FUNDAMENTALS OF ECOTOXICOLOGY

The Science of Pollution

FOURTH EDITION

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CHAPTER 1

Introduction

On the day of the patients' victory at court, someone wrote a headline: "The Day that Tomoko Smiled." She couldn't possibly have known. Tomoko Uemura, born in 1956, was attacked by mercury in the womb of her outwardly healthy mother. No one knows if she is aware of her surroundings or not.

Smith and Smith (1975)

1.1 HISTORIC NEED FOR ECOTOXICOLOGY

It is natural and responsible to periodically reconsider the wisdom of our evermore complex and encompassing system of environmental regulations. Do United Nations treaties and European Union directives encroach too much on the sovereignty of nations? Have environmental regulations grown too costly for developed countries or too stifling for developing countries? It may be difficult at first glance to understand why national sovereignty should not be more respected or why significant amounts of the money now spent on environmental regulation should not be reallocated to the global economic crisis, critical social problems, medical research, technological innovation, education, reinvigorating space exploration, or other worthwhile endeavors, e.g., Lomborg (2001). But, just 50 years ago, it was easy to understand: Tomoko Uemura's mother understood.

Tomoko Uemura was born with severe and permanent neurological damage after her mother had unknowingly consumed mercury-laden fish. Tomoko was barely aware of her surroundings during her pain-filled 21 years of life. Although Tomoko's mother grew to understand the consequences of inattention to pollution, what she could not grasp as her personal tragedy unfolded was how the conditions leading to her daughter's agonizing life were allowed to come into existence in the first place.

Explanation of Tomoko's, and related, tragedies must begin with events that emerged a little more than half a century before she was born. At the close of the nineteenth century, complex changes were occurring unevenly across many countries. All grew out of the unprecedented shifts in human population size and distribution, and our singular talent for extracting resources and energy from the environment. This was a time of shortsighted exploitation of natural resources and cavalier attitudes toward worker health. Population expansion brought widespread land and soil degradation through farming, foresting, mining, smelting, and other activities. With expansion to fill all available frontier regions such as in the western United States, the option was no longer open to move to an unsullied area after despoiling local natural resources. Widespread degradation left the development of a sound knowledge base and practices for resource conservation as the only available alternative.

Perhaps soil degradation and eventual conservation is the clearest and most global illustration of this point. McNeill (2000) points out that two of the three historical surges in soil erosion overlapped with this period. The first did not, having occurred in the Middle East, India, and China circa 2000 BC–AD 1000. The second surge (1490s–1930s) did, starting with the European expansion into North America, South America, South Africa, Northern Africa, Australia, and New Zealand. The third, and ongoing, surge encompassed most of the world beginning in the 1950s. In addition to soil erosion, mining during the nineteenth and early twentieth centuries produced wide swaths of metal-contaminated soils in broad regions such as the Akita Prefecture of Japan, Silesia region of Poland, Ontario Province of Canada, and western United States, typically tainting local agricultural produce and waters.

Out of necessity, natural resource conservation encompassing land, water, wildlife, and fisheries resources became essential in developing countries. Typical of legislation that started to emerge during this period, the United States passed its first wildlife conservation act, the Lacey Act (1900). Society's ultimate embrace of a conservation ethic was clearly articulated in the 1905 revelation of U.S. President Theodore Roosevelt's head of the new Department of Forest Service, Gifford Pinchot:

> *Suddenly the idea flashed through my head that there was a unity in this complication – that the relation of one resource to another is not the end of the story.... All of [the] separate questions fitted into and made up the one great problem of the use of the earth for the good of man.*
>
> **Pinchot (1947)**

And another general movement materialized to cope with harm occurring to humans exposed to chemicals. Global, albeit uneven, trends in urbanization and industrialization brought with them harmful chemical consequences to human well-being that required redress.

People were gathering together in cities of a size never seen before in history. In addition to the infectious disease risks that emerged as large cities came into existence, urban air pollution–associated health risks appeared, becoming one of the first blatant pollution problems needing attention. As the twentieth century unfolded, coal burning was pervasive for industrial and domestic heating purposes. An archetypal consequence was the December 1952 London "fog" episode that killed 4000 Londoners outright (Anderson, 2009). Although this was an extreme case, appalling air pollution was being experienced in other cities including those in Europe (e.g., Athens, the Ruhr region of Germany, and numerous cities in Soviet-dominated countries), North America (e.g., Chicago, Mexico City, Pittsburgh, and St. Louis), and Asia (e.g., Calcutta). Large cities improved air quality temporarily by switching from coal to oil; however, the appearance of automobiles brought unhealthy air pollution back in the form of photochemical smog (McNeill, 2000).

Industries contributed substantially to city air pollution. Indeed, the term acid rain was first coined in 1872 by Angus Smith who identified it as the cause of extensive vegetation death around industrialized Newcastle and Liverpool (Markham, 1995). A 1930 air pollution episode precipitated by a brief inversion over an industrialized Belgian town in Meuse Valley increased death rates 10-fold and the sickened citizens with histories of respiratory illness (Anderson, 2009). This scenario played out yet again in Donora, Pennsylvania, when an October 1948 inversion held zinc smelter smoke close to the ground, increasing death rates by sixfold and sickening hundreds of residents. In Siberia, lung cancer rates of forced-labor residents skyrocketed when the Norilsk nickel mines and smelters came into existence in 1935 in support of Soviet industrial plans (McNeill, 2000).

Some of the first pieces of environmental pollution legislation (such as, the U.K. Clean Air legislation of 1956 and 1963) aimed at controlling health effects of air pollution in and around large cities and industries. In many cases, a local problem was resolved temporarily by building taller smoke stacks that spread pollutants over wider areas. They became a problem for another day.

On related fronts, labor rights and industrial hygiene movement leaders fought for a more evenhanded **corporatism*** during industrialized society's nonage.† An exemplary figure of this time was Alice Hamilton who founded the science of occupational health. Her career as an advocate, beginning circa 1910, included negotiation to control U.S. workplace poisons such as mercury (hatter industry), phosphorous (match manufacture), benzene (general industrial solvent), and radium (watch face painting) (Hamilton, 1985). She successfully added chemical agents to the list of workplace dangers needing resolution. As the industrial hygiene movement matured into the 1930s, employers, employee representatives, scientists, and government officials came together to resolve early industrial indiscretions and to assure future adherence to the principles of evenhanded corporatism. This coalescing of responsible and affected parties would eventually be adopted by those attempting later to cope with pollutants in the general environment. An expectation of a safe work environment was eventually established. As a final contributing social movement, awareness and political action emerged about harmful chemicals in foodstuffs and drugs. That movement established the expectation of safe foods and medicines, and in 1906, resulted in the creation of a new U.S. Food and Drug Administration.

To summarize, concepts, approaches, and institutions appeared during the first half of the twentieth century for addressing pressing problems of natural resource conservation, urban air quality, industrial workplace hygiene, and harmful chemicals in food and drugs. The associated social evolution established an approach and ethic that would next extend outward to address harmful chemicals in the general environment. Environmental pollutants became a serious social issue to resolve during the second half of the twentieth century.

Blended into these historical demographic and industrial trends midway through the century was the **Green Revolution,** which began in the 1940s and quickly spread throughout the world (Evenson and Gollin, 2003). The key goal of this revolution was to improve crop production through an integrated application of high-yield crop strains, chemical fertilizers, and chemical biocides. It too brought unique resource conservation, worker safety, food safety, and general pollution issues as the second half of the century began.

So an explanation can now be provided to Tomoko's mother about conditions that allowed her daughter's life to be so painful and brief. Tomoko was born just as society moved beyond the pale in its activities within natural systems that it depended on. Society was becoming aware of its mistakes and realizing that it had new responsibilities to carefully regulate toxicants in the general environment. Too late for Tomoko, beliefs, behaviors, and laws were poised for necessary change‡ but had not yet changed. Change in our environmental ethic had not come soon enough for many such as Tomoko.

One of the newest fads in Washington – and elsewhere – is "environmental science." The term has political potency even if its meaning is vague and questionable. Lacking specific definition, it embraces every science – physical, natural, social – for all of them deal with man's surroundings and their influence and impact upon him.

