7. ECOTOXICOLOGICAL EFFECTS

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7.1 INTRODUCTION

Ecotoxicology is the study of toxic effects of substances on species in ecosystems and involves knowledge of three main disciplines: toxicology, ecology and chemistry (Figure 7.1). Truhaut [2] coined the term ecotoxicology and included effects on humans in his definition, man being part of ecosystems. The current tendency is to include the effects of chemicals on all species in the biosphere in the definition of ecotoxicology [3]. However, in this section, we will not consider effects on man. Environmental risk assessment (ERA) shares many methodological aspects with human health risk assessment (HRA). However, there are a number of fundamental differences between ERA and HRA related to the scope of ERA which covers ecosystems and the biosphere. Fundamental aspects of ERA are discussed in the next section.

Ecotoxicological effects are changes in the state or dynamics at the organism level, or at other levels of biological organization, resulting from exposure to a chemical. These levels may include the sub-cellular level, the cellular level, tissues, individuals, populations, communities and ecosystems, landscapes and finally, the biosphere. The number and variety of interactions increases dramatically with increasing levels of biological complexity.

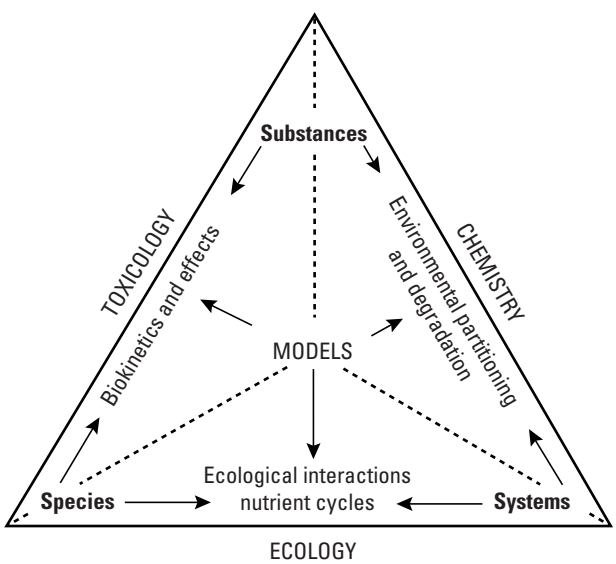

Figure 7.1. Ecotoxicology is a multi-disciplinary study into the toxic effects of substances on species in complex systems [1]. With permission.

Chemists are primarily interested in molecules and fate processes, toxicologists in biokinetics, modes of toxic action and effects in one or a number of standard test species, whereas ecologists are interested in the structure and function of ecosystems, effects, interactions and recovery at the population and ecosystem level, as well as in population genetics, biogeography, physiology and evolution. Due to the complexity of ecosystems, models are needed to describe the interactions between substances and species (toxicology), between substances and systems (chemistry) and between species in systems (ecology), as well as to account for the overall integration of these interactions (Figure 7.1). These models require input from mathematics, statistics and informatics.

Although the scientific backgrounds, interests and goals of the scientific disciplines differ, a synthesis of these disciplines is observed in the context of risk assessment. Normally, a sequence of research problems can be identified in the process of environmental risk assessment: the preliminary, the refined and the comprehensive stages [4]. Given the wide variety of research questions and topics (Table 7.1), this synthesis does not take place automatically. This chapter aims to illustrate how these disciplines can be integrated in ecotoxicology and are key to our methods for the risk assessment of chemicals.

This chapter will concentrate on ecotoxicological approaches used for the risk assessment of industrial chemicals. In Section 7.2 we will address some fundamental aspects of ERA. In Sections 7.3-7.5 we will introduce the core aspects of aquatic toxicity, sediment toxicity and terrestrial toxicity. For the aquatic environment the focus will be on freshwater species rather than on marine species. Readers interested in site-specific risk assessment, in effects beyond the population level, or in marine ecotoxicology, are referred to Suter [5], Suter et al. [6] and Hoffman et al. [7]. Two other subjects, i.e. factors modifying toxicity and mixture toxicity are presented in Sections 7.6 and 7.7. Sections 7.8 and 7.9 focus on ecotoxicogenomics and endocrine disruption. How PNECs are derived is presented in Section 7.10, whereas the assessment of PBT and

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Table 7.1. "Disciplines" of ecotoxicology and some of their research topics.

Chemistry	Toxicology	Ecology	Mathematics
Exposure assessment	effects assessment	community structure	environmental fate models
Transport	modes of toxic action	community functions	pharmacokinetic models
Partitioning	bioaccumulation	population dynamics	LC50 and NOEC statistics
Transformation	biotransformation	nutrient/energy cycling	species-species extrapolation
SARs/QSARs	extrapolation	various interactions	population and ecosystem models

vPvB substances is given in Section 7.11. Section 7.12 provides some concluding remarks. Selected references are provided in Section 7.13.

7.2 FUNDAMENTAL ASPECTS OF ERA

7.2.1 Taxonomic diversity

ERA deals with millions of species rather than just one, as in the case of HRA. Estimates of the total number of species on earth vary from 10 to 100 million [8], and approximately 1.5 million species have been taxonomically classified. Some of the large taxonomic groups are given in Table 7.2. The majority of phyla is found in the marine environment. The largest taxonomic groups are the insects, spermatophytes, molluscs and fungi. The mainly marine phyla of porifera and echinodermata (5000 species each) belong to smaller taxonomic groups. Among the vertebrates (a total of 45,000 species), fish species account for 23,000, amphibians 2500, reptiles 5000, birds 8500, and mammals 4500 species respectively per taxon.

In ERA, effects on species from a few taxonomic groups are studied using a limited set of tests. This raises the following question: how do we select species for testing from among the 1.5 million taxonomically classified species? The current minimum requirement for ecotoxicological testing in risk assessment with fish, daphnids and algae is nothing but a gross simplification of an ecosystem. In practice it would be impossible to test a representative sample (e.g., 1%) of such a variety of species. In fact, the current trend in ERA is to generate more information from less testing. The practice in ERA is to be pragmatic: species are selected on the basis of their ecological function (trophic level), their morphological structure, and their route of exposure [10].

Table 7.2. Numbers of classified species of some large taxonomic groups of the plant and animal kingdom [9].

Regnum vegetabile		Regnum animalia	
Algae	20,000	Protozoa	46,000
Lichens	20,000	Porifera	5,000
Fungi	100,000	Coelenterata	10,000
Bryophyta	23,000	Plathyhelminthes	12,000
Pterydophyta	11,000	Nematoda	10,000
Spermatophyta	250,000	Mollusca	120,000
		Annelida	8,000
		Arachnida	30,000
		Crustacea	35,000
		Insecta	750,000
		Diplopoda	7,200
		Echinodermata	5,000
		Chordata	45,000

Table 7.3. Selection criteria for an ecotoxicity test.

Chemistry
The species should be representative in terms of:
– ecological function (trophic level)
– route of exposure
– morphology
The species should:
– be easy to keep under laboratory conditions
– be easy to feed and to breed
– have a large reference database
The test should be:
– applicable to a wide range of chemicals
– short, predictive, sensitive and cheap
– statistically sound, i.e. produce a quantifiable concentration-effect relationship within the test period
– useful for risk assessment
– internationally validated by various laboratories
– standardized, i.e. give reproducible results when carried out according to good laboratory practice (GLP)
– accepted by the regulatory and scientific communities

Table 7.4. Criteria for selecting ecological endpoints [5]. With permission.

1. Biological relevance
2. Public relevance
3. Unambiguous operational definition
4. Accessibility to prediction and measurement
5. Susceptibility to the hazardous agent

Table 7.5. Unacceptable effects according to Stephan [12]. With permission.

1. Unacceptable reduction in survival
2. Unacceptable reduction in growth
3. Unacceptable reduction in reproduction
4. Unacceptable level of avoidance
5. Unacceptable percentage of gross deformities or visible tumours in organisms
6. Unacceptable concentrations of toxic residues in consumed tissues
7. Unacceptable flavour in consumed tissues

Practical aspects (Table 7.3) are important, as are social, economic and recreational factors.

7.2.2 Toxicological endpoints

In ERA, the goal is to protect populations and ecosystems, rather than individuals of certain species. It may be assumed that by protecting most of the species, the functioning of ecosystems is also protected [11]. Suter [5] postulated that ecological endpoints should satisfy five criteria (Table 7.4). This, however, leaves open the question of how to achieve an acceptable level of ecosystem protection. In routine toxicity testing, only a very limited number of species are tested and protection of all other species is assumed by extrapolating the results from toxicity testing on important endpoints: survival, growth and reproduction. This extrapolation should also protect ecological interactions, habitat factors, keystone species and functional groups. The terms "unacceptable" and "important" are value judgements and often lead to much debate. Stephan [12] has given seven major unacceptable effects that pollutants can directly or indirectly have on important species (Table 7.5).

Due to the large taxonomic diversity, life cycles vary greatly. Reproduction and growth depend on the species itself, time (e.g., food availability) and space (e.g., climatic conditions, soil-type, etc.). A further complication is the fact that species such as amphibians and insects undergo metamorphosis during transition from the larval to the adult stage. This affects their intrinsic sensitivity to pollutants, but may also affect the routes and magnitude of exposure.

To what extent then should ecosystems be protected? To protect all species in an ecosystem is problematic for two reasons. It is impossible to guarantee that the most sensitive species is tested [13] and the associated cost would be tremendous. Furthermore if testing results in very conservative Predicted No Effect Concentrations (PNECs) this would imply a ban on most human activities which would not be acceptable to society as a whole.

In practice, the protection of species and ecosystem function is assumed by establishing either the most sensitive species of the relevant toxicity data and applying safety factors, or a relevant statistic of the toxicity data set, such as a certain cut-off percentage p when the toxicity data are described by a theoretical distribution function; known as species sensitivity distributions (see Section 7.10.2). In both cases, additional assessment factors can be applied to extrapolate from single-species laboratory data to a multi-species ecosystem [14]. Some science-policy papers [15,16] have explained the use of a

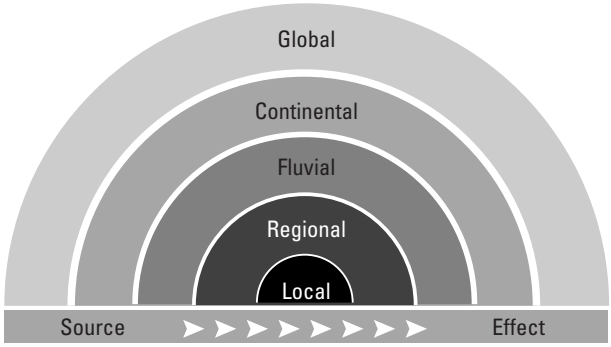

Figure 7.2. Five levels of scale at which environmental problems occur [15].

cut-off percentage as follows: the protection of all species at all times and places is not deemed necessary because ecosystems can tolerate some stress and occasional adverse effects. A reasonable level of protection can be provided by using a small cut-off percentage p of the species, pragmatically defined as 5% based on chronic toxicity data [17]. There are several problems with the cut-off percentage, e.g., the possibility that economically or ecologically important species fall within this 5% category.

Very pragmatic choices have been made in ERA to protect species, ecosystems, ecosystem functions or processes that have been successful in improving ecosystem quality, as demonstrated by cases such as the river Rhine [18].

7.2.3 Spatial scales

An understanding of the scale of environmental problems is key to effective risk assessment and remediation (Figure 7.2). Scale is linked to the area that a species needs to be able to maintain a stable population and the likelihood of exposure to a chemical in that area. Viable home ranges can range from very small for a microbe, to entire oceans for a blue whale.

Pollutant emissions may occur on a local scale, but due to redistribution and transport, the effects may become apparent on a global scale. The large-scale distribution of chemicals, however, should not be confused with the occurrence of effects, i.e., adverse effects may be restricted to certain sensitive populations or ecosystems which may occupy relatively small areas. Several examples can illustrate this scale dependency.

Pollution caused by heavy metals, many pesticides, and industrial chemicals exhibit their effects at the *fluvial scale* and/or *regional scale*. Indoor pollution caused by consumer products and air pollution in cities are examples of pollution on the *local scale*.

Acidification is the process whereby harmful effects occur as a result of pollution from the atmosphere with acid-forming substances and ozone. Acidification leads to damage to forests, heath land, aquatic ecosystems, agriculture, buildings and materials. Acidification arises from acid-forming substances such as sulfur dioxide, nitrogen oxides and ammonia. Oxygen radicals are formed from volatile organic compounds and nitrogen oxides. These react with the oxygen present in the air to form ozone. The harmful effects of ozone in the populated environment appear to be very similar to those of acid-forming substances and exert their influence on a *continental scale*. Other spatial examples include the impact of long-range transport and the effects of persistent organic pollutants (POPs) such as DDT and PCBs [19].

Finally, some pollutants can exert effects on the entire biosphere. Although the ozone layer in the stratosphere only contains minute quantities of ozone it has an important function. It absorbs ultraviolet (UV) radiation from the sun, which is harmful to man and ecosystems. As has become clear in recent years, the ozone layer is being depleted by a number of substances, such as chlorofluorocarbons (CFCs), which exert their effects on a *global scale*.

7.2.4 Temporal scales

Over the last two decades, our awareness of the importance of the spatio-temporal aspects of environmental pollution has increased. ERA deals with the sustainability of ecosystems, with large-scale effects, long-term processes and long recovery times (Figures 7.3 and 7.4). In HRA we are mainly concerned with individuals with a maximum exposure period of approximately 70 years. The generation time of man (approximately 25 years) is long compared to many other species (Table 7.6). This certainly goes for politicians, whose "generation time" is even shorter (approximately 5 years), while they make decisions that sometimes affect many generations to come [1]!

In ERA we are concerned with effects on a variety of temporal scales. Time scales are relatively long in relation to higher levels of biological organization [5,20], biological processes or evolutionary processes (Figure 7.3). This wide variety poses specific problems in ecotoxicity testing. In ERA, the hazard of a chemical is initially deduced from short time (acute) toxicity tests (e.g., see Section 7.3.3). However, depending on the

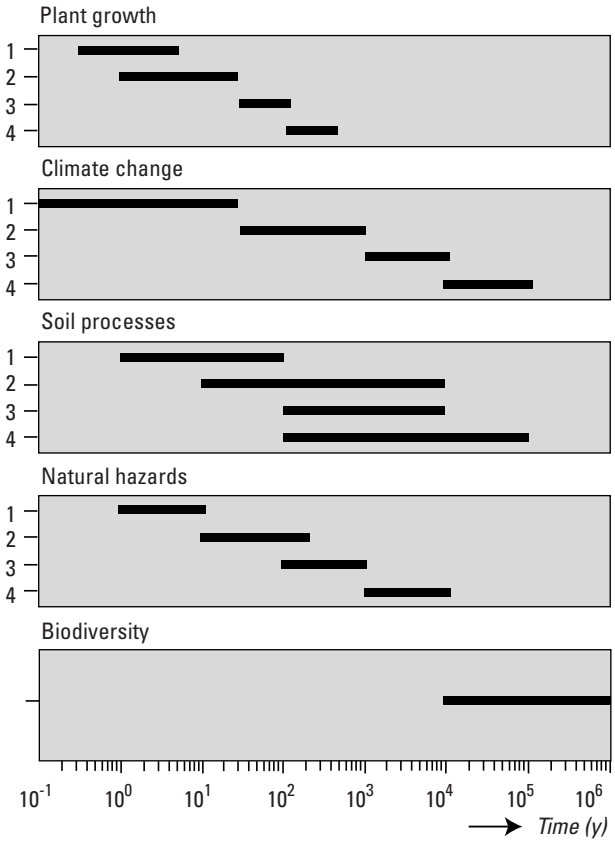

Figure 7.3. Timescales of processes affecting sustainability of ecosystems. *Plant growth:* (1) length of one growth cycle of annual crops, including rotation up to 5 years, (2) length of one growth cycle of perennial crops, (3) length of growth cycle of production forest and (4) average biomass turnover rates of tropical rainforest. *Climate change:* (1) time scales of meteorological fluctuations: decades (smallest time unit used in simulation models of plant growth), seasonal and annual changes variations up to 30 years, the minimum record length for reliable assessment of climatic parameters, (2) historical climate changes (cf. Little Ice Age 1500-1850 AD) (3) Holocene (cf. climatic optimum 6000 years BC) and (4) Pleistocene, stadial/interstadial and glacial/interglacial oscillations. *Soil processes:* (1) time needed for complete erosion of topsoil, (2) time needed for severe nutrient depletion by leaching in humid tropics, (3) the same for the temperate zone and (4) time needed for formation of fully developed topsoil. *Natural hazards:* (1) frequency intervals between moderate floods in alluvial areas, (2) the same for major disastrous floods, (3) frequency intervals for andesitic volcanic ash falls and (4) the same for destructive volcanic eruptions. *Biodiversity:* time needed for restoration of macrofauna and macroflora biodiversity by evolution after major disturbance. From Fresco and Kroonenberg [20]. With permission. Copyright Elsevier.

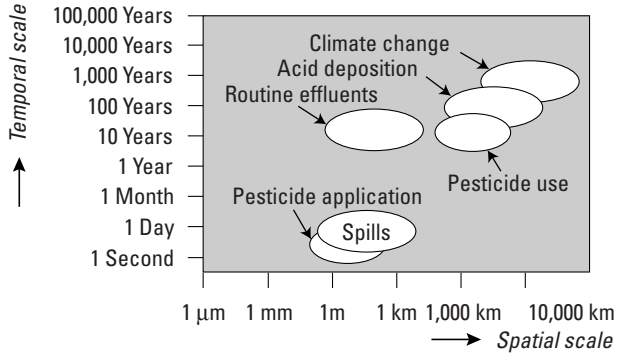

Figure 7.4. Chemical anthropogenic hazards on spatial and temporal scales. From Suter[5]. With permission.

typical generation time of a species and the mode of action (MOA) of the substance concerned, the distinction between short-term and long-term (chronic) toxicity is sometimes arbitrary. When specific hazards are identified, e.g., when a substance has effects on growth, reproduction and development, longer toxicity tests can be performed covering a partial or a full life cycle. The effects of some chemicals may be observed in the sensitive early life stages (ELS), such as embryonic development or neonates, for which ELS tests are developed. The impact of these effects on the viability of the population can be analyzed (see Sections 7.3.4 and 7.3.5).

7.2.5 Complexity of exposure

In ERA, exposure assessment is often restricted to external exposure: concentrations in media such as water, soil, sediment and air. These external concentrations, predicted environmental concentrations (PECs), are related to external effect levels (PNECs). In addition, ERA deals with a wide variety of species and factors influencing actual exposure which complicates exposure assessment (Table 7.7). When comparing the effects of chemicals under different exposure conditions or on different species, the bioavailability of the chemical needs to be taken into account (see Chapter 3).

In some cases it is more useful to determine the internal exposure concentration in species that have been exposed. Internal effect concentrations can then be compared with "critical body residues" (CBRs) associated with the onset of mortality for specific classes of chemicals, such as narcotics or polar narcotics. Chemicals with the same mode of action will have a relatively narrow range of critical body concentrations [21,22]. The internal dose can be estimated from external exposure with toxicokinetic models [23] or

Table 7.6 Generation times for some species.

Species	Generation time
Bacteria	≈ 0.1 d
Green algae (*Chlorella* sp.)	≈ 1 d
Waterflea (*Daphnia* sp.)	≈ 10 d
Snails (*Lymnaea* sp.)	≈ 100 d
Rats	≈ 1 y
Politicians	≈ 5 y
Man	≈ 25 y

Table 7.7 Summary of factors contributing to the complexity of exposures in ERA.

Niche-partitioning	Exposure time
Abiotic factors	Non-linearity
Surface/volume area	Consumption patterns
Life history	Feeding and growth rate
Behaviour	Biotransformation

with relatively simple partitioning models [24,25]. This may make it possible to move from a purely descriptive external exposure to internal exposure with toxicological relevance. Once this step is taken, extrapolation to chemicals with similar modes of action (MOA) immediately becomes possible [26-28]. It should be noted that the use of the CBR concept for general risk assessment needs improvement. Most notably the CBR distribution for narcotics is still quite wide, both between different chemicals and between species or phyla. Uncertainty is reduced by using lipid normalization, but is also related to the quality and interpretation of some of the original studies [29]. It is also not always easy to define the MOA of a substance using the currently available tools [30,31]. Hopefully, the development of structural alerts, read across and other methods (Chapters 9-11) will help to improve the use of this concept in risk assessment.

Niche partitioning

Once a chemical enters the environment, partitioning and degradation processes take place (Chapter 3). Species in specific ecological niches may be exposed intensively, depending on the chemical's fate and behaviour. Benthic species, for instance, that burrow in the sediment, such as the lugworm, are in intense contact with pollutants that partition to the sediment particles that they ingest. Exposure assessment in sediments and soils is complicated and, in many cases, predictions can only be made for certain groups of chemicals under a variety of assumptions such as equilibrium-partitioning between pore water and soil or sediment [32,33]. For the sake of simplicity, environmental exposure models often assume a homogeneous distribution of chemicals in a limited number of narrowly defined compartments. Nature, however, is not homogeneous but heterogeneous with many niches occupied by a great variety of species adapted to these niches.

Figure 7.5 shows the relationship between the atmospheric fallout of pollutants and concentrations in eel in Sweden. It shows that aerial transport can lead to high residues of bioaccumulating substances in fish. Similar observations were made in monitoring studies on pesticides in rain [35]. These monitoring studies showed the presence of high concentrations of some volatile pesticides in rain where water quality standards were exceeded by more than a factor 100. Thus, habitats or niches, such as shallow lakes which are highly dependent on rain, may be intensively exposed. This also applies to lichens, bryophytes and fungi living on trees or in the top horizons of soils with a wide variety of bacteria, plant and animal species present in these niches. Persistent organic pollutants (POPs) can be transported and deposited in vulnerable ecological niches. In the Canadian arctic, POPs have been shown to accumulate in the food chain from lichen to caribou to wolf [36].

Exposure time

In routine toxicity testing in ERA, the classical dose or concentration-response model is used where exposure time is kept constant. The exposure time in such tests depends on the species and its generation time (Table 7.6). Exposure time is an important variable, often crucial to toxicity (Sections 7.3.1 and 7.3.2). If the temporal dynamics of the endpoint that is studied are included, the statistical power of the test increases and effects can be expressed as functions of both exposure concentration and exposure time [37]. This then allows additional toxicokinetic parameters of the tested species to be estimated such as the elimination rate of a chemical [38], but can also provide input in models for population dynamics (Section 7.3.5). Despite the obvious advantage of gaining more insight with the same toxicity tests, little progress has been made with applications in regulatory toxicity testing.

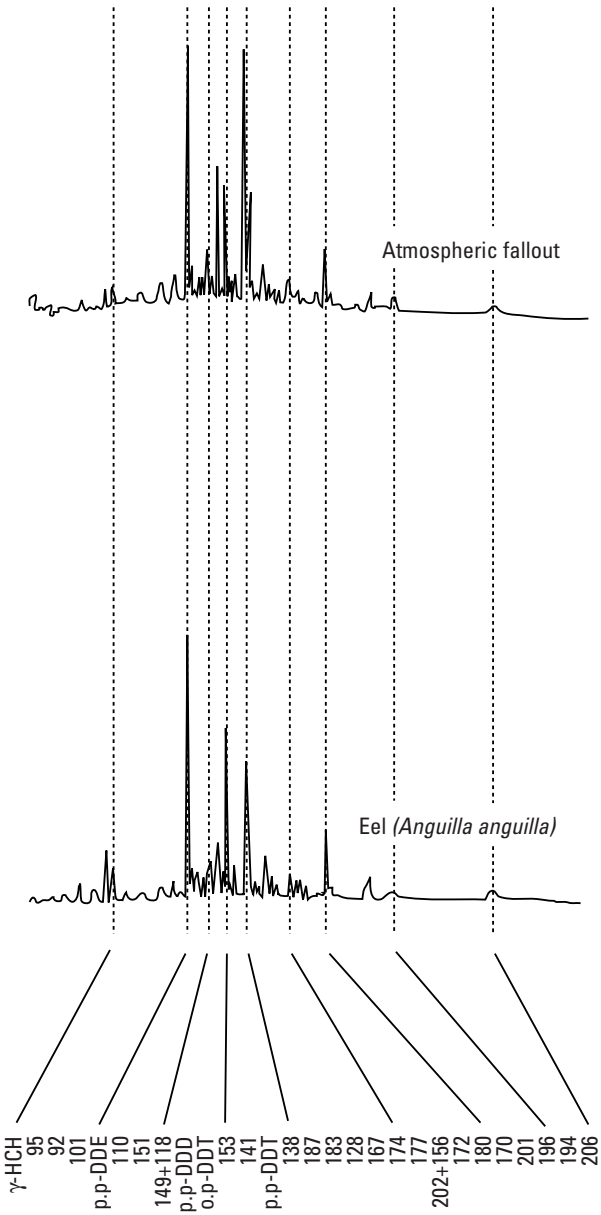

Figure 7.5. Composition and distribution of persistent pollutants in atmospheric fall-out and eel in Swedish lakes. Numbers represent 25 PCB congeners. From Larsson, Hamrin and Okla [34]. With permission. Copyright Elsevier.

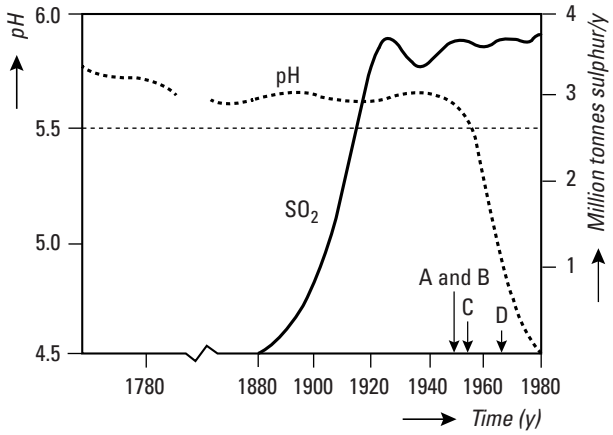

Figure 7.6. Non-linear changes in pH due to emissions of SO2 and extinction of smallmouth bass (A), lake whitefish (B), longnose sucker (C) and lake trout (D) in the period from 1760-1980. Modified from Stigliani [39]. With kind permission of Springer Science and Business Media.

Abiotic factors

The magnitude of external exposure is subject to large spatio-temporal fluctuations. These fluctuations may be caused by varying emissions of the chemicals but a number of abiotic factors may also be involved, such as soil type and climate, e.g., wind speed, temperature, humidity, and rainfall (see Section 7.6). The geographic and temporal variations in river flows may also differ and affect the actual exposure situation. It is clear that photochemically degradable chemicals or readily biodegradable chemicals may do much more harm in cold, northern climatic regions than in tropical areas with much more sunshine.

Non-linearity

Long-term observations of emissions and exposure to chemicals show that unpredictable changes may occur (Figure 7.6). These changes are related to changes in a number of capacity controlling properties (CCPs) e.g., the cation or anion exchange capacity, pH, redox potential, organic matter content, soil texture, salinity and microbial activity [39]. Acidification, climate change, pollution-induced reductions in microbial activity, and lowering of the groundwater table are among the factors that may alter CCPs. These changes in CCPs may alter the bioavailability of pollutants by several orders of magnitude (Section 7.6 and Chapter 4) and may thus lead to unexpectedly strong ecological effects.

Surface area/volume ratio

So far we have dealt with factors modifying external exposure. Let us now turn to internal exposure, for which chemicals have to pass external barriers (Chapter 3). Chemicals can pass through biological barriers, e.g., the cell membrane, lungs, gills, skin, cuticle, etc. by diffusion. For soil-dwelling species with firm cuticles or exoskeletons, such as many arthropods, direct dermal

Table 7.8. The relationship between surface area and volume of species.
For the sake of simplicity, the shape of species is taken to be cubic.

Edge (mm)	Surface area (mm^2)	Volume (mm^3)	Surface/ Volume ratio	Examples
0.001	$6x10^{-6}$	10^{-9}	6000	cells/bacteria
0.01	$6x10^{-4}$	10^{-6}	600	algae (*Chlorella* sp.) and fungi (*Penicillium* sp.)
0.1	$6x10^{-2}$	10^{-3}	60	protozoans (*Paramecium* sp.)
1	6	1	6	nematodes and crustaceans (e.g. *Ceriodaphnia dubia*)
10	$6x10^{2}$	10^{3}	0.6	earthworms/small fish (e.g. guppy)
100	$6x10^{4}$	10^{6}	0.06	rainbow trout/pigeon
1000	$6x10^{6}$	10^{9}	0.006	sharks/cows

uptake of pollutants from the soil does not seem to be an important exposure route. However, research carried out with spiders suggested that dermal exposure to contaminated soil may be important even for these species. For soft-bodied organisms living in close contact with the soil, such as earthworms, uptake via the skin is important [40]. This means that earthworms and probably other soft-bodied soil organisms such as protozoans, tardigrades, nematodes and enchytraeids, take up chemicals mainly via the body wall [41]. For these species, the toxicity of chemicals in the soil is mainly determined by the pore water concentration (Section 7.5). This pore water concentration can be derived from the total concentration using sorption data [32,33].

Dermal uptake via diffusion depends on the permeability of this barrier to the chemical, the concentration gradient and the surface area over which diffusion takes place. The larger the surface area and concentration gradient, the greater the transport rate. The transport rate is inversely related to the length of the diffusion path (Fick's law). This equation can be written as follows:

$$M = DA\,(C_1 - C_2)\,/\,L \qquad (7.1)$$

where

- M = the rate of diffusion (mol/s)
- D = the diffusion coefficient (m^2/s)
- A = the surface area over which diffusion takes place (m^2)
- $(C_1\text{-}C_2)\,/\,L$ = the concentration gradient, i.e., the difference in concentrations (mol/m^3) divided by the length (L) of the diffusion path (m)

Diffusion is more efficient in cells or tissues with short diffusion paths, i.e., in tissues where the surface area/ volume ratio is high (unicellular species or specialized tissues such as the lungs or gills). In nature, the permeability of external biological barriers varies widely. The same applies to surface area/volume ratios which depend on the size of the species (Table 7.8). Thus, diffusion, i.e., exposure, is much faster in small species than in large species. Large species often have special adaptations to accelerate diffusion processes for gas exchange, e.g., internal or external gills or lungs. These adaptations also affect the rate of chemical uptake. Furthermore, the toxic effects of chemicals which affect cell membranes, e.g., surface-active chemicals, will be greater in small species or tissues with high surface area/volume ratios. This is why many surface-active chemicals have bactericidal and algicidal properties and are relatively toxic to fish.

Consumption patterns

The consumption pattern of species (including man) differs widely. There are omnivorous, carnivorous and herbivorous species and many food specialists, such as caterpillars, mites, ticks and some bird species. Their average daily consumption patterns, i.e., the food chains, are largely unknown, both qualitatively and quantitatively. In order to illustrate the importance of consumption patterns to exposure assessment, fish consumption will be used as an example. In Table 7.9 a comparison is made between man and a fish-eating bird, the cormorant (Figure 7.7). The average daily consumption of fish (wet weight; wwt) in The Netherlands and Japan is 10 g and 96 g, respectively [42], while the cormorant's daily intake is 400 g to 750 g.

Table 7.9. Fish consumption patterns and daily intakes of hexachlorobenzene (HCB) in The Netherlands (NL), Japan and in the cormorant *(Phalacrocorax carbo).*

	NL	Japan	Cormorant	
			male	female
Body weight (kg)	70	70	2	3
Fish consumption (kg_{wwt}/d)	0.01	0.1	0.5	0.5
Fish consumption (70 kg_{bw})[a]	0.01	0.1	17.5	11.6
Intake of HCB[b] (mg/kg_{bw}·d)	0.03	0.3	50	33.3

[a] Fish consumption expressed in terms of the body weight of man (70 kg).

[b] The Swedish product standard for HCB (200 µg/kg fish) was used for the calculations [32].

When the fish consumption of cormorants is expressed in terms of human body weight, it can be concluded that their daily consumption is enormous (11.6 to 17.5 kg fish per day). It is more than 100 times the average daily fish consumption in Japan and more than 1000 times the average in The Netherlands. The second conclusion is that the exposure of food specialists to pollutants can be extremely high, which should be taken into account in risk assessment for secondary poisoning (Section 7.10.3).

