7 Modes and Mechanisms of Action

Although risk assessments may be entirely empirical, an understanding of the underlying mechanisms by which effects are induced can improve an assessment's efficiency and credibility. This requires employing the concepts of mode of action and mechanism of action, and extending them from toxicology to the analysis of effects of other agents. These concepts are important to the analysis of effects of mixtures (Chapter 8), development of quantitative structure–activity relationships (Section 26.1), use of toxicokinetic models to extrapolate among species (Section 26.2.7), and other aspects of ecological risk assessment.

7.1 CHEMICAL MODES AND MECHANISMS

Mechanisms of action (MoAs) are the specific means by which chemicals induce effects in organisms. Examples include narcosis, respiratory uncoupling, and inhibition of calcium uptake. Modes of action are more general and phenomenological; a mode of action implies a common toxicological outcome but not necessarily the same underlying mechanism. Examples include acute lethality, tumorigenesis, feminization, teratogenesis, and hatching failure. Note that the terms mechanism of action and mode of action are not used consistently in the literature, and there is no agreed dividing line between them.

MoAs are important in ecological risk assessment, because chemicals with a common MoA should behave similarly and may even be used interchangeably in some models. For example, they should be fit by the same quantitative structure–activity relationship (QSAR), they are expected to cause the same effect at the same molar concentration at a site of action, they should have a concentration-additive or dose-additive combined effect when they appear as a mixture, species are expected to have the same relative sensitivity to chemicals with the same MoA, if acclimation to a chemical occurs it is likely to occur with other chemicals with the same MoA, species sensitivity distributions and other exposure–response models are expected to have the same slope for chemicals with the same MoA, etc.

The potential advantages of mechanism-based assessment are not easily achieved. Most ecotoxicology is based on only whole-organism responses; without observations of suborganismal effects, the MoA is not apparent. (However, the organismal responses are the mechanisms by which effects on populations and communities are induced.) Further, exposures are expressed as external concentrations or applied doses, so the concentration at the site of action is unknown. Toxicokinetic modeling can estimate site of action concentrations, but they are not well developed for ecological species of concern and in most cases the site of action is unknown or uncertain. Many chemicals have multiple MoAs. Most information on MoA is derived from acute lethality tests, and the MoA may be different for nonlethal effects at lower doses and longer exposures (Slikker et al. 2004a,b). In addition, the MoA may depend on the route of exposure because of effects at the portal of entry or toxicokinetic

differences such as first-pass hepatic metabolism. An example of a chemical with multiple MoAs is lead, which causes acute lethality and chronic stress in fish by acting on calcium ion channels on the gills (Niyogi and Wood 2004), but also causes neurotoxic effects in extended aqueous exposures, and constipation and gut impaction in dietary exposures (Woodward et al. 1994a,b). Another example is organophosphate pesticides, which are acutely lethal by cholinesterase inhibition, but some are also androgen receptor antagonists, possibly having chronic effects by that mechanism (Tamura et al. 2003). MoAs often do not correspond to chemical classes (Figure 7.1), so identification of MoAs requires specific studies (Russom et al. 1997).

Interest in developing and using knowledge of MoA in ecological risk assessments has been increasing (Escher and Hermens 2002, 2004). Mechanistic toxicology references have been available for mammals for some time (Boelsterli 2003), and are increasingly available for other taxa such as fish (Schlenk and Bensen 2001) and arthropods (Korsloot et al. 2004). Lists of modes and MoAs have been published (Russom et al. 1997; Wenzel et al. 1997; Escher and Hermens 2002). Although they are a good start, they are limited to common mechanisms of acute lethality.