Klopsteg (1966)

* Corporatism is "the belief that all parts of society [are] necessary to its harmonious functioning, and that therefore all parts should cooperate to see to the welfare of each part." (Clark, 1997)

† In the United States, these movements were embedded in what has been called the **Progressive Era** that occurred between the late 1890s until the United States' entry into the World War I in 1917. Occurring during the shift from agrarian to a more urban and industrialized state, the goal of Progressive Era was to transform democracy into a more just political system by replacing customs and dubious beliefs—self-evident intuitions—with modern ones, including innovations based on sound scientific reasoning and technology. A scientific lens was systematically focused on social and political issues such as those concerning race, women's voting rights, reasonable limits of capitalism, labor rights, and immigration.

‡ This objective explanation is likely very unsatisfactory to anyone who, if only for a moment, imagines themselves in place of Tomoko or her mother. For the interested reader, a subjective narrative for the Minamata poisonings is provided in one of the best photojournalism works to date, *Minamata* (Smith and Smith, 1975). The Smiths' book and Carson's *Silent Spring*, were major catalysts for what would become a global movement to control environmental pollutants.

The vastness of the earth has fostered a tradition of unconcern about the release of toxic wastes. Billowing clouds of smoke are diluted to apparent nothingness; discarded chemicals are flushed away in rivers; insecticides "disappear" after they have done their job; even the massive quantities of radioactive debris of nuclear explosions are diluted in the apparent infinite volume of the environment … [But] we have learned in recent years that dilution of persistent pollutants even to trace levels detectable only by refined techniques is no guarantee of safety. Nature is always concentrating substances that are frequently surprising and occasionally disastrous.

Woodwell (1967)

After World War II, the **dilution paradigm** (the solution to pollution is dilution) was gradually replaced by the **boomerang paradigm** (what you throw away can come back to hurt you). Two widely publicized epidemics of heavy metal poisoning from contaminated food had occurred in Japan. By the 1950s, enough organic mercury was transferred through the marine food web to poison hundreds of people in Minamata Prefecture. Nearly a thousand people, including Tomoko Uemura, fell victim to **Minamata Disease** before Chisso Corporation halted mercury discharge into Minamata Bay. In a major mining region of Japan (Toyama Prefecture), citizens were slowly being poisoned from 1940 to 1960 by cadmium in their rice. This outbreak of what became known as **itai-itai disease** was linked to irrigation water contaminated with mining wastes.*

In 1945, open-air testing of nuclear weapons began at Alamogordo, New Mexico, and nuclear bombs exploded over Hiroshima and Nagasaki later that same year. Nine years later, the Project Bravo bomb exploded at Bikini Atoll, dropping fallout over thousands of square kilometers of ocean including several islands and the ironically named fishing vessel, *Lucky Dragon* (Woodwell, 1967). The Marshall Islands of Ailinginae, Rongelap, and Rongerik received radiation levels of 300–3000 rem† within 4 days of this detonation (Choppin and Rydberg, 1980). Fourteen years later, Hempelmann (1968) would report elevated prevalence of nodular thyroids in Marshallese children (78% of the 19 exposed children vs. 0.36%–1.7% of unexposed children) notionally caused by the 1954 detonation radiation.

Fallout radioactivity in the air here increased sharply yesterday to 17.4 measured units, almost twice Sunday's 8.25 micromicrocuries per cubic meter of air. A micromicrocurie is one millionth of a millionth of the radiation strength of a gram of radium.

***Washington Post* (1961)**

On a broader scale, the hemispheric dispersal and unexpected accumulation of fission products in foodstuffs from these and subsequent detonations eventually created concern about possible long-term health effects. Initially, fallout had elicited only brief comment such as the snippet above taken from the December 12, 1961 weather page of the *Washington Post*. But concern grew quickly as the public read more thoughtful articles such as the 1963 *Washington Post* front-page exposé reporting "Persons living within 400 miles of the Nevada nuclear test site have been exposed during the last dozen years to far more radiation from radioactive iodine

* The name, itai-itai, which literally means "ouch-ouch," reflects the extreme joint pain associated with the disease. Doctors gave the disease this moniker based on the exclamations of patients as they came into clinics and hospitals for help.

† A **rem** or **R**oentgen **e**quivalent **m**an is a measure of radiation that takes into account the differences in biological effects of various types of radiation. It relates the radiation dose received to its potential biological damage. As such, it is a convenient unit for defining allowable radiation exposures, e.g., the average person receives approximately 0.360 rem (360 mrem) of radiation annually. The rem has been replaced as the official unit by the sievert (Sv). (1 rem = 0.01 Sv.) In contrast, the **curie** used later is a straightforward measure of radioactivity. One curie is 2.2×10^6 disintegrations per minute (dpm). Although still used widely as in this book, the curie has been replaced as the official unit of radioactivity by the **becquerel** (Bq). One curie is 3.7×10^{10} Bq.

than hereto realized" (Simons, 1963).* From 1960 to 1965, human body burdens of ^{137}Cesium increased rapidly worldwide and then slowly decreased as the United States, former Soviet Union, France, United Kingdom, and China bowed to public pressure to cease open-air testing (Shukla et al. 1973).

Unreported discharge of radionuclides occurred in addition to these overt releases. Most were kept from the general public for reasons of national security. On the northwest coast of England, a fire in the Windscale plutonium-processing unit released 20,000 Ci of radioactive iodine (^{131}I) to the surrounding area (Dickson, 1988). Release of ^{131}I is particularly disconcerting because it concentrates in the thyroid, greatly increasing cancer risk. After atmospheric release, ^{131}I in fallout can contaminate local vegetation, be ingested by dairy cattle, and accumulate in thyroids of dairy product consumers. At the secret Soviet Chelyabinsk 40 military plant in the Urals, plutonium processing had furtively discharged 120 million curies to a nearby lake and enough to the Techa River to induce radiation poisoning in citizens living downriver (Medvedev, 1995). A 1957 storage tank explosion at this same facility released 18 million curies of radioactive material, forcing approximately 11,000 people to evacuate a 1,000-km^2 area (Trabalka et al. 1980; Medvedev, 1995). From 1944 to 1966, knowledge of releases from the U.S. Atomic Energy Commission's Hanford Site in Washington State was kept from the general public. The complex released 440,000 curies of ^{131}I into the atmosphere between 1944 and 1947 (Stenehjem, 1990). An estimated 20,000 curies were released to the Columbia River on May 12, 1963, from the Hanford K-East reactor (Stenehjem, 1990).

Concern about pollutant effects to nonhuman species was also growing. Recollect from our discussion of trends during the first half of the century that a natural resource conservation ethic had come into being and institutions were established to ensure adherence to basic conservation principals. Pesticides such as the then widely used DDT† (dichlorodiphenyltrichloroethane or 1,1,1-trichloro-2,2-di-(4-chlorophenyl)-ethane) began accumulating in wildlife to alarming concentrations, resulting in direct toxicity and sublethal effects. The indisputable success of DDT in combating insect vectors of human disease such as malaria, yellow fever, and encephalitis, had encouraged its broadened use to control agricultural and nondisease-related pests. Responsible government agencies—the U.S. Department of Agriculture and Public Health Service, for instance—had found little evidence for concern in the early 1940s as DDT use spread and intensified. However, U.S. Department of Interior did express concern by the mid-1940s about widespread DDT application and possible harm to wildlife (Nelson and Surber, 1946). Then, in 1954, Professor Wallace at Michigan State University noticed dead and dying robins after DDT spraying on campus to control Dutch elm disease (Carson, 1962). Hunt and Bischoff (1960) and Dolphin (1959) documented from 1957 to 1960 the overt death of Western grebes (*Aechmophorus occidentalis*) after bioaccumulation of the DDT-related pesticide, DDD (1,1-dichloro-2,2-bis[*p*-chlorophenyl] ethane) through a freshwater food web (Clear Lake, California). Following excessive DDD application to this lake, enough accumulated in the grebes' brains to cause axonic dysfunction and death. Dolphin (1959) described a 1949 administration of DDD to control the nonbiting gnat, *Chaoborus astictopus*, of Clear Lake as "involving introducing approximately 40,000 gallons of a 30% DDD formulation … from drum-laden barges"! In 1962, Rachel Carson brought these and many other events together in a remarkably well-crafted and factually accurate exposé, *Silent Spring*.