Life histories

There is an overwhelming variety of species (Table 7.2). Many plants and especially some parasitic fungi, such as rusts (Uredinales) and smuts (Ustilaginales), have very complicated life histories. The same applies to parasitic nematodes, mites and insects. Many insects, such as butterflies (Lepidoptera), stoneflies (Plecoptera), mayflies (Ephemeroptera), dragonflies (Odonata) and midges (Diptera), undergo a metamorphosis with concomitant changes in the niches they occupy. Many amphibian and oviparous fish species go through a number of different embryonic and post-embryonic stages each with their own exposure patterns (Figure 7.8). Particularly the early life stages appear to be very sensitive to pollutants. Frogs, toads and many insect species undergo transformations which take them from an aquatic to a terrestrial life-cycle stage. This has consequences for both their direct exposure routes (exposure via air, water and soil) and their indirect exposure routes, i.e., their food consumption patterns. In other words, life-history patterns are extremely important in ecotoxicological testing. The diversity in life histories is huge. Unfortunately, qualitative and quantitative information is often not used or lacking.

Figure 7.7. A food specialist: the cormorant *(Phalacrocorax carbo)*. Courtesy of P. Van Der Poel, Huizen, the Netherlands.

Feeding and growth rates

Many abiotic factors can modify the feeding and growth rates of species and may also determine the type of diet and hence exposure. Feeding rates determine the uptake rate of chemicals, whereas individual growth rates or rates of cell division may be seen as "internal dilution processes" for body burdens of chemicals. For many species data on feeding and growth rates are lacking but reasonable approximations are available in the literature [43,44].

Behaviour

The behavioural responses of organisms to toxicants may modify subsequent exposure. The most commonly reported example is avoidance of contaminated food, soil or water. However, toxicants may also go unnoticed or attract organisms. Migration, hibernation, isolation, breeding and the formation of resistant structures such as plant seeds or the winter eggs (ephippia) of daphnids all affect the actual exposure of organisms. There is little behavioural data for many species, but avoidance behaviour is now recognized in several guidelines.

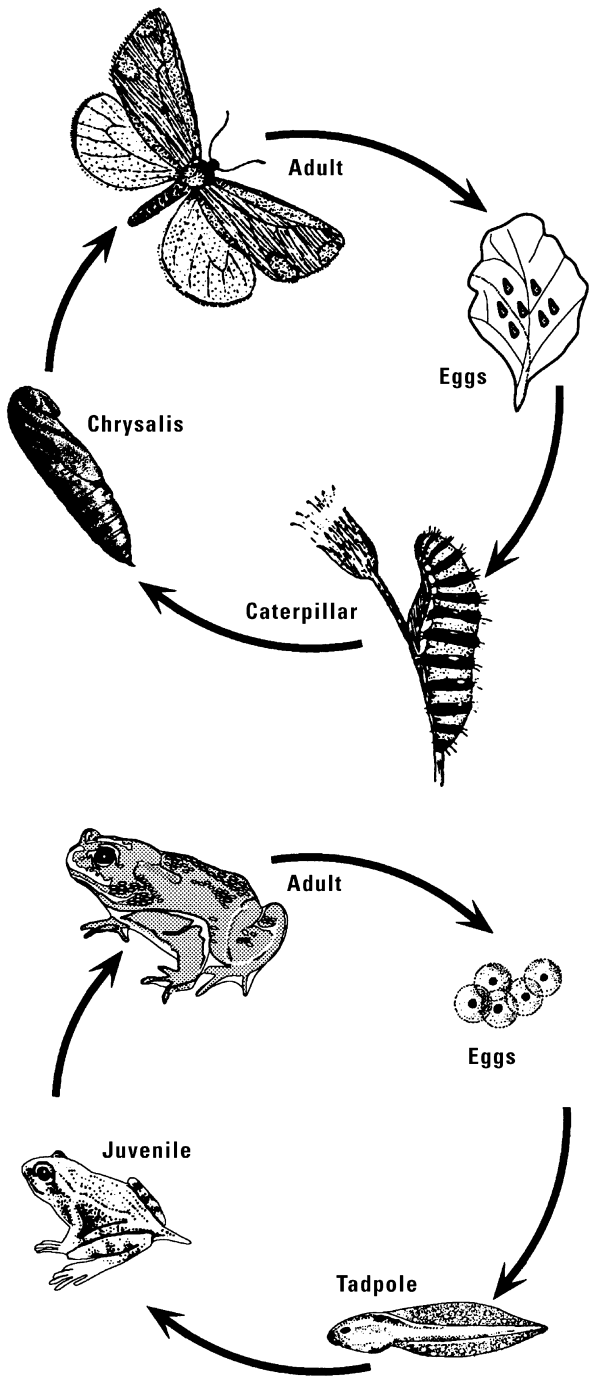

Figure 7.8. Life cycles of an insect and amphibian species with concomitant changes in exposure patterns.

Biotransformation

The biotransformation of toxicants is of essential importance. It may result in detoxification and elimination of the metabolite, or it may enhance toxicity through the formation of toxicologically active metabolites (Chapter 3). Biotransformation patterns vary between organisms and may be modified by a number of abiotic factors, such as temperature. However, the absence of empirical biotransformation rates and information on formed metabolites is mostly lacking which hampers risk assessment. This is generally recognized as an important field of study. Information on rates and routes of biotransformation has been compiled to provide a basis for models that predict which metabolites are formed [45].

Conclusions

In conclusion, there are many factors in ERA which are crucial for the calculation of external and internal exposure concentrations. In ERA there is no single PEC but a variety of PECs. These PECs are species dependent and are influenced by a large number of biotic and abiotic factors. Most of them are unknown and where they are known, they are often not quantified. This lack of information prevents the calculation of actual received dose or internal exposure concentrations. It is mainly for this reason that in ERA exposure predictions are restricted to predictions of external concentrations (PECs) in media such as soil, sediment, air and water. Exposure predictions may go beyond this level for only a few species.

7.3 AQUATIC TOXICITY

7.3.1 Exposure systems

In aquatic toxicology, exposure is of particular importance. Contrary to mammalian or avian toxicology, where the toxicant is often administered directly to the organism via food or injection which leads to a known internal dose, exposure in the aquatic environment is much more complicated. In most aquatic toxicity tests the toxicant is dissolved in the test medium. The test organisms build up an internal concentration through the skin and particularly through the gills, by partitioning between water and the organism (Chapter 3). Because the internal concentration of the toxicant is usually not known, toxicity is expressed as external concentration in the exposure medium, rather than as internal concentration.

Because the actual concentration of the chemical together with the duration of exposure is of prime

importance in determining whether an adverse effect will occur or not, concentration and exposure time must be considered carefully. Maintaining stable exposure concentrations is a problem in aquatic toxicity testing which is why particular attention is devoted to this subject. Exposure to volatile chemicals, degradable chemicals, adsorptive, highly bioaccumulative chemicals and chemicals with low water solubility poses great problems in practice. Therefore, various methods have been developed for exposing aquatic organisms to such substances in order to test for ecotoxicological effects, with varying degrees of success. Four general types of toxicant delivery systems are used in toxicity testing: (1) static, (2) renewal, (3) flow-through, and (4) food.

Static exposure systems

Static exposure systems are much simpler in design and operation than flow-through systems. They generally consist of exposure vessels in which the test organisms are subjected to the same test solution for the duration of the test. The test substance is administered once only and the solution is not changed or renewed. Such systems are only generally used for acute tests with a few exceptions, and then generally for technical reasons (e.g., the alga growth inhibition test) [46]. The advantages of this type of exposure system are its simplicity, reduced handling stress to the organisms compared with renewal techniques, and low cost. Static systems are generally used where:

- The test substance is known to be highly soluble and stable in aqueous solution.
- The test substance is not expected to be toxic at the limit test concentration.
- A multi-component test substance is tested using a water-accommodated fraction.
- A very small quantity of the test compound is available.
- Disposal of the test solutions is critical.

Nevertheless, there are a number of problems that commonly arise in static systems:

- Decrease in the concentration of the test material through loss due to evaporation, transformation, sorption, biodegradation or bioaccumulation in the test species. If the exposure concentrations deviate by more than 80-120% of nominal, they should be expressed relative to the geometric mean of the measured concentrations at the start and end of the test.
- Low dissolved oxygen concentrations occur if the test material has a high biochemical oxygen demand (BOD) or as a result of the accumulation and microbial degradation of faecal material. This can be circumvented by the use of aeration and oxygen measurements, unless the substance is expected to be volatile.
- Starvation, where feeding is not possible because it could interfere with the bioavailability of the toxicant.

Owing to these limitations, static exposure systems are generally used in short-term tests (< 96 h), with non-volatile or slowly degradable chemicals with a low bioaccumulation potential and a low loading (biomass/volume of water) of test organisms. Box 7.1 shows the consequences of high loading of test vessels. The results of simulation studies can be seen in Figure 7.9.

Renewal exposure systems

Renewal or semi-static exposure systems are a compromise between flow-through and static exposure systems. The apparatus used is essentially the same as in a static system; however, instead of exposing the test organisms to the same solution throughout the test, the test organisms are periodically transferred to fresh solutions or a proportion of the solution is removed and renewed with fresh test solution. Renewal exposure systems allow feeding and the test can be prolonged indefinitely. Renewal exposure systems are mainly used with small organisms (e.g., *Daphnia* spp.) that could be flushed out of flow-through systems or are very sensitive to currents (e.g., copepods). They are also useful when only a limited amount of test material is available but a prolonged test is required. Although static-renewal systems circumvent some of the disadvantages of static systems, some disadvantages remain:

- Frequent handling of the test organisms increases stress and the possibility of injury.
- The concentration of the test material may not be constant throughout the test.
- It is more labour-intensive than static tests.

Flow-through

Flow-through or continuous-flow exposure systems are designed to expose the test organisms to a relatively constant concentration of the toxic material and control water flowing into and out of the exposure chambers. The flow may be continuous or intermittent. Flow-through systems are able to maintain a constant concentration of the test material, a constant water temperature, and maintain the dissolved oxygen concentration in water at between 60 and 100% saturation. In the case of fish, the flow should preferably be 6 litres of test water per gram of fish per day. In addition, a flow rate of at

Box 7.1. Consequences of high loading of test vessels for the exposure concentration under static exposure conditions

Basic information

n-octanol-water partition coefficient (K_{ow})	= 100,000
Mass of fish (M)	= 0.001 kg
Fat content of fish	= 5%
Bioconcentration factor (BCF) ≈ 0.05 x K_{ow}	= 5000
Volume of test vessel (V)	= 1L
Test concentration (C_w) in water (at t = o)	= 1 mg/L

Mass balance equation at t = o

No bioaccumulation, total mass of toxicant in water: C_w x V = 10 mg

Mass balance after prolonged exposure

We assume t ≈ ∞ and no losses due to (a) volatization, (b) biotic or abiotic degradation and (c) adsorption to the wall of the test vessels. Therefore the chemical only partitions between fish and water. The mass balance equation then becomes:

(mass in fish)	+ (mass in water)	= total mass
(BCF x M x C_w)	+ (C_w x V)	= 1 mg
(5000 x 0.001 x C_w)	+ (C_w x 1)	= 1 mg

Result

In this example C_w becomes 0.16 mg/L. With a higher loading of fish (0.01 kg/L) or when testing superlipophilic chemicals with a K_{ow} of e.g., 1,000,000, the concentration in water would drop to 0.02 mg/L.

least five times the test chamber volume per 24 hours is needed. Many types of toxicant delivery systems have been designed for use in flow-through exposure systems. Peristaltic and syringe pumps are widely used for the delivery of concentrations of a toxic chemical to aquatic organisms [47]. Another common system is the proportional diluter, a gravity-fed system, which delivers a series of more or less constant concentrations of the test material. First developed by Mount and Brungs [48], the proportional diluter has been modified and improved for a wide variety of applications.

Flow-through systems are the preferred method for aquatic toxicity studies on fish, particularly if the test substance is not stable or is poorly soluble. For volatile substances they should be used in conjunction with a closed system. In some cases a headspace inside such a system is acceptable depending on the Henry constant of the substance. The major disadvantage of flow-through systems and proportional diluters is their complexity; they require considerable attention and maintenance if they are to function properly, such as frequent verification of the actual concentrations of the test compound. However, once functional the fluctuation of test substance concentration is generally much lower than in static or semi-static tests, thereby increasing confidence in the results of the study.

Food

Highly bioaccumulative substances are usually poorly water soluble which is problematic in the standard test systems for both toxicity tests and bioaccumulation tests designed to determine the bioconcentration factor. For substances with a log K_{ow} > 4.5 (decimal logarithm of the *n*-octanol-water partition coefficient), it is difficult to achieve a constant exposure level that is high enough to easily measure toxicity or bioaccumulation. Test concentrations that exceed the solubility level or are supplemented with a large amount of solvent (OECD Guidelines recommend a maximum of 100 mg/L) may result in an underestimation of true toxicity levels due to physical effects. In risk assessment, tests with effect levels above the solubility level are considered to be invalid.

In dietary tests, fish are fed chemical-spiked food at a fixed concentration over a specific period of time, depending on the expected half-life of the chemical. At the end of the food exposure period, the remaining animals are provided with uncontaminated diet and

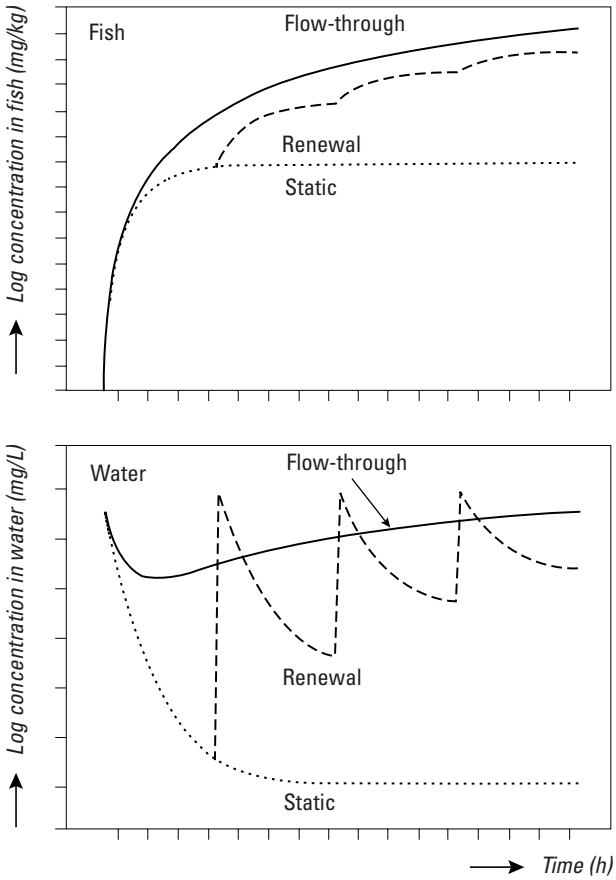

Figure 7.9. Simulations of concentrations of a non-volatile persistent chemical in fish and water in a static, a renewal and a flow-through system. It is assumed that no losses occur due to volatilization, adsorption and degradation of the chemical. Courtesy of D. De Zwart, National Institute for Public Health and the Environment, Bilthoven, The Netherlands.

analyzed to establish a depuration curve. From these data, the half-life, dietary assimilation and bioaccumulation factor can be easily derived [49]. It is also possible to determine whether the (final) toxicity at a given exposure level was observed under steady-state conditions or not. It should be noted that there is currently no standard procedure for performing a dietary-based toxicity test for fish, although exposure to contaminants in food may be commonplace in *Daphnia* reproduction tests due to the time of exposure of the food source (algae) to the test substance.

7.3.2 Analysis of toxicity tests

Lethal or sublethal effects of chemicals are typically analyzed in a setup with a series of containers or tanks with increasing concentration of a chemical and must include a control. The response of the organism to the increasing concentration of the chemical is used to determine the endpoint of interest. For acute toxicity tests, mortality is expressed as the median lethal concentration (LC50), which is the estimated concentration of the test material that will kill or immobilize 50% of the test organisms in a predetermined period of time. Similarly, median effect concentrations (EC50) can be calculated for any specified effect. For EC values, the endpoint has to be specifically defined. If an asymptote has been reached in the toxicity-time curve (Figure 7.10), the final value is called the incipient or ultimate LC50, or threshold lethal concentration. Because this value eliminates the influence of time of exposure, the result can only be compared to similar L/EC50 values and its use cannot easily be extended to determining ecological significance in terms of population effects.

A variety of methods can be used to calculate LC50 and EC50 values and their confidence limits, of which the non-parametric and the parametric methods are most commonly applied. The most common parametric methods are based on transforming the concentration levels so that the transformed concentration-mortality relationship has a known concentration-effect relationship [50,51]. The nonparametric methods, such as the Spearman Karber method, use the monotonicity of the concentration-mortality curve to generate an empirical curve from which LC50 and EC50 estimates can be obtained. Reviews are provided by Hoekstra [52] and Newman and Unger [3].

Another summary statistic that is commonly used in toxicity tests for regulatory testing is the no observed effect concentration (NOEC). This assumes that there is a concentration (threshold) of a toxicant below which no adverse effect is expected (Figure 7.11). The threshold concentration-response curve climbs at the threshold concentration; the response is zero up to that point and increases beyond that point. The NOEC is determined by hypothesis testing, e.g., by the Williams test or a post-analysis of variance (ANOVA), such as Dunnet's multiple comparison test [50]. In a statistical analysis of variance, the NOEC is determined by comparing the responses of the exposure concentrations with the control (unexposed) responses to test the zero hypothesis that they are the same as the control responses. Such an analysis will produce the lowest observed effect concentration (LOEC), i.e., the lowest concentration whose mean response differs significantly from the control. The NOEC is defined as the test concentration directly beneath the LOEC. In some cases no effects are observed

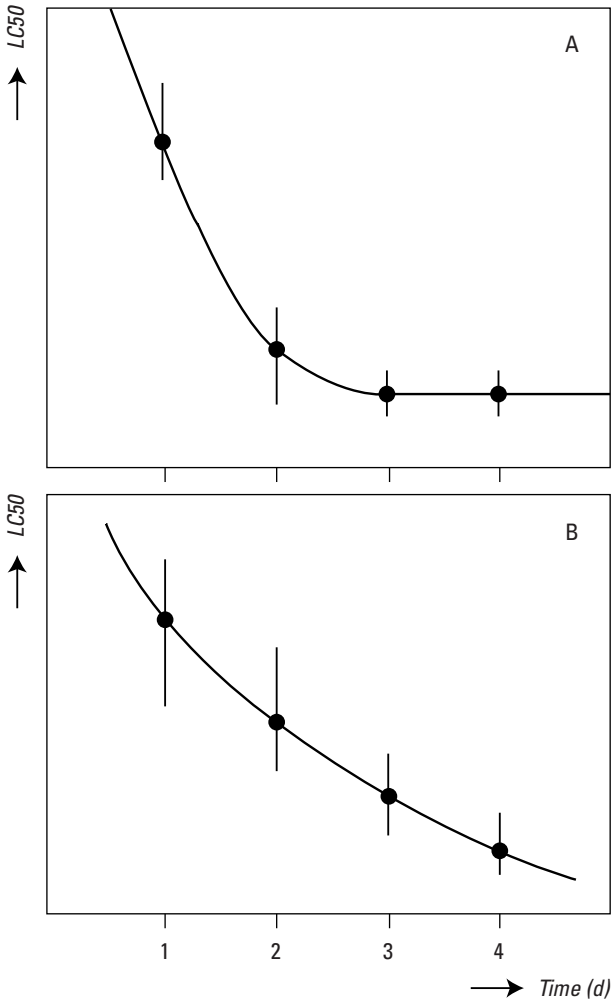

Figure 7.10. LC50 values and their 95% confidence limits vary with time. Two LC50-time curves are shown. The incipient LC50 for chemical A, i.e. the concentration of a chemical which is lethal to 50% of the test organisms as a result of exposure for periods sufficiently long for acute toxicity essentially to have ceased, is reached within 3 days. For chemical B the asymptotic part of the LC50-time curve is not reached and prolonged testing is necessary to estimate its incipient LC50.

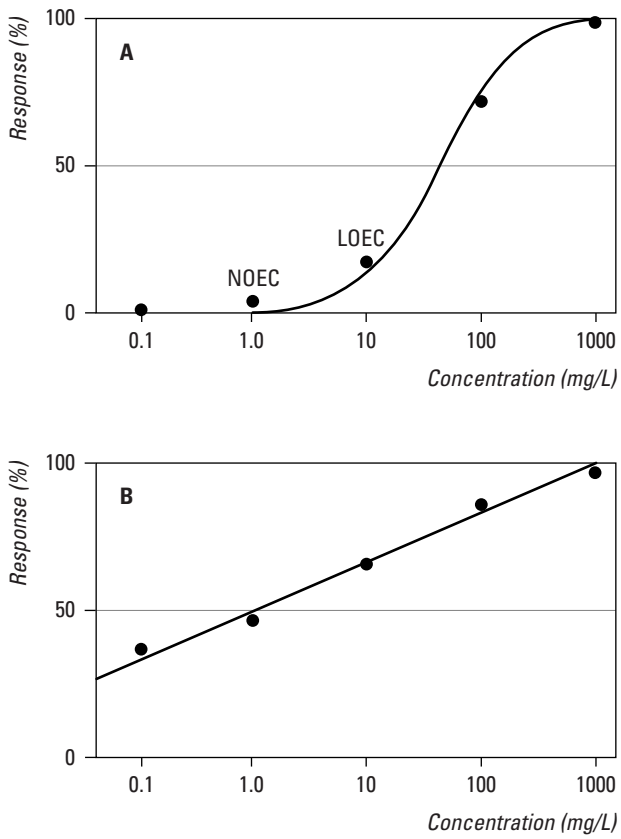

Figure 7.11. Typical examples of concentration-response functions for chemicals with (A) and without (B) a threshold. For chemicals with a threshold a No Observed Effect Concentration (NOEC) can be determined. The nonthreshold concentration-effect curve shows that there is no threshold concentration below which exposure is relatively harmless. Most carcinogens and mutagens are non-threshold chemicals.

at the highest test concentration. It is statistically incorrect to designate this as a NOEC and it should be reported as no effect at the highest concentration tested. When this value coincides with the solubility limit of the test substance this should be specified. Sometimes results are reported as the maximum allowable toxic concentration (MATC), which is the geometric mean of the LOEC and NOEC.

When no threshold is observed experimentally, it implies one of the following:

- There is no theoretical basis for the existence of a threshold, as in the case of genotoxic carcinogens and mutagens. Zero response occurs only at zero dose or concentration (see also Chapter 6, Section 6.5.3).
- Although there might be a threshold, experimental limitations have kept it from being identified.

The nonthreshold concentration-effect curve shows that there is no threshold concentration below which exposure is relatively harmless. As the concentration increases so does the probability of an adverse effect. The relationship between concentration and response is a straight line (Figure 7.11).

The NOEC based on hypothesis testing suffers from a number of disadvantages [37,53]. First, the ANOVA design is more concerned with avoiding having

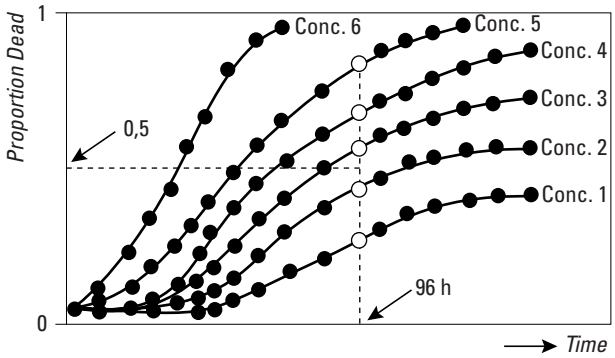

Figure 7.12. Time-concentration-response diagram to illustrate the increased power of analyzing toxicity data when using time to death data, instead of only 96-h data to calculate the LC50. From Newman and Unger [3]. With permission.

to state that a concentration is toxic when it is not (with an arbitrary Type I error not based on biological significance) than with avoiding having to state that a concentration is not toxic when it is (Type II error). However, in risk assessment, we are concerned with the latter [5]. In addition, the NOEC itself does not give any information on the concentration-effect curve. The NOEC can only be one of the tested concentrations for which no confidence limits can be calculated.

These disadvantages can be partly overcome in several ways: first, by regression analysis of the concentration-effect relationship. The great advantage of this is that after deriving an equation for the concentration-effect relationship, a concentration can be estimated which corresponds to a specified degree of an adverse effect. This key aspect is utilized in the benchmark dose approach (BMD), described in Section 6.5.3 of this book. In this approach, adapted here for ecotoxicology, the NOEC is replaced by a critical effect concentration derived from the concentration-effect curve, corresponding to a prescribed small effect considered non-adverse, such as an EC5 or EC10. The BMD approach, however, is not always an improvement if the variation in response between animals in relatively small dose groups is large [54].

The second approach is to utilize the temporal dynamics of effects in a time-response approach (reviewed in [3]). Instead of only reporting the survival of test animals at a single point in time (e.g., 96 hours), survival can be monitored during the entire experiment (Figure 7.12). The advantages of time-response approaches are that, due to increased statistical power, additional biological factors can be taken into account such as sex, temperature or acclimation history. To fit survival-time data, an analysis is needed that differs from estimating the LC50. Several nonparametric and parametric methods can be used to fit the survival curves [3,50]. In the parametric models, the shape of the survival curve is described by a hazard model. Hazard models are used to analyze a variety of phenomena, ranging from mechanical component failure to cancer incidence. A special application of the hazard model is used to analyze the mortality probability, related to accumulation of the chemical in an organism [38,55]. Essentially, it combines the CBR concept [21] with time-response modelling. Although internal concentration can be treated as a hidden variable in this approach, if available, it improves the modelling of the accumulation-related increase in mortality over time (Figure 7.13).

Regardless of the outcome of a statistical test, it is still necessary to draw a separate conclusion about the biological importance of the observed effect [5]; hence, a statistically significant effect is not the same as a relevant biological effect.

7.3.3 Short-term toxicity

Introduction

Laboratory toxicity tests with fish, invertebrates or algae are usually single-species tests in which the toxicity of a chemical is measured through mortality, decreased growth rate and lowered reproductive capacity. These tests have been highly standardized and applied to a select group of organisms. A distinction should be made between acute and chronic tests. Acute toxicity can be defined as the severe effects suffered by organisms from short-term exposure to toxic chemicals. The objective of acute toxicity testing is to determine the concentration of a particular chemical that will elicit a specific response or measurable end-point from a test species in a relatively short period of time, usually 2 to 7 days. In chronic toxicity tests, effects are studied over prolonged periods of exposure, often over entire life cycles and usually the endpoints are primarily sublethal (such as growth) or measurements of reproductive output. Subchronic studies are of longer duration than acute exposure but generally do not exceed a period equivalent to one-third of the time taken for a species to reach sexual maturity. Short test duration is not synonymous with acute toxicity. This can best be explained by using the algae growth inhibition test as an example. Both acute and chronic endpoints can be obtained from toxicity tests with algae because algae have relatively short life cycles (Table 7.6) so the EC50 is used as an acute endpoint and the NOEC/EC10 as a

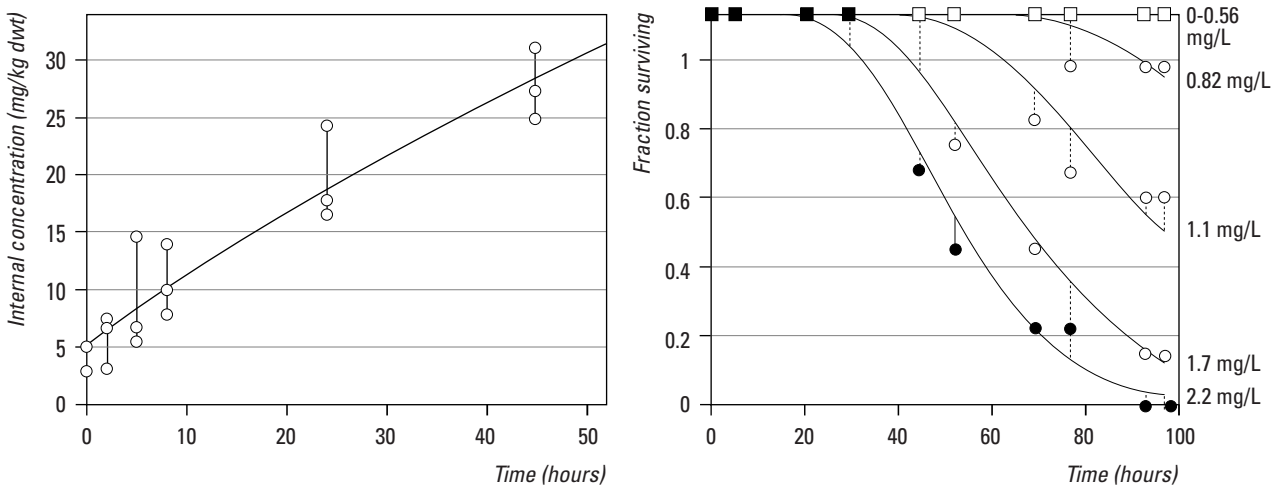

Figure 7.13. Combining body residue analysis (left panel, at 0.1 mg/L) with time-to-death mortality data for cadmium (right panel, 0-2.2 mg/L) in a dynamic energy budget model for *Daphnia magna*. From Jager et al. [55]. With kind permission of Springer Science and Business Media.

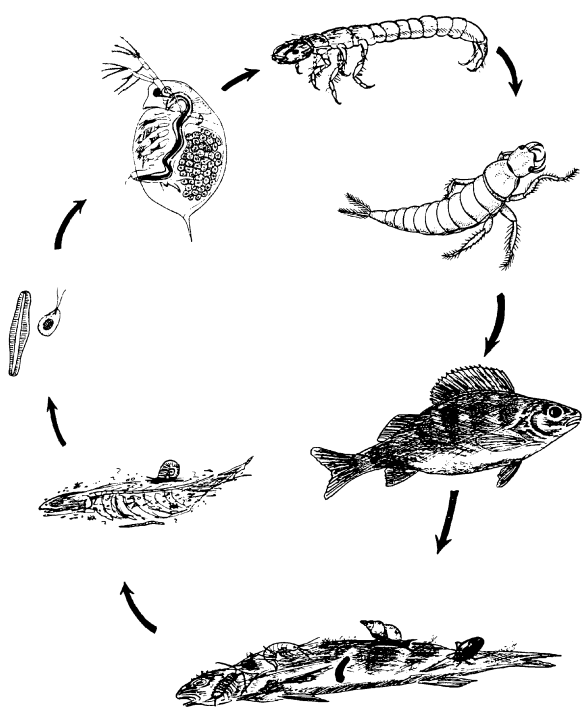

Figure 7.14. Simplified aquatic food chain consisting of primary producers (various species of algae), herbivores (daphnids), primary carnivores (caddisfly larvae), secondary carnivores (beetle larvae), tertiary carnivores (fish) and decomposers, i.e., detritus-feeding animals such as snails and amphipods and bacteria and fungi (species not drawn to the same scale).

chronic endpoint. Furthermore, short-term or episodic exposure may lead to chronic effects, for example the occurrence of neurotoxic effects in organisms after shortterm exposure to certain organophosphate insecticides.

Acute toxicity tests have two general applications in environmental risk analysis. One application is in determining acute toxicity. The objective here is to provide a basic set of data for three trophic levels (algae, daphnids, fish) which can be used in conjunction with a large assessment factor to estimate PNECs of a specific chemical. For risk assessment, the concentration-response curves can also be used to determine the biological response of the species at a given environmental concentration.

The second type of application is toxicological screening. The purpose of screening is to determine whether the chemical or solution being tested is biologically active with respect to the endpoint being measured. Essentially, screening tests provide "yes or no" answers, i.e., a chemical is toxic or non-toxic, mutagenic or non-mutagenic, and so on, at the concentration tested, usually a regulatory threshold.

Tests with animals

Freshwater invertebrate and fish species commonly used for acute toxicity studies [47,56,57] are chosen to represent different functional groups such as herbivores, carnivores or decomposers (Figure 7.14). The endpoints measured in these studies can include any response that an organism or population may exhibit as a

Table 7.10. Characteristics of the acute immobilization test with daphnids [59].