Classifications are incomplete because of the extremely large number of potential MoAs. For example, for each of the many hormone receptors in the various animal and plant taxa, there are at least four MoAs: two involving the hormone receptors and two involving regulation. Agonists are chemicals that bind to, and activate, the receptor (i.e., they act like the hormone), and antagonists bind to, but do not activate, the receptor and block the hormone. They may be quite specific. For example, a chemical may be an agonist or

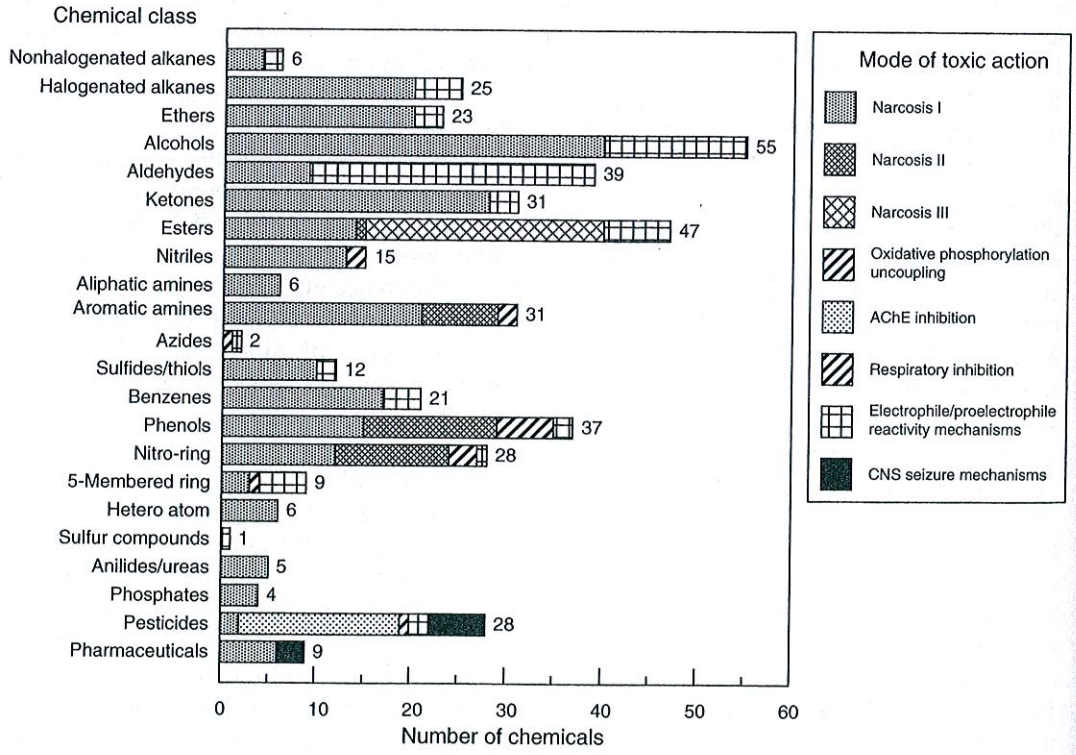

FIGURE 7.1 A chart showing that members of a chemical class may have different mechanisms of action (MoAs). (From Russom, C.L., Bradbury, S.P.S., Broderius, J., Hammermeister, D.E., and Drummond, R.A., *Environ. Toxicol. Chem.*, 16, 948, 1997. With permission.)

antagonist of estrogen receptors on different cells in an organism (Safe et al. 2002). Regulators are chemicals that affect the mechanisms that regulate the production of hormones. They may either upregulate or downregulate. Hence, the mechanisms of endocrine disruption alone are too numerous to address given current knowledge and resources.

One solution to this surfeit is to define a few categories of chemicals that have a simple common MoA [inert (baseline narcotic or narcosis I) and less inert (polar narcotic or narcosis II)] that can be used to predict toxicity of most organic chemicals and a few broad categories that can be used to estimate an interval within which toxicity will fall (reactive chemicals and specifically acting chemicals) (Verhaar et al. 1992, 2000). The simple four-bin categorization is popular in Europe for assessment of aquatic risks from organic chemicals (De Wolf et al. 2005).

A few MoAs and categories of mechanisms are discussed here because of their potential importance in the practice of ecological risk assessment:

Narcosis: Also termed baseline or inert toxicity, narcosis is the least toxic mechanism and is common to all organic chemicals. It results from the nonspecific disruption of cell membranes due to partitioning of the toxicant into the lipid bilayer. It causes death at a relatively constant membrane molar concentration and is fully reversible prior to death. It occurs in at least two distinct classes of chemicals. Baseline or nonpolar narcosis is caused by nonionic organic chemicals that partition to both storage and membrane lipids. Polar narcosis is caused by organic chemicals such as phenols and anilines with an ionic component, which causes them to partition more readily to the phospholipids of cell membranes than to storage lipids. Polar narcotics are more toxic on a body residue basis, because of this preferential partitioning to the site of action. Narcosis is the most common MoA; Russom et al. (1997) estimated that 71% of a sample of 225 industrial organic chemicals comprised narcotics in acute lethality tests of fathead minnows.