* The basis for this newspaper article was a 1962 *Science* article by Lapp who examined the risk of infant thyroid cancer as a consequence of open-air testing. Estimates were done for the area surrounding the Nevada test site and also for a distant location in Troy, New York, that received considerable fallout (circa 2–4 μCi·^{131}I sq. mi.$^{-1}$) on April 26, 1953, from a Nevada detonation.

† DDT was an extremely important tool for disease control throughout the world. Indeed, Paul Müeller was awarded the 1948 Nobel Prize in medicine for discovering its value as an insecticide. Its importance in the context of disease vector control is often overshadowed by our present understanding of its adverse effects on nontarget species if used indiscriminately. Indeed, UN Environmental Programme delegates at the May 17, 2004 Stockholm Convention on POPs conceded that a complete DDT ban was unreasonable in malarial regions of the world. The World Health Organization now prescribes careful reintroduction of DDT for malaria control in developing countries (Lubick, 2007b).

The men who make pesticides are crying foul. "Crass commercialism," scoffs one industrial toxicologist. "We're aghast," says another. "Our members are raising hell," reports a trade association.... Statements are being drafted and counter-attacks plotted.

Lee (1962)

Notwithstanding attacks upon the work of this "quiet women author" (Lee, 1962), Rachel Carson succeeded in drawing the public's attention to the consequences of pesticide accumulation in wildlife (Souder, 2012). Although relatively nontoxic to humans, it had become clear that DDT and DDE (dichlorodiphenyldichloroethylene or 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethylene) inhibited Ca-dependent ATPases in the shell gland of birds, resulting in shell thinning and increased risk of breakage of eggs after being laid (Cooke, 1973, 1979). Birds at higher trophic levels were particularly vulnerable because DDT and DDE were resistant to degradation and accumulated in tissue lipids. The result was an increase in concentration with each trophic exchange in a food web. Reproductive failure of raptors and fish-eating birds became widespread. For instance, the average number of offspring per pair of osprey (*Pandion haliaetus*) nesting on Long Island Sound dropped from 1.71 young/nest (1938–1942) to only 0.07–0.40 young/nest by the mid-1960s (Spitzer et al. 1978).* Reproductive output of raptor populations decreased similarly in Alaska (Cade et al. 1971) and other regions of North America (Hickey and Anderson, 1968). Reproduction of brown pelicans (*Pelecanus occidentalis*) on the South Carolina coast from 1969 to 1972 fell below that needed to maintain viable populations (Hall, 1987). Ratcliffe (1967, 1970) reported the same downward trends for falcons (*Falco peregrinus*) and other raptors in the United Kingdom.

As the adverse effects of DDT became apparent, and perhaps more importantly, insect pests began developing resistance to DDT, agricultural chemists began synthesizing a complex arsenal of new pesticides (Figure 1.1). A wide array of pesticides differing in mode of action, toxicity, and environmental persistence came into existence soon after the 1939 introduction of DDT.

From among all of these instances of harm from pollutants in the general environment, the Minamata poisonings in Japan and DDT accumulation in raptors and fish-eating birds became the two events that most captured the public's attention and accelerated a paradigm shift (dilution paradigm to boomerang paradigm) (Figure 1.2). Together, they drew attention away from giddy industrialization and the well-intended Green Revolution to the consequences of inattention to pollutants in the environment. They were among the first issues to give impetus to the science of ecotoxicology.

1.2 CURRENT NEED FOR ECOTOXICOLOGY EXPERTISE

Today, we are hearing and seeing dire warnings of the worst potential catastrophe in the history of human civilization: a global climate crisis....

Gore (2006)

Pollution is not in the process of undermining our well-being. On the contrary, the pollution burden has diminished dramatically in the developed world.

Lomborg (2001)

* Osprey populations have rebounded. Ambrose (2001) reports that fewer than 8,000 breeding pairs existed in the United States in 1981 but that number increased to 14,246 pairs by 1994.

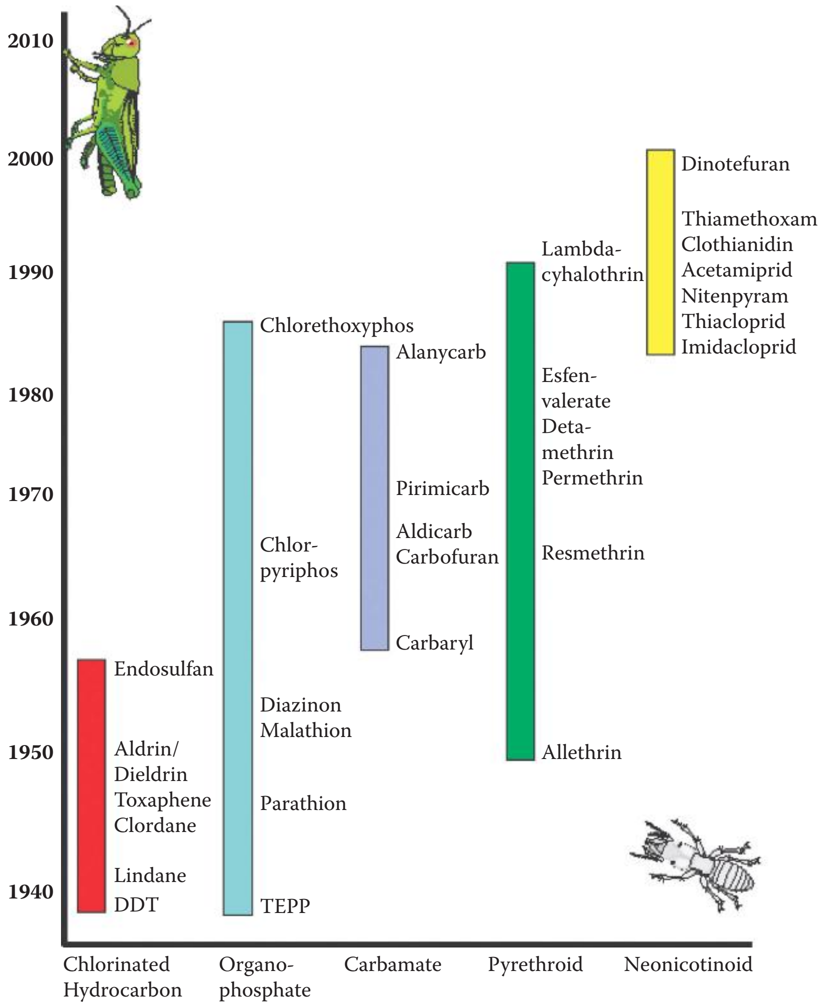

Figure 1.1 The general development chronology of the five major types of organic insecticides. The information in this figure was derived from Casida and Quistad (1998) (Figure 1), Jeschke and Nauen (2008), and Jeschke et al. (2011). The neonicotinoids are plotted with patent year, i.e., imidaclopid (1985), thiaclopid (1985), nitenpyram (1988), acetamiprid (1989), thiamethoxam (1992), and dinotefuran (1994).

Everyone would like to feel that the problems just described reflect early mistakes in our global techno-industrial revolution that will not be repeated.* That would be an unequivocal mistake. Our population size and technological ingenuity have created a world containing harmful pollutants that can no longer be diluted to harmless levels: the existence of real risk from pollutants now requires due diligence. Techno-industrial progress and environmental legislation proceed unevenly within and among countries, creating ample opportunity for repetition of past mistakes. And novel problems continue to emerge despite our increased diligence and complex regulations. It would be

* This premise is sufficiently prevalent to warrant a label, the **Bad Old Days Incongruity**. The assumption is often made that the worst environmental issues are now past and present issues are being handled adequately with existing legislation and technology. This assumption does not stand up to evidence such as that described herein. The adverse impacts of present and emerging issues are as serious—arguably much more serious in many instances—than those of the past. Increased attention and sophistication in dealing with these issues is required as the world's population increases and our techno-economic systems become more complex and far reaching.