Test species	*Daphnia magna, Daphnia pulex* or any other suitable *Daphnia* species less than 24 h old
Test duration	usually 48 h
Test system	static, semi-static or renewal test in tubes or beakers, with at least 2 ml of test solution per animal
Feeding	no food
Light/temperature	light-dark cycle of 16:8 h at constant temperature between 18-22°C
Endpoints	immobility
Parameter	EC50

Table 7.11. Characteristics of the fish acute toxicity test [60].

Test species	juveniles of various fish species, e.g. guppy *(Poecilia reticulata)*, zebrafish *(Brachydanio rerio)*, fathead minnow *(Pimephales promelas)* or rainbow trout *(Oncorhynchus mykiss)*
Test duration	usually 96 h
Test system	static and renewal test (maximum loading 1.0 g fish/L) and flow-through (higher loading can be acceptable)
Feeding	no food
Light/temperature	light-dark cycle of 16:8 h at constant temperature between 20-25°C (warm water fish species) and 13-17°C (cold water fish species)
Endpoints	survival
Parameter	LC50

result of chemical exposure. However, the end-point most commonly used in acute toxicity studies using invertebrates, such as daphnids and fish, is death (LC50) or immobilization (EC50). These end-points are easily determined, have obvious biological and ecological significance and are amenable to concentration-effect analysis (see also Section 6.5.3). The characteristics of routine acute toxicity tests with daphnids and fish are presented in Tables 7.10 and 7.11 respectively.

The general set up of short-term toxicity tests usually consists of five test concentrations, a control, a solvent control if needed, and 10 to 20 organisms for each concentration or control. Although short-term toxicity testing is generally seen as a simple routine matter, it is relatively complicated (Table 7.12). Therefore, highly standardized test protocols have been developed by international organizations. Recommended procedures are provided by e.g., the Organization for Economic Co-operation and Development (OECD; see Chapter 16), the International Organization for Standardization (ISO), the US Environmental Protection Agency (USEPA), the American Society for Testing and Materials (ASTM), and Environment Canada. Through the harmonization of test guidelines [58-60], the OECD plays an important role in the international arena of chemicals control. Most industrialized countries adopt OECD test guidelines once they are officially approved.

Tests with plants

The development of testing procedures to study the toxic effects of chemicals on aquatic plants has centred on unicellular algae and duckweed [46, 61]. A short summary is given in Table 7.13.

Because of their short generation times, phytotoxic effects can be measured over several generations in a relatively short period. The parameters generally measured in phytotoxicity studies are photosynthesis and population growth. Effects on photosynthesis can be measured by a number of well-established methods including O_2 production, $^{14}CO_2$-uptake, photosynthetic pigment concentration, ATP production, and cell counts [61-63].

Effects assessment using growing populations requires repeated counting of cells or fronds (leaf-like part of a plant), or determination of biomass over a period of time, several times the generation period of the organisms. In the data analysis the main emphasis is on the inhibitory effects on the population growth rate [63, 64] (see Section 7.3.5 for an explanation of basic population dynamics). The growth rate in the exponential

Table 7.12. Important aspects of a test protocol with fish.

Biological aspects
Ecology of test species
Acclimation
Treatment of unhealthy fish
Age at testing
Feeding
– type of food
– amount of food
– frequency of feeding
Loading (density)
Sample size
Randomization
Duration of test
Control mortality
Physical aspects
Temperature
Light/dark regime
Holding facilities
Materials
Shape/volume of test vessel
Chemical aspects
Source of water
– dissolved oxygen (DO)
– pH
– hardness
– particulate matter
– complexants
– impurities
Carrier solvent
– type
– concentration
Test compound
– solubility
– stability
– volatility
– BOD
– bioaccumulation potential
– chemical detection method
Exposure conditions
– static/renewal/flow-through
– replacement time
– stability of DO and pH
– test concentration: nominal or measured
– test concentration: stability over time

phase of growth (Figure 7.15) for each toxicant concentration is calculated with linear regression:

$$r = [ln\ (N_2 / N_1)]\ /[t_2 - t_1] \tag{7.2}$$

where

r = the exponential growth rate (1/d) from time t_1 to t_2
N_2 = cell number in the exponential growth phase at time t_2
N_1 = cell number in the exponential growth phase at time t_1
t_2, t_1 = time (d)

The effects of a chemical on inhibition of the average specific growth rate can be calculated as follows:

$$\%\ \text{inhibition} = \frac{r_0 - r_t}{r_0} \cdot 100 \tag{7.3}$$

where r_0 is the growth rate in the control and r_c is the growth rate in the presence of the toxicant at concentration c. For the biomass increase (yield) in the same period, a similar equation is used. As an alternative, growth inhibition can also be modelled with a threshold model [65,66]. This model assumes no effects at concentrations below a certain threshold, and the hazard is modelled as proportional to the concentration above the threshold. An example of this method is given for *Daphnia magna* in Figure 7.21 in Section 7.3.5.

Microbial tests

Bacterial processes are extremely important with regard to nutrient cycling, secondary productivity, biodegradation and metabolism, as are the ecological consequences of toxicity-induced stimulation or inhibition of these processes (Figure 7.16).

Some tests are performed with isolated bacterial and fungal species to study the bactericidal or fungicidal effects (concentrations causing mortality) and the bacteriostatic or fungistatic properties (concentrations preventing growth and proliferation of cells without killing them). Species often used are *Vibrio fischeri*, a saltwater species used in the Microtox test [56], *Pseudomonas putida*, *P. fluorescens* and the ciliate *Tetrahymena sp*.

In microbial ecotoxicology, attention is primarily focused on functional approaches, i.e., the effects on microbial processes. Functional tests include the study of toxic effects on the carbon cycle, especially the effects of heterotrophic bacteria on mineralization (the biological process of transforming organic matter by

Table 7.13. Characteristics of the microalgae or cyanobacteria growth inhibition test [46].

Test species	unicellular green algae, *(Pseudokirchneriella subcapitata* or *Desmodesmus subspicatus)*, diatoms *(Navicula pelliculosa)* or cyanobacteria *(Anabaena flos-aque* or *Synechococcus leopoliensis)*.
Test duration	72 h (short-term chronic test)
Test system	static test in Erlenmeyer flasks with 100 ml test solution on a rotary or oscillatory shaker
Medium	synthetic nutrient-enriched medium
Light/temperature	constant light at constant temperature between 21-24°C
Endpoints	inhibition of population growth/biomass and yield
Parameter	EC50 (50% inhibition of growth or yield), EC10/EC20, NOEC

complete oxidation into carbon dioxide, water and other inorganic compounds). Other tests focus on the nitrogen cycle: nitrogen fixation (the process of fixing molecular nitrogen into organic matter), ammonification (the release of ammonium from organic matter), nitrification (the conversion of ammonia to nitrite and nitrate), and denitrification (the anaerobic conversion of nitrate to atmospheric nitrogen and N_2O). Specific enzyme activities can be measured as well, but in practice they are of little value for monitoring adverse effects.

In addition to their function in ecosystems, microbial activity in sewage treatment plants (STP) is essential. To protect the microbial activity of STPs, microbial toxicity tests can be used to derive a no effect level for microorganisms. The current presence of pharmaceuticals for veterinary and human use, including antibiotics, raises concerns about microbial inhibition in STPs [67,68]. Routine tests with bacterial strains or inocula are generally carried out to study the inhibition of respiration, nitrification, growth or changes in bioluminescence [69].

An example of a routine respirometric test is given in Table 7.14. The EC50 values in such tests can be derived by non-linear regression [70]. In some cases, specific biodegradability tests (OECD guidelines 301-302) can also be used to derive NOECs for microbial toxicity. A more detailed discussion of biodegradation and how it can be predicted is given in Chapters 3 and 9 (Sections 3.5 and 9.4.3).

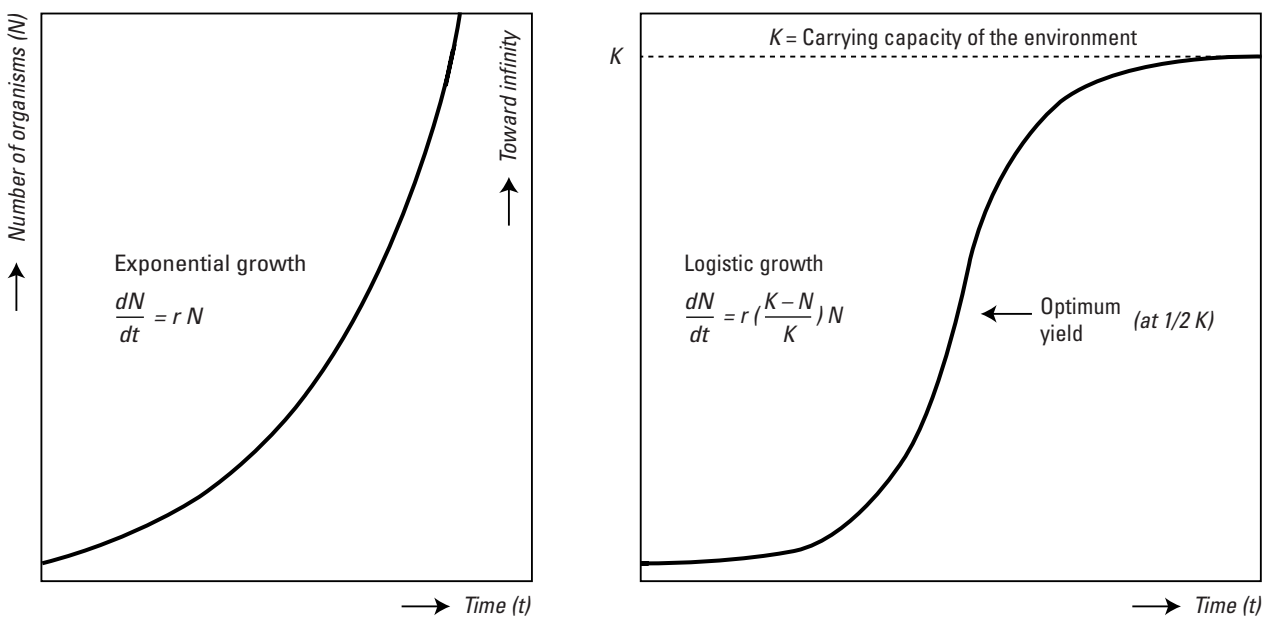

Figure 7.15. Basic forms of population growth: exponential growth (left) and logistic growth (right).

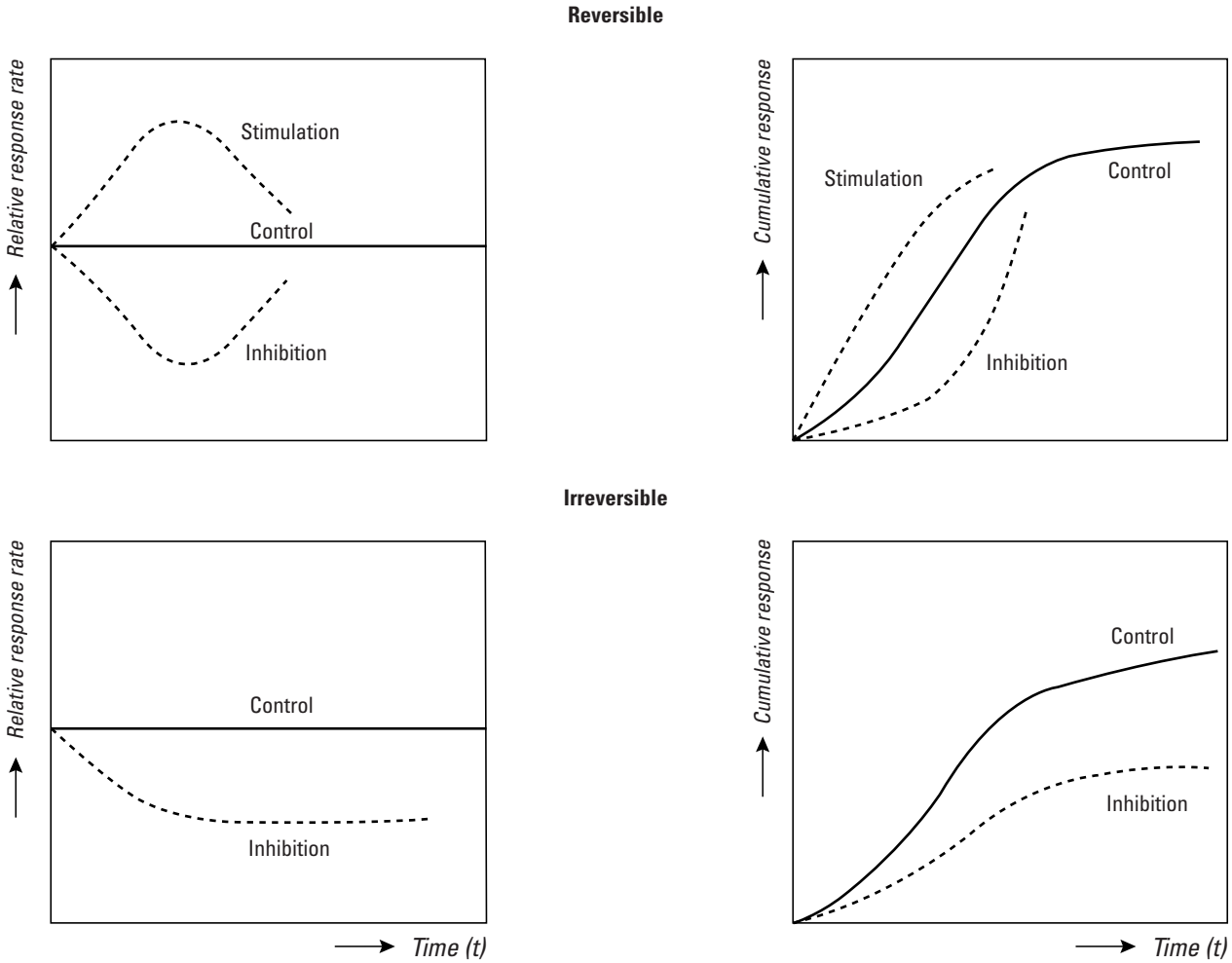

Figure 7.16. Types of microbial responses.

7.3.4 Long-term toxicity

Introduction

The aim of chronic toxicity testing is to determine whether prolonged exposure to chemicals will have significant adverse effects on ecosystems. For the aquatic environment, this is accomplished by estimating chronic toxicity threshold concentrations for a number of selected species inhabiting the ecosystem. From these data, the chronic threshold concentration for the aquatic ecosystem or PNEC can be predicted with fixed or calculated extrapolation or uncertainty factors (Section 7.10).

Apart from survival, chronic toxicity studies are based on end-points like individual growth (body length and body weight), abnormal development (teratogenicity), hatching time, hatchability, reproduction (total number of young, brood frequency, etc.) and behavioural aspects, etc. These data are then subjected to concentration-effect modelling or hypothesis testing to derive the NOEC.

Three categories of tests are commonly used to predict the chronic effects of toxic chemicals on aquatic organisms (Table 7.15). Data from these categories of tests can be used to estimate the PNECs (Section 7.10).

Partial and full life-cycle tests

In life-cycle tests, groups of test organisms are exposed to a series of concentrations of the test chemical over one or more generations. In fact, most algal toxicity studies are life-cycle tests, but this term is generally used in the context of fish and invertebrate studies. Life-cycle tests begin with the eggs, larvae or juveniles and continue until the test organisms have (or should have) reproduced. The tests can continue through several generations, if desired. Chemical concentrations range from those

Table 7.14. Characteristics of the activated sludge respiration inhibition test [69].

Test species	inoculum from aerobic sewage sludge
Test duration	3 h
Test system	static test in a BOD bottle
Medium	synthetic medium with inorganic nutrients and peptone, meat extract and urea
Light/temperature	dark at 20°C
Endpoints	inhibition of the respiration rate (oxygen consumption of micro-organisms expressed as mg O_2/L·h)
Parameter	EC50 (50% inhibition of the respiration rate)

Table 7.15. Types of chronic toxicity studies.

Life-cycle toxicity tests measure the effects of chronic exposure to a chemical on reproduction, growth, survival, and other parameters over one or more generations of a population of test organisms
Sensitive life stage tests measure the effects of chronic exposure on survival and growth of the toxicologically most sensitive life stages of a species, for example, eggs and larvae of fish
Sublethal chronic toxicity tests measure the effects of chemicals on various biochemical or physiological functions or on histology of individual organisms

with significant adverse effects on survival, growth and reproduction to at least one which has no significant effect on these parameters, compared with the controls. The species that can be used in life-cycle toxicity tests are limited to those which can complete their life cycles under laboratory conditions. Rand [47] has listed those animal species most commonly used in life-cycle toxicity tests.

Due to the cost and length of time required for full life-cycle tests for some species, certain routine reproduction tests do not cover the entire life cycle but are partial life-cycle tests. Only the most important partial life-cycle tests with invertebrates will be discussed here, together with some basic principles of population dynamics. For a more extensive review of invertebrate studies see Persoone and Janssen in Calow [56].

Tests with daphnids

The best known partial life-cycle test is the chronic reproduction test with daphnids (Figure 7.17). According to Persoone and Janssen in Calow [56] there are five reasons for selecting this species:

1. They are broadly distributed in freshwater bodies and are found in a wide range of habitats.
2. They form an important link in many aquatic food chains (they graze on primary producers and are food for many fish species).
3. They have a relatively short life cycle and are relatively easy to culture in the laboratory.
4. They are sensitive to a broad range of aquatic contaminants.
5. Their small size means that only small volumes of test water and little bench space are required.

The entire lifespan of *D. magna* or *D. pulex* takes approximately 60 days [72]. The life-cycle test with *D. magna* or *D. pulex* takes 21 days (Table 7.16). After approximately 10 days the first brood will appear and subsequent broods will normally be produced at intervals of 2 to 3 days (Figure 7.18). The young are separated from the parents and the total number of offspring is treated as a reproduction parameter.

Another widely accepted chronic reproduction test, especially in the US, is the test with *Ceriodaphnia dubia*. This 7-day bioassay was developed by Mount and Norberg [73]. It is a cost-effective bioassay, and is frequently used as an invertebrate bioassay in the USA [74]. In the *Ceriodaphnia* reproduction test, three broods are normally produced on days 3, 5 and 7. The experimental design and the statistical analysis of the data are comparable with the *Daphnia* test, but the test has several advantages, although there is no OECD guideline for *Ceriodaphnia. Ceriodaphnia* are distributed widely throughout Europe, Asia and North America, are easy to culture and the exposure period is much short-

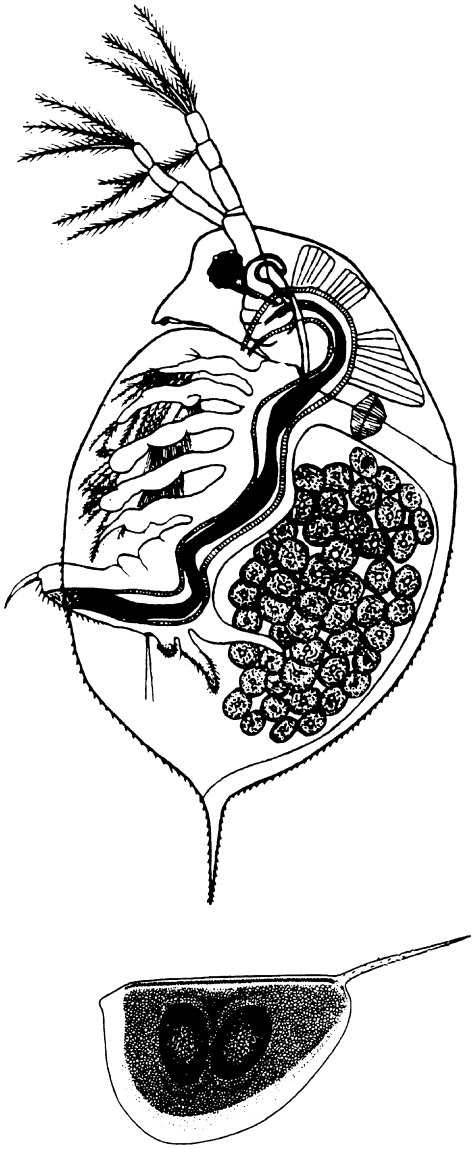

Figure 7.17. *Daphnia* with normal eggs in the brood pouch and ephippia (winter eggs).

er (1 week instead of 2 to 3 weeks). Acute and chronic sensitivity to a broad array of substances was found to be comparable to that of *Daphnia* sp. [75].

Tests with sensitive life stages

Considerable time and expense is involved in conducting ELS toxicity tests, especially for fish. Methods have been developed for utilizing tests with the most sensitive life stages to predict chronic toxicity threshold concentrations. Figure 7.19 gives a representation of the various life stages of an oviparous fish: salmon. After gametogenesis (the production of sperm and egg cells) fertilization takes place. This is accompanied by swelling of the egg through water uptake. The egg membrane becomes relatively impermeable. Within the egg membrane the fertilized egg cell divides and differentiates through a number of different embryonic stages (embryogenesis) until the eggs hatch. Once the eggs are hatched an alevin with a yolk sac or a yolksac larva (also known as eleuthroembryo) appears. The alevin feeds itself using its internal food source: the yolk deposited in the yolk sac. During further development this yolk is resorbed and the so-called swim-up fry start to catch and ingest food, progressing to the juvenile and finally adult stages. At this reproductive stage maturation of the ovary and testes occurs, producing mature egg and sperm cells (gametogenesis). Many other fish species develop in a similar manner, although some show marked differences, e.g., viviparous fish, such as guppies.

According to McKim [76] and Van Leeuwen et al. [77] it is generally the early life stages of fish which are most sensitive to chemical toxicants. This susceptibility results from a potential for exposure and responsiveness: the intrinsic susceptibility or sensitivity, in its strictest sense. McKim [76] showed that estimates of chronic toxicity threshold concentrations calculated from ELS tests were not significantly different from those calculated from entire life-cycle toxicity tests. An OECD test guideline for fish ELS studies is available [78].

A standard fish ELS test (FELS, Table 7.17) starts with freshly fertilized eggs, which implies that FELS tests exclude any potential effect of a chemical on the process of gametogenesis, or on the process of fertilization (Figure 7.19). FELS tests are terminated after swim-up fry have been fed for a given period of time. The length of the feeding period is also species-dependent.

The great advantage of FELS tests is that they save time and money compared with full or partial life-cycle studies with fish. Thus, estimates of chronic toxicity thresholds can be made for more chemicals and for a wider variety of species from different habitats and trophic levels than are possible with life-cycle toxicity tests. However, as several life stages are covered in the test it is still a sensitive assay, and may be preferable to the juvenile fish growth test (OECD guideline 215) which determines effects on juvenile fish growth during a 28-day exposure period. Although compared with full life-cycle studies, embryolarval tests reduce the time required to produce information on the toxicity of chemicals, they remain laborious. To further reduce the exposure time, short-cut methods are needed. This is why several procedural variations are used [58,79]. The short-

Table 7.16. Characteristics of the *Daphnia* reproduction test [71].

Test species	neonates of *Daphnia magna*
Test duration	21 d
Test system	semi-static (renewal at least three times a week) or flow-through, with 50-100 ml test solution per animal
Feeding	green unicellular algae obtained from a laboratory culture, ration between 0.1 and 0.2 mg C/(daphnid.day)
Light/temperature	light/dark cycle of 16:8 h at constant temperature of 18-22°C
Endpoints	reproduction (total number of offspring), parent survival and time to production of first brood
Parameter	LC50, EC50 and NOEC

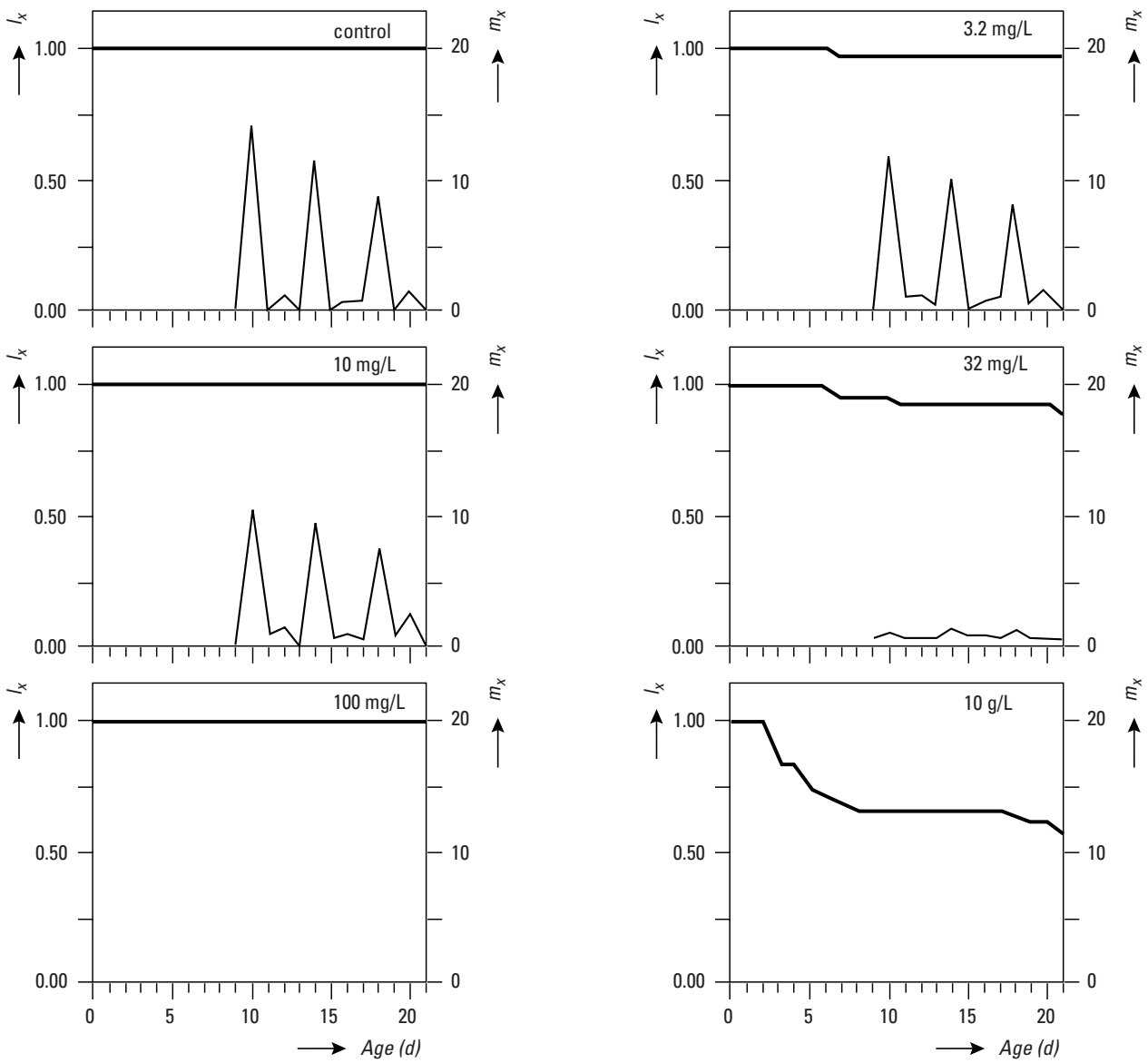

Figure 7.18. Life table of *Daphnia magna* at various concentrations of bromide: survivorship curve l_x (—) and fertility curve m_x, (–). From Van Leeuwen, Rijkeboer and Niebeek [66]. With kind permission of Springer Science and Business Media.

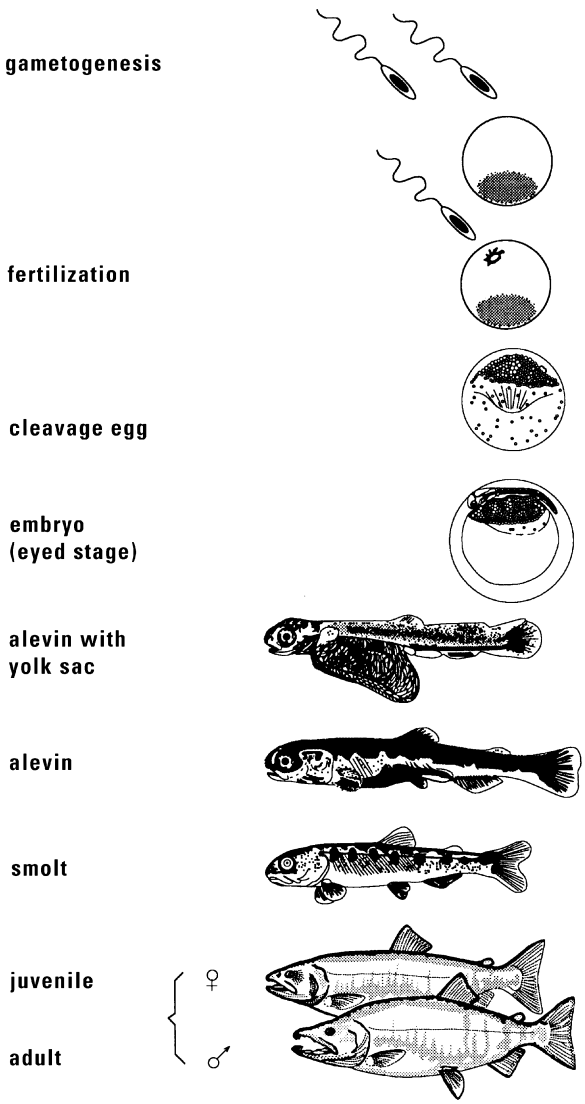

Figure 7.19. Life cycle of a salmonid fish.

term toxicity test on embryo and sacfry stages (OECD guideline 212) can be used as an alternative to a full FELS test. It can be considered a chronic test because it covers sensitive life stages from egg to sacfry. However, it is expected to be less sensitive than the full FELS test.

7.3.5 Population dynamics

Population dynamics are relevant to the study of toxic effects in ecosystems and their recovery after exposure. Although the focus of ERA is on the protection of populations, relatively little attention has been devoted to population dynamics. The effects of chemicals on population dynamics are increasingly integrated in strategies for higher-tier toxicity testing [80,81]. The same principles of population dynamics apply to both sediment-dwelling organisms and terrestrial populations (Sections 7.4 and 7.5) and are discussed in this section. Some examples will be given to illustrate the current lack of ecological realism in single-species toxicity tests.

The most commonly performed population toxicity experiments are tests with algae and *D. magna*, although soil invertebrates such as *Orchesella cincta* or *Folsomia candida* or the nematode *Plectus acuminatus* may also be used [82-86]. These experiments focus on effects on either the exponential or logistic growth of populations. When a population is subject to a constant schedule of birth and death rates it will gradually approach a fixed or *stable-age distribution*, whatever the initial age distribution may have been, and will then maintain this stable age distribution indefinitely (Lotka theory). When the population has reached this stable age distribution, it will increase in numbers according to the simplest model for population growth: *exponential growth*. The exponential growth model has a constant per capita growth rate *(r)*, which is independent of the population density (Figure 7.15), resulting in unbounded exponential growth. For some periods of time, exponential growth can be observed for fast growing micro-organisms, algae and daphnids (Figure 7.20). Unbounded growth is not found in nature for prolonged periods of time. A simple model which captures the essential features of an environment with finite resources is *logistic growth* (Figure 7.15). Population growth decreases as population density increases. Here the effective per capita growth rate has the density dependent form $r(1\text{-}N/K)$: this is positive if $N<K$, negative if $N>K$, and thus leads to a generally stable equilibrium value at $N=K$. K may be thought of as the *carrying capacity* of the environment, as determined by food, space, predators, or other things; r is the *intrinsic growth rate*, free from environmental constraints.

What is the relationship between age and population growth? We know that both birth and death rates vary with age. In fact, there are four basic concepts. First, every population has an age distribution that indicates the proportion of the population in various age classes. Second, every population has a growth rate. Third, in every population there is a regime of *age-specific mortality*, often depicted by the survivorship curve, which describes the probability of surviving from age 0 to age x or beyond. The fourth concept is that of *age-specific fertility*, often represented by the fertility, fecundity or maternity function. The study of population

Table 7.17. Characteristics of the fish early-life-stage test [78].