Disruption of ion regulation: Many metals induce toxic effects in aquatic organisms by disrupting ion channels on respiratory surfaces involved in the regulation of inorganic ion levels, particularly Na^+ and Ca^{++}. This is the basis for biotic ligand models (Chapter 26). This mechanism also includes disruption of channels involved in neural sodium and potassium pumps. In addition to heavy metals, disruptors of these channels include some neurotoxins produced as defensive chemicals. Well-known examples include cardioglycosides produced by foxgloves (*Digitalis* spp.) and tetrodotoxin from puffer fish.

Reactive oxygen generators: Some chemicals such as the herbicide paraquat and quinones such as tetrachlorohydroquinone can undergo redox cycling in which they reduce oxygen to reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical. These reactive oxygen species cause nonspecific effects including degradation of membrane lipids and proteins.

Uncouplers: Some chemicals, including pentachlorophenol and dinitrophenol, uncouple the oxidative phosphorylation pathway that generates adenosine triphosphate (ATP) in mitochondria. Consequences of uncoupling include reduced useful energy (ATP), increased oxygen consumption, and excess heat generation.

Photosynthesis inhibitors: Some herbicides and other chemicals block photosynthetic electron transport.

Cholinesterase inhibition: Organophosphate and carbamate pesticides act by inhibiting the enzyme acetylcholinesterase, leading to the accumulation of acetylcholine in synapses, and overstimulation of the cholinergic nervous system.

Endocrine disruption: This is a category of mechanisms which includes chemicals that mimic endocrine hormones (agonists), that occupy hormone receptors without activating them (antagonists), and that act on the systems that regulate endocrine hormone production.

The best-known environmental endocrine disruptors are estrogen agonists and antagonists, but all hormone systems are potentially subject to disruption by chemicals in the environment. Ecdysone-inhibiting pesticides such as diflubenzuron are examples of ecologically significant endocrine disruptors.

Pheromone disruption: This category of mechanisms is analogous to endocrine disruption but affects hormones involved in intraspecies communication. For example, endosulfan inhibits reproduction in red-spotted newts by blocking the production of pheromones (Park et al. 2001).

In many cases, the MoA for a chemical is identified in terms of inhibiting a particular organ or organ system without knowledge of the specific mechanism. Hence, many toxicology texts are organized in terms of organ systems (e.g., Schlenk and Bensen 2001). Such MoAs are defined by where the effect occurs (e.g., hepatotoxicants) rather than how they occur (e.g., respiratory uncoupling). In particular, many chemicals have been identified as immunotoxic in fish, but the mechanisms are often unclear (Rice 2001). Immunotoxicity serves to illustrate the difficulty of interpreting MoAs based on organ system performance. While changes in immune function clearly have implications for disease resistance and tumor suppression, it is extremely difficult to translate a change in immune system condition into probabilities of survival. Even when realistic experimental studies showing immunotoxicity can be related to an actual disease outbreak, it is difficult to prove that the magnitude of outbreak was due to the toxic effect. For example, more than a decade after the 1988 mass mortality of European harbor and gray seals associated with phocine distemper infections, and after several observational and experimental studies, the role of immune disruption by polychlorinated biphenyls (PCBs) has continued to be a contentious issue (O'Shea 2000a,b; Ross et al. 2000).