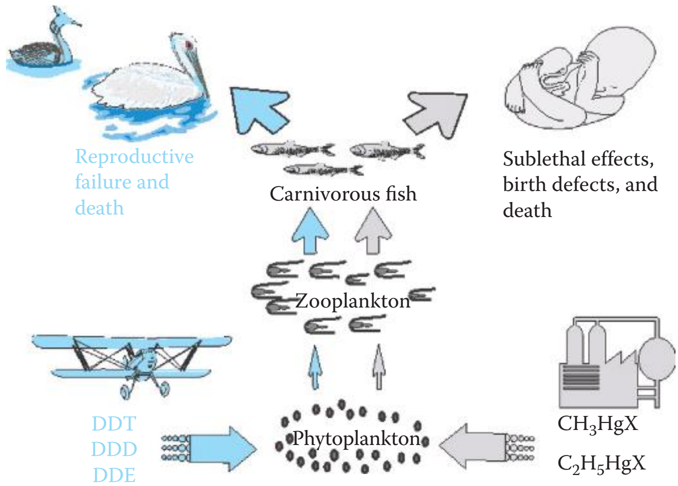

Figure 1.2 Two of the first pollutants to draw attention to the inadequacies of the dilution paradigm were DDT and methylmercury. They accelerated the emergence of the boomerang paradigm. Both chemicals were returned to humans or to valued wildlife species by transfer through food webs.

absurd to argue that, because early problems have been solved, attention and resources can now move from environmental pollution* to other important issues. As we approached and then entered the present millennium, problems extended more and more frequently to transnational and global scales (Figure 1.3). Many now are imbedded in **laggard systems** (Kerr, 2007), that is, systems with adverse effects that only slowly approach a critical state after conditions become established for their emergence. Adverse consequences of our present problems are equivalent to, or often more serious than, those of historic problems although they may manifest more subtly. This makes wise decisions difficult to reach for the public and mandates an increasingly more subtle and granular understanding of ecotoxicological phenomena. It also requires a level of international cooperation that is unfamiliar, and intermittently discomforting, for citizens of many countries. Effective attention must be given to a wide range of contaminants.

Nuclear materials still require our attention and resources. The core of Three Mile Island Reactor Unit 2 (Harrisburg, Pennsylvania) melted on March 28, 1979, releasing 3 Ci of radiation (Booth, 1987). In 1986, nearly 30 years after the Chelyabinsk 40 explosion in the Urals, the Chernobyl Reactor 4 core melted down in the Ukraine, creating the largest radioactive release in history (301 million curies as estimated by Medvedev [1995]). Fallout from Chernobyl spread rapidly across the Northern Hemisphere. The estimated 350,000 m^3 of waste from uranium (and rare earth element) mining and processing operations near the Estonian coastal city of Sillamäe remains and continues to release high levels of radon gas decades after Estonia won back its independence from the Soviet Union (Raukas, 2004). Three reactors in the Japanese Fukushima Daiichi nuclear power facility melted down on March 11, 2011 (Dauer et al. 2011), releasing enough radioactive material to eventually kill a projected 130 humans via fatal cancers (Ten Hoeve and Jacobson, 2012). The International Atomic Energy Agency (2007) estimates that the world's output of nuclear power will double by 2030 as countries' fossil fuel–dominated energy strategies shift to encompass more options. Increased presence of nuclear power stations in our landscape seems inevitable.

* Even a careful reading of Lomborg's book from which the above, superficially contrary quote was taken reveals that his appraisal embraces a central theme of increased thoughtfulness in balancing the benefits and adverse consequences of our activities, that is, "… it is absolutely vital for us to be able to prioritize our efforts in many different fields, e.g., health, education, infrastructure and defense, as well as the environment" (Lomborg, 2001).

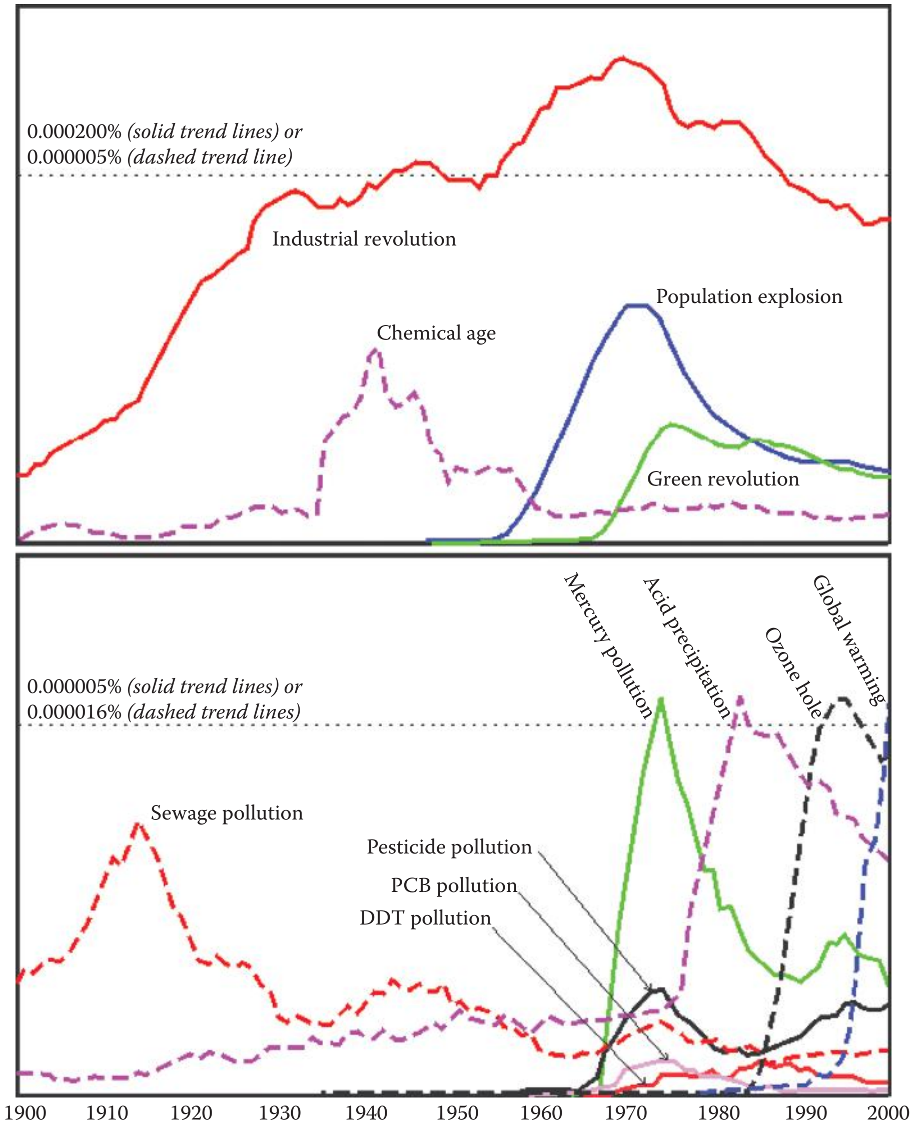

Figure 1.3 Results of an NGram analysis of several bigrams relevant to ecotoxicology. The top panel is the prevalence of bigrams related to social trends that were found in the millions of English books scanned to date by Google (see ngrams.googlelabs.com). The vertical axis reflects the occurrence of the bigram as a proportion of all of the bigrams in these books. The bottom panel depicts the same for bigrams related to impacts of environmental pollution. Clearly, a sequence of events occurring in the twentieth century established an awareness of a sequence of pollution types. An important theme to note is the expending scale for each in the temporal sequence of pollution types. For example, sewage pollution was usually associated with a water body. The more recent pollution types, e.g., the ozone hole or global warming, encompass scales of a continent and the entire biosphere. (Modified from Figure 8.1 in Newman, M. C., *Quantitative Ecotoxicology,* 2nd Edition, Taylor & Francis/CRC Press, Boca Raton, FL, 2013.)

Chemical wastes require continued attention and funding. A myriad of environmental issues remain in affiliated countries after the collapse in 1991 of the Soviet Union (Tolmazin, 1983; Edwards, 1994). Wastes from Soviet-era metal mining and smelting activities in Poland remain extensive and only partially remediated (Figure 1.4). Tributyltin (TBT), an antifouling agent in

Figure 1.4 Pervasive metal contamination exists in Poland's Olkusz mining region. Lead and zinc have been mined here since the medieval ages; however, industrial activities surged through the 1960s and 1970s in a manner typical of the times. Similar, widespread metal contamination was produced around the Avonmouth (UK), Gusum (north Sweden), Copperhill (Tennessee), and Flin Flon (Canada) smelters. In the early 1980s, releases at the Polish mining region decreased as production demand abated, and modern electrofilters and scrubbers were installed (top panel). (See Łagisz et al. [2002], Stone et al. [2001, 2002], and Niklińska et al. [2006] for details about ecotoxicological consequences.)

marine paints, has decimated coastal mollusk populations throughout the world (Bryan and Gibbs, 1991; Leung et al. 2006) yet has only recently been effectively regulated through international agreement. Mercury in fish and game remains a concern as new sources appear such as mercury used in South American gold mining (e.g., de Lacerda et al. 1989; Branches, 1993; Reuther, 1994; Alho and Vieira, 1997; Malm, 1998; Wade, 2013) (Figure 1.5). Subsurface agricultural drainage in the San Joaquin Valley of California brought selenium in the Kesterson Reservoir and Volta Wildlife Area to concentrations causing avian reproductive failure (Ohlendorf et al. 1986). Such high selenium concentrations from irrigation and other sources remain a global issue (Lemly, 2004). Efforts to avoid harm to humans (Ember, 1980; Settle and Patterson, 1980; Millar and Cooney, 1982) and wildlife by reducing lead in products such as gasoline and lead shot have been effective only since the late 1970s. Even well into the 1980s, debate continued about lead's effects and the

Figure 1.5 Mercury being used to extract gold from ore mined in Portovelo, Ecuador (June 2007). Pulverized ore is agitated with a puddle of mercury (arrows) to form a gold–mercury amalgam, which in this picture, is being placed into a piece of cloth. Mercury is hand-squeezed from the amalgam and remaining mercury is driven off by heating with a propane torch. This process results in substantial human exposure and mercury release into the environment. (Note that the person wringing the mercury from the amalgam has a silver bracelet into which the mercury will also readily dissolve to form a silver–mercury amalgam.) (Courtesy of Lane, K., College of William & Mary.)

need for federal regulation (Anderson, 1978; Marshall, 1982; Anonymous, 1984a; Ember, 1984; Putka, 1992).* Regardless, lead acid battery fabrication and recycling plants in Kenya still result in abnormally high blood lead levels in workers (Were et al. 2012). In late 2007, concern about lead emerged again as high concentrations were measured in toys manufactured by China: China's booming economy overran its capacity to maintain adequate quality control on these imports.

There is little reason to suspect that pollution events such as those that emerged in Europe, North America, and Asia during the 1970s and 1980s will not continue to emerge. The need remains for vigilance and accurate assessment of associated risk. As an infamous instance of confused risk communication, the controversy about the Hooker Chemicals and Plastics Corporation's waste dump sites at Hyde Park and Love Canal became hysterical as the public waited for a clear statement of risk from authorities and scientists (Culliton, 1980; Anonymous, 1981, 1982; Smith, 1982). Similar instances involving high perceived risk and uncertainty will inevitably appear in the future, requiring accurate estimation and communication of real risk. As an example that no one wishes to risk repeating, poor risk estimation and communication resulted in the December 2, 1984, explosion of a storage tank at a Union Carbide pesticide plant (Bhopal, India) and release of a methyl isocyanate cloud that killed 2,000 people and harmed another estimated 200,000 (Anonymous, 1984b; Heylin, 1985; Lepkowski, 1985). The impact on human life from this overnight catastrophe far exceeded that of the 1956 Minamata poisonings.

The intense activities associated with petroleum production, transport, and consumption also require our close attention. Recent examples to support this statement are easily found. On March 16, 1978, the *Amoco Cadiz* supertanker ran aground at Portsall (France) and released

* Relative to our slow acceptance of lead's adverse effect, Tackett (1987) provides a revealing quote by Benjamin Franklin (July 31, 1786). "This my dear Friend is all I can at present recollect on the Subject. You will see by it, that the Opinion of this mischievous Effort from lead is at least above Sixty Years; and you will observe with Concern how long a useful Truth may be known and exist, before it is generally receiv'd and practis'd on." Indeed, Michaels (2008) describes acute lead toxicity in hundreds of workers only a few decades ago that was related to lead-based gasoline additive production. As recently as one decade ago—200 years after this quote was made, the value of reducing lead in gasoline was being actively questioned.

roughly 209,000 m^3 of crude oil (Ellis, 1989). On March 24, 1989, March 24, 1989, the *Exxon Valdez* spilled 41,340 m^3 of crude oil into Prince William Sound. The oil covered an estimated 30,000 km^2 of Alaskan shoreline and offshore waters (Piatt et al. 1990). Marine bird populations are still recovering from this spill (Lance et al. 2001). From August 2, 1990 until February 26, 1991, the largest oil release to have ever occurred at that time was deliberately spilled by Iraqi troops occupying Kuwait. Half a million tons (roughly equivalent to 522,000 m^3) of crude oil from the Mina Al-Ahmadi oil terminal were pumped into the Arabian Gulf (Sorkhoh et al. 1992). Plumes of contaminating smoke from the intentional ignition of Kuwaiti oil wells by the Iraqi troops were visible from space (Figure 1.6). Beginning on April 20, 2010, and lasting for 84 days (Atlas and Hazen, 2011), the *Deepwater Horizon* drilling rig blowout released between four and five million barrels (approximately 715,442 m^3) of oil into the Gulf of Mexico, exceeding the 1990 Kuwaiti spill in size and easily displacing the 1989 *Exxon Valdez* spill as the largest oil spill in U.S. history (Camill et al. 2010; Kerr et al. 2010).* No fundamental change has occurred since the twenty-first century began that would exclude these kinds of releases happening at the current frequency into the near future.

Other smaller or more diffuse, but incrementally as damaging, events also require expertise in ecotoxicology. Beyond the intentional release described above, the Arabian Gulf receives 67,000 m^3 of oil annually from smaller leaks and spills (Sorkhoh et al. 1992). Before the *Exxon Valdez* spill in Alaska, a 1978 act of sabotage to the trans-Alaska pipeline had released 2540 m^3 of oil onto land near Fairbanks. In October 2001, 1081 m^3 of oil gushed from a hole shot in the trans-Alaska pipeline by an intoxicated man. The average number of oil spills and volume per spill in or around U.S. waters from 1970 to 1989 were 9,246 and 47,000 m^3, respectively, with no obvious downward

Figure 1.6 Kuwaiti oil wells set afire by Iraqi troops as seen from a U.S. space shuttle flight. Oil wells are seen burning north of the Bay of Kuwait and immediately south of Kuwait City. (Courtesy of NASA.)

* It also exceeded the June 1979 Mexican IXTOC 1 well oil spill that lasted 9 months and released three and a half million barrels (556,430 m^3) into the southern Gulf of Mexico (Kerr et al. 2010).

trend in either through time (Table 8 in Gorman, 1993). Three more events seem typical of those we should expect into the foreseeable future. On November 8, 2007, an estimated 220 m^3 of bunker fuel was spilt into San Francisco Bay from a container ship that struck the Bay Bridge due to a navigational mix-up. A week later, 4921 m^3 of oil was spilt near the Strait of Kerch when the Russian tanker, *Volganeft-139*, broke up in a north Black Sea storm. Reports by MSNBC suggested inadequate ship maintenance as a significant contributor to what the Krasnodar region's governor, Alexander Tkachyov, referred to as an "ecological catastrophe" (MSNBC News Services, 11/12/2007). In contrast to the previously discussed 1957-to-1960 loss of a few hundred grebes on Clear Lake to DDD, this one wreck quickly killed 30 thousand oiled seabirds and damaged critical habitat along a major bird flyway. One month after these two oil spills (December 7, 2007), an estimated 12,520 m^3 of crude oil spilled from the single-hull supertanker, *Hebei Spirit*, after it struck a crane on a barge off the west coast of Korea, resulting in Korea's largest spill. Clearly, real risk for harmful oil spills will be present into the near future and require our full attention.

Chemicals from our agricultural activities also require continued diligence in the twenty-first century. At the time Rachel Carson was writing *Silent Spring* (*circa* 1960), annual production of synthetic organic chemicals was 43.9 billion kilograms. Worldwide production had climbed to 145.1 billion kilograms by 1970 (Corn, 1982). By 1985, U.S. use of pesticides roughly doubled from the 227 million kilograms used in 1964 (Figure 7 in Gorman [1993]). Growing dependency on a complex array of pesticides has now become a global trend that is particularly problematic for countries with rapidly developing economies.

> *As we approached the* [Chinandega, Nicaragua] *airport the now familiar stench of chemicals became overpowering. As we walked down the airstrip to the health clinic in the airport complex, I glanced across the adjacent fields toward the nearby dwellings of town. Nothing was moving in the open space, not birds, not insects, none of the creatures normally found in such abundance in the tropical climates. Here indeed, Rachel Carson's prophecy seemed all too real.*
>
> **Murray (1994)**

Many persistent pesticides banned in developed countries are still used in developing countries (Simonich and Hites, 1995). Less persistent, but more toxic, pesticides are also used under inadequate regulation in many developing countries, inflicting more harm to humans and ecological entities than the harm from DDT described by Rachel Carson (Murray, 1994; Roth et al. 1994; Castillo et al. 1997, 2000; Henriques et al. 1997; Ecobichon, 2001; Murray et al. 2002; Wesseling et al. 2005).

> *In light of the growing evidence that many chemicals disrupt hormones, impair reproduction, interfere with development, and undermine the immune system, we must now ask to what degree contaminants are responsible for dwindling animal populations.*
>
> **Colborn et al. (1996)**

Unsuspected effects of conventional contaminants are being discovered and brought to the public's attention by diverse means such as the bestselling book, *Our Stolen Future* (Colborn et al. 1996). Reflecting a welcome maturation of our collective environmental ethic, *Our Stolen Future* was taken seriously by industries instead of receiving the indignation and counterattacks that greeted *Silent Spring*. This is important because much uncertainty remains about pollutant effects to endocrine systems. An important instance underscoring the need for increased diligence involves one of the most widely used agrochemicals today, atrazine. Despite decades of controversy, it has now been determined to harm aquatic organisms with key effects involving endocrine system dysfunction (Rohr and McCoy, 2010).

And unsuspected movements of conventional pollutants are also being elucidated. **Persistent organic pollutants** (POPs)* are particularly disconcerting because they are now known to disperse widely and accumulate to high concentrations in wildlife (e.g., Weber and Goerke, 2003; Tanabe, 2004; Lubick, 2007a) and humans (e.g., Landrigan et al. 2002; Lorber and Phillips, 2002; Pronczuk et al. 2002; Solomon and Weiss, 2002) (Figure 1.7) in regions far removed from their points of release. In response to this new understanding, the 2004 Stockholm Convention called for a gradual, international elimination of the most prominent, including polychlorinated biphenyl, dioxins, furans, and nine pesticides. Also the European Union's Registration, Evaluation, and Authorization of Chemicals (REACH) directive has included as one important aim the reduction of POPs (Tanabe, 2004).

> *China is choking on its own success. The economy is on a historic run, posting a succession of double-digit growth rates. But the growth derives, now more than at any time in the recent past, from a staggering expansion of heavy industry and urbanization that requires colossal inputs of energy, almost all from coal, the most readily available, and dirtiest, source.*
>
> **Kahn and Yardley (2007)**

New situations of concern continue to emerge in which conventional contaminants cause undeniable harm. Often, the stage of a country's economic development is a major contributor. Certainly, the unethical shipping of toxic waste from developed nations to underdeveloped African countries that occurred in the 1980s is one blatant example in which this was the case (Vir, 1989; Lipman, 2002; Simpson, 2002). Another is China's current economic growth as described in the aforementioned quote. China has begun to conscientiously balance economic growth and environmental stewardship (Liu and Diamond, 2005; Aunan et al. 2006; Fu et al. 2007; Zhang, 2007), but major obstacles remain. Features of the current Chinese historic economic growth, such as the **Township**

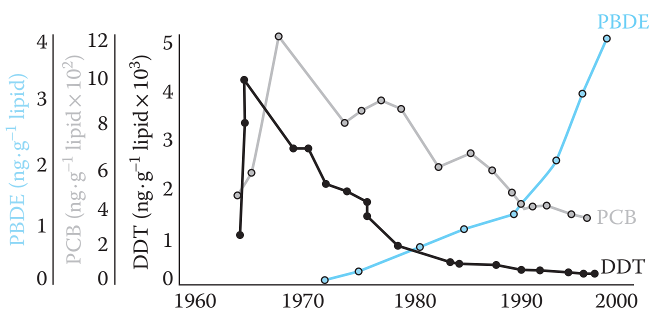

Figure 1.7 Trends in three persistent organic pollutants (POPs) in Swedish breast milk. DDT was banned in Sweden in the early 1970s, and accordingly, concentrations drop through the decades after peaking in the mid-1960s. A less obvious decline is seen for polychlorinated biphenyl (PCB) concentrations, resulting in continued concern about adverse PCB effects on humans and wildlife. Concentrations of brominated POPs used as fire retardants (polybrominated diphenyl ethers [PBDE]) increased rapidly through the decades; however, considerable effort is now being spent in the European Union to move away from PBDE use (Birnbaum and Staskal, 2004; Betts, 2007; Blum, 2007). Note that the scales for DDT, PCB, and PBDE are ng·g^{-1} of milk lipid ×10^3, ×10^2, and ×10^0, respectively. (Data extracted from Figures 3, 9, and 11 in Solomon, G. M. and Weiss, P. M., *Environ. Health Perspec.*, 110, A339–A347, 2002.)

* POPs are those organic pollutants that are long-lived in the environment. Many tend to increase in concentration as they move through food webs. Often, **Persistent, Bioaccumulative Toxicants** or Persistent, Bioaccumulative Chemical is the specific term used for POPs that tend to biomagnify in food webs.

and Village Enterprise (TVE) sector,* create circumstances with the potential for creating much more environmental damage than typical during such an economic surge. Other challenges to environmental compliance need resolution as China's economy booms (McElwee, 2011). One issue is the current tendency for regulators responsible for enforcement to be paid through, and consequently answerable to, local officials who might favor development over environmental protections. A third, less apparent, issue is deeply rooted guanxi† that may obligate a regulator to local affiliates. This obligation might take precedence over, or sway interpretation of, loosely written central government regulations. Despite these impediments, effective environmental concern is now crucial in many regions of China that have unique problems. For instance, the extensive pollution in Baotou (Inner Mongolia) is one linked to China's growth to dominate global rare earth element mining‡ (e.g., Yongxing et al. 2000; Zhang et al. 2000; Li, 2006; Wen et al. 2006) (Figure 1.8).

Yet another recent instance involved economically driven changes in conventional pesticide use in Central America (Figure 1.9). For several decades preceding the 1980s, agro-economic forces moved Nicaraguan agriculture away from a modest importation of arsenate-based (1920s cotton production) and then organochlorine pesticides. The amounts of pesticide imported and the toxicities of those imported pesticides increased dramatically when Nicaraguan agriculture shifted from large cotton plantations to smaller farms growing a range of export crops (Murray, 1994). Lax regulation based on ineffective pesticide use registration was pervasive. The Pan American Health Organization (2002) estimated that 97.5% of all pesticide use in Nicaragua from 1992 to 2000 was unregistered. The surge in acute human poisonings shown in the top panel of Figure 1.9 resulted from this regulatory failure and unsafe handling by small farmers who were accustomed to less toxic pesticides. A more recent, but similar, trend is clear in the bottom panel.

> *PBDEs, used as fire retardants in furniture, are structurally similar to the known human toxicants PBBs, PCBs, dioxins, and furans. In addition to having the similar mechanisms of toxicity in animal studies, they also bioaccumulate and persist in both humans and animals.*
>
> **Blum (2007)**

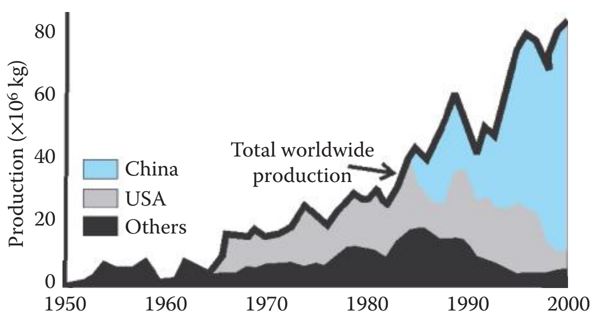

Figure 1.8 China's rapid rise to dominance in rare earth element production (redrawn from Figure 1 of the USGS Fact Sheet 087-02 [USGS, 2002]). These elements are essential components of modern color television screens, liquid-crystal displays of computer screens, optical networks, glass polish systems, and magnet technologies.

* The **TVE sector** comprised 20 million small factories throughout the Chinese countryside (Tilt, 2006). These small factories are responsible for much of the economic upswing in China but also are the most difficult to control relative to pollution. Drawing on World Bank analysis of China, Tilt (2006) states that the TVE sector accounts for 60% of the "air and water pollution, endangering human health and posing a serious threat to agro-ecosystems."

† As applied here, guanxi (关系) refers to a tradition of behaviors within a personal network of relationships in which members perceive a mutual obligation and capacity to ask for a favor in return. Guanxi is important and widespread in China.

‡ The **rare earth elements** include La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y. They are the lanthanides with atomic numbers from 57 to 71 plus scandium (atomic number 21) and yttrium (atomic number 39).

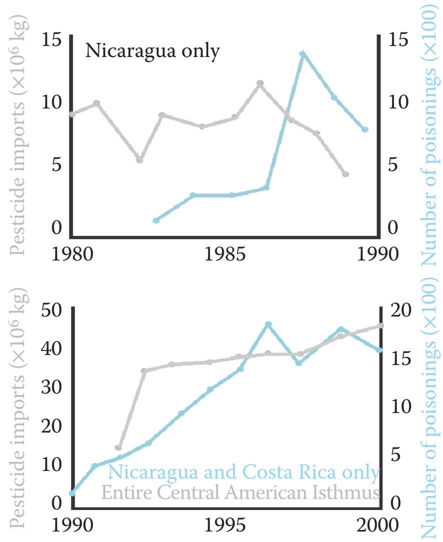

Figure 1.9 Pesticide use and effects in Central American countries. Exemplary of trends during the 1980s are those shown in the top panel for Nicaragua (from Figure 3-3 and Table 4-3 of Murray [1994].) The bottom panel depicts the same themes today of increasing pesticide importation into the Central American isthmus (Pan American Health Organization, 2002) and consequent acute human poisoning, e.g., that for Costa Rica and Nicaragua. (From Wesseling, C. M. et al. *Toxicol. Appl. Pharm*., 207, S697–S705, 2005.)

And releases of new chemicals are becoming a concern. Brominated fire retardants, chlorination by-products, fluorinated chemicals, synthetic estrogens, hormones from livestock production, alkylphenol ethoxylates and their degradation products, manufactured antimicrobial products, pharmaceuticals, and constituents of personal care products* are important examples of heretofore ignored contaminants that are presently discharged in large quantities (Hale et al. 2001; Meyer, 2001; Daughton, 2004; Chapman, 2006). Being unconventional contaminants, most are inadequately regulated (Daughton, 2004). And novel wastes occasionally emerge such as those associated with nanotechnologies (Daughton, 2004; Chapman, 2006) and electronic wastes (Widmer et al. 2005). Only during the last few years have ecotoxicologists begun to take adequate notice of the potential, widespread impacts of these materials in natural systems.

Contaminants amenable to atmospheric transport have become especially disconcerting because of the spatial scale over which they have an impact. This is certainly a major concern with many POPs. Acid rain is now a transnational problem (Likens and Bormann, 1974; Likens, 1976; Cowling, 1982), damaging both aquatic (Glass et al. 1982; Baker et al. 1991) and terrestrial (Cowling and Linthurst, 1981; Ellis, 1989) ecological systems. With an estimated 69% of China's energy derived from coal, acid rain has become extensive in Asia (Larssen et al. 2006). Chlorofluorocarbons (CFCs) used as propellants and coolants have been linked to ozone depletion in the stratosphere (Zurer, 1987, 1988; Kerr, 1992), and efforts are being made to greatly reduce their use (Crawford, 1987). But, despite the milestone 1987 Montreal Protocol call for complete elimination of CFC use by 2000, efforts by lawmakers were still underway into the mid-1990s to delay, and even avoid, any U.S. reduction of CFC emissions (Lee, 1995). Such common delay tactics result in unnecessary harm later.

* **Pharmaceuticals and Personal Care Products** released into the environment, especially from wastewater treatment facilities, are often referred to collectively as PPCPs.

The above monotonous litany of problems is not intended to convince the reader that techno-industrial advancement is incompatible with environmental integrity and human well-being. Rather, it is intended to establish two simple truths. First, by the mid-twentieth century, the dilution paradigm failed with clearly unacceptable consequences to human health and ecological integrity. Second, expertise in ecotoxicology is now critical to our well-being. The Minamata poisonings and eggshell thinning by DDT were the first blatant signs—not the high water marks—of unacceptable environmental pollution. The approaches and ethics established during the resource conservation, workplace health, and food safety movements were modified to address pollution issues faced by society.

Major environmental problems remain and new ones appear annually that are as, or more, significant than historical problems. Appropriately, environmental themes are so interwoven into our culture that associated concepts are communicated to the general public under catchy titles such as *Silent Spring, Our Stolen Future*, or *An Inconvenient Truth*. Expertise in ecotoxicology is essential for weighing the costs and benefits of the innumerable technological and industrial decisions affecting our lives. Nonmarket goods and services, and natural capital (Odum, 1996; Prugh, 1999) must be included in decision making. Such services provided pro bono by nature are estimated to be in the range of $33 trillion annually, twice the annual gross domestic product of the countries of the earth (Rousch, 1997). Investment of time, thought, and resources to avoid damage to service-providing natural systems is economically, as well as ecologically, wise behavior. Environmental regulations reduce human suffering, foster sustainable economic prosperity, and allow responsible environmental stewardship.

> *… growth and the environment are not opposites – they complement each other. Without adequate protection of the environment, growth is undermined; but without growth it is not possible to support environmental protection.*
>
> **Lomborg (2001)**

VIGNETTE 1.1 Emergence and Future of Ecotoxicology

John Cairns, Jr.
Virginia Institute and State University

Quite naturally, toxicology began with concerns about adverse effects of chemical substances on humans. The focus of toxicology gradually extended to organisms domesticated by humans and then to wild organisms of commercial, recreational, or aesthetic value. Unfortunately, these toxicity tests are all homocentric, commendable but inadequate when human society is dependent on an ecological life-support system. The close linkage between human health and the health of natural systems makes an ecocentric toxicity component essential. Figure 1.10 illustrates the dimensions of this challenge and shows that, despite remarkable progress in the field of ecotoxicology over the last four decades, there is still a long way to go.

In its earliest stages, environmental toxicology depended on short-term laboratory tests with single species that were low in environmental realism but with satisfactory replicability. However, such tests used neither endpoints nor levels of complexity characteristic of ecosystems. Neither did they include cyclic phenomena and many types of variability that are the norm for the complex, multivariate systems known as ecosystems. In short, the “eco” was seriously deficient in the field of ecotoxicology. Although simple tests were often used as surrogates for more important properties, toxicity tests at lower levels of biological organization, such as single species, were not readily validated at higher levels of biological organization, such as communities, ecosystems, or landscapes.

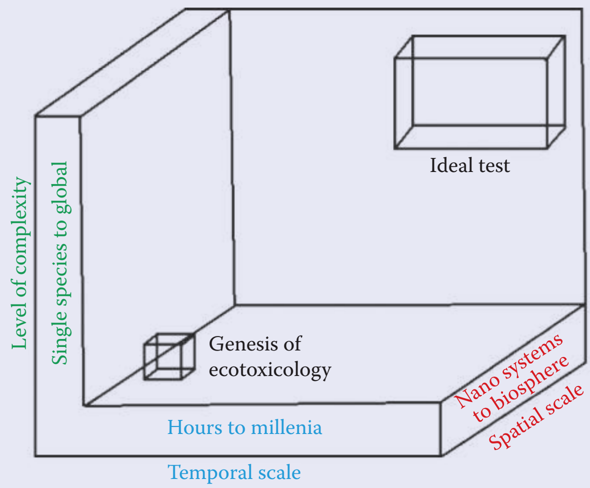

Figure 1.10 Schematic depiction of the genesis of ecotoxicology and the ideal goal about which one can,at present, only speculate. All three major components should be matched to the problem of interest to achieve the goal.

RESHAPING THE PLANET

Arguably, the impetus for the development of ecotoxicology resulted from the unmistakable environmental transformations that occurred in the twentieth century on a scale unique in human history. The world has become increasingly humanized, and ecosystems have become more fragmented and diminished in aggregate size. Consequently, ecosystems have begun to lose resilience and need greater protection from threats to their integrity. This situation is an uncontrolled "experiment" on a planetary scale for which the outcome is uncertain. Although environmental change has been the norm for 4 billion years, the planet has been altered by the human species for 4 million years; however, the rate and intensity of change that occurred in the last century are a cause for deep concern. One might reasonably assert that ecotoxicology is an attempt to provide some rules for the planetary game human society is playing. Almost every sport has elaborate rules that are discussed in great detail by fans. The human, therefore, needs to recognize the natural laws that determine the outcome of the game of life in which all are participants.

FUNCTIONING DESPITE UNCERTAINTY

Both the human condition and the "tools of the trade" (ecotoxicology) are constantly changing. Theories and practices once thought to be sufficient have been shown to be inadequate, often with stunning rapidity. Ecotoxicology can make major contributions in reducing the frequency and intensity of environmental surprises by (1) determining critical ecological thresholds and breakpoints, (2) developing ecological monitoring systems to verify that previously established quality control conditions are being met, (3) establishing protocols for the protection and accumulation of natural capital, (4) providing guidelines for implementing the **precautionary principle**,* (5) developing guidelines for anthropogenic wastes that contribute to ecosystem health, and (6) responding to environmental changes with prompt remedial ecological restoration measures when evidence indicates that an important threshold has been crossed.

* The cautious policy or position that, even in the absence of any clear evidence and in the presence of high scientific uncertainty, action should be taken if there is any reason to think that harm might be caused.

NATURAL CAPITAL AND INDUSTRIAL ECOLOGY

Natural capital consists of resources, living systems, and ecosystem services. Natural capitalism envisions the use of natural systems without abusing them, which is essential to sustainable use of the planet. Sustainable use of the planet requires a mutualistic relationship between human society and natural systems, and affirms that a close relationship exists between ecosystem health and human health. Natural capitalism deals with the critical relationship between natural capital—natural resources, living systems, and the ecosystem services they provide—and human-made capital (Hawken et al. 1999).

Industrial ecology is the study of the flows of materials and energy in the industrial environment, and the effects of these flows on natural systems (White, 1994; Graedel and Allenby, 1995). The essential idea of industrial ecology is the coexistence of industrial and natural ecosystems. Properly managed, industrial ecology would enhance the protection and accumulation of natural capital in areas now ecologically degraded or at greater risk than necessary. However, the most attractive feature of industrial ecology may be that it would involve temporal and spatial scales greater than those possible with even the most elaborate microcosms or mesocosms. To optimize the quality and quantity of information generated by these hybrid systems, some carefully planned risks must be taken, and regulatory agencies must be sufficiently flexible to permit them. Because some ecologic damage is inevitable under these circumstances, ecotoxicologists must be knowledgeable of the practices commonly used in ecological restoration. Even industrial accidents can be a valuable source of ecotoxicological information if they are immediately studied by qualified personnel and the information widely shared. Regulatory and industrial flexibility in assessing experimental remedial measures would also enhance the quality of the information base. The obstacles to achieving this new relationship between industry and regulatory agencies boggle the mind, yet there seems to be no comparable, cost–effective means of acquiring the needed ecotoxicological information over such large temporal and spatial scales. Convincing the general public and its representatives of the values inherent in this approach will be a monumental task, but the consequences of making ecotoxicological decisions with inadequate information are appalling.

SPECULATIONS

Failure to react constructively to unsustainable practices does not always lie in not knowing what is wrong or even in not knowing what to do about it but, rather, in the failure to take this knowledge seriously enough to act on it. Thus, ecotoxicologists have a responsibility to raise public literacy about their field to ensure that the information they generate is taken seriously and used effectively. Ecotoxicologists and other environmental professionals must be aware that their data, predictions, estimates, and knowledge will be used in a societal context that is embedded in an environmental ethos (or set of guiding beliefs). Because science is often idealized as independent of an ethos, there is some level of tension between the essentially scientific task of estimating risk and uncertainty, and the value-laden task of deciding what level and type of risks are acceptable. If sustainable use of the planet becomes a major goal for human society, it is difficult to visualize how the mixture of science and value judgment can be avoided. One hopes that the process of science, with its priceless quality control component, can remain intact for data generation and analysis while producing better and more relevant information to be used with intelligence and reason in making value judgments. However, sustainable use of the planet will require a major shift in present human values and practices. At the same time, ecotoxicology will be evolving, possibly rapidly, so that keeping system and order in the mixture will be difficult.

Although some of the trends in ecotoxicology briefly described here seem probably inevitable, the direction of the field will almost certainly be determined by environmental surprises, which are ubiquitous and unlikely to occur in convenient places at convenient times. Worse yet, the temptation will be strong to study them entirely with existing methodology, which will probably be inadequate.

A multidimensional research strategy is needed that emphasizes ecosystem complexity, dynamics, resilience, and interconnectedness, to mention a few important attributes. However, major obstacles exist to the development of such a program in the educational system, governmental agencies and industry, and with a citizenry increasingly suspicious of science and academe in general. Society depends primarily on its major universities for the generation of new knowledge; however, this function has become a commodity produced for sale, which means the research direction is all too often a function of marketability. An unfortunate consequence is that writing grant proposals (now often termed contracts) consumes an ever-increasing proportion of the time of ecotoxicologists and other environmental professionals. Ecotoxicologists may often postpone visionary, long-term projects whose outcomes are highly uncertain for short-term projects of severely limited scope determined by the perceived needs of the funding organization rather than being truly exploratory undertakings.

Some counter trends exist to these discouraging developments, often occurring outside of "mainstream" science. A number of new journals are challenging the fragmentation of knowledge, and publications are espousing the consilience ("leaping together") of knowledge. Increasingly, within academe, the focus is on issues that transcend the capabilities of a single discipline or even a few disciplines. In addition, environmental professionals, such as ecotoxicologists, are finding ways to minimize the effects of budgetary constraints. However, it still seems likely that one or more major environmental catastrophes will be needed to persuade decision makers that a major shift in approach is needed to cope more effectively with the ecotoxicological and other uncertainties, which human society now faces and are likely to increase substantially in the future.

Much evidence is also available on other major issues affecting ecotoxicology, including the following:

1. Climate change, including global warming: Rainfall patterns are changing and mountain snow, the source of water for many rivers, is decreasing. Markedly reduced flow in major rivers means far less dilution of the wastes discharged into them. Major floods displace contaminated sediments and probably the ecological partitioning of the chemicals associated with them. Unstable climate conditions will weaken many species, making them more vulnerable to toxic stress. Drought-stressed terrestrial plants and animals will also often be more sensitive to toxicants. Finally, because ecosystems are structured to optimize energy and nutrient flow, loss of keystone species will alter both ecosystem function and structure. All these factors must be carefully considered when designing and interpreting ecotoxicological tests at different levels of biological organization.
2. Peak oil and "tough oil" (e.g., tar sands): Once oil supplies have peaked globally, petroleum availability will rapidly decