need to control such pest, thus protecting those communities, and the ecological side-effects derived from the application of chemicals for controlling the pest. Thus, the need for identifying mitigative strategies under an adaptive management regime and their comparison with the 'do-nothing' approach. Since most of our knowledge on forest impacts comes from studies carried out in North America and Europe, it would be desirable to compare such impacts with those in other parts of the world, e.g., the more diverse forests of Japan, India or Australia.

Impacts on Aquatic Ecosystems

Pesticides impacts on freshwater communities are quite well understood and have been studied more widely than in any other ecosystem (see Chapter 6). Under normal circumstances, most pesticide residues in soil move eventually into water bodies found in the landscape. However, studies on this topic are biased towards fish and macro-invertebrates, whereas field studies about the effects of pesticides on macrophytes and microorganisms are scarce. Moreover, fungicides have received very little study [1], even though they are routinely applied to agricultural systems [2]. Of course, the effects of mixtures and multiple stressors in aquatic systems are still an important topic of research which has not been developed sufficiently. One area that requires more attention in future investigations is the effect of pesticides on ecosystem function as well as the services provided by the aquatic systems, whose potential losses have not been quantified yet. In connection with this objective, studies on ecosystem-based traits have already been proposed [3]. Another area that deserves more attention is recovery from pesticide exposure. It is not known which processes weigh more in the recovery of communities in freshwaters (recolonisation, compensatory effects of nutrients, higher temperatures?), and how widespread is the occurrence of long-term effects in the field.

A common problem encountered when evaluating the impacts of toxic pollutants in ecological risk assessments, whether they are pesticides, pharmaceuticals or any other chemical, is that predictions are based on rough extrapolations from effects at low levels of organization (e.g., individual or population effects measured by EC50s or NOECs). At the same time, measurements such as NOEC are unreliable and should be replaced by more accurate alternatives such as the no-effect concentration (NEC) [4,5]. Direct testing of individual chemicals or mixtures of chemicals using micro- and mesocoms is one effective way to overcome our current deficient approaches, and thus reduce our dependence on extrapolating results from lower levels of biological organization for both risk assessment and regulation. This is particularly relevant in the case of the emergent pollutants of concern (see Chapter 7), for

which very few ecotoxicological data are available in the first place. It follows from this that we need to develop improved empirical and experimental methodologies for evaluating field-based observations (and mesocosms) of anthropogenic stressors at higher levels of biological organization in both terrestrial and aquatic systems. Only then we will be able to determine the real impacts at community and ecosystem levels without flawed extrapolations [6], and improve our risk assessment modelling [7].

If freshwater ecosystems are the recipient of the first load of residues emanating from industrial processes, urban wastes or agricultural practices, eventually all pollutants enter the oceans. Those which are persistent or bioaccumulate in organisms will almost inevitably end up at the top of the marine food chain, and therefore will impact our fisheries. From the start, coastal ecosystems receive the bulk of the pollution *via* the discharges of rivers into estuaries and adjacent coasts. Marine currents spread these contaminants not only between the pelagic and benthic communities but also up and down the coastline, reaching coral reefs and finally the open ocean, where pelagic ecosystems represent the ultimate frontier. The complexity of these interactions in regard to chemical pollution is well described in Chapter 8, where the effects of ecotoxicants are analysed from the cellular and tissue level to the community level in progressive steps, culminating in the integrative assessment of overall impacts using various methods. The lack of information on how individual responses are transferred to ecosystem level is the major hurdle in the latter assessments: although bioassays have improved our understanding of the effects of pollutants, it is difficult to extrapolate their results to the ecosystem, as they lack ecological 'realism' [8]. As indicated above, this calls for new approaches to evaluate the effects at the highest level of organization, as well as for agreed methods for integrative assessment.

A similar approach was followed in Chapter 9 to describe the effects of a wide range of pollutants in coral reefs, an ecosystem often described as the 'rainforest of the oceans' due to its high biodiversity. Here, more than in any other ecosystem, is where our ecotoxicological knowledge is still lacking considerably. Since reefs are often exposed to multiple stressors simultaneously and for prolonged periods there is a need for information on the effects of chronic exposure to pollutants and stressor interactions (e.g., climate change factors) on a broad range of species and life history stages. In particular, there is concern that prolonged exposure to herbicide mixtures may trigger a cascade of unknown effects in the delicate and complex food web associated with the corals [9]. At the same time, more effort needs to be directed towards characterisation of the reef system's potential to recover from and adapt to ever increasing anthropogenic contaminants in a globally changing environment.

Finally, because pelagic processes (see Chapter 10) are important to both the surface layer (plankton) and benthic communities, further research in the ecotoxicology of these ecosystems should be directed towards integrating, not dividing, our understanding of the effects of pollutants in the different environmental compartments. There is a need for more research on how contaminants affect both primary producers and microbial loop components. Current knowledge is limited to effects on single algal species, and there is virtually no knowledge of impacts in more complex systems that include bacteria and protists. Although there is a better understanding of how contaminants affect some zooplankton species (e.g., calanoid copepods), there is an urgent need for more research on mesozooplankton species, including metamorphosing stages of numerous invertebrate taxa.

The chapters in this book provide a comprehensive review of the known ecological impacts of a wide variety of pollutants in all major ecosystems of the planet at the present time. Ignorance about the world is indeed the greatest enemy of humankind, so by bringing these facts to the public we hope will encourage the new generations to take more care in managing our activities. Only by knowing where we stand can we start thinking about solutions to minimise the release of contaminants and device capable remediation systems that may reduce or eliminate completely the major chemical threats to the environment. It is from this perspective that this book was conceived in the first place.

ACKNOWLEDGEMENTS

The editors would like to acknowledge the contribution of all authors of this book in preparing this concluding chapter.

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APPENDIX

ADDITIONAL REFERENCES TO CHAPTER 4

Included here are a number of references consulted to write this chapter, but that have not been cited in the text for reasons of insufficient space.

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Review of Pesticide Impacts on Non-Target Communities

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