Some chemicals have both nutrient and toxic MoAs. All metals are toxic at sufficient exposure levels. For macronutrient metals such as calcium, iron, potassium, and sodium, toxic levels are so high that they are more commonly deficient than toxic. However, for many micronutrients, including chromium, cobalt, copper, manganese, molybdenum, nickel, selenium, vanadium, and zinc, toxicity is a more common concern, particularly in industrialized nations. A typical exposure–response relationship for these nutrient metals is illustrated in Figure 7.2. Risk assessment is particularly difficult for elements such as selenium that have a

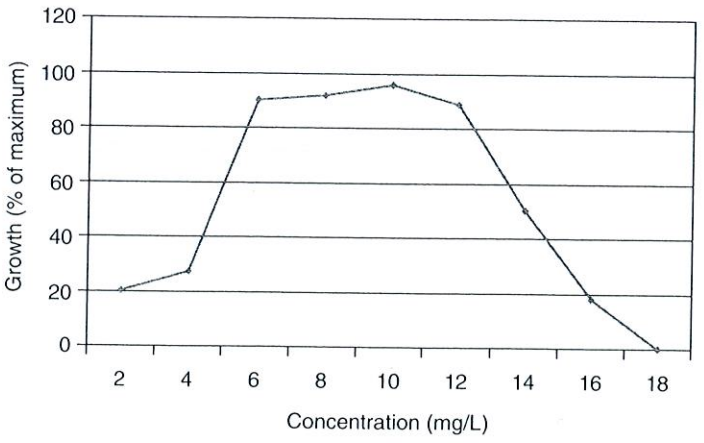

FIGURE 7.2 An exposure–response relationship of a micronutrient metal. Growth is inhibited by deficiency at very low concentrations, and regulated over a wide range of concentrations resulting in optimal growth; however, at high concentrations toxicity reduces growth.

narrow optimal range. If a precautionary approach is employed, benchmark levels for protection from toxicity may be set in the deficient range. Ammonia is another nutrient that commonly achieves toxic concentrations in the environment. In some cases, chemicals may have a nutrient MoA in organisms at all levels of exposure but have adverse effects on communities at high levels. Increased loading of ecosystems with nutrient elements, particularly nitrogen and phosphorous, cause changes in species composition and relative abundances which may be considered adverse. At high levels in aquatic ecosystems, the increased production can result in anoxia due to autotrophic and heterotrophic respiration that exceeds photosynthesis and aeration.

7.2 TESTING FOR MECHANISMS

Tests can be used to identify mechanisms by which chemicals might induce effects. In vitro tests for mechanisms cannot be used to estimate effects but can potentially allow the screening of chemicals so that testing can be focused on the most relevant tests of the most hazardous chemicals. Examples include the Ames *Salmonella* bioassay to detect mammalian mutagens and the fish liver slice test to detect estrogenic chemicals. Escher and Hermens (2002) provide in vitro tests for their classification of aquatic ecological MoAs. Such tests are currently not a component of regulatory testing schemes, although research to make them more acceptable is ongoing. Alternatively, conventional tests can be modified or supplemented to yield information on the MoA. For example, observations of the behavior of fathead minnows during 96 h lethality tests distinguished three syndromes that corresponded to narcosis I, narcosis II, and either cholinesterase inhibition or electrophile reactivity (Drummond et al. 1986; Drummond and Russom 1990; Russom et al. 1997).

7.3 NONCHEMICAL MODES AND MECHANISMS

The concepts of mode of action and MoA are applicable to other hazardous agents and may be clarified by those applications. A mode of action may be defined as a response at one level of organization that has a common implication at higher levels. For example, acute lethality is a common mode of action resulting from high levels of exposure to chemicals, harvesting, explosions, high temperatures (as in passage of organisms through a cooling system), crushing by vehicles, etc. Each of these examples has a different mechanism. This organism-level mode of action, however, has the same implication for population abundance or production and would be represented in the same way in a population model. Other examples of organism-level modes of action include reduced fecundity, increased developmental deformity, and reduced growth. Modes of action for populations include reduced abundance of older-age classes (from harvesting or slowly bioaccumulating chemicals), changes in sex ratio (from harvesting or endocrine disrupting chemicals), and local extinction (many mechanisms). Multiple mechanisms can induce these effects, but they have the same implications for the population's role in the community. Modes of action for ecosystems include reduced primary production, structural diversity, and nutrient retention. As with the other levels, all of these modes of action could result from multiple mechanisms. Careful consideration of MoAs is necessary when estimating risks from multiple activities and agents affecting a particular site or population (Chapter 8).