Test species	zebrafish *(Brachydanio rerio)*, fathead minnow *(Pimephales promelas)*, rainbow trout *(Oncorhynchus mykiss)* and a variety of other species
Test duration	all tests begin with freshly fertilized eggs and may end at the early fry stage, but the test duration depends on the species and the temperature of the water (normally 28 d for fathead minnow and zebrafish and 60-90 d for rainbow trout)
Test system	renewal or flow-through test
Feeding	with commercial fish food starting at the transition of the yolk sac larval stage and the swim-up fry stage
Light/temperature	species-dependent
Endpoints	survival, growth (length and weight) and developmental (teratogenic) effects, time till hatching and end of hatching, yolk resorption, histopathology and behavioural effects may be included as well
Parameter	LC50, EC50, LOEC and NOEC

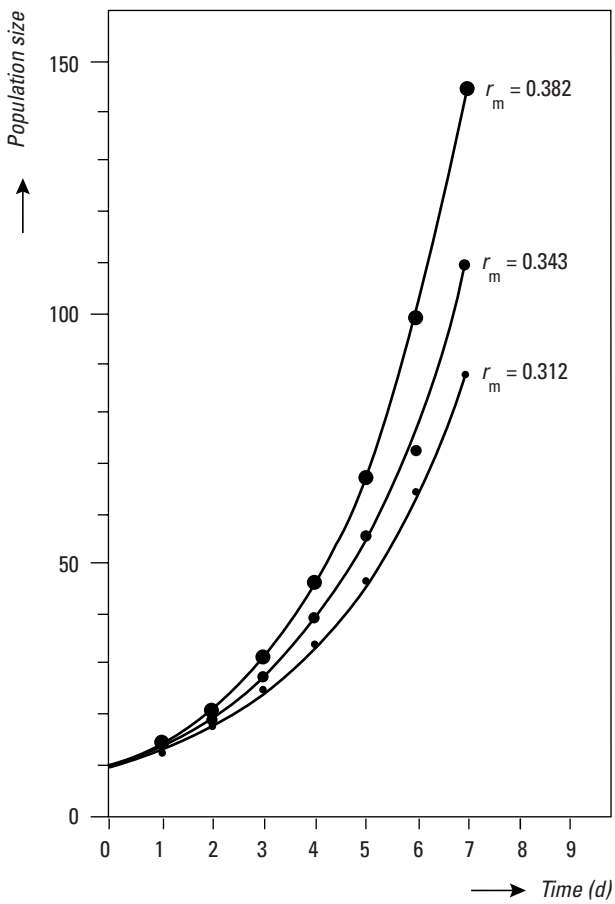

Figure 7.20. Exponential growth of *Daphnia magna* populations with different intrinsic rates of increase, derived from Box 7.2.

growth in relation to age structure is called *demography*. Figure 7.18 shows an example of a demographic study with survivorship and fertility curves for *D. magna*.

Population dynamics concepts can also be incorporated in tests with cohorts (isolated generations), i.e., tests in which a fixed number of individuals are exposed from the juvenile through the adult period. In cohort studies separate measures of age-specific survival and fecundity are combined in a life-table and used to estimate the intrinsic rate of natural increase. The intrinsic rate of increase *(r)*, the growth rate of an exponentially increasing population, can be calculated with the Euler-Lotka equation:

$$\sum_{x=0}^{\infty} l_x m_x e^{-rx} = 1 \qquad (7.4)$$

where l_x is the probability of surviving to age x, m_x is the age-specific fecundity (number of female offspring per surviving adult at age x) and x is time expressed in days.

In Box 7.2, three hypothetical life-table experiments (A, B and C) are shown. The experiments which started with newborn (< 24 h) daphnids show no mortality (l_x remains 1 in all three experiments). The total number of young produced in four broods in each experiment over a period of 21 days was 70. In standard *Daphnia* toxicity protocols, effects on reproduction would not have been detected, because the total number of newborn daphnids is the only measure of toxic effects on reproduction. But if we use basic population dynamics, dramatic adverse ecological effects can be demonstrated. If the intrinsic rate of increase is calculated by successive approximation using Equation 7.4, three different values for r are obtained (Box 7.2.). If these values are substituted in the equation for exponential growth (Figure 7.15), it appears

Box 7.2. Demonstration of the consequences of a delay in reproduction on the intrinsic rate of increase *(r)* in three hypothetical 21-d life-table studies (A, B and C) with *Daphnia magna*.
Note that there is no parental mortality (l_x remains 1) for all three experiments.

Time (d)	l_x	m_x (A)	m_x (B)	m_x (C)
1	1	0	0	0
2	1	0	0	0
3	1	0	0	0
4	1	0	0	0
5	1	0	0	0
6	1	0	0	0
7	1	10	0	0
8	1	0	10	0
9	1	0	0	10
10	1	0	0	0
11	1	15	0	0
12	1	0	15	0
13	1	0	0	15
14	1	0	0	0
15	1	20	0	0
16	1	0	20	0
17	1	0	0	20
18	1	0	0	0
19	1	25	0	0
20	1	0	25	0
21	1	0	0	25
T[a]		70	70	70
r[b]		0.382	0.343	0.312

[a] T is total number of young after 21d.

[b] r is calculated by successive approximation from Equation 7.4. For the examples A, B, and C the following set of equations is obtained:

Life-table study A: $10 \times e^{-7r} + 15 \times e^{-11r} + 20 \times e^{-15r} + 25 \times e^{-19r} = 1$ (r = 0.382)
Life-table study B: $10 \times e^{-8r} + 15 \times e^{-12r} + 20 \times e^{-16r} + 25 \times e^{-20r} = 1$ (r = 0.343)
Life-table study C: $10 \times e^{-9r} + 15 \times e^{-13r} + 20 \times e^{-17r} + 25 \times e^{-21r} = 1$ (r = 0.312)

that population growth is greatly affected (Figure 7.20). The special importance of toxicity-induced effects on the age of first reproduction is often overlooked [87]. Apart from the age of first reproduction, there are several other aspects which greatly influence population growth (Table 7.18). Some of these parameters can be measured in the laboratory, others cannot.

Experiments with populations may provide a good alternative to life-cycle studies, especially when the population-level response depends on the sensitivity of different life-cycle stages or variables. The integration of effects on different life-history traits can only be studied in this way. Population toxicity studies begin with small, exponentially growing populations. The underlying assumption for projecting future growth, with either the exponential or the logistic growth model, is that the population has a stable age structure. At low population densities, growth will proceed exponentially and the stable-age structure can be calculated with:

$$c_x = (l_x e^{-rx}) / \left(\sum_{x=0}^{\infty} l_x e^{-rx} \right) \qquad (7.5)$$

Table 7.18. Factors affecting population growth.

Age at first reproduction
Brood size
Brood frequency
Length of reproductive period
Condition of neonates
Emigration and immigration
Predation and competition e.g. for food and space

where c_x is the proportion of the total population in the x^{th} age class. If r is 0, the stable age distribution will have exactly the same shape as the survivorship curve. Furthermore, the equation shows that as r increases, the younger age classes become an increasing proportion of the population. An example of a population toxicity experiment is shown in Figure 7.21. The test began with exponentially growing populations of 20 daphnids, composed of different ages. The stable-age distribution was calculated with Equation 7.5 (where r = 0.3 and l_{21}

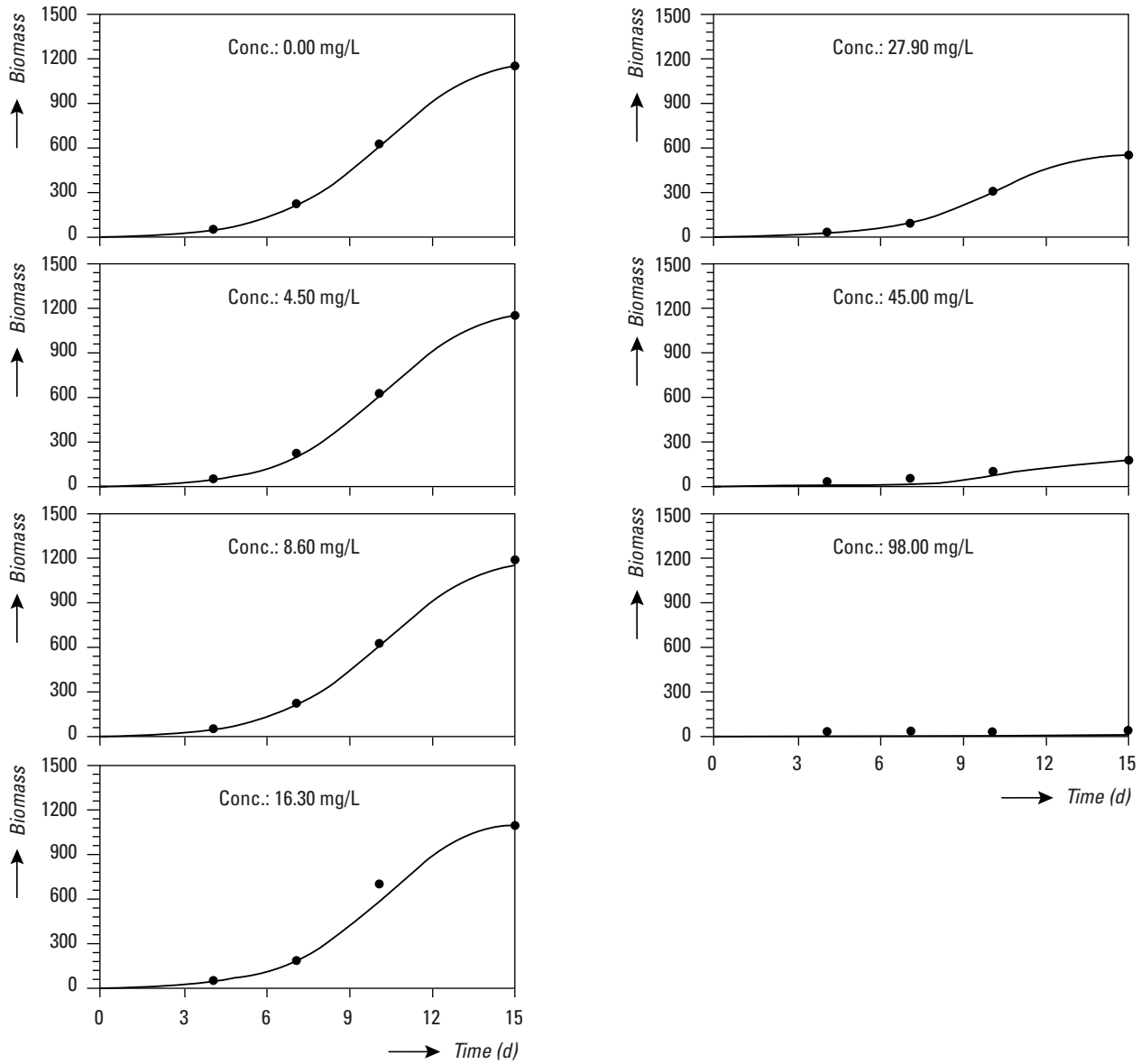

Figure 7.21. The effects of bromide on the logistic growth of *Daphnia magna* populations [66]. Biomass represents the number of daphnids per test container, circles represent the observed and lines the expected values based on model calculations after Kooijman et al. [88]. With permission. Copyright. Elsevier.

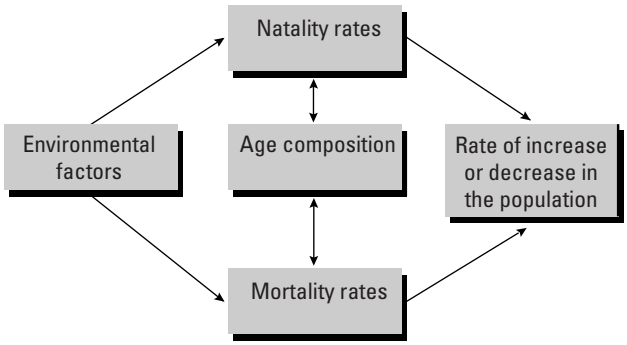

Figure 7.22. The relationship between environmental factors, age distribution and population growth.

= 1, Box 7.2, study C), giving the following stable-age distribution: 5, 4, 3, 2, 2, 1, 1, 1, and 1 daphnid(s) aged 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7, 7 to 8, and 8 to 9 day(s), respectively.

Population growth is often far from logistic when tests are prolonged after populations have attained their numerical maxima. This may be ascribed to the absence of an instantaneous reaction to changes in population density, i.e., time lags are likely to occur which causes populations repeatedly to "overshoot" and then "undershoot" their equilibrium densities. In field situations environmental conditions are never constant and logistic growth can only be observed over short periods of time. Furthermore, in natural populations the age structure is almost constantly changing, because populations do not increase for long in an unlimited fashion. These relationships are shown in Figure 7.22.

Toxic effects in ecosystems often include a change in species composition [88-90]. The problem of life-history strategy may be viewed as that of the optimum allocation of an organism's energy to growth, maintenance and reproduction [38]. In evolutionary terms an organism will devote energy to growth and maintenance only if this will increase its reproductive contribution to future generations. Growth is important in many species because fecundity increases with size, and competition for territories may favour larger individuals. When explaining ecosystem changes, many authors refer to life-history strategies such as the *r* and *K*-species theory. If *K*-species are more sensitive than *r*-species then in contaminated ecosystems *r*-species will replace *K*-species. The *r* and *K*-theory originates from MacArthur and has been formulated most clearly by Roughgarden [91]:

1. In a population that is repeatedly reduced to a low density by some exogenous factor, a genotype with a high intrinsic rate of population growth *(r)* will prevail.
2. In a population constantly occurring in a state of high density with strong competition, a genotype with a high equilibrium density *(K)* will prevail, even if it has a low intrinsic growth rate.

There are examples showing that the theory is correct, but as is often the case in ecotoxicology, there are also examples showing that the theory cannot be generalized. Interactions with other species and differences in the sensitivity of species play an important role in the dynamics of species in a community experiencing toxic stress [89,92]. This shows that detailed case studies are needed to disentangle the various biological and toxicological factors that influence the response of interacting populations to chemical stress. To improve the predictability of the effect of substances on ecosystems, these studies should go hand-in-hand with theory and model development [92].

7.3.6 Multi-species studies

Ideally, PNECs should be determined through studies of exposed ecosystems that are representative of the ecosystems to be protected. However, full scale field tests are expensive and complex. Intermediate methods between laboratory and field tests may contribute to more effective and cost-effective higher-tier risk assessment. Uncertainty about the ecological effects of a chemical can be addressed with indoor microcosm experiments, outdoor micro/mesocosm tests, or a combination of these. Multi-species tests (MS tests) aim to determine fate processes and how these affect bioavailability to different species, and species interactions. They are ecologically more relevant than single-species tests (SS tests), but may be harder to interpret due to system-specific conditions that may not be easy to extrapolate to different conditions. MS tests require that the natural conditions of ecosystems are well documented and that many basic ecological, physicochemical and toxicological data are available (Chapter 1). Unfortunately, in most cases, these data are not available (see Figure 12.1 in Chapter 12). This lack of information hampers the interpretation of observations in MS tests. In many cases PNECs are based on single-species toxicity data. SS tests have some major advantages:

- They are rapid, easy to conduct and not too expensive.
- They can be standardized.

Table 7.19. Some shortcomings of single-species tests.

1. They utilize genetically homogeneous laboratory stock test populations
2. They examine only the responses of individuals, which are averaged to give a mean response for the test species instead of population responses
3. They use species of unknown relative sensitivities and species that may not be indigenous to the receiving ecosystem
4. They are mostly conducted under experimental conditions that are not similar to natural habitats
5. Distribution and degradation processes are often ignored
6. Indirect toxic effects resulting from various ecological interactions are not taken into account
7. Toxic effects on basic ecological processes are often not studied
8. They do not consider recovery rates of populations or ecosystems
9. Cumulative effects of multiple stresses coupled with varying chemical/physical properties are often not studied

- They are relatively easy to replicate.
- Their interreplicate and intertest variability is usually lower compared to micro/mesocosms.
- They are valuable screening tools.
- They are an appropriate way to determine toxicological effects on survival, growth, reproductive success, behaviour and a variety of other individual characteristics.

SS tests also have some serious limitations (Table 7.19) which impair the proper scientific assessment of chemical impacts on ecosystems [93,94].

Comprehensive, system level tests, or MS tests, are complex by their nature. There are a wide variety of potential measurements that can be made, which are generally subdivided into fate, functional parameters and structural parameters. Knowledge of the physicochemical and toxicological properties and fate influencing the exposure conditions is essential. In order to understand the potential routes of exposure of various species, it is useful to obtain a mass balance of the chemical under investigation. Thus, measurements of the chemical in soil, water, sediment and biota should be made. Measures of critical by-products, degradation products or metabolites of the chemical should be included if they are expected to be toxic, or if their measurement is required to complete the mass balance.

The relevant endpoints for effects assessment are structure and function, and may include effects on genetic variability, or the probability of extinction of certain species. Ecosystem functions, such as respiration or primary production (Figure 7.23) are often regarded as less sensitive than structural parameters (species composition), because the species responsible for an ecosystem function may be replaced by less sensitive species capable of maintaining the same functional processes (functional redundancy). However, some herbicides can have a pronounced direct effect on oxygen production in an ecosystem, which is noticeable at lower concentrations than the ensuing changes in species composition [96]. This indicates that both functional and structural aspects need to be considered in MS tests.

Measurements at the species level are often focused on representatives of various trophic levels, functional groups, or otherwise important species. At community level, measurements often include species composition and abundance, presence of important taxa, biodiversity indices, and other functions.

In a survey of MS tests, Emans et al. [97] and De Jong et al. [98] listed a number of criteria to evaluate MS tests (Table 7.20). The frequency of sampling should be sufficient to allow development of time-concentration relationships in the critical phases. The spatial and temporal distribution of samples will depend on the test system, the objective of the study and the chemical. Many MS tests do not meet the quality criteria listed above [97,99], but experience in this field is rapidly growing. MS tests are used in higher-tier risk assessment of chemicals, mainly of pesticides [80,81,100-104], but they also have some drawbacks (Table 7.21).

Multivariate techniques are recommended to analyze the effects of chemicals on model ecosystems. Different approaches (e.g., Principal Component Analysis, Similarity Analysis) are available in general statistical packages or as specific programs that have their own pros and cons. The Principal Response Curves is a multivariate technique especially designed for the analysis of microcosm and mesocosm data at a community level [105,106]. Its advantage over other techniques is that its output is easy to communicate. The differences in community composition between

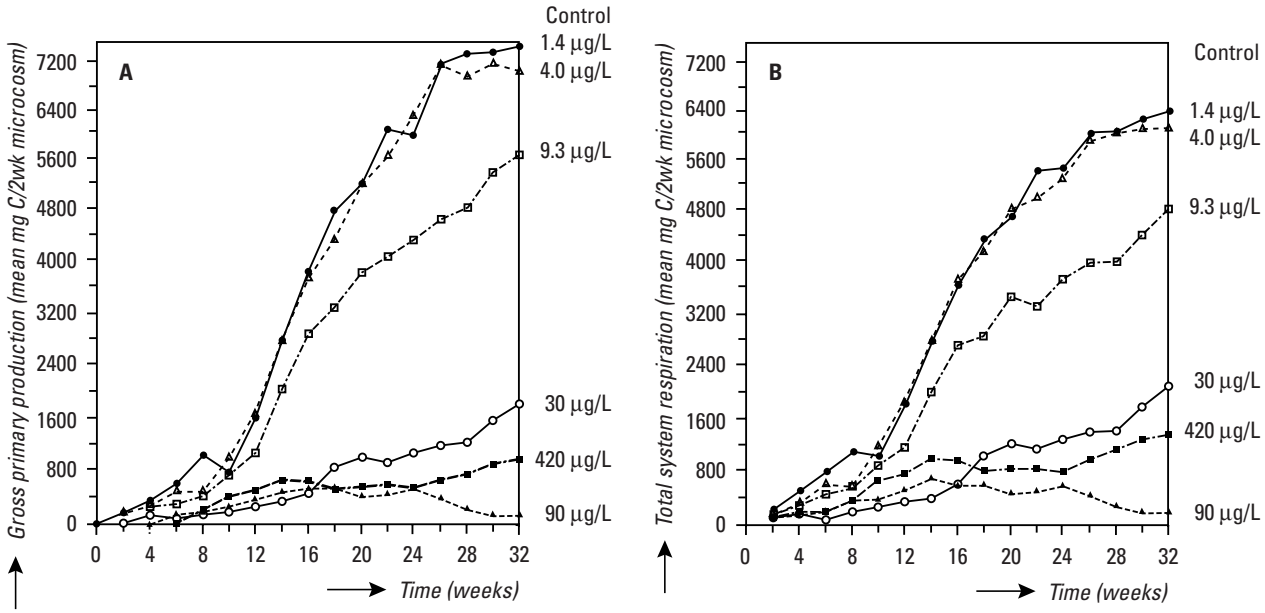

Figure 7.23. Bi-weekly gross primary production (A) and total system respiration (B) as measured in a microcosm test with copper. From Hedtke [95]. With permission. Copyright Elsevier.

the control and the treatments are displayed as a graph. This graph makes it possible to identify which species are affected most, how much the effect differs from the control, and how this evolves in time over the course of the experiment (Figure 7.24).

Modelling studies have shown that selection pressure, differences in interspecies sensitivity and competition between species for resources play an important role in understanding the effects of chemicals in micro or mesocosms. Long-term toxicant effects on sensitive species will influence the competition between species for resources and will lead to the replacement of sensitive species by more competitive species that are more tolerant to the chemical, with a decrease in species diversity [89], as observed in metal-stressed nematode communities [107]. Single pollution events disturb the competition and predator-prey relationship between species [92], leading to changes in species composition that are sometimes are predictable based on laboratory toxicity data [108].

Table 7.20. Criteria for the validity of multi-species tests [97,98]. With permission.

1. The system should represent a realistic community
2. The experimental setup and conditions should be well described, including physicochemical parameters such as pH, temperature and hardness
3. Several taxonomic groups should be exposed to well-described test concentrations for a longer period
4. In each experiment several concentrations should be tested, consisting of one control and at least two test concentrations
5. Each test concentration should have at least one replicate
6. The concentration of the test compound should be measured several times during the experiment
7. Apart from effect parameters like population density and biomass, effect parameters on higher integration levels such as species diversity and species richness should be determined. The endpoints should be in accordance with the mode of toxic action
8. A distinct concentration-effect relationship should be obtained
9. A reliable multi-species NOEC should be derived

Table 7.21. Some difficulties of using multi-species tests [104]. With permission.

1. Costs.
   These are relatively high.
2. Standardization.
   Standardization (harmonization) of MS tests is difficult because the type of study to be performed depends on the question to be answered and may differ from chemical to chemical, from application to application, and from site to site. This will hinder the *mutual acceptance of data*, and therefore increase the costs to industry. The need for standardization is doubtful when we consider the lack of ecological and environmental realism it would imply. However, no standardization at all may lead to hidden subjectivity related to, amongst other things, the taxa included in the experiments (e.g. macrophytes, fish and amphibian species).
3. Lumping of variables.
   In view of the laboriousness of monitoring all species in a community, in MS tests often only lumped variables (e.g. functions, total algal biomass and oxygen production) are observed and many other effects, i.e. reduction in species diversity, may thereby escape notice. As a result extinction of species, i.e. "genetic erosion" may occur. Multivariate statistical methods can be of help to study the diversity and density of all species present in a MS test [105,106]
4. Rapid divergence.
   Some experimental communities tend to diverge rapidly in their development so that only coarser kinds of acute effects stand a reasonable chance of being detected [111].
5. Stability of exposure concentration.
   Stress in MS tests usually decreases rapidly after inoculation, because the toxic chemical is (biologically) degraded or becomes less available in other ways. In single-species tests, the level of stress is usually kept fixed by continuous or intermittent replacement of the test media. Supplying a continuous dose in an MS test resolves the problem only partially [110], but the administration of the chemical can be made to mimic the actual field exposure situation.
6. Adaptation.
   There may be processes which modifies the susceptibility of species (e.g. adaptation or selection of resistant individuals). The quantitative importance of such processes is hard to assess and interpret. Individuals that survive because of their resistance to one chemical may be more vulnerable to another [111]. This process does allow to study the impact of a chemical on community composition and related secondary effects.
7. Replication.
   If any effects are found in the variables observed, there is the problem of disentangling them from the scatter or of avoiding errors of the second kind in the statistical analysis of the results [100,102,111]. Experimental standardization and improved statistical techniques have greatly improved the replicability of MS systems [105,106].
8. Extrapolation.
   As one MS study cannot be representative of all ecosystems, caution is needed when extrapolating the results to other communities or ecosystems. In this respect MS tests do not differ from standardized SS tests.

Types of multi-species tests

MS tests encompass a broad range of bioassays, ranging from small laboratory microcosms made up of artificial assemblages of a few species [107], to more natural microcosms up to 10 m^3 or mesocosms up to 10^4 m^3, or even larger natural systems such as an entire lake or a section of a watercourse which is deliberately contaminated with a chemical to determine concentrationeffect relationships [56,102]. Essentially, there are two basic types of MS tests (Table 7.22). Microcosm and mesocosm tests provide ways of studying potential pollutants in systems which simulate parts of the natural environment (i.e., the macrocosm) but which are also open to experimental manipulation.

Microcosms are often used to study contaminant effects on community structure and function. Microcosms can be used indoors or outdoors. Due to their size (from a few litres to several hundreds of litres), some aspects of natural systems may not be mimicked in full, such as presence of all trophic levels. Nevertheless, essential characteristics such as diversity, competition and primary productivity can easily be studied. The standardized

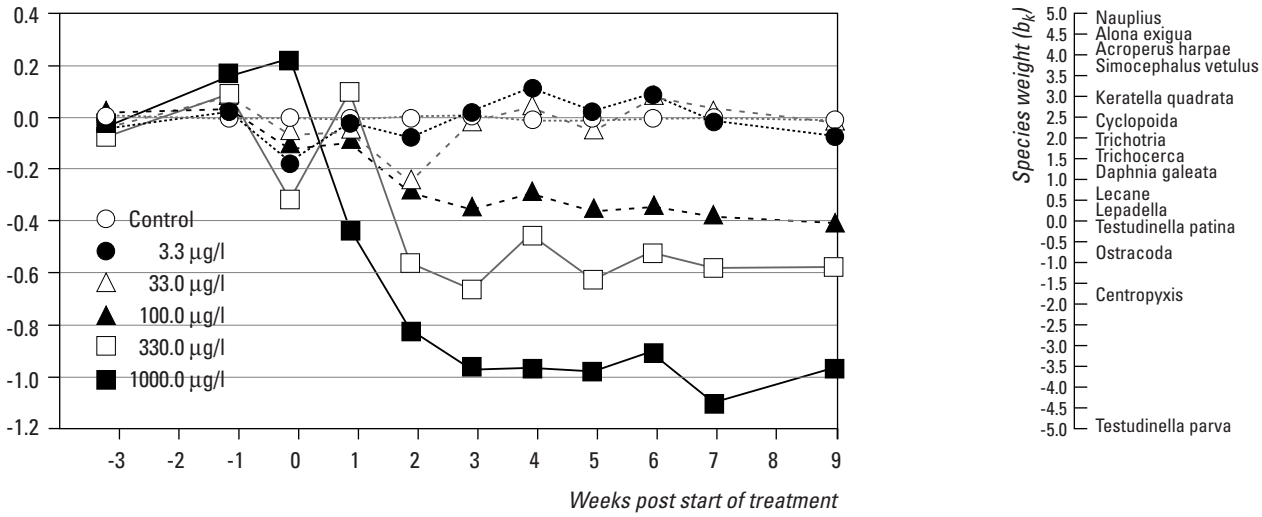

Figure 7.24. Multi-variate statistical analysis (principal response curves) of a freshwater microcosm zooplankton community, after treatment with the fungicide Carbendazim. The curves represent the time course of the effect of treatment on the zooplankton community. The species weight (b_k) explains the affinity of the taxon with the curve. Taxa indicated with a positive species weight are expected to decrease in abundance, relative to the controls, and taxa with negative weights are expected to increase. From van den Brink et al. [90]. With permission. Copyright Elsevier.

aquatic microcosm (SAM) originally developed by Taub [110], has been extensively evaluated. It is a multitrophic level community test with more than 15 biotic components (microalgae, pelagic and benthic invertebrates). The test system is static and consists of a series of 3-L glass jars, containing an artificial medium and a sand substrate (Figure 7.25). The test is carried out in triplicate with a total of 6 treatments over 63 days. The abundance of algae, macro-invertebrates and micro-invertebrates as well as nutrient dynamics and chemical fate, are among the recommended endpoints. The design and analysis of microcosm tests has greatly improved, such that former concerns about cause-effect relationships, replication and divergence of test results [104] have been sufficiently addressed [80, 105,106].

The difference between mesocosms and microcosms is mainly their size. Many mesocosm studies have been performed in artificial ponds, enclosures in lakes or oceans, or artificial streams. Artificial ponds (10m x 5m x 1m) contain sediment and are colonized by algae, macrophytes and macro-invertebrates, and can be stocked with invertebrates and fish [80,102,103]. Studies normally take approximately five months. Endpoints include chemical fate processes, dissolved oxygen, algal biomass, composition and the abundance of phytoplankton and zooplankton, as well as macro-invertebrates, snail reproductive success, and fish survival and growth. Experimental stream ecosystems are used to study lotic ecosystems, with an emphasis on the specific benthic macrofauna. For an overview, see Kennedy et al. in [7].

Concluding remarks

SS tests and MS tests both have their place in ERA. The use of MS tests in regulatory testing is of greatest value if used in combination with tests that can provide data

Table 7.22. Types of multi-species tests [80,103].

Microcosms: experimental tanks/ponds or bench-top systems with a water volume of between 10^{-3} and 10 m^3, or experimental streams less than 15 m in length
Mesocosms: outdoor experimental tanks/ponds with a water volume between 1 to 10^4 m^3 or experimental streams greater than 15 m in length

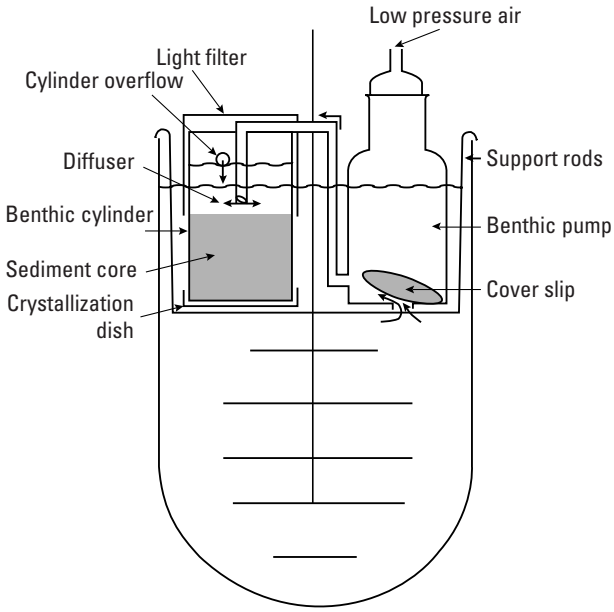

Figure 7.25. Diagram of a single test vessel in an experimental microcosm test system.

on fate, population interactions and ecosystem processes. Adequate fate and population models can be used to fill the gap between SS and MS testing, i.e., between environmental chemistry, toxicology and ecology. This will also serve the regulatory needs.

Multi-species indoor or outdoor tests can play an important part in elucidating the role of environmental factors that may modify the exposure and susceptibility of species. The decision whether or not a field study is required should be based on data obtained from preliminary and refined effects assessment and on data used for exposure assessment, e.g., degradation rates and partition coefficients between air, water and soil, or sediment. Therefore, these studies should not be seen in isolation but should be incorporated in a tiered scheme of testing, consisting of various stages: preliminary, refined and comprehensive assessment (Section 7.10.4).

It should be noted that the bioavailability of the test compound and the distribution of sensitivities of a number of important species should be known before a field test can be carried out. If, based on this information, there is some degree of risk or uncertainty, a field study may be necessary. Such a study should include sensitive and representative organisms. Several guidance documents are available for tiered testing strategies, with MS tests or field tests incorporated in higher tier testing [80,81,103].

7.4 SEDIMENT TOXICITY

7.4.1 Introduction

Most of the experimental work in aquatic toxicology has focused on the potential effects of dissolved pollutants on pelagic organisms. It has been well established that pollutants entering the aquatic environment partition to suspended particles and sediment [112,113], depending on their partition coefficients. Sediment constitutes an important compartment of aquatic ecosystems. Where a substance is likely to be found in sediments at harmful levels, due to its known chemical and toxicological properties and use pattern, a risk assessment addressing its fate and effects on benthic organisms should be performed. This should, in particular, consider the chemical's association and degradation in sediment and its toxicity to benthic organisms. Due to chemical loading of the sediment, tumours or liver neoplasm's have been observed in bottom feeding fish like carp [114], probably caused by polyaromatic hydrocarbons (PAH). Other sublethal effects, such as deformities in the mandibles or antennae of relatively tolerant sediment-dwelling taxa like chironomids [115] and setal abnormalities in oligochaetes [116] have also been observed. These effects are indicative of chemically polluted sediment areas.

Sediment and soil have a large capacity to retain environmental contaminants, especially persistent hydrophobic organic chemicals, or positively charged divalent or trivalent ions. Consequently, soil and sediment may act as a sink for, and a source of, toxic chemicals through the sorption of contaminants to particulate matter. Sediment can serve as a historical record of change due to both manmade pollution and natural environmental causes [117]. Surface water contamination disperses over time and space, and the chemical that is sorbed to the sediment can become a hazard to aquatic communities (both pelagic and benthic) which may be undetected from observations of contaminant concentrations in the water column. Even if the quality of the overlying water is improved, e.g., due to emission reduction, the polluted sediment may still act as a long-term threat to the organisms exposed to it [118].

In the following sections the principles of assessing the toxicity of chemicals in sediment are discussed with reference to the source of exposure, i.e., from sediment or interstitial water. Several methods for measuring this toxicity have been proposed. Internationally agreed water-sediment test guidelines are described only for chironomids, [119,120], but ASTM guidelines are available for other species [121]. Sediment toxicity tests

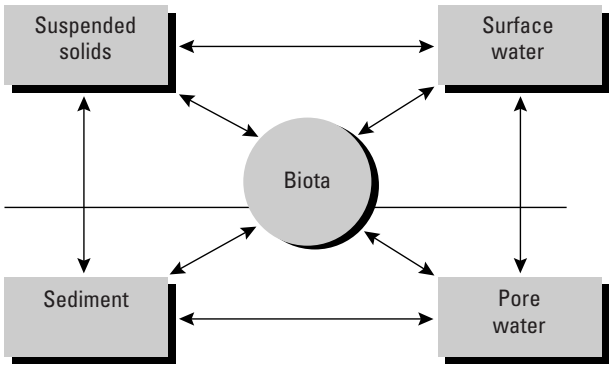

Figure 7.26. Compartments and their interrelationships in the sediment-water system [122]. With permission.

often differ with regard to the source and preparation of the sediment. The biological procedures and toxicological responses, however, are similar to those for water column (nektonic) organisms such as fish and daphnids. The emphasis is on the predictive goal, i.e., how can we derive sediment quality objectives (SQOs) or LC50, EC50, or NOEC values for chemicals or groups of chemicals?

7.4.2 Exposure systems

The design of test systems to determine the toxicity of chemicals to benthic organisms needs to take into account how the test chemical is introduced and distributed. It must also address sediment characteristics and the habitat, physiology and feeding modes of the test organism. Figure 7.26 illustrates how the different compartments of a sediment-water system might contribute to the contaminant uptake by sediment inhabiting benthic, epibenthic and pelagic organisms. Experimental information on the relative significance of the various uptake pathways for aquatic organisms is somewhat conflicting. Benthic and epibenthic organisms like polychaetes and many arthropod species can accumulate strongly adsorbing inorganic compounds like cadmium, and very lipophilic organic compounds like PCBs and PAHs. The main routes of exposure of organic contaminants for soil inhabiting invertebrate species can be pore water or ingestion of soil particles or for sediment dwellers, pore water, overlying water and ingestion of suspended solid or sediment particles [123-126].

Using equilibrium partitioning models, and taking into account environmental factors and sediment parameters, such as particle size and organic carbon content, NOECs for sediments can be estimated from (pore) water NOECs for individual compounds [26,32,33,124,127]. These models are presented in more detail in Chapter 3. Quantitatively the mode and kinetics of contaminant uptake from sediment can vary considerably between species, depending on factors such as feeding, habitat, activity and metabolism of the organism, developmental stage, season and history of exposure. Furthermore, the biological community itself strongly influences the physicochemical environment in the sediment and thus the bioavailability of contaminants by various processes:

- Primary productivity influences pH conditions which in turn influence metal chemistry.
- Sulfate reduction to sulfide by bacteria facilitates metal sulfide formation [128].
- Biological activity influences redox conditions and metal redox conversions.
- Production or degradation of organic matter may influence complexation of the contaminants.
- Bioturbation influences sediment-water exchange processes and redox conditions.
- Oxygen consumption leads to anaerobic conditions which can favour dehalogenation reactions [129] but inhibit mineralization processes.

Thus the bioavailability of pollutants depends on several abiotic as well as biotic factors. This is one of the main obstacles in experimental ecotoxicological research with sediments. Experimental manipulation of sediments may drastically influence the bioavailability of the test compound and therefore its toxicity. A basic understanding of a chemical's fate is, therefore, a prerequisite. Various exposure systems are available for testing sediment toxicity. Exposure systems can be entirely aquatic, but when experiments are carried out with pore water such systems should be used with care, depending on the species used and the duration of the study. Chronic studies using sediment organisms in sedimentfree conditions can cause severe stress (e.g., *Lumbriculus variegatus*, annelid worms, will tie themselves in knots when tested without substrate).

7.4.3 Effects assessment

This section provides a short review of methods which can be used to derive NOECs for sediments. It includes test methods for assessing the toxicity of sediment and setting quality objectives and methods for determining the toxicity of chemicals to sediment-dwelling organisms. Eight methods were evaluated at an OECD workshop [112] as being potentially useful for deriving sediment quality objectives (Table 7.23). The first three methods

Table 7.23. Potentially useful methods for deriving sediment quality objectives according to the OECD [112].

1. Equilibrium partitioning
2. Interstitial water quality
3. Spiked sediment toxicity
4. Reference concentrations
5. Apparent effects threshold
6. Screening-level concentrations
7. Sediment quality triad
8. Tissue residues

listed in Table 7.23 were recommended by the OECD [112] for the development of numerical sediment quality criteria on the basis of seven evaluation criteria (Table 7.24) and are discussed further. The remaining five were not recommended for this purpose, which does not mean that they are inadequate. The section ends with specific considerations for site-specific effects assessment.

Sediment quality objectives

The equilibrium partitioning (EP) approach derives NOECs or sediment quality objectives (SQOs) from aquatic NOECs or water quality objectives (WQOs) by predicting interstitial water concentrations and appropriately normalized sediment concentrations [32,33,124]. This method can be used as a screening method to assess the risk to sediment organisms. If exposure levels exceed the SQO, tests with benthic organisms should be part of a refined risk assessment. The formula for deriving the SQO for a particular chemical is:

$$SQO = K_p \cdot WQO \quad (7.6)$$

where SQO is the sediment quality objective (mg/kg dry wt), K_p is the solids-water partition coefficient (L/kg dry wt), and WQO is the effects-based water quality objective (mg/L). For comparative reasons, the solids-water partition coefficient is often adjusted with respect to organic carbon (OC) content (f_{oc} = %OC/100) and an organic carbon partition coefficient is thus defined:

$$K_p = K_{oc} \cdot f_{oc} = C_s / C_w \quad (7.7)$$

where K_{oc} is the OC-normalized K_p, and C_s and C_w are the chemical concentrations in solids and water, respectively. Typical values for OC content in sediment are in the range of 4 to 6%. The standard value for f_{oc} in sediment is set at 0.05. Where only the organic matter (OM) content is known, f_{oc} can be derived as follows: $f_{oc} = 0.6 \times f_{om}$. For neutral organic chemicals K_{oc} is often estimated from the K_{ow} using Log K_{oc} = Log K_{ow} – 0.21 [130]. The EP approach is based on the observation that interstitial water concentrations are more closely correlated than bulk sediment concentrations with toxicity to or bioaccumulation of environmental contaminants in benthic organisms (Figure 7.27). The EP method [32,124] assumes that:

1. The concentrations in sediment and interstitial water are in equilibrium.
2. The concentrations in any of these phases can be predicted using appropriate partition coefficients and concentrations in one phase.
3. The effect concentrations in sediment can be predicted using adequate partition coefficients and effect concentrations.
4. The WQO provides an appropriate effect concentration for deriving sediment quality objectives.

Table 7.24. Evaluation criteria for methods for deriving sediment quality objectives according to the OECD [112].

1. *Chemical specificity:* can the method be used to derive a concentration for a specific chemical?
2. *Causality:* are the observed effects caused by a specific chemical?
3. *Chronic effects:* does the method consider chronic toxicity endpoints?
4. *Bioaccumulation:* does the method consider food chain accumulation and ingestion of contaminated sediment for benthos and fish?
5. *State of development:* is the method validated, used and ready for use?
6. *Bioavailability:* how generally applicable is the method across sediment types? Are sediment quality objectives a function of the bioavailable phase?
7. *Applicability:* is the method applicable to bedded sediment or suspension?

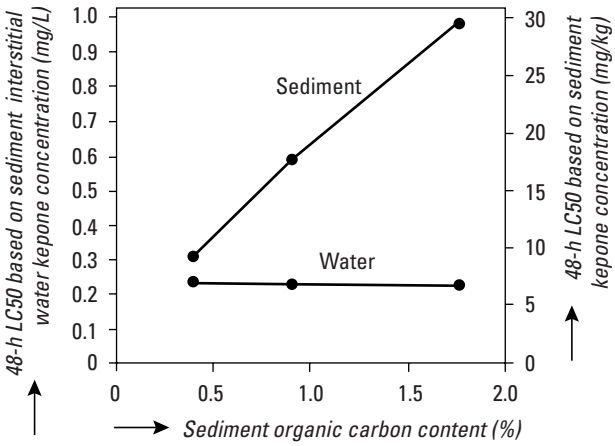

Figure 7.27. Plot of 48-h LC50 values based on interstitial water and sediment kepone concentrations versus sediment organic carbon content for the midge *Chironomus tentans*. From Ziegenfuss, Renaudette and Adams [131]. With permission.

The advantage of the EP method is that its theoretical basis is well established. It has been tested for non-ionic hydrophobic chemicals and metals [25,26,32,124] and has been applied for derivation of SQOs in the absence of test data (see Chapter 11, Section 11.3.3). The procedure for normalizing sediment concentrations requires a model for chemical partitioning, K_p, which can relate solid phase and liquid phase concentrations. At present there are models available for non-ionic organic chemicals, certain metals and a few ionic organic chemicals. The EP method can be applied to all chemicals (including metals) for which a series of aquatic NOECs or WQOs (also known as water quality criteria or standards) are present, and for which reliable K_p values are available. K_p values for most heavy metals vary with environmental conditions (Section 7.6), which complicates extrapolation to other conditions and the general application of the method. It can be used for marine and freshwater sediments and between sites. Examples of the calculation of SQOs are given in Chapters 4 and 11.

The *interstitial water quality* method is similar to the EP method except that interstitial water concentrations are measured instead of being predicted. This is difficult where substances are present at low levels or have low solubility. Non-depleting solid phase extraction methods are used to measure freely dissolved concentrations of lipophilic substances in interstitial water [132-134]. The *tissue residue* approach seeks to relate chronically acceptable chemical concentrations in benthic organisms to chemical concentrations in sediment using the EP approach. It has the same relative strengths and weaknesses as the interstitial water toxicity method but is less developed in terms of sediment quality criteria. One of its strengths is that it allows a predictive approach using sediment exposure-driven bioaccumulation [26,27,33].

Table 7.25. Test phase systems studied in predictive and empirical sediment toxicity studies.

1. Elutriate (water-extractable)
2. Extractable (solute other than water)
3. Interstitial or pore water phase
4. Whole sediments
5. *In situ*

In the *spiked sediment toxicity* approach, LC50s or NOECs in sediment are derived from experimental dose-response data generated in the laboratory. The SQO is then derived based on the experimental data (see Section 7.10). The test organisms are exposed to sediments spiked with a range of concentrations. The toxicological endpoints are those normally studied in aquatic toxicity tests. With this method it is assumed that spiked sediment in the laboratory behaves similarly and shows similar effects to natural *in situ* sediments. The major limitation of this method is that this assumption may not be true (Section 7.4.2). The advantages of the method are that it is chemical specific, demonstrates a clear causality, and reflects the bioavailability of the test compound. The use of artificial sediment is preferred over the use of natural sediment because of the reproducibility of results in spiking studies, unless site-specific effects are studied. Spiking the sediment can occur via the sediment itself (preferred) or via the water phase. In both cases, care should be taken with regard to equilibration time between the different phases, especially for poorly soluble compounds [120,121]. The sediment should be characterized in terms of particle size, organic matter content, and cation or anion exchange capacity. For natural sediment, additional parameters can be reported such as pH, ammonium and nitrogen content.

Site-specific approaches

The practical aspects of sediment sampling, storage, the collection of interstitial water, elutriates, spiking, sediment dilution and other conditions of exposure are discussed by the OECD [112] and Burton et al. [121,135]. The *sediment quality triad* is designed to evaluate the overall quality of the sediment of specific sites [136,137]. It compares: (a) chemical concentrations

Table 7.26. Representative freshwater and sediment toxicity tests [121,122,135].

Biological level	Assay/organism/community	Endpoint
Amphibians	*Xenopus laevis*	embryolarval survival, terata
Fish	*Salmo gairdneri*	embryolarval survival, growth, terata
	Pimephales promelas	embryolarval survival, growth, terata
	Brachydanio rerio	embryolarval survival, growth, terata
Zooplankton	*Colpidium campylum*	growth
	Brachionus sp.	survival
	Protozoan colonization	structure indices, respiration
	Daphnia magna	survival, reproduction
	Ceriodaphnia dubia	survival, reproduction
Benthic invertebrates	*Panagrellus redivivus*	survival, growth, moulting
	Caenorhabditis elegans	survival
	Tubifex tubifex	survival
	Stylodrilus heringianus	survival, avoidance, reworking rate, growth
	Hyalella azteca	survival, growth, reproduction
	Pontoporeia hoyi (*Diporeia* sp.)	survival, avoidance
	Corbicula fluminea	survival, growth
	Anodonta imbecilis	survival
	Chironomus tentans	survival, growth, emergence
	C. riparius	survival, growth
	Hexagenia limbata	survival, moulting frequency
	macrobenthic community	community/population indices
Microbes	*Vibrio fischeri*	luminescence
	alk. phosphatase	enzyme activity
	dehydrogenase	enzyme activity
	β-Galactosidase	enzyme activity
	β-Glucosidase	enzyme activity
Phytoplankton	Pseudokirchneriella subcapitata	population growth, ^{14}C-uptake
	natural phytoplankton	fluorescence, structure-species abundance
Macrophytes	*Lemna minor*	growth (fond number), chlorophyll-*a*, biomass
	Hydrilla verticillata	shoot length, root length, dehydrogenase-activity, chlorophyll-*a*, peroxidase

in sediment, (b) toxicological responses in the laboratory, and (c) benthic community health. It has some major advantages as it combines chemical and biological observations under laboratory and field conditions. It is widely used, but it is not chemical specific, i.e., unknown mixtures of chemicals and other stressors are often considered and therefore it cannot show causality. The test phases normally used in sediment toxicity studies (Table 7.25) each have their own strengths and weaknesses. These problems can be partly overcome by performing a toxicity identification and evaluation (TIE), linked to a triad analysis. In a TIE, toxicity-based fractionation procedures are used to identify specific contaminants as causative toxicants [138]. Sediment pore water is isolated from sediment and toxicity experiments are conducted with it. This can involve concentrating the isolate (e.g., using solid phase extraction methods) and subsequent dilutions. Solid phase micro-extraction methods offer a relatively quick and cheap way of characterizing the pollution profile of organic substances that are bioavailable and also give an indication of the bioaccumulation potential [139]. Depending on the scope of the TIE research, one or several test species can be used to test the toxicity of pore water [140].

In their excellent review on freshwater sediment toxicity, Burton et al. [121] describe a number of

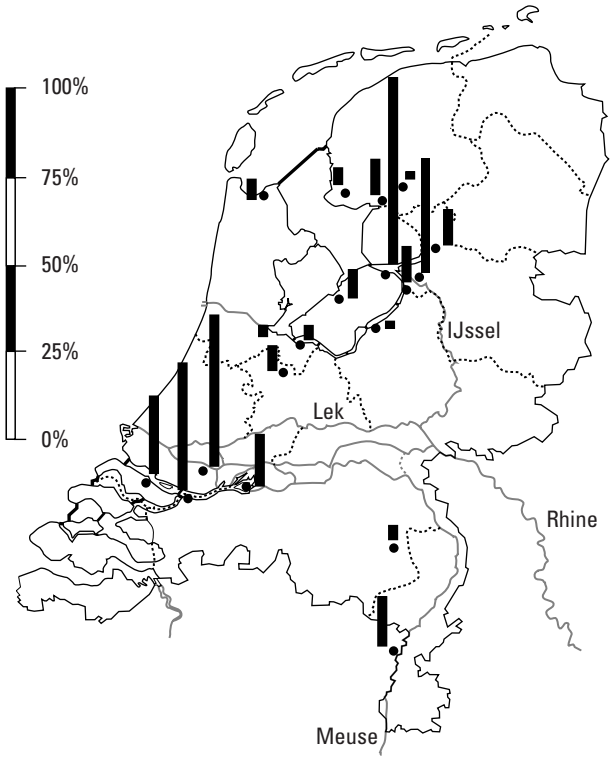

Figure 7.28. High frequencies of mandible malformations in midge larvae as observed in sediments in The Netherlands. Data from Van Urk and Kerkum [115].

approaches, the practical difficulties and various species used to assess freshwater sediment toxicity (Table 7.26). From Table 7.26, it may be concluded that standard test species like bacteria, algae, daphnids and fish are commonly used to assess the toxicity of aqueous fractions of contaminated sediments. Tests with pore water extracts or elutriates are often carried out and semistatic sediment-water systems are also used. In these systems direct contact with contaminated sediment particles is taken into account.

Some problems are associated with TIE with regard to the influence of extraction techniques and their relevance to the bioavailability in the in-situ situation. The same applies when only pore water exposure is considered, ignoring exposure to sediment by contact or ingestion, which could be especially relevant for lipophilic substances with low water solubility. Various tiered decision-making frameworks for sediment contamination have been developed to address these problems [141,142]

7.4.4 Sediment toxicity testing

Good quality water and sediment are typified by species of macro-invertebrate taxa like mayflies (Ephemeroptera), caddisflies (Trichoptera) and stoneflies (Plecoptera). By contrast, dominance of the tubificid oligochaete species *Limnodrilus hoffmeisteri* and *Tubifex tubifex* is recognized as an indication of polluted sediment. Based on the results of field studies [115], midge larvae (Chironomidae) are used as test organisms for spiked sediments (Figure 7.28). There are also internationally harmonized guidelines available [119,120]. A test guideline with the benthic oligochaete *Lumbriculus* using spiked sediment is available in draft form [143].

Other benthic organisms with different morphological structures and different environmental behavioural and trophic properties are also used, such as oligochaetes, polychaetes, nematodes, bivalves, burrowing mayflies and crustaceans, such as amphipods and isopods, as well as several plant species [58, 121]. ASTM has published a number of standard techniques for 10 and 28-day tests with marine and freshwater amphipods (e.g., *Hyalella azteca*) polychaetes, oligochaetes *(Tubifex tubifex)* and mayflies, that are reviewed in Burton et al. [121].

Table 7.27. Characteristics of a chironomid toxicity test [119,120].

Test species	Chironomus riparius, *C. tentans* or other species, e.g. *C. yoshimatsui.*
Test duration	20-65 d , depending on species and growth rate
Test system	Static (in some cases semi-static or flow-through) with elutriates or sediment and water at a layer depth ratio of 1:4 in 600 ml glass beakers
Feeding	at least three times a week with a commercial fish food
Endpoints	larval emergence, growth and survival
Temperature	20-25°C, depending on species.
Parameter	EC50, LC50 and NOEC/LOEC

Table 7.28. Susceptibility of Chironomus riparius life stages to dieldrin (nominal concentrations in mg/L) [122].

Parameter	Life stage	Concentration
96-h LC50	egg	>100
96-h LC50	2nd larval stage	5.2
96-h LC50	3rd larval instar	12.6
96-h LC50	4th larval instar	17.9
23-d NOEC	egg-4th larval instar	0.1

Short and long-term toxicity tests compare the survival, growth, reproduction or other toxicological endpoints among a range of benthic, epibenthic and pelagic organisms. These organisms are exposed to experimentally contaminated (spiked) solid or liquid phases in a test system in order to determine the effects of chemicals or groups of chemicals on sediment-dwelling organisms. As an example of a sediment toxicity test, the subchronic test with *Chironomus riparius*, is given in Table 7.27. As with fish, chironomids exhibit differences in susceptibility at various life stages (Table 7.28).

In conclusion, sediment toxicity studies have matured. Much work has been done to standardize the assessment of sediment toxicity, both for routine toxicity testing with relevant benthic organisms and for the assessment of the toxic effects of a polluted sediment. Some pitfalls remain. Sediment is a very heterogeneous environment and exposure and bioavailability in the field may differ from that in the laboratory. Manipulation of sediment may drastically influence bioavailability and thus toxicity, indicating that risk assessment requires careful consideration of the physicochemical processes at work in sediments.

7.5 TERRESTRIAL TOXICITY

7.5.1 Introduction

Soil contamination is widespread and thousands of polluted sites have been identified in industrialized countries. The importance of soil as a key component of ecosystems is now widely recognized. Several countries have already established soil quality objectives and programs for site-specific risk assessment [144].

Due to their public appeal, adverse effects of contamination of the terrestrial environment are often discussed in terms of the decline and recovery of populations of rare plant species, such as orchids, mammals such as otters *(Lutra lutra)*, bats (e.g., *Myotis dasycneme*), and various species of birds, such as terns (*Sterna* sp.), eider ducks *(Somateria mollissima)*, cormorants *(Phalacrocorax carbo)*, partridges *(Perdix perdix)*, peregrine falcons *(Falco peregrinus)*, goshawks *(Accipiter gentilis)* or little owls *(Athene noctua)*. Environmental protection is often directed towards protecting soil organisms, but protecting soil functions is at least as important in view of the sustainability of land use.

From an ecological point of view, the main functions of soil are those associated with the decomposition of organic matter, mineralization of nutrients, and synthesis of humic substances. Essential parts of the carbon, nitrogen, phosphorus and sulfur cycles take place in the soil. The root zone (rhizosphere) in particular, is closely involved in soil processes. Soil organisms mainly contribute to litter breakdown. This is done by soil invertebrates and soil microbes in concert. The vertical distribution of species varies greatly. The highest density of species is found in the topmost layer of the soil profile. Apart from the role of soil in nutrient cycles, the soil formation process (Figure 7.3) is essential for supporting plant life and in stabilizing mineral particles.

Although the terrestrial environment is crucial for the human population, the soil has only recently become an important topic for ecotoxicologists. First we will turn our attention to exposure assessment in experimental systems.

7.5.2 Exposure systems

Soil contains solid, liquid and gas compartments, each of different and varied composition. The solid compartment is composed of mineral particles and organic material, the liquid one is made up of water with dissolved nutrients and dissolved organic carbon, while the gas compartment consists of different gases and volatile organic substances. These constituents are arranged in a certain order and according to particle size in a certain texture and structure. Particle size influences the total surface area. Soil is an extremely heterogeneous environment, both horizontally and vertically. As a consequence, physical, chemical, and biological characteristics vary, thus creating a wide variety of habitats for soil-dwelling species. This complexity and heterogeneity greatly affect actual exposure situations.

In toxicity tests with terrestrial organisms, different exposure systems are used depending on the way in which organisms are exposed. The three major uptake routes are: (1) ingestion and oral uptake of food or soil

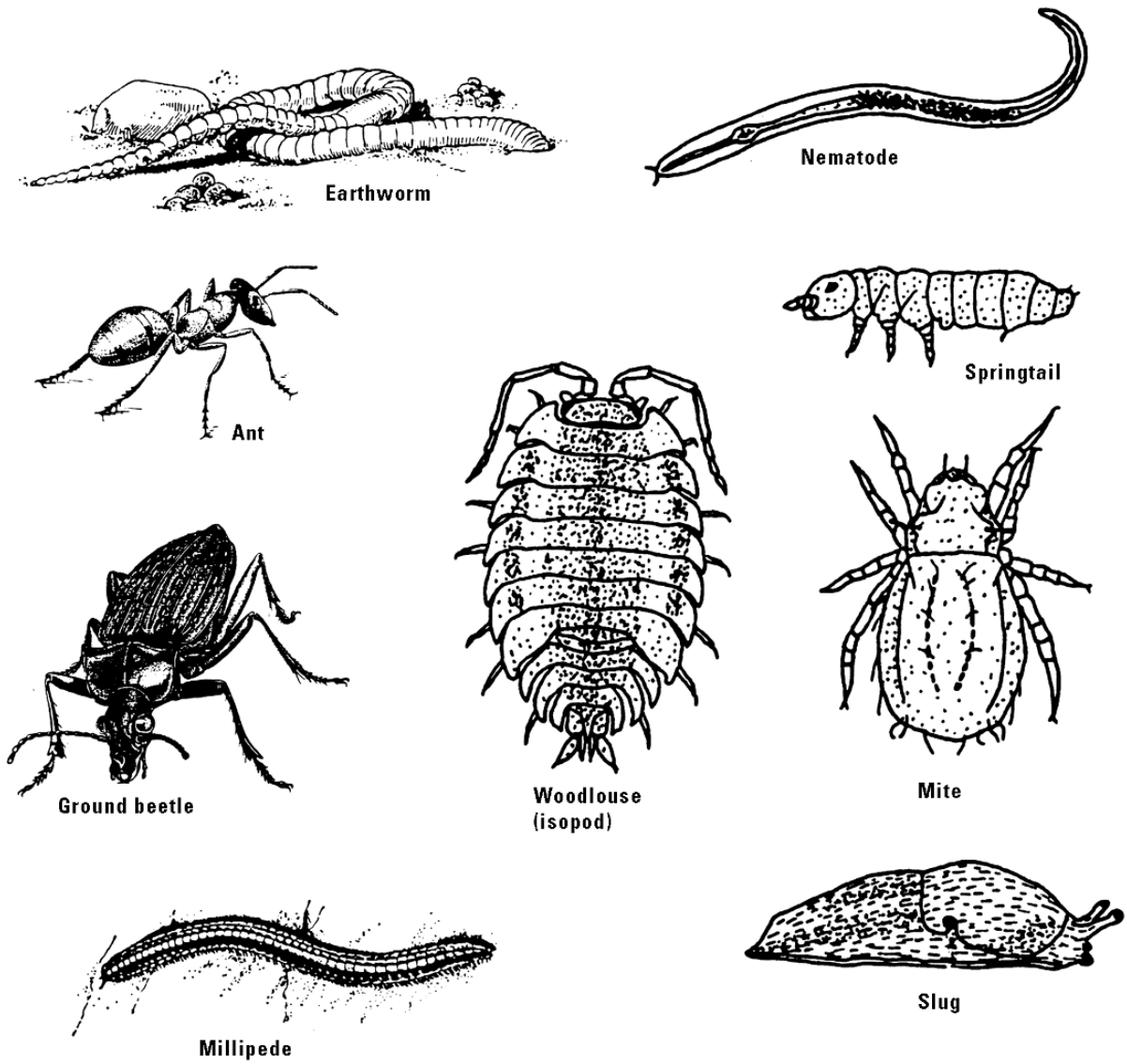

Figure 7.29. Some representatives of the soil invertebrate fauna (not drawn to the same scale). Modified from Van Straalen and Van Gestel in Calow [56]. With permission.

particles, (2) dermal uptake of pollutants from the soil or plant surfaces, and (3) respiration (via stomata, tracheae and lungs).

Effects on micro-organisms are mostly studied by exposing the indigenous microflora of a clean soil by introducing the test chemical into a soil sample [145] or by isolating micro-organisms or microbial communities and testing them in artificial substrates. In invertebrate toxicity tests a species-specific exposure method is often used in relation to the expected uptake route in the field. Tests with birds or mammals can be used to study oral, inhalation or dermal toxicity. The bioavailability of the chemical tested will differ for each exposure pathway, which is reviewed in the next section.

Chemicals mixed with the soil

For soil-dwelling species such as bacteria, fungi, soil invertebrates (e.g., protozoans, earthworms, enchytraeids, mites, and nematodes; see Figure 7.29), and most vascular plant species, tests in soil seem to best simulate

Table 7.29. Composition of OECD artificial soil [146,151].

Industrial quartz sand	70%
Kaoline clay	20%
Sphagnum peat	10%
Water content (% of the water holding capacity)	40-60%
pH (by addition of $CaCO_3$)	7.0±0.5

natural exposure routes. Although arthropods normally do not ingest mineral soil, many live in close contact with it and take up chemicals from the soil/air interface. This is thought to be mediated via a water film. The way the substrate is prepared allows the dose to be expressed as a concentration per mass unit of dry soil, i.e., in mg/kg. The substrates used in soil tests vary from natural materials taken from the field, to soils artificially created out of commercially available materials [146]. The type of soil will have a major influence on bioavailability, i.e., the distribution of chemicals over the solid, gas and pore water phases, and will greatly affect toxicity.

As with aquatic sediments (Figure 7.27) the soil toxicity of many organic chemicals is often directly related to the organic matter content of the soil. This has been demonstrated for earthworms and a variety of other species [147,148]. With heavy metals, bioavailability may depend on soil pH, organic matter content, cation exchange capacity (CEC) and clay content [149]. Quantification of the contribution made by each factor to the toxicity and bioavailability of metals to earthworms is difficult [147], but modern statistical and mathematical models have greatly helped to improve our understanding [41,150]. For purposes of standardization, the use of artificial soil is recommended, for earthworms, enchytraeids and soil arthropods (Table 7.29).

Direct and indirect application

Topical dosing is generally applied to mammals and arthropods [152]. The toxic solution is applied directly to a predetermined area of the body surface after immobilization of the animal. This method of topical application allows the dose to be expressed as an absolute amount per animal. From a toxicological point of view, this is a preferred exposure method, since any effect can be directly related to the dose; disrupting factors such as consumption, movement and other activity can be eliminated as sources of variation. However, in a field situation the actual dose received is usually unknown.

Arthropods and plant seeds may also be dosed by contact with a chemical through immersion in a solution. The time of immersion is standardized. The dipping technique is easy to carry out but it has the disadvantage that the dose received is unknown. Effects are expressed in terms of the concentration of the chemical in the dipping solution. Plants and invertebrates, such as earthworms, nematodes and protozoans, may also be tested in aqueous solutions of the test chemical. In these tests, the species are treated as aquatic organisms and the aqueous phase is considered to be the most important route of exposure and the interaction with the soil solid phase or air is neglected. Both topical and whole-body exposure techniques are mainly restricted to laboratory research methods. According to Van Straalen and Van Gestel in Calow [56] other routes of exposure may also be important in the field situation [147].

An important exposure route for species on agricultural land treated with sprayed chemicals is residual uptake. Surfaces coated with films of pesticides will act as a source of uptake by organisms as they move over the surface, especially high surface-activity species, such as predatory mites, spiders, beetles, and springtails. In experiments organisms may be present during application of the toxicant, in which event the effect is caused both by direct and residual exposure. More frequently, however, the treated surface is allowed to dry and organisms (often arthropods) are placed on the treated surface for the test. Surfaces used in such tests include plant leaves, sand, natural soils, or artificial substrates such as filter paper or glass [153]. The bioavailability of the residue depends very much on the nature of the substrate, its tendency to adsorb the chemical, and its moisture content. For the purposes of standardization the use of an inert material that will neither adsorb nor react with the chemical, i.e., glass or sand, is recommended, but effective doses established in this way are very difficult to apply to field situations, as inert surfaces do not resemble the natural situation [154].

Chemicals added to food

Dietary uptake of chemicals via food is a well-known exposure route of mammals and birds (Chapter 5). Dietary uptake is a direct route for chemicals sprayed on leaf surfaces, acting as stomach poisons in phytophagous invertebrates, as well as for chemicals associated with dead organic matter which have an effect on saprotrophs. Dietary exposure may also occur via the food chain, e.g., predatory birds or mammals feeding on fish (Table 7.9) or mammals, herbivores feeding on various species of plants, or microbivorous arthropods feeding on fungi

that concentrate chemicals from the soil. In these toxicity tests, chemicals are homogeneously mixed with the food, and the effective dose is expressed per dry mass of food. Other dietary routes are via drinking water or sucrose solutions. If the test animals take in the amount fed completely, or if consumption can be determined by weighing the food left, the dose can be expressed in mg/kg body weight. This allows comparison with doses taken up via other routes, e.g., topical application. The food used in feeding experiments largely depends on the species. The uptake efficiency of chemicals added to the diet is highly variable. Effective concentrations are difficult to compare between species because they will be influenced by the type of food used and the physiological condition of the animal. Avoidance of contaminated food is a common response in some arthropods but is also found for birds [155].

Exposure via the air

When organisms are tested for their susceptibility to gaseous air pollutants, exposure units must be airtight before they are flushed with a known concentration of the chemical in air. Plant exposure to gases is controlled in open top chambers, which is the preferred method [156]. Several pesticides, especially those applied as fumigants, exert their effects through aerial exposure and are tested in this way on various plant and animal species such as flies, fleas, and ticks, etc. [148,153]. When some pesticides are sprayed on a surface, actual exposure may actually be via the air, as the chemical evaporates from the surface film to reach toxic concentrations above the surface.

7.5.3 Effects assessment

Soil toxicity tests have been developed as a means to provide hazard information for the terrestrial environment. This hazard information can be used to derive soil quality objectives, similar to the way in which sediment quality objectives are derived. Sites that are polluted may need remedial treatment to prevent risks to man or the environment. Due to the high costs associated with soil remediation, it is essential to have efficient laboratory test methods to indicate potential hazards and use in-situ bioassays to determine the risks at specific sites. Several large-scale programmes have been devised to provide such methods [157-159]. The triad framework for site-specific risk assessment for sediments can be adapted for soils. By combining information on measured concentrations, toxicological responses in the field or laboratory and soil community composition, conclusions can be drawn on the risks to the soil ecosystem [159].

Situations where sediments are removed from water bodies and deposited on land for reasons of water management or remediation, require special attention. Once these sediments are put on land, the physicochemical conditions change dramatically due to water loss and the predominantly aerobic conditions, thereby influencing biodegradation and the mobility of the contaminants.

7.5.4 Soil toxicity testing

Microbial tests

Bacteria are by far the most numerous organisms in soil, varying from 10^6 to 10^9 cells/g [56]. Although bacteria are dominant in soil, fungi, as a group, also play an essential role in the decomposition of organic matter. This is because of their ability to develop in the soil by means of hyphae and through the use of enzymes capable of degrading a variety of persistent substances such as lignin. In the same way as for sediments, toxicity tests can be carried out with micro-organisms from a clean soil. Soil functional microbial tests are carried out with freshly sampled soil containing an active microflora consisting of numerous species. These functional tests are thought to be more representative of the soil ecosystem. The microbial processes studied are essentially the same as those described in Section 7.3.3.

For site-specific risk assessment to deal with chronic soil pollution, the ability of micro-organisms to develop pollution-induced community tolerance (PICT) is studied relative to control sites [160-162]. Microbial communities can develop tolerance to specific chemicals, due to the loss of sensitive species and genetic or physiological adaptation. The ability of bacteria to use a variety of specific substrates in micro-well plates is compared between control sites and polluted sites. When the bacteria from a polluted site show a higher metabolic activity on specific substrates, after pre-exposure to specific chemicals, than bacteria from a control site, community tolerance is increased and a strong causal link between the pollutants and microbial functions of a specific site is established.

Vascular plants

The available toxicity data for terrestrial plants are highly diverse. Plant tests have been reviewed by Kapustka and Reporter in Calow [56] and Klaine et al. [62]. Two plant groups have been used extensively in developing rapid partial life-cycle tests, *Arabidopsis* and *Brassica*.

Substances can be taken up by the plant via the soil, via soil splash on the leaves, and through direct

Table 7.30. Characteristics of the terrestrial plant growth test [163].

Test species	a minimum of three species should be selected for testing, at least one from each of the following categories: rye grass *(Lolium perenne)*, rice *(Oryza sativa)*, oat *(Avena sativa)*, wheat *(Triticum aestivum)*, sorghum *(Sorghum bicolor)* (category 1), mustard *(Brassica alba)*, rape *(Brassica napus)*, radish *(Raphanus sativus)*, turnip *(Brassica rapa)*, Chinese cabbage *(Brassica campestris)* (category 2) and vetch *(Vicia sativa)*, mung bean *(Phaseolus aureus)*, red clover *(Trifolium pratense)*, fenugreek *(Trifolium ornithopodioides)*, lettuce *(Lactua sativa)* and cress *(Lepidium sativum)*
Test duration	plants are harvested usually 14-21 d after 50% emergence in the controls
Test system	static system, the test substance is dissolved in a solvent and mixed with natural soil or applied to soil surface
Light/temperature	suitable for growth
Endpoints	emergence and growth (wet weight)
Parameter	LC50 (emergence) and EC50 (growth)

deposition on leaves and other above-ground parts of the plant. Standard tests differentiate between the two main exposure pathways [163-164]. This recognizes the need to evaluate the effects of plant protection products that are sprayed on non-target plant species.

The most common type of phytotoxicity test is the seedling emergence and growth test [163]. Four to five plant species are commonly used (Table 7.30). The seed germination tests, often promoted as representing a sensitive and critical stage in the life cycle, is rather insensitive to many toxicants. This is caused by two factors: first, many chemicals are not taken up by the seed; and second, the embryonic plant derives its nutritional requirements internally from the seed storage materials, essentially making it isolated from the environment. The early growth test yields relevant information on exposure via the soil. The endpoints that are reported after a 14 to 21-day growth period are biomass of the plant [165], as well as shoot height and visible detrimental effects.

The vegetative vigour test [164] evaluates the effect of a spray application of a substance on shoot weight or shoot height, after a 21 to 28-day growth period from treatment. In addition, visual differences with the control with regard to chlorosis, necrosis, wilting or deformations can be reported.

Soil invertebrates

Harmonized soil toxicity test using invertebrates are available for earthworms, enchytraeids, Collembola, snails and insect larvae. Earthworms are commonly used because of their great ecological importance. International guidelines include the OECD acute earthworm toxicity test [146] and the OECD reproduction test [151]. For these two tests, *Eisenia andrei* or *E. fetida* are recommended. These are not actual soil-dwelling species, but are commonly found in compost and dung heaps, and can be cultured easily in the laboratory on a substrate of horse manure or cow dung. According to the guidelines, other soil-dwelling species may also be used. Instead of *E. fetida*, it is suggested to use the soil inhabiting *Apporectodea caliginosa* to improve the ecological relevance of the reproduction test. However, due to its slow reproduction cycle and the need to collect test individuals from the field, this species is not recommended for routine toxicity testing [166]. Therefore the two *Eisenia* species are recommended for practical reasons.

The acute toxicity screening test consists of the 2-day filter paper contact test, and the 14-day artificial soil test, scored on the survival endpoint. The filter paper contact test is a toxicity-screening test, but it has no predictive value for the effect of chemicals in the soil. The results obtained from the artificial soil test (Table 7.31) can easily be applied to natural soils using sorption data (Section 7.4.3). This test is reasonably capable of predicting effects in the field.

The earthworm reproduction test lasts for 8 weeks [151]. The parent animals are exposed for four weeks and then removed from the system and mortality and growth determined (Table 7.32). After another 4 weeks, the total number of off-spring produced is recorded. Another long-term invertebrate test is the 6-week enchytraeid reproduction test, with a similar test design as for the earthworm reproduction test [167].

Several other soil invertebrate toxicity tests have been developed for a number of major soil invertebrate taxa: oribatid mites, nematodes, isopods, staphilinid beetles,

Table 7.31. Characteristics of the acute artificial soil test with earthworms [146].

Test species	*Eisenia fetida* and *E. andrei*
Test duration	14 d
Test system	static test in test jars with 750 g (wet weight) of OECD artificial soil
Light/temperature	low light intensity (400-800 lux) at 20°C
Endpoints	survival
Parameter	LC50

Table 7.32. Characteristics of the reproduction test with earthworms [151].

Test species	*Eisenia fetida* and *E. andrei*
Test duration	pre-incubation (at least one day), exposure of adults to treated soil (4 weeks) followed by incubation of cocoons in untreated soil (4 weeks)
Test system	static test in test jars with OECD artificial soil
Light/temperature	low light intensity (400-800 lux) at 20°C
Food	oatmeal, cow or horse manure (dried and ground)
Endpoints	survival and growth of adults (after 4 weeks of exposure) and reproduction i.e. the total number of offspring per adult worm (after a further four weeks)
Parameter	NOEC and/or ECx (EC10, EC50) for reproduction, LC50, % of initial weight.

centipedes, millipedes, Collembola, and interactions between nematodes and between predatory mites and nematodes [166]. Some of these species are listed in Table 7.33. Additional information on test procedures can be found in Van Gestel and Van Straalen [148] and in Løkke and Van Gestel [166].

Beneficial arthropods

A special group of invertebrates are the "beneficial" arthropods that may improve the productivity of agricultural soils. There is commercial interest in designing and applying plant protection products in such a way that "beneficials" are least affected. Among the beneficials are pollinators such as the honey bee *(Apis mellifera)* and predatory and parasitic species that attack pest species. There is an internationally harmonized test guideline available for the effects of substances on honey bees [152] which is based on the guideline of the European and Mediterranean Plant Protection Organization (EPPO).

The Hymenoptera contain a large number of parasitic species. The female insect deposits an egg in or on a host (usually an insect egg or larva), which is then gradually eaten as the offspring develop. Within the order of the Coleoptera, the families Carabidae (ground beetles), Staphylinidae (rove beetles) and Coccinellidae (ladybirds) contain representatives that are commonly found on agricultural land and are recognized for their predation of pests (Figure 7.30). Among the various arthropod groups, other predators such as spiders and predatory mites are also important. The families Erigonidae and Linyphiidae (money spiders) are important groups with a great species diversity. Guidelines for evaluating the side effects of pesticides to non-target arthropods have been published [153] which may be useful for other categories of substances as well.

The array of methods used in testing terrestrial invertebrates is wide because different tests have been developed with different aims. Many methods still differ in relation to the medium to which the chemical is applied (different types of soil, contact surfaces), and the influence on bioavailability (see Section 7.6). This is why the OECD will continue to review and further harmonize terrestrial ecotoxicology guidelines.

Tests with birds and mammals

There has long been public concern about the effects of pollutants on mammals and birds. Bird and mammalian toxicity tests therefore have a much longer tradition than tests with soil invertebrates, for example. Mammalian toxicity data are required mainly to determine the potential risk to humans. Toxicity is determined using

Table 7.33. Overview of selected laboratory tests using terrestrial invertebrates, evaluated according to three criteria[a] according to Van Gestel and Van Straalen [148]. With permission.

Tests species		A	B	C
Protozoans	*Colpoda cuculus*	+	+	–
Nematodes	*Plectus acuminatus*	+	+	–
Isopods	*Porcellio scaber*	+	–	±
	Trichoniscus pusillus	+	–	±
Mites	*Platynothrus peltifer*	+	–	±
Collembola	*Folsomia candida*	+	+	+
	Orchesella cincta	+	±	±
Enchytraeidae	*Enchytraeus albidus*	±	+	+
Lumbricidae	*Eisenia fetida*	–	+	+
Molluscs	*Helix aspersa*	+	±	±
Hymenopteran parasites	*Encarsia formosa*	±	+	–
	Trichogramma cacoeciae	+	±	–
Beetles	*Bembidion lampros*	+	±	±
	Aleochara bilineata	+	±	±
Predatory mites	*Phytoseiulus persimilis*	±	+	–
Spiders	*Oedothorax apicatus*	±	–	±
Honey bees	*Apis mellifera*	+	+	±

[a] A: ecological relevance, B: potential for standardization and culture by different laboratories, C: potential to derive environmental quality criteria from the test results.

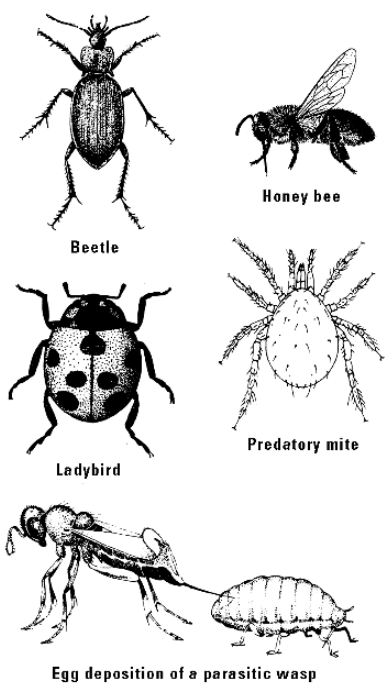

Figure 7.30. Some representative "beneficial" arthropods (not drawn to the same scale).

terrestrial mammals such as the laboratory rat and at least one other species (mouse, rabbit, guinea pig or dog) in order to test for skin and eye irritation and skin sensitization, and to determine acute, repeated dose and reproductive toxicity. As the principles of laboratory toxicity testing of wild mammals and mammals used for HRA do not differ, see Chapter 6 for more information.

In ERA it can be important to obtain toxicity data for birds, since rats and mice do not necessarily provide reliable surrogate data. Many carbamate and some organophosphate insecticides are distinctly more toxic to birds than to mammals. This reflects biochemical and physiological differences between these two taxonomic groups. These additional mammalian laboratory toxicity studies may reduce the uncertainty in ERA, but from an ecological, ethical and cost point of view the need seems questionable, particularly in view of the enormous uncertainty surrounding ERA for other taxonomic groups (Table 7.2).

Bird toxicity studies may be required for the notification of new chemicals or for environmental risk assessment of existing chemicals. In most countries these tests are obligatory for the registration of pesticides. In the US, the USEPA requires a series of tests on pesticides

Table 7.34. Observations in the USEPA avian oral dose LD50 test [168].

– Survival, body weight and food consumption
– Gross necropsies (optional). When performed, all dead birds should be examined, as well as a sufficient number of survivors in order to provide a characterization of gross lesions. Inspections of the gastro-intestinal tract, liver, kidneys, heart, and spleen should be made
– Other signs of intoxication should be described as to what was observed when and for how long

Table 7.35. Characteristics of the short-term OECD avian dietary test [171].

Test species	the Mallard duck *(Anas platyrhynchos)*, the northern bobwhite quail *(Colinus virginianus)*, the Japanese quail *(Coturnix coturnix japonica)*, pigeons *(Columba livia)*, ring-necked pheasant *(Phasianus colchicus)* and red-legged partridges *(Alectoris rufa)*
Test duration	usually 11 d, acclimatization (3 d), exposure to a diet containing the test substance (5 d) and exposure to the basal diet free of the test substance (for a minimum of 3 additional days)
Test levels	5 dietary levels and 2 control groups
Food	commercial food type
Observations	mortality, body weights, food consumption, signs of toxicity, and tissues from poisoned birds or from birds killed at the end of the test may be subjected to pathological, biochemical and residue examination
Parameter	LC50 and, if appropriate, an estimated NOEL

arranged in a tiered system that may progress from basic laboratory studies to applied field studies. Typically, the initial requirement is for two avian laboratory tests, an acute oral LD50 study and a dietary LC50 study. Additional avian reproduction toxicity data may be required with high PEC/NEC ratios or in the event of frequent application or persistence, which may result in long-term exposure. Given the urgent need for reduction of the use of animals, it has been proposed to reduce the number of tests to a modified acute toxicity test and a reproductive toxicity test, and use more efficient test protocols [168].

Determination of the avian single-dose oral LD50 follows the same principles described for mammals (Chapter 6). The species normally tested are the Mallard duck *(Anas platyrhynchos)*, the northern bobwhite quail *(Colinus virginianus)* or the Japanese quail *(Coturnix coturnix japonica)*. Normally five treatment levels are tested plus an additional control group. If necessary, a vehicle control group is included as well. The number of birds per treatment level is 10. In the single-dose oral LD50 test (Table 7.34) food is withheld from all birds for at least 15 hours prior to oral dosing. After administration of the test material the birds should have free access to a standard ration of food and water. Food consumption is monitored. The observation period is 14 days. This period must be extended if toxic signs persist or birds continue to die on the last day of the observation period. The results are expressed as LD50 (mg/kg_{bw}). If possible, NOELs should be reported as well. To reduce animal use in this test, a more efficient test protocol has been proposed [169], based on an "up and down" procedure [170]. This procedure is based on a stepwise reduction of the dose to two birds with a fixed factor. Each time the two birds survive, the dose is increased with a factor x. If one bird dies, the dose is reduced by a factor $\sqrt{x}$, or increased by a factor $\sqrt{x}$ if the previous dose was lower. The procedure stops when two deaths occur. The LD50 is then calculated as the geometric mean of the relevant doses. Although the results are less precise than when classical methods are used, far fewer birds are needed.

The avian dietary toxicity test is part of the OECD test guidelines. The aim of this test guideline is to determine the acute dietary LC50 (Table 7.35). A practical problem with the dietary study is the incorporation of the test chemical in food. This can raise certain difficulties related to uniform mixing of the substance in the diet, its volatility and, for pesticides, its formulation. The dietary study might be omitted by using information from a reproduction study for dietary exposure [172].

Long-term toxicity testing is occasionally carried out with birds where a long-term effect is suspected, or with

Table 7.36. Characteristics of the OECD avian reproduction test [172].

Test species	recommended species: the Mallard duck *(Anas platyrhynchos)*, the northern bobwhite quail *(Colinus virginianus)* and the Japanese quail *(Coturnix coturnix japonica)*
Test duration	approximately 34 weeks, exposure to a diet containing the test substance (for a minimum of 20 weeks), collection of eggs (over a 10-week period), followed by incubation and hatching of the eggs, the young are maintained for 2 weeks
Test levels	a minimum of 3 dietary concentrations and 1 control group
Food	commercial food type
Observations	mortality and signs of toxicity, body weights of adults and of the young at 14 days of age, food consumption of adults and young, gross pathological examination of adult birds, egg production, cracked eggs, egg shell thickness, viability, hatchability and effects on young birds, the residue analysis of selected tissues is optional
Parameter	NOEC (mg/kg diet)

chemicals that produce a delayed effect (e.g., certain organophosphorous pesticides). An avian reproduction test guideline is also provided by the OECD (Table 7.36). The aim of the reproduction test is to determine the NOEL (mg/kg diet) for the parameters studied. If a carrier is used for test diets, the same vehicle should be added to the diets of birds in the control group. After an exposure period of 20 weeks, the birds are induced by photoperiod manipulation to lay eggs. The eggs are collected, artificially incubated and hatched. Currently, improvements to this test are being discussed with a 10 week exposure period and fewer birds per treatment.

In test guidelines for birds, particular emphasis is placed on lethal effects. However, animal welfare concerns are pushing towards a reduction in test animals and improved statistical design. The trade-off is that with reduced testing set ups, the ability to detect the effects of chemicals may be less than with classical methods. Clearly, an optimum balance needs to be found between statistical power, animal welfare and sufficient safety for the environment. Examples of how bird and mammalian toxicity studies can be used to derive PNECs for soil and water are given in Section 7.10.3.

Multi-species tests

Model ecosystems, microcosms or micro-ecosystems are designed to simulate certain aspects of real ecosystems and therefore go beyond standardized tests for standard setting or testing of polluted soil. These systems can be used to study effects on individual species, predator-prey relationships, competition for resources, soil functions and biodiversity [173,174].

Multi-species tests with bacteria, plants and invertebrates have not reached the stage of international harmonization. Several terrestrial model ecosystems (TMEs) have been described [174-176] and an attempt has been made to standardize them [177]. The TMEs contain a soil column, soil micro-organisms, invertebrates, sometimes plants or even a small tree. The system may be either closed or open to the ambient air, and may contain intact core samples from a natural habitat or reasonably standardized soil. The effects of pre-treatments, such as drying, sterilizing, inoculation, litter type, age of litter, etc., can have a significant impact on the behaviour of the system and need to be thoroughly investigated.

Different types of TMEs (or integrated soil microcosms) exist. They may be composed of intact soil columns with intact soil cores, indigenous invertebrates and mixed plant flora, or they may be assembled systems consisting of sieved soil, selected introduced and indigenous invertebrates, and perhaps a single plant species [173,174]. The natural situation is approached more closely in the first type, but the second type offers more possibilities for replication under controlled laboratory conditions. Rainfall may be simulated and leachate can be collected and analyzed. Activities of saprotrophic invertebrates can, for example, be easily assessed in terms of system functions [175] such as leaf litter fragmentation and nutrient conversion (Figure 7.31). Uncertainties attached to laboratory-field extrapolation can be partly avoided by carrying out experiments under semi-field or field trial conditions [56], or by using more complex TMEs as a bridge between laboratory and field testing (Figure 7.32).

TMEs and associated modelling [178] offer good potential for improving our ability to predict effects on soil. More scientific research is needed to understand

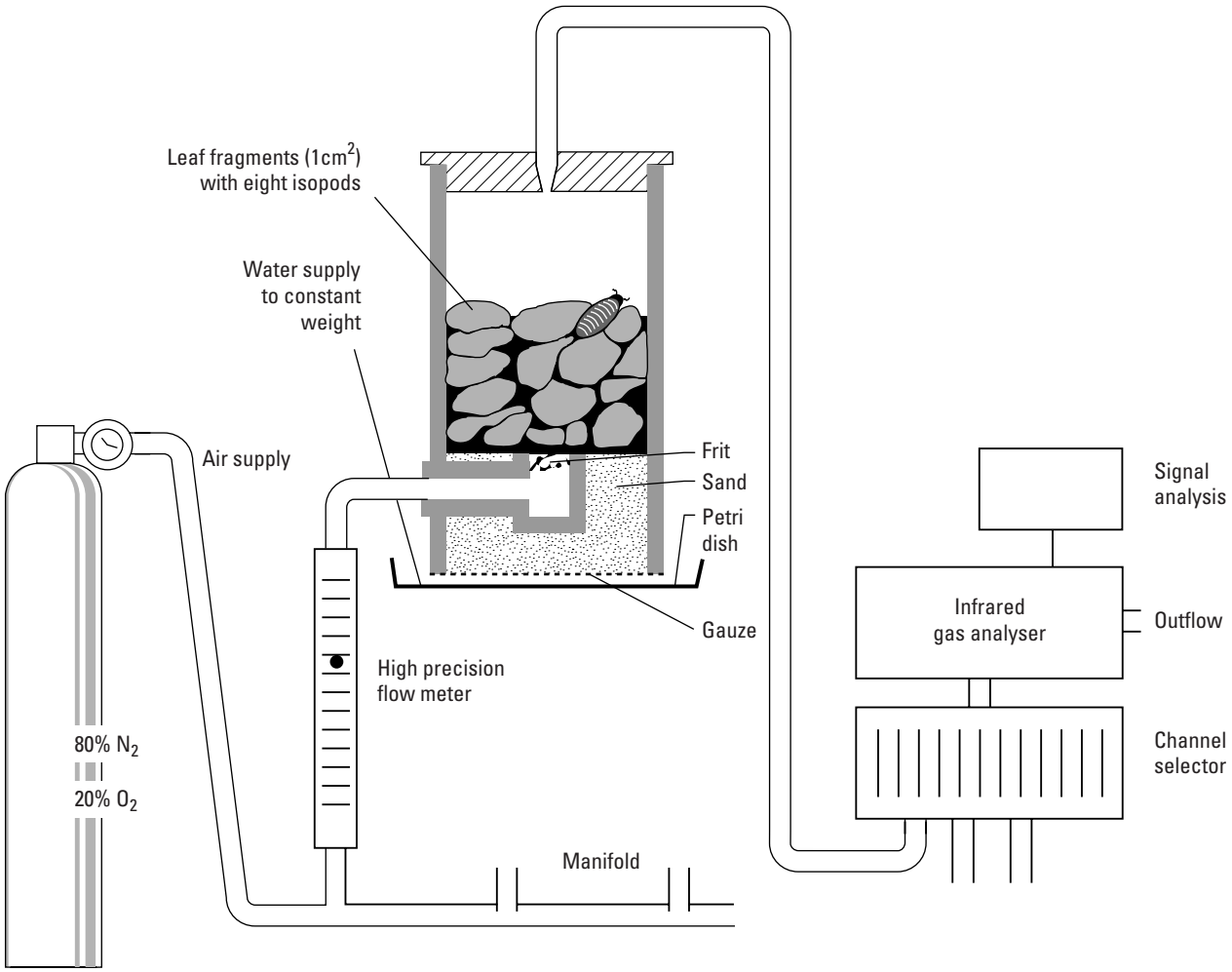

Figure 7.31. Diagram of equipment designed to measure CO_2 production in a flow-through system. The microcosm contains an amount of litter supplemented with isopods, placed on the basal layer of sand. From Van Wensem [175]. With kind permission of Springer Science and Business Media.

the complexity of terrestrial ecosystems and improve the assessment tools for regulatory soil ecotoxicology.

7.6 FACTORS MODIFYING TOXICITY

7.6.1 Introduction

Modifying factors can be defined as any characteristic of the organism or its environment that affects the toxicity of a particular chemical. The initial topics discussed in the previous sections on aquatic, sediment and terrestrial toxicity included exposure systems. Exposure and exposure systems are extremely important. Exposure systems affect the direct or indirect bioavailability of the test chemicals. Exposure systems affect the behaviour of the exposed test species and the behaviour of the test species may affect the bioavailability of the chemical. Even in very simple artificial laboratory test systems, modifying factors can dominate the results of the toxicity test. Numerous modifying factors have also been summarized in Table 7.12. An extensive review of the literature on modifying factors has not been attempted; only major factors are described in more detail in the following sections. Bioavailability issues are also addressed in Chapter 3.

7.6.2 Abiotic factors

Oxygen concentration

Lloyd [179] published a guide for estimating the lethal level of ammonia, based on research with rainbow trout

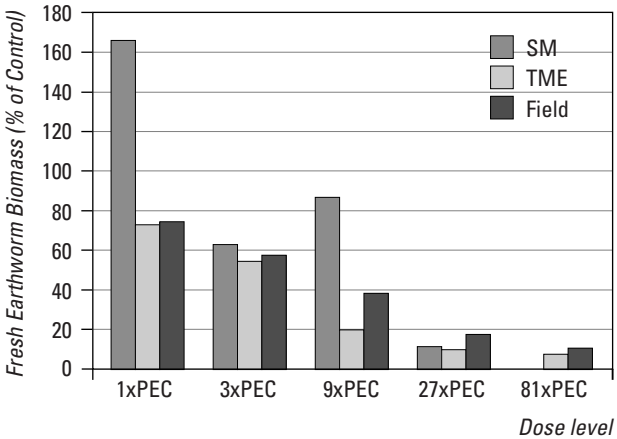

Figure 7.32. Effects of a fungicide on earthworm biomass in a comparison between a soil microcosm (SM), a terrestrial model ecosystem (TME) and a field experiment. From Edwards [174]. With permission. Copyright Elsevier.

Table 7.37. Effects of oxygen on the toxicity of ammonia to rainbow trout. From Lloyd [179].

Oxygen saturation (%)	LC50 (mg/L N)
81	42
62	34
41	25
30	21

Table 7.38. Effects of pH on the toxicity (LC50 in µmol/L) of chlorophenols to fish. From Hermens (unpublished results).

Chemical	pH = 6	pH = 8
4-Chlorophenol	60	71
Pentachlorophenol	0.44	3.4

and the chemical behaviour of ammonia in water. It was shown that low oxygen saturation levels increased the aquatic toxicity of ammonia (Table 7.37). Depletion of oxygen also favours the activity of anaerobic bacteria, reducing conditions (speciation of heavy metals), and affects the breakdown of organic chemicals.

Redox potential (Eh)

Decreasing redox potential (more reducing conditions) mobilizes oxide-sorbed toxic chemicals as it dissolves iron and manganese oxides. Increasing redox potential (more oxidizing conditions) mobilizes heavy metals by dissolving metal sulfides [179]. The influence of acid volatile sulfide (AVS) has been studied extensively [180]. Sulfides of cadmium, copper, nickel, lead, and zinc have lower sulfide solubility product constants than sulfides of iron and manganese, which are naturally formed as a product of the bacterial oxidation of organic matter in sediments. Manganese and iron will be displaced when metals are in a sediment with manganese and iron monosulfides. Because these sulfides have low solubility, sediments with an excess of AVS will have very little metal activity in the interstitial water and the expected toxicity will be low. The metal/AVS ratio is indicative of toxicity. The vast majority of sediments found in the environment have metal/AVS ratios <1.0 and toxicity is predicted to be low. For sediments with metal/AVS ratios >1.0 toxicity is less certain.

Temperature

Temperature affects the solubility of chemicals in water, it influences the form of some chemicals (e.g., ammonia), and governs the amount of oxygen dissolved in water. It also affects biochemical processes such as mineralization. Temperature also affects the activity of cold-blooded animals up to a certain maximum, which is species dependent. There is no single pattern for the effects of temperature on the toxicity of pollutants. The toxicity of metals (e.g., zinc) generally increases with increasing temperature, whereas the aquatic toxicity of pesticides can be positively, negatively [47] or not correlated with temperature [181].

Hydrogen ion concentration

The behaviour of weak acids and bases depends on the extent to which they exist in the neutral or charged state. This is determined by the pK_a value of the chemical and the pH. The pH affects the toxicity of ionized chemicals. Generally, chemicals are more toxic in their neutral unionized state. Pentachlorophenol (pK_a = 4.69) and, to a lesser extent, 4-chlorophenol (pK_a = 9.37) are more toxic at low pH values (Table 7.38). These chlorophenols are weak acids. In normal pH ranges they are dissociated ($HA \Leftrightarrow H^+$ and A^-) in water. The presence of the ionized toxic form increases with pH as log $[A^-]$ / [HA] = pH- pK_a, resulting in a higher LC50. Similarly, the toxicity of ammonia (pK_a = 9.35) increases with pH as the proportion of ammonia in the toxic unionized state (NH_3) increases. Lowering pH also increases heavy metal solubility which enhances bioavailability and thus ecotoxicity (Figure 7.33). Lowering pH also reduces the cation exchange capacity of soil, and alters the soil

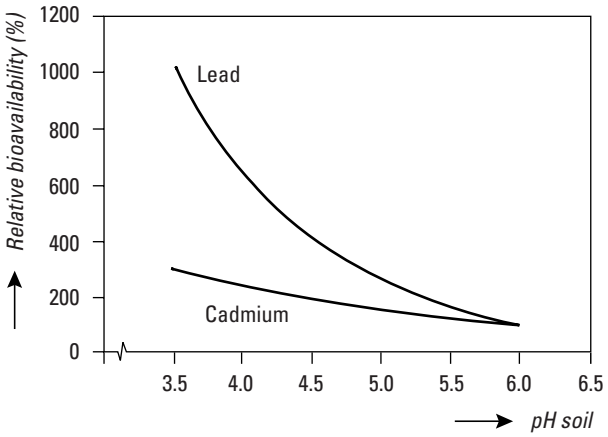

Figure 7.33. Estimated relationship between the relative bioavailability of cadmium and lead for the earthworm *Lumbricus rubellus* and soil pH, with pH 6.0 taken as 100%. From Bergema and Van Straalen [182]. With permission.

microbial population. Changes in microbial activity affect the biodegradability of chemicals and may also affect their bioavailability.

Water hardness

Calcium and, to a lesser extent, magnesium are the predominant dissolved cations in fresh water and are chiefly responsible for water hardness. Water hardness affects the speciation of heavy metals in a complicated manner. The aquatic toxicity of heavy metals such as cadmium, copper, lead, zinc and nickel decreases with increasing hardness (Figure 7.34).

Cation or anion exchange capacity (CEC or AEC)

Soil with a low CEC or AEC has a poor capacity to retain cations (e.g., metals) or anions (e.g., organic anions) by sorption. CEC and AEC are important soil properties which depend on inorganic clay mineral content and type, organic matter content, and soil pH [184].

Clay and organic matter

High clay and organic matter (OM) content reduce the bioavailability of many organic chemicals and heavy metals, and thereby toxicity (Figure 7.27). Decreasing OM content reduces CEC, soil buffering capacity, the sorption of toxic organics, and soil water-holding capacity, it also alters physical structure (e.g., increases soil erodibility) and decreases microbial activity. Clay and OM content are among the most important soil and sediment capacity-controlling properties. In fact, they determine the cation exchange capacity. Regressions or "reference lines", as they are known,

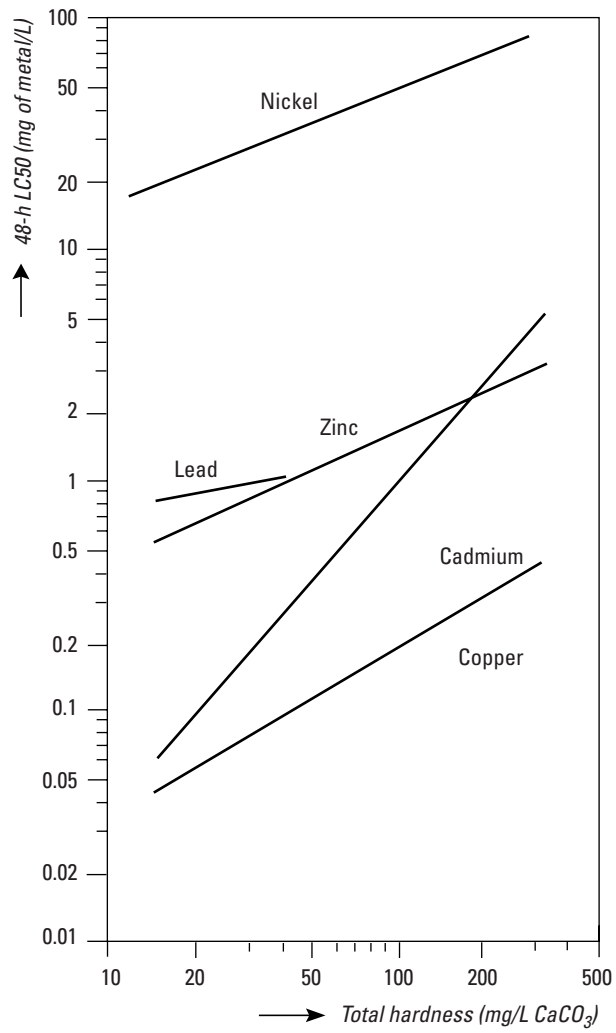

Figure 7.34. Relationship between total hardness of water and 48-h LC50 of some heavy metals in rainbow trout. From Brown [183]. With permission. Copyright Elsevier.

were originally developed to correct for background concentrations in different soil types, but are now applied as a bioavailability correction [185]. In The Netherlands, these relationships are used as correction factors to compare measured concentrations of heavy metals and organic chemicals (C_{obs}) in different types of soils, each with their own respective quality standards:

$$QS \geq C_{obs} \frac{a + 25b + 10c}{a + bL + cH} \tag{7.8}$$

where QS is the quality standard for "standard soil", i.e., soil or sediment with a 25% clay content (w/w)

and a 10% organic matter content (w/w), C_{obs} is the observed concentration of the contaminant in soil, L is the measured percentage of clay (fraction < 2μ) in the soil or sediment, H is the measured percentage of organic matter (humus) in the soil under investigation, and a, b and c are constants whose values depend on the specific contaminant under consideration. For example, for cadmium these values are 0.4, 0.007 and 0.021, respectively. The constants for the various metals are derived from measurements in undisturbed soil taken from nature reserves, and therefore are not indicative of bioavailability. It should be noted that the pH, an important factor which determines the bioavailability of metals, is not included in the equation. For organic chemicals the clay content is not considered important and standard soil is simply defined as soil with 10% organic matter.

Salinity

Increasing salinity can make toxic chemicals more soluble by altering the ion exchange equilibrium, increasing soluble complexation, and decreasing chemical thermodynamic activities in solution. It can also reduce microbial activity. For metals, toxicity increases with decreasing salinity. For organophosphorous insecticides, the opposite was found [186].

7.6.3 Biotic factors

Biotic characteristics also constitute important modifying factors. Food availability influences the energy budget of species. The allocation of energy to maintenance, growth and reproduction can be affected by both toxicants and food availability [38]. Lack of food generally makes species more sensitive to the effects of chemicals [181,186].

Sometimes it is difficult to distinguish between biotic and abiotic factors because in practice complicated interactions take place. Jagers op Akkerhuis [187] showed a strong positive correlation between spider activity and deltamethrin-induced toxicity. He demonstrated that pesticide toxicity was determined by walking activity through increased residual uptake via the cuticle. Walking activity itself was greatly affected by temperature and humidity.

The most important biotic factors are the test species themselves. There are clear differences in sensitivity. These can be explained on the basis of taxonomy, (i.e., morphological and physiological differences), trophic level, (i.e., the niche they occupy), and the exposure routes of the chemicals. Life stage (Section 7.3.4) and size, (i.e., surface/volume ratio (Section 7.2.5)), intrinsic rates of increase (r or K-species, Section 7.3.5) all affect the susceptibility of species. The same is true in relation to a number of factors, such as nutrition, health, population density, parasitism and acclimation, all of which can be controlled during toxicity testing (Table 7.12).

7.6.4 Biotic ligand models to predict toxicity of metals

All of the abiotic factors mentioned above have an influence on the form in which metals are present, and this affects the bioavailability of metals and their effect on organisms. Predicting the toxicity of metals has evolved to much more than adjusting for the influence of Mg^{2+} and Ca^{2+} content. Chemical equilibrium models can be used to predict in which forms metals are present in the water column, also called metal speciation. Different water characteristics lead to differences in metal speciation that in turn affects the acute toxicity of the metals. The method is an extension of the gill surface interaction model and the free ion activity model (FIAM), where the free ion is responsible for the toxicity. The free ion concentration is calculated with chemical equilibrium models, but other reactive metal species can also bind to the critical sites and thus need to be incorporated [188,189]. Biotic ligand models (BLMs) have been developed to predict the effect of these complex abiotic-biotic interactions on metal accumulation and toxicity (Figure 7.35). The development of BLMs is reviewed by Niyogi and Wood [189] and Paquin et al. [190]. The term “biotic ligand” refers to a discrete receptor or site of action in an organism where accumulation of metal leads to toxic effects. Acute toxicity of the metal is related to the critical metal accumulation at the biotic ligand. However this critical concentration or critical burden may be receptor-specific instead of a whole-tissue concentration or burden [189].

The BLM can provide an estimate of the amount of metal accumulation at the biotic ligand site for a variety of chemical conditions and metal concentrations. BLMs for various metals have been developed for algae, daphnids and fish [191,192]. The current focus of BLM research is on predicting chronic toxicity [193,194], since for risk assessment purposes the application of a pragmatic acute-chronic ratio to a mechanism-based BLMs is not appropriate.

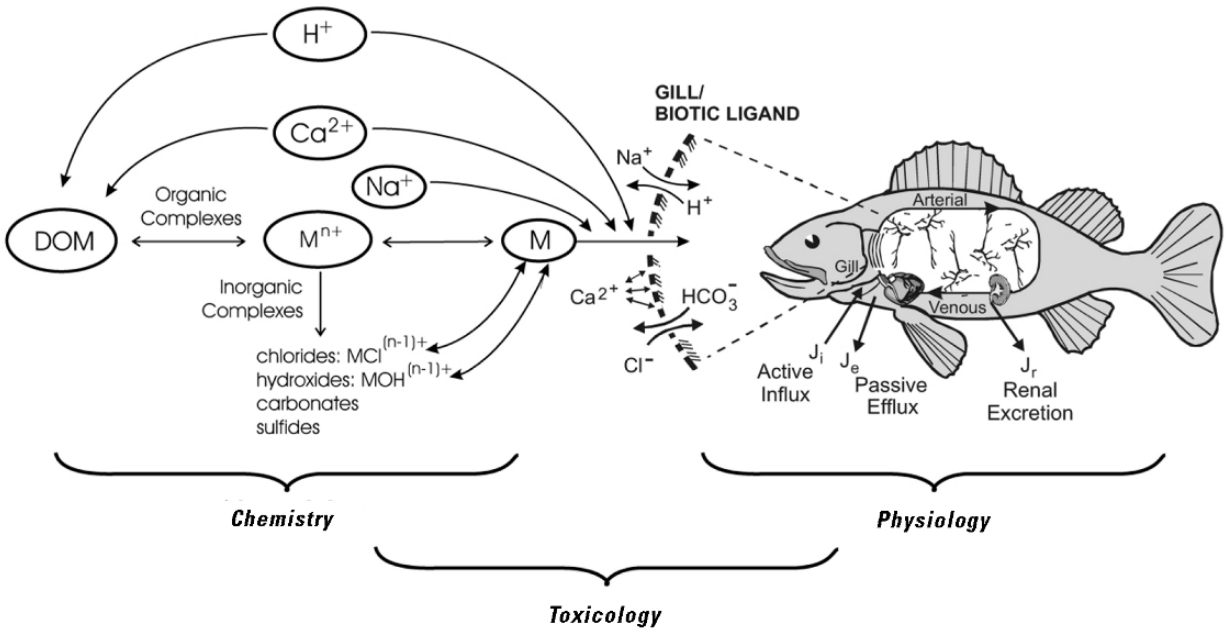

Figure 7.35. Diagram of the biotic ligand model (BLM) and the relation between chemistry, physiology and toxicology in the BLM approach. From Paquin et al. [190]. With permission. Copyright Elsevier.

7.7 MIXTURE TOXICITY

7.7.1 Mixture toxicity scales

Much of the information available on the ecotoxicity of substances relates to chemicals tested simply under laboratory conditions, or considered separately in field studies. Yet, it is uncommon to find an aquatic or terrestrial ecosystem which is polluted by a single toxicant. Usually several harmful substances are present together in significant quantities in polluted soil, sediment or surface water. This possibility of organisms being exposed to several chemicals simultaneously requires consideration of the possible interactions between the chemicals themselves and their effects on the organisms.

Table 7.39 gives four types of joint action with respect to quantal responses. A joint action is defined as similar or dissimilar depending on whether the sites of primary action of the two chemicals are the same or different, and as interactive or non-interactive depending on whether one chemical does or does not influence the biological action of the other. Other terminology is given in Figure 7.36. In most practical applications of mixture toxicity, the concepts of concentration-addition and response addition are most frequently used.

Concentration-addition is used for chemicals with a similar mode of action. The joint effect of such a mixture is calculated with concentration-addition rules [188,189]. Almost every hydrophobic chemical can exert at least a narcotic, non-specific toxicity often called baseline toxicity [30,33]. The toxicity of mixtures of narcotic chemicals can be calculated using concentration-addition rules. For chemicals with different modes of toxic action that do not interact at the target site or receptor, mixture toxicity can be described by response addition [195]. For mixtures where interaction between the tested chemicals does occur, the theory is well developed [196] but toxicological confirmation is not strong.

Table 7.39. Four types of joint action of chemicals according to Plackett and Hewlett [195]. With permission.

	Similar joint action	Dissimilar joint action
Interaction absent	simple similar action or *concentration addition*	independent action or *response addition*
Interaction present	complex similar action	dependent action

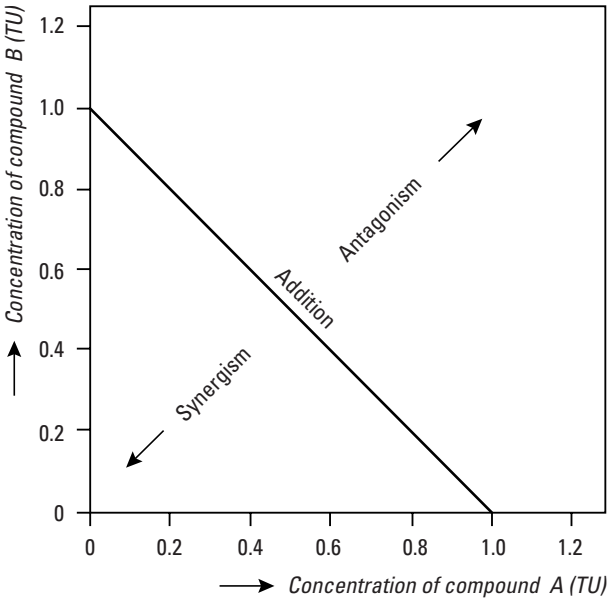

Figure 7.36. Possible toxicological interactions in a mixture of two chemicals.

Quantitative structure-activity relationships (QSARs) can be used in the classification of many pollutants into a small number of groups of compounds with a similar mode of toxic action (see also Chapters 10 and 11). When this classification is applied to mixtures of more than two chemicals, problems can arise because the different pairings can fall into different classes of joint action, and other joint actions may be possible between different pairs. Therefore, a mathematical description of the joint toxicity of a mixture of n compounds ($n \geq 2$) is possible only in a few cases.

Effects in LC50 experiments can be predicted in mixture toxicity studies by using compounds with a similar mode of action (concentration-addition). In a mixture of two compounds 50% mortality will be produced if both compounds are present at a concentration of 0.5 of their respective LC50 values. The ratio of a chemical's concentration and its LC50, i.e., c/LC50, is termed the toxic unit (TU) [197]. In a mixture of 10 chemicals the same effect will be observed when each chemical is present at a concentration of 0.1 TU, i.e., an equitoxic mixture with for each chemical equal fractions of their LC50s. Thus, concentration-addition means that the LC50 of a mixture M is described by the sum of the concentrations of n individual compounds (expressed as fractions of their LC50), equalling unity, and the effect would be equal to the effect of 1 TU. In mathematical terms this can be expressed as:

Table 7.40. Classification of mixture toxicity [198].

M	Classification of mixture potency
> n	antagonism
n	no-addition
1 to n	partial addition
1	addition
<1	supra-addition

$$M = \sum_{x=1}^{n} \frac{c_i}{LC50_i} = 1 \qquad (7.9)$$

It should be noted that the effective concentration of the mixture M is assumed to be unity in equitoxic mixture studies. In field situations, equitoxic mixtures never occur, but the principle holds for studying the effect of mixtures.

When compounds in a mixture have different modes of action the situation becomes much more complicated. The effect can be predicted in only one case, i.e., when the compounds in the mixture have dissimilar modes of toxic action, and the tolerances are fully positively correlated (independent action). When an LC50 experiment is performed 50% mortality will be observed if one of the compounds is present at a concentration which equals its LC50. In equitoxic mixtures this means that every single compound will be present at its LC50 concentration, which means that the sum of the concentrations equals the number of compounds *(n)* present in the mixture *(M=n)*. In fact, there is no combined effect at all; the toxicity of the mixture does not exceed that of the compound present at the highest toxic concentration. This situation is therefore called "no addition". When the compounds interfere toxicologically, i.e., interaction occurs, the toxicity of the mixture may vary from partially additive, if M lies between 1 and n, to antagonistic or supra-additive, when M is either greater than n or smaller than 1.0 (Table 7.40). Antagonism occurs when one compound diminishes the toxic effects of another. Supra-addition or potentiation is the opposite effect: one compound increases the toxicity of another.

Most studies on the combined effects of compounds have been performed with mixtures of only a few compounds. The exception to this are aquatic toxicological studies in which complete additivity has been proven for mixtures of many compounds (n = 50) with a similar mode of action [199].

Table 7.41. Toxicity of equitoxic mixtures of chemicals having similar modes of toxic action[a]

Mixture	Species	Criterion	n	M	Reference
1	*P. reticulata*	14-d LC50	50	0.9	[199]
2	*D. magna*	48-h EC50	50	1.2	[200]
3	*D. magna*	16-d LC50	25	1.5	[200]
4	*D. magna*	16-d EC50[b]	25	1.5	[200]
5	*D. magna*	16-d EC50[c]	25	0.6	[201]
6	*P. reticulata*	14-d LC50	11	1.0	[199]
7	*P. reticulata*	14-d LC50	17	1.1	[202]
8	*P. reticulata*	14-d LC50	9	1.0	[202]

[a] Mixtures 1-5 comprise chemicals with a limited chemical reactivity (narcotic chemicals), mixtures 6-8 show results of experiments with chlorophenols, anilines and reactive organic chemicals, respectively.

[b] Reproduction.

[c] Growth.

7.7.2 Mixture toxicity studies

Many mixture toxicity studies have been carried out with only two chemicals. This information was published in a report by the European Inland Fisheries Advisory Commission [198]. Mixture toxicity experiments have also been performed with 50 narcotic compounds with fish *(Poecilia reticulata)* and daphnids *(D. magna)*. The results are summarized in Table 7.41 for equitoxic mixtures. The experiments affirm the assumption of concentration-additivity (M = 1). Mixtures 3, 4 and 5, however, show an M value which deviates quite considerably from 1, but the value suggests additivity rather than non-additivity (Table 7.40). Deneer et al. [203] showed that in mixtures consisting of narcotic chemicals, compounds present at concentrations as low as 0.0025 TU will still contribute to joint toxicity. The concentration-addition model was still valid at these very low concentrations. Clear examples of concentration-addition were also provided in fish toxicity studies with equitoxic mixtures of chlorophenols, anilines and reactive organic chemicals (Table 7.41). The toxicity of a mixture of 18 triazine herbicides to algae was very well predicted by concentration-addition (Figure 7.37).

Table 7.42 shows the results of experiments with mixtures of compounds with different modes of action. In cases where chemicals have strictly dissimilar modes of action, Faust et al. [207] showed that response-addition rules could predict algal toxicity due to a mixture of 16 chemicals (Figure 7.38). The joint effect of chemicals where response-addition rules are expected to apply are often underestimated by these rules. Complex, larger mixtures often show a tendency to behave as mixtures with a similar mode of action, best described by concentration-addition rules [208,209]. This may be caused by the absence of true response-addition of the compounds involved. It may also be caused by the combined effects of non-specific chemical activity, e.g., several neurotoxic pesticides also show minimal toxicity due to their anaesthetic action. This combined effect may result in partial response additivity which is hard to distinguish from concentration-addition in large mixtures. In studies with *D. magna* carried out by Enserink et al. [210], the combined effect of equitoxic mixtures of

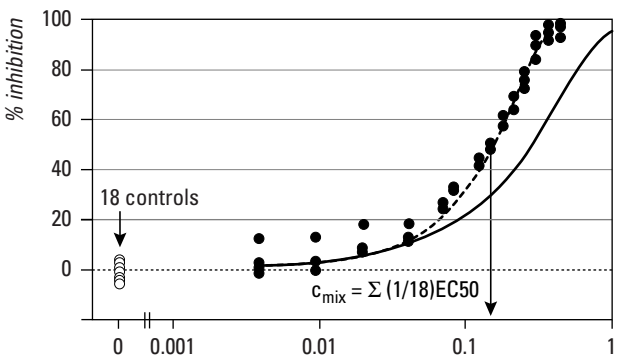

Figure 7.37. Observed and predicted toxicity to algae of a mixture of 18 triazine herbicides, mixed in the ratio of their individual EC50 values. Dashed line: prediction according to concentration-addition; solid line: prediction according to response-addition (independent action). Open symbols: controls, filled symbols: observed toxicity. From Faust et al. [204]. With permission. Copyright Elsevier.

Table 7.42. Toxicity of equitoxic mixtures of chemicals having different modes of toxic action.

Mixture	Species	Criterion	n	M	Reference
9	*P. reticulata*	14-d LC50	8	1.1-1.7[a]	[205]
10	*P. reticulata*	14-d LC50	24	2.3	[205]
11	*D. magna*	16-d LC50	14	1.1	[206]
12	*D. magna*	16-d NOEC[b]	14	1.9	[206]

[a] Results of 5 experiments with different mixtures.
[b] Reproduction.

eight heavy metals was near complete concentration-addition. These results were in accordance with effects of equitoxic mixtures of six metals on survival, body weight and reproduction of *Ceriodaphnia dubia* [211].

In conclusion, concentration-addition strictly applies to chemicals with similar modes of toxic action and response-addition (independent action) predicts well for strictly dissimilarly acting chemicals [205]. Chemicals with different modes of toxic action can often almost behave according to concentration-addition. The most important conclusion from experimental studies on the combined effects of heavy metals and organic chemicals is that mixture toxicity is a reality. Chemicals exert their detrimental effects in equitoxic mixtures at very low concentrations, at fractions of 0.0025 of their LC50, i.e., at or below their NOEC [203]. This raises major questions about the quality criteria set for single compounds. In fact, it has been shown that mixtures of heavy metals at levels of their water quality criteria induce adverse effects on crustaceans and fish [210,211]. The consequences for the risk management of chemicals should then be taken into account.

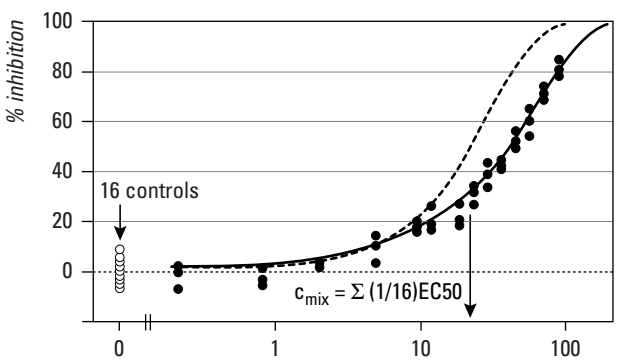

Figure 7.38. Observed and predicted toxicity to algae of a mixture of 16 dissimilarly acting substances, mixed in the ratio of their individual EC50 values. Dashed line: prediction according to concentration-addition; solid line: prediction according to response-addition (independent action). Open symbols: controls, filled symbols: observed toxicity. From Faust et al. [207]. With permission. Copyright Elsevier.

A very pragmatic way of dealing with mixtures in the context of quality objectives is to calculate the ratio of the ambient concentration and the quality objective for each compound. The sum of these fractions is the scaled risk quotient *RQ* for the mixture:

$$RQ = \frac{C_a}{EQO_a} + \frac{C_b}{EQO_b} + \frac{C_c}{EQO_c} + \cdots \qquad (7.10)$$

with C_x ambient concentrations for substance *x*, and EQO_x, the quality objective for substance *x*. If the RQ is > 1, the environmental quality objective for the mixture is exceeded. If RQ <1, the environmental quality objective is not exceeded. This procedure is often used, but can lead to misleading results for the following reasons. It is assumed that the quality objectives (if present) are derived in a comparable way for each substance. In reality, the EQOs are often not based on comparable data sets and similar effects. It is inappropriate for substances that have dissimilar modes of action and therefore do not follow simple concentration-addition rules. This limits the practical use of this rule for structurally similar substances that have the same mode of toxic action. The risk to ecosystems further to exposure to mixtures can be assessed by linking mixture toxicity rules to the SSD concept [212]. The SSD concept is explained in Section 7.10.2.

7.8 ECOTOXICOGENOMICS

Experimental data can be supplemented or even replaced by the use of *in vitro* tests, (Q)SARS, and read-across methods, as explained in Chapters 6, 10 and 11. The

rapidly developing application of molecular biology in ecology and ecotoxicology *("genomics")* holds promise for developing alternatives to *in vivo* testing as well [213]. In addition, genomics could be used for prioritization of chemicals, guiding experimental design and providing insight into the molecular and mechanistic background to toxicological effects [214,215].

The field of genomics aims to elucidate how the genome of a species translates into biological functions. Genomics consists of many disciplines and methods including sequencing, identifying the function of specific genes, gene expression by studying mRNA transcription *("transcriptomics")*, protein expression *("proteomics")*, and metabolite characterization *("metabolomics")*. The term ecotoxicogenomics was coined to cover the application of these methods to ecotoxicology [216]. At present, the available methods do not yet allow the use of genomics in regulatory testing. However, this may change [215,217]. The challenge faced in ecotoxicogenomics is to get a grip on the relationship between the toxicological stimulus, gene transcription and expression and the ensuing metabolic changes, and the relationship between dose/concentration and effect.

Gene expression is usually altered as a result of toxicity. Single gene biomarkers exist for classes of chemicals, such as induction of hepatic vitellogenin mRA by oestrogen-like compounds, or up regulation of cytochrome P450 1A by binding of planar aromatic compounds to the aryl hydrocarbon receptor [215]. The significance of changes in gene expression in terms of concentration response for risk assessment purposes may be difficult to interpret, since the mRNA is not always transcribed and many proteins are modified after translation. This is essentially the same "significance" debate on the use of biomarkers in risk assessment [218].

Transcriptomics deals with studying changes in genome-wide expression through quantification of mRNA, possibly extending to many thousands of genes at the same time [216]. With this method, transcripts that are up and down regulated as a result of experimental conditions can be identified. There is a need to analyze the transcriptome in reaction to non-toxicological and toxicological stimuli in order to interpret the toxicological "fingerprint" compared to the control organisms. A more advanced method is where the transcriptome can be unambiguously related to specific genes (profiling), but this requires that the genome of the species is sequenced.

Proteomics refers to the total evaluation of protein profiles in a cell or specific tissue. This can provide the linkage between gene regulation and the phenotypical changes in response to a chemical or class of chemicals.

Metabolomics describes the overall characterization of the dynamic metabolic reaction to a toxic or physiological stimulus. Both proteomics and metabolomics refer to functional entities within the tissue of the cell and offer a more integrated assessment than that based on genes or gene products [215,216]. The challenge is to use these methods to provide insight into the mode of toxic action, act as evidence for the absence of effects (decision for no further testing), or to replace or complement further testing [214]. The application of ecotoxicogenomics in regulatory testing has been elaborated by Tyler et al. [215] and Ankley et al. [217].

7.9 ENDOCRINE DISRUPTION

Endocrine disrupting compounds cause functional changes of the endocrine system through a variety of mechanisms. It is one of the aspects of reproductive toxicology. Endocrine disruption may result in adverse effects in an organism or its progeny. Effects on reproduction and development are especially of interest due to their possible effects at the population level (see previous section). Following the seminal book on endocrine disruption by Colborn et al. [219], many new tests have been developed (for an overview, see [220]) with an emphasis on the steroid sexual hormone system of vertebrates, but none has yet been approved by the OECD at the time of writing this chapter. The goal of these studies is to determine if modulation of endocrine activity leads to serious long-term adverse effects that cannot be detected with other toxicity tests. Some effects on early life stages could lead to delayed population effects that can only be detected in life-cycle tests with one or more consecutive generations.

Endocrine disruption can be studied in *in vitro* studies, mammalian screening assays or human health studies for repeated-dose, carcinogenicity and reproductive toxicity (see Chapter 6). The difficulty with endocrine disruption in ecotoxicology is that species from different phyla have different endocrine systems that may not react to a chemical in the same way that mammals do. *In vitro* screening assays have been developed that are mostly based on cell lines or receptors from mammalian tissue, such as estrogenic androgenic, progestagenic and thyroidal receptor binding assays. An example of a fish-specific estrogen activity assay is the induction of the egg yolk protein vitellogenin in cultured hepatocytes from fish liver. Batteries of *in vitro* screening assays have been used to identify endocrine modulating effects in both man and wildlife for a specific group of chemicals, such as brominated flame retardants [221].

Table 7.43. Stages in risk assessment and required effects information [4].

Tiers	Stages	Effects data
Tier-1	preliminary or initial	short-term toxicity
Tier-2	refined	chronic toxicity
Tier-3	comprehensive	(semi) field data

Table 7.44. Assessment factors applied in aquatic effects assessment [14].

Available information	Assessment factor[a]
At least one short-term L(E)C50 for each trophic level (base set: algae, *Daphnia* and fish)	1000
One long term NOEC(fish or *Daphnia*) in addition to base set	100
Two long term NOECs from two trophic levels (fish, *Daphnia* or algae) in addition to base set	50
Three long term NOECs from three trophic levels (fish, *Daphnia* or algae) in addition to base set	10
Species sensitivity distribution method	5-1, case by case
Field data or model ecosystems	case by case

[a] Many additional rules are available to cover different situations, leading to adaptations of the appropriate assessment factor [14].

In vivo screening assays are based on changes in vitellogenin levels to signal estrogenic or anti-estrogenic effects, or androgenic effects (21-d fish screening assay, draft OECD guideline). Other proposed fish tests are tests on sexual development, reproduction and a two-generation full life-cycle test, allowing effects on the F2 generation to be studied. Amphibian metamorphosis of *Xenopus laevis* is under the influence of thyroid hormones, and has been proposed as a 21-d study. Adverse effects on thyroid activity can be developmental disturbance, histopathological effects on the thyroid or thyroid hormone levels [222].

Confirmatory tests are all based on reproduction studies and have been proposed for *D. magna* (enhanced OECD 211 guideline), copepods and mysids. These tests can detect effects on invertebrate hormone systems such as ecdysteroids.

Apart from improvements in test design, endocrine modulating effects need to be identified efficiently and with sufficient coverage of phyla in the animal kingdom.

7.10 DERIVATION OF PNECs

According to the OECD [4] effects assessment can be divided into three stages, depending on the type of information available (Table 7.43). *Preliminary effects assessment* is the stage at which only reliable QSAR estimates or a few LC50 or EC50 values from short-term studies are available. *Refined or intermediate effects assessment* can take place if a few NOECs from chronic tests are available and, finally, *comprehensive effects assessment* is the stage at which field studies, multi-species toxicity studies (or many chronic test results) are available. At each stage different methods may be applied to arrive at a PNEC for the environment. PNECs are derived based on a number of important assumptions. These assumptions are critical to this analysis although their validity has not been thoroughly substantiated:

1. The species selected for testing are representative of the sensitivities of species found in ecosystems.
2. The chronic toxicity threshold determined for the most sensitive species is the chronic toxicity threshold for ecosystems.
3. Species and species-level properties of ecosystems are the most sensitive to toxic chemicals.

Effects assessment does not go beyond the preliminary stage for most chemicals because of the lack of basic toxicity data. This means that, in practice, precise predictions about effects at the ecosystem level can hardly ever be made. Yet, PNECs can always be predicted even at the preliminary stage. This means that chemicals can always be compared on the basis of little data, provided that the assessments are carried out consistently (Chapter 1). Effects assessment involves many uncertainties and

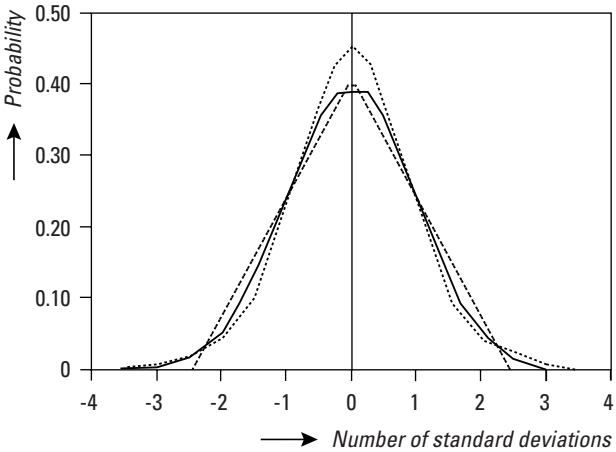

Figure 7.39. Probability density functions for standard log-logistic (·····), log-normal (——) and triangular distributions (- - -) (m = o, s = l) of species' sensitivities. Modified from Health Council of The Netherlands [10].

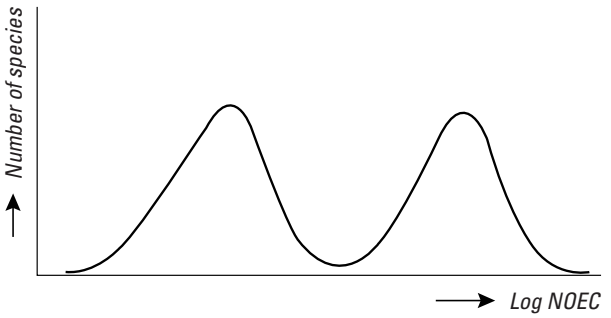

Figure 7.40. For chemicals with a specific mode of toxic action, e.g. certain herbicides and insecticides, a bimodal distribution of species sensitivities may be found instead of a log-logistic or log-normal distribution. In such cases HC5 calculations can be made for both the target and non-target species.

many extrapolations are made from a few species to many species, from acute to chronic effects, from the laboratory to the field, etc. [1,5,223].

7.10.1 Preliminary effects assessment using assessment factors

For the estimation of PNECs, assessment factors or uncertainty factors can be applied to the available toxicity data to account for the different sensitivities of other, untested species in ecosystems. When only a limited set of toxicity data is available, a *constant assessment factor* is used to adjust the effects concentration (laboratory LC50, EC50, NOEC, etc.) to PNECs for ecosystems (Table 7.44). Assessment factors may be used to extrapolate from concentrations with acute effects to NOECs, from a few NOECs to a representative sample, and from the lowest chronic NOEC to the field situation. For each extrapolation step a factor of 10 is suggested. If a data set contains LC50 values for algae, daphnids and fish, the PNEC is estimated from the lowest LC50/(10 x 10 x 10).

The assessment factors presented in Table 7.44 are largely based on a report dating from 1984 [224] which have subsequently been updated in different regulatory frameworks, e.g., in the EU [14]. Although the assessment factors may differ between these frameworks, there is agreement about the magnitude of these factors.

Assessment factors are not based on any theoretical model but are based on experience with chemical effects assessment. They are useful but provide only an approximate means of deriving PNECs. Assessment factors should be used with care with *acute data* since specific modes of toxic action may not be detected in acute toxicity tests (e.g., pesticides, neurotoxicants, cell division inhibitors), or for chemicals with high log K_{ow} values that significantly bioaccumulate. The test results must be evaluated to confirm, for example, that the test concentration does not exceed solubility limits and that the duration of the test is sufficiently long in relation to the log K_{ow} value or LC50-time curve (Figure 7.10). The assessment factor approach is suggested for extrapolation of a limited set of laboratory toxicity data not only for aquatic species, but also for terrestrial and sediment invertebrate species and for birds and mammals.

7.10.2 Refined effect assessment using species sensitivity distributions

PNECs can be calculated using assessment factors (Table 7.44), but they can also be calculated with species sensitivity distributions (SSDs). The variation in sensitivity of species to a contaminant, described by a statistical or empirical distribution function of responses is called a species sensitivity distribution (SSD). The input for these calculated extrapolation models are LC50s or NOECs from a number of representative species. For the derivation of environmental quality objectives, it is common to use NOECs. Especially for data-rich substances, the SSD method can be used to analyze patterns in species sensitivity and derive quality objectives based on statistical theory instead of fixed assessment factors. The use of SSD in ecotoxicology is reviewed by Posthuma et al. [225]. The SSD approach is based on five critical assumptions:

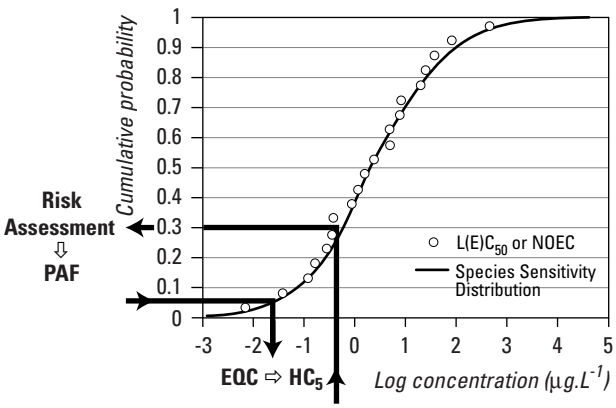

Figure 7.41. Cumulative species sensitivity distribution (SSD), with the toxicity data for different species (dots) and the fitted SSD (line). SSDs can be used in two ways: for calculating risk at a specific concentration (expressed as potentially affected fraction, PAF), or by calculating an environmental quality criterion (EQC) for a certain cut-off value, e.g. the 5th percentile (HC5). From Posthuma et al. [225]. With permission.

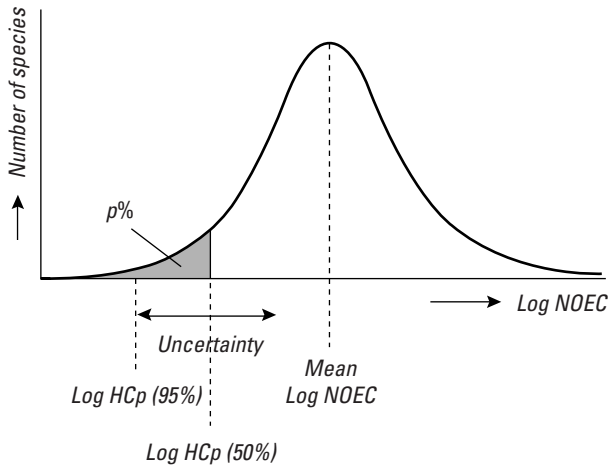

Figure 7.42. The normal density function and estimation of the concentration at which the NOEC of no more than 5% of the species within an ecosystem is exceeded (HC5). The HC5 can be calculated at two levels of confidence: 50% and 95%. The 50% confidence estimate of the HC5 is the "most probable" estimate, whereas the left 95% confidence limit of the HC5 is the "safer" value [226]. With permission. Copyright Elsevier.

1. The sensitivities of a (selected) set of species can be described by some distribution, usually a parametric distribution function, such as the triangular, normal or logistic distribution (Figure 7.39).
2. Since the true distribution of toxicity endpoints is not known, the SSD is estimated from a sample of toxicity data.
3. The distribution should adequately describe the observed sensitivity of species. In the case of chemicals with a specific action, a bi-model pattern of target species versus non-target species is often observed (Figure 7.40). In which case, it could be more appropriate to use the target species distribution for the SSD calculation.
4. The SSD can be used for setting or deriving environmental quality objectives, and for risk assessment using measured or predicted environmental concentrations (Figure 7.41). The 5th percentile of a chronic toxicity distribution has often been chosen as a concentration which is protective for most species in a community, but the cut-off value of 5 is a policy decision. This concentration is called the HC5. The complementary value of *p* has become known as the 95 % (100-p) protection criterion. This is considered to be an acceptable approach for protecting the structure and function of aquatic ecosystems [10,14,16,225].

The available SSD methods use different assumptions regarding the shape of the species sensitivity distribution (SSD). Aldenberg and Jaworska [226] assume a log-normal distribution, but a log-logistic distribution [227] or log-triangular distribution is also possible (Figure 7.39). The methods of Aldenberg and Jaworska were based on earlier models by Aldenberg and Slob [227], Kooijman [228], Van Straalen and Denneman [185], and Wagner and Løkke [229].

The log HC5 is estimated with:

$$\log \mathrm{HC5} = \bar{x} - K_s \cdot s \qquad (7.11)$$

where

- HC5 = the hazardous concentration for 5% of species
- $\bar{x}$ = the sample mean of log NOEC data for *m* species
- K_s = the one-sided extrapolation constant for a logistic or normal distribution, dependent on *m*
- s = the sample standard deviation of log NOEC values for m species.

The uncertainty in the estimated HC5 can be calculated at a lower (95%), a median (50%) and a higher (5%)

Box 7.3. The calculation of HC5 and FCV values with different extrapolation techniques, using experimental chronic and subchronic NOECs (mg/L) of sodium bromide (NaBr), dimethoate and pentachlorophenol (PCP) for 11 different test species [232]. With permission. Copyright Elsevier.

Test species	NaBr	Dimethoate	PCP
Pseudomonas fluorescens (bacteria)	3200	320	1.0
Microcystis aeruginosa (bacteria)	3200	32	1.0
Scenedesmus pannonicus (algae)	3200	100	0.1
Lemna minor (higher plants)	3200	32	1.0
Daphnia magna (crustaceans)	10	0.032	0.1
Culex pipiens (insects)	100	0.32	3.2
Hydra oligactis (hydrozoans)	1000	100	0.032
Lymnea stagnalis (mollusks)	10	10	0.0032
Xenopus laevis (amphibians)	32	1.0	0.032
Poecillia reticulata (fish)	32	0.1	0.1
Oryzias latipes (fish)	320	0.32	0.032

The HC5 values are calculated according to Aldenberg and Jaworska [226] using the software ETX 2.0 [230], and the FCV values according to Erickson and Stephan [233] using the software ETX 1.3a [231]. All species were used in the calculations.

Results [mg/L]	NaBr	Dimethoate	PCP
HC5 (5-95% confidence limits)	4.3 (0.29-21)	0.019 (0.00057-0.16)	0.004 (0.00041-0.016)
FCV	5.45	0.019	0.0023

confidence level (Figure 7.42). The corresponding K_s values for each confidence level depend on the toxicity data sample size and are implemented in software for calculating the HC5 [230,231]. The K_s values for a log-logistic distribution [227] do not differ very much from those for the log-normal distribution at the same level of confidence [226]. Consequently, the calculated HC5 values are in the same range. Sample calculations are shown in Box 7.3. It is generally recognised that a diversity of taxonomical groups needs to be considered for deriving HC5 values (Table 7.45).

Erickson and Stephan [233] presented a method based on the triangular distribution to estimate a final chronic value (FCV) which applies the 5% cut-off to taxonomic genera, instead of species. Therefore, the FCV is an estimate of the 5th percentile concentration of chronic toxicity values for genera. The FCV is preferably calculated from chronic NOEC values for at least eight different animal families. Chronic values for species are combined to estimate mean chronic values for each genus. From the cumulative distribution of these

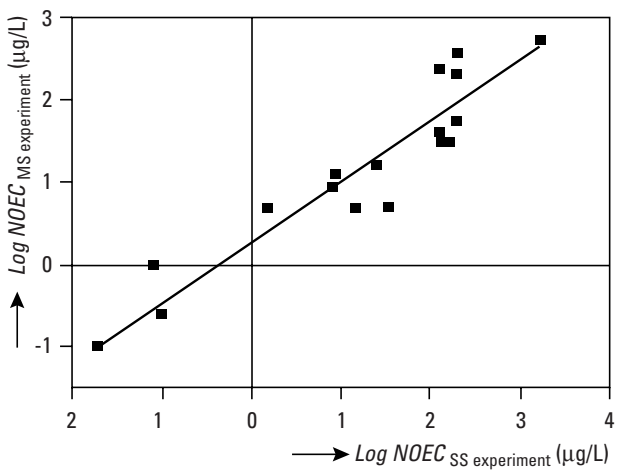

Figure 7.43. Model-II regression of $NOEC_{ms\ experiment}$ on $NOEC_{ss\ experiment}$ for similar or related species, corresponding effects parameters and similar exposure concentrations, based on 17 data pairs: log $NOEC_{ms\ experiment}$ = 0.750 x log $NOEC_{ss\ experiment}$ + 0.263; r = 0.935. From Van Leeuwen, Van De Plassche and Canton [104]. With permission.

Table 7.45. Information requirements for using statistical extrapolation in the EU based on species sensitivity distributions [14], based on similar requirements in the USA [233] except for algae and higher plants.

1. Class Osteichthyes, frequently tested species including salmonids, minnows, bluegill sunfish, channel catfish, etc)
2. A second family in the phylum Chordata (may be in the class Osteichthyes or an amphibian, etc.)
3. A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)
4. An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
5. A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.)
6. A family in any order of insect or any phylum not already represented
7. Algae
8. Higher plants

genus means, the HC5 is estimated from the lowest four genus means by a non-parametric or graph method. As a variation on the original method, where only data for specified animal families were used (Table 7.45), single-species data (of plants and animals) may be used as input in the equation for comparison with the other methods, in which event the calculated FCV is considered to be equivalent to the HC5.

The method has some advantages over the other SSD methods. Deviations from the assumed distribution restricted to the upper part of the distribution will have little impact on the calculation if only the lowest data in a sample are used. Another advantage of using only the lowest data is that it allows the inclusion of test results with "greater than" values, which are excluded in other approaches [233]. A comparison of HC5 values with the FCV is made in Box 7.3. It shows that the differences are relatively small at the same level of confidence.

Criticisms have addressed statistical issues, how representative SSDs are of species in different ecosystems, the inability of SSDs to deal with species interactions and issues related to environmental quality [234,235].

Verification of SSD methods with species sensitivities in microcosms, mesocosms or semi-field studies showed that HC5 values predicted from single-species tests generally do not significantly differ from NOECs derived from field studies [97,236]. Species tested in multi-species experiments appeared to be equally sensitive as similar or related species in single-species experiments when tested for corresponding parameters (Figure 7.43). These results were partly confirmed by the comparison of laboratory SSDs for chlorpyrifos with field-derived SSDs (Figure 7.44). This shows that the use of SSDs for standard setting can produce field-relevant results and makes better use of the available data instead of focusing on the lowest available test result for a specific endpoint.

7.10.3 Effects of secondary poisoning

Only methods for direct toxic effects have so far been described. A point of major concern is the effect of biomagnification (accumulation in the food chain) which may lead to indirect toxicity, i.e., to secondary poisoning. Most birds and mammals in aquatic and terrestrial ecosystems are predating organisms at the end of a food chain and thus may be exposed to high concentrations in their diet (Table 7.9). A simple approach has been developed to estimate NOECs for predating animals such as fish-eating and worm-eating birds and mammals [237,238]. Where no data are available on toxicity for

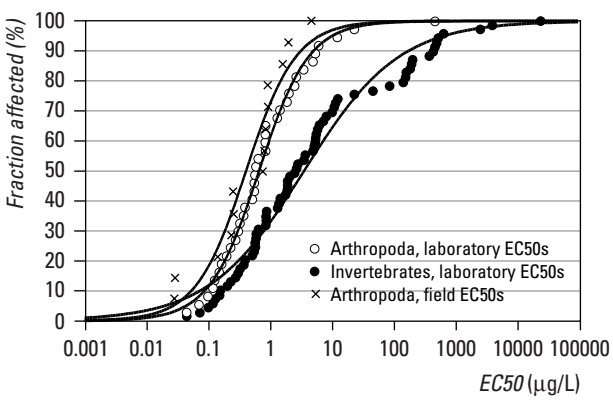

Figure 7.44. Comparison of SSD curves for chlorpyrifos between the laboratory and a semi-field test, based on acute LC/EC50 values. From Van den Brink et al. [108]. With permission.

Table 7.46. Relationship between mg/kg diet (dry laboratory chow diets) and mg/kgbw according to Lehman [14,239].

Animal	Body weight (kg)	Food consumption (g/d)	Conversion factor mg/kg diet to mg/kg_{bw}·d
Mouse	0.02	3	0.15
Rat (young)	0.10	10	0.10
Rat (old)	0.40	20	0.05
Guinea pig	0.75	30	0.04
Rabbit	2.0	60	0.03
Dog	10.0	25	0.025

wild mammal or bird species, the subchronic toxicity for laboratory mammals (mg/kg_{bw}) is used and converted to concentrations in the diet (mg/kg diet) using a conversion factor based on the consumption rate of the species. This method is only advisable when no other toxicity data for birds or mammals are available:

$$NOAEL_{diet} = NOAEL / F \quad (7.12)$$

where $NOAEL_{diet}$ is the estimated dose expressed as the concentration in the diet (mg/kg diet), NOAEL is the chronic or subchronic value for laboratory mammals expressed in mg/kg_{bw}·d and F is the consumption rate (kg_{diet}/kg_{bw}·d). According to Lehman [239] the conversion factor is approximately 0.10, but this factor varies considerably, depending on the animal studied (Table 7.46). If only acute toxicity data are available an application factor of 90 to 3000 (Table 7.47) may be used to extrapolate from acute to chronic toxicity, but it should be stressed that this may lead to large errors. Different approaches are available to derive a $NOAEL_{diet}$ for taxonomic groups such as birds and mammals, based on either an assessment factor approach [240] or an SSD-based approach [241].

To avoid secondary poisoning, the concentration of chemicals in the food should be below the NOAEL in dietary toxicity tests with animals that are representative of fish-eating or worm-eating birds or mammals. The diet is assumed to consist completely of fish or earthworms. The NOAEL is considered the maximum concentration in food which will not lead to adverse effects.

The maximum concentration in the food of fish-eating predators can be converted to a maximum concentration in water that will protect predators, based on the bioconcentration and biomagnification in the food chain:

$$NOEC_{pred} = \frac{NOAEL}{BCF_{fish} \cdot BMF_1} \quad (7.13)$$

where the $NOEC_{pred}$ is the external no observed effect concentration for fish or worm-eating birds or mammals expressed as mg/L (water) or mg/kg (soil). The BCF is expressed as L/kg wwt for fish. The BMF [-] in this equation is a correction for the fact that fish can accumulate substances from food as well (Table 7.48), thereby exceeding the level if fish were to be exposed in the water phase only (see Chapter 3 on bioaccumulation). To account for food chains in the marine environment, this route can be extended by one extra biomagnification step. This step represents the biomagnification from fish to fish-eating birds and mammals that serve as prey for top-predators. Thus the route of exposure is uptake by aquatic organisms (e.g., small fish), biomagnification by fish, biomagnification by fish-eating predators and finally, consumption by the top-predator. Equation 7.13 is extended with an additional BMF and then becomes:

$$NOEC_{pred} = \frac{NOAEL}{BCF_{fish} \cdot BMF_1 \cdot BMF_2} \quad (7.14)$$

BCFs are derived from experimental data, or where data are lacking, from estimates. The BCF in fish can be estimated using QSARs from Chapter 9 [14]; e.g.

$$\log BCF_{fish} = 0.85 \cdot \log K_{ow} - 0.70 \quad (7.15)$$

For substances with a log K_{ow} higher than 6, a parabolic equation can be used.

see next page (7.16)

Table 7.47. Assessment factors used to derive PNECs for birds and mammals to assess the effects of e.g. secondary poisoning [14].

Available information	Duration of test	Assessment factor (oral) applied to the lowest value
LC50 bird	5 d	3000
NOEC bird	chronic	30
NOEC mammal, food	28d	300
	90 d	90
	chronic	30

Table 7.48. Default BMF-values [-] for organic substances, used in assessment of secondary poisoning [14].

log K_{ow} of substance	BCF (fish)	BMF_1	BMF_2
< 4.5	< 2000	1	1
4.5 - < 5	2000-5000	2	2
5 – 8	> 5000	10	10
> 8 – 9	2000 – 5000	3	3
> 9	< 2000	1	1

The biomagnification factor (BMF) is preferably measured, but defaults can be used for organic substances estimated from the relationship between K_{ow}, BCF and the BMF of the substance (Table 7.48). Similar equations can be derived for protecting worm-eating birds or mammals [14] based on measured or estimated BCFs for earthworms (see Equation 3.58, Chapter 3).

Comparison of the $NOEC_{pred}$ with PNECs for surface water or soil can reveal whether secondary poisoning could constitute a critical pathway. This occurs when these values for fish-eating birds and mammals are lower than the PNECs for direct toxic effects in water or soil. Examining secondary poisoning by using worm and fish-eating birds and mammals is a clear simplification of food webs occurring in nature (Figure 7.45). Large errors may occur for superlipophilic chemicals that are not well predicted by existing relationships (Equation 7.16, Chapters 3 and 9). Furthermore, the use of these simple models does not mean that other birds or mammals feeding on other species are not at risk, even though the value for $NOEC_{pred}$ should be protective. Therefore, the $NOEC_{pred}$ values for fish-eating birds and mammals should be considered as indicative of secondary poisoning. Alternative approaches to bioaccumulation in risk assessment are discussed in Chapter 3.

7.10.4 Comprehensive assessment

In ecotoxicology discussions on the advantages and disadvantages of single-species testing are still relevant [13,96-106,111,234]. Acute toxicity tests are the first step towards understanding the toxic effects of chemicals in ecosystems. Chronic tests are the second step and provide a reference point closer to the actual NEC at ecosystem level (Table 7.43). Much aquatic ecotoxicological research has been devoted to finding the most susceptible species [13], but the responses have been shown to be chemical specific, i.e., dependent on the nature of the chemical. It is not surprising that research carried out to select the most suitable combination of aquatic species [232] has led to the conclusion that the toxic potential of a chemical can be reasonably predicted from a test set with an alga, a crustacean and an egglaying fish species. Nevertheless, NOEC values obtained from SS tests are often in the same order of magnitude as those derived from more labour-intensive and expensive MS tests [97,236].

$$\log BCF_{fish} = -0.20 \cdot \log K_{ow}^{\,2} + 2.74 \log K_{ow} - 4.72 \qquad (7.16)$$

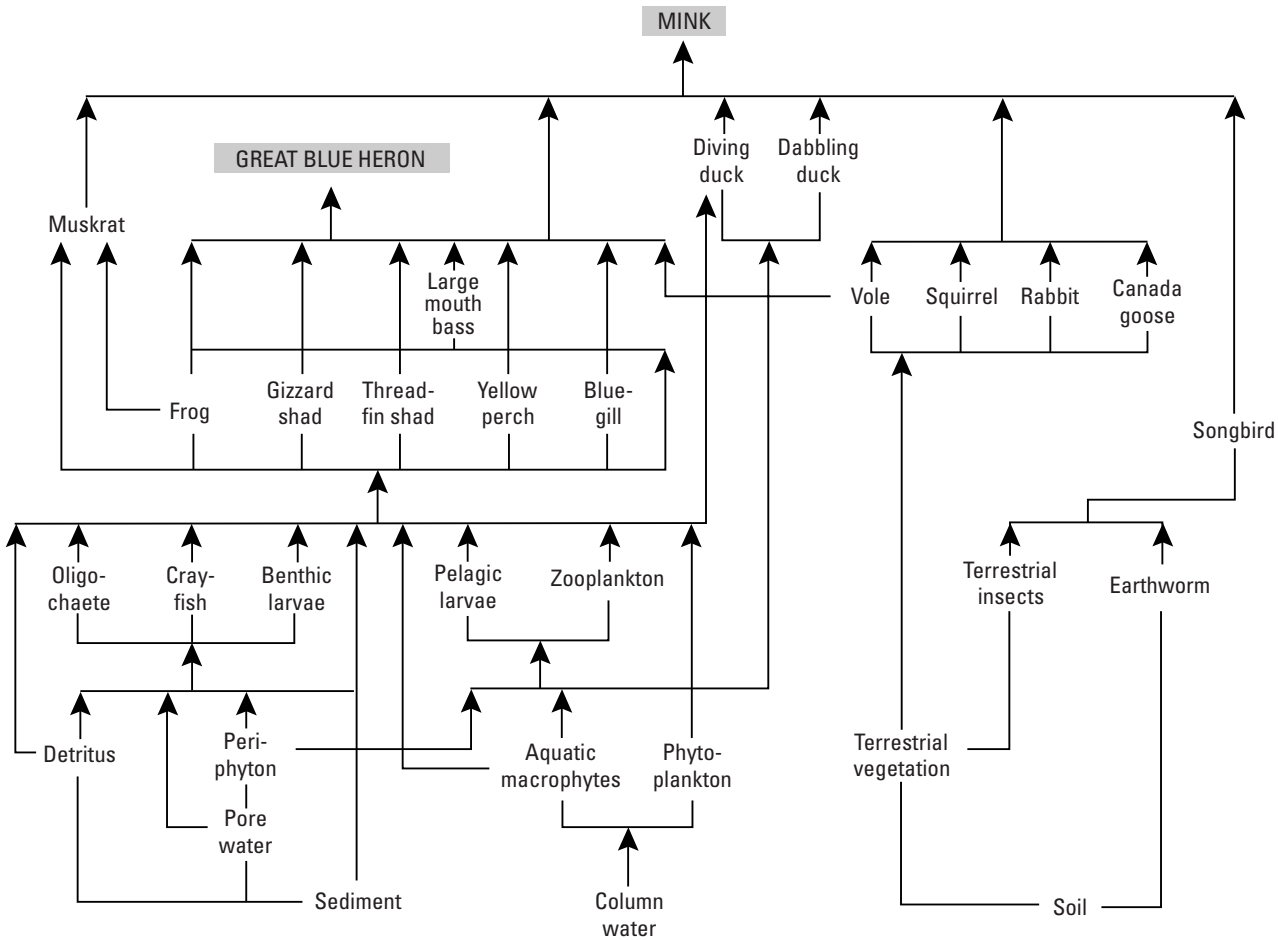

Figure 7.45. Diagrammatic representation of the routes of exposure of mink and great blue heron to contaminated terrestrial and aquatic environments. From Macintosh et al. [242]. With permission.

The studies normally carried out at the comprehensive stage are system level tests (MS tests). The best MS tests are field studies, but experimental microcosm and mesocosm studies provide a more cost-effective and efficient alternative. When the margin of safety is small, i.e., where the PNEC is close to the PEC, the effects of chemicals may need to be tested in more complex studies, as in higher tier testing for pesticides. Comprehensive tests may be appropriate when the economic consequences of a preliminary risk management decision are too great. In either case, additional information from MS tests should assist in environmental decision making because [80,81,94]:

- The overall impact of a chemical on populations within a community may be different from what was predicted from laboratory single-species tests due to poorly understood *interactions* between populations and their environment.
- Ecosystem studies may provide an opportunity to evaluate the ability of populations and communities within the affected ecosystem to recover from stress. The rate at which *recovery* occurs is a measure of the permanence of the effect.
- Ecosystem studies often provide *more realistic exposure conditions* with regard to the bioavailability and fate of the chemical, e.g., volatilization, adsorption and degradation. In this way better information can be obtained about the predicted environmental exposure concentration. MS tests thus provide more realistic evaluations of fate and effects.

It has been argued in many papers that (semi-)field studies may provide the ultimate answer in effects assessment (Section 7.3.6). Their role in the risk management is still relatively limited. Effects in the field are difficult to interpret and much depends on the questions that need answering. Field tests can only

Table 7.49: PBT and vPvB criteria according to Annex XIII of the REACH Regulation [246]

Property	PBT criteria	vPvB criteria
Persistence[1]	$T_{1/2}$ > 60 days in marine water, or $T_{1/2}$ > 40 days in fresh or estuarine water, or $T_{1/2}$ > 180 days in marine sediment, or $T_{1/2}$ > 120 days in fresh or estuarine sediment, or $T_{1/2}$ > 120 days in soil.	$T_{1/2}$ > 60 days in marine, fresh or estuarine water, or $T_{1/2}$ > 180 days in marine, fresh or estuarine sediment, or $T_{1/2}$ > 180 days in soil.
Bioaccumulation[2]	BCF > 2000 L/kg	BCF > 5000 L/kg
Toxicity	NOEC < 0.01 mg/L for marine or freshwater organisms, or substance is classified as carcinogenic (category 1 or 2), mutagenic (category 1 or 2), or toxic for reproduction (category 1, 2 or 3), or there is other evidence of chronic toxicity, as identified by the classifications: T, R48, or Xn, R48 according to Directive 67/548/EEC.	-

[1] The assessment of persistence in the environment is based on available half-life data collected under adequate conditions, which must be described by the registrant.

[2] The assessment of bioaccumulation must be based on measured data on bioconcentration in aquatic species, which may be freshwater or marine species.

provide clear answers to specific questions, but very often the questions cannot be formulated clearly because of the relatively limited amount of standard physicochemical and ecotoxicological data available, although much progress has been made [58].

Because so little is known about the variations in ecosystem susceptibility to chemicals (community to community extrapolation), it is not correct to propose a single extrapolation factor between a MS test and other ecosystems. The use of microcosms provides a reasonable alternative, which has many advantages over true field studies [96-99]. Effects assessment for most chemicals will, in most cases, still be based on extrapolation methods used for preliminary and refined effects assessment, i.e., on acute and chronic single-species toxicity data. From the validation of several of these extrapolation methods, by comparing MS NOECs with extrapolated data [97,108,236], it appears that the use of extrapolation methods leads to equal or lower rather than higher values than the MS NOECs derived from field studies and microcosm studies.

The general view is that, if little data are available, which is the case for more than 99% of chemicals, only a preliminary effects assessment is possible, in which event assessment factors can be used (Table 7.45). For more data-rich substances, it is now accepted to use the extrapolation methods of Aldenberg and Jaworska [226] and the USEPA [16] as a good basis for determining PNECs.

Modern statistical methods could make even more use of the existing data, by mining the information hidden in the sensitivity of species for chemicals that we *do know* a lot about. These sensitivity patterns can be used to estimate the uncertainty for those chemicals where we lack this information [241,243].

Both laboratory and field work are needed to provide more insight into the complexity of ecosystems and to improve the way in which PNECs are derived for environmental risk assessment.

7.11 ASSESSMENT OF PBTs AND vPvBs

PBT substances are chemicals that pose specific risks to ecosystems and human health, due to their persistence in the environment, their bioaccumulative properties in food webs and their toxicity. A special class of chemicals are those that are very persistent and very bioaccumulative (vPvB). For these substances, it is recognized that accumulation in the environment and food webs is highly likely, but unpredictable levels could occur in man or the environment over long time periods. PBTs such as the insecticide endosulfan, the aromatic hydrocarbon anthracene, and the flame retardant octobromodiphenyl-ether, have been associated with negative health and

ecological effects, due to chronic exposure to these substances. This experience with PBT/vPvB substances has shown that they can give rise to specific concerns that may arise due to their potential to accumulate in parts of the environment:

- The effects of such accumulation are unpredictable in the long-term.
- Such accumulation is practically difficult to reverse as cessation of emission will not necessarily result in a reduction in chemical concentration.

PBTs and vPvBs distribute between air, water and soil or sediment. These properties also mean that these substances can reach remote areas and contaminate food webs in pristine areas. Many persistent chemicals have been found in the arctic due to this process of *long range transport*, for which screening models are available [244].

The combination of persistence and bioaccumulation, which can give rise to toxic effects after a longer time and over a greater spatial scale than chemicals without these properties, makes PBTs a group of special concern. Methods and tools, such as the PBT profiler, have been developed to screen chemical inventories for PBT properties. These screening tools can be used in the absence of chemical-specific data [245].

The properties of the PBT/vPvB substances lead to a increased uncertainty in the estimation of risk to human health and the environment by applying quantitative risk assessment methodologies. For PBT and vPvB substances a "safe" concentration in the environment is difficult to establish with sufficient reliability. Therefore, a separate PBT/vPvB assessment is required under REACH [246] in order to take these specific concerns into account. Registrants are required to perform this specific PBT/vPvB assessment in the context of their chemical safety assessment. A general introduction on the assessment of PBTs and vPvBs is given in Chapter 12, Section 12.3.5.

The PBT and vPvB assessment in the REACH regulation [246] consists of a screening assessment and a definitive assessment [220]. The screening assessment for biodegradation uses a limited set of biodegradation tests or model predictions. Bioaccumulation is screened based on the *n*-octanol-water partition coefficient, physicochemical indicators such as molecular weight, and maximum diameter and octanol solubility. Toxicity is screened based on the available aquatic and bird or mammalian toxicity data or estimated toxicity.

If the substance fulfils the criteria for a potential PBT/vPvB (Table 7.49), a definitive assessment should be conducted. The definitive assessment should be based on measured data for biodegradation, bioaccumulation and long-term toxicity tests for aquatic organisms and by evaluating the classification of the substance for human health hazards. Detailed guidance for the assessment of PBT and vPvB substances will become available in the Technical Guidance Document [220].

7.12 CONCLUDING REMARKS

Chemical substances and their by-products are being release into the environment, on a worldwide basis, at increasing levels. It is estimated that up to 90% of these chemicals have not been adequately evaluated for their aquatic toxicity towards algae, daphnids and fish (Chapter 1 and 12). Terrestrial and sediment toxicity data are also very scarce. Few ecotoxicological studies have been reported addressing adverse effects at higher levels of biological organization, i.e. the population or ecosystem level.

Applying the current risk assessment paradigm and meeting the associated data-generation requirements, combined with the increased need to evaluate the potential effects of thousands of industrial chemicals [246], are big challenges for the chemical industry, national and international regulatory agencies and associated stakeholders [214]. The long-term solution to these challenges will not be to generate more hazard data more quickly but rather to determine which specific effects data, groups of chemicals, and exposures are essential for assessment and appropriate management of the risks. Testing to cover all data gaps according to a generalized checklist approach ("box ticking") should be prevented [214]. In fact, a complete review of all available scientific evidence data will not provide clear, definitive answers to the risk management questions that regulators must address. Uncertainty inevitably remains. Steensberg [247] has discussed this before: "We, correctly, believe that we have not understood anything at a fundamental level unless we have understood the mechanism of causation. And we often think, incorrectly, that such understanding is a prerequisite of wise action. It is often necessary to make a decision on the basis of knowledge sufficient for action but insufficient to satisfy the intellect."

Regulators and ecotoxicologists must avoid the cult of a search for complete answers to the pressing health and ecological problems before it takes action against them. To do otherwise is to erect a barrier to timely and intelligent action [247]. That is why the next part (Part IV) of this book (Chapters 8-11) is entirely devoted to data, data estimation methodologies and testing strategies.

7.13 FURTHER READING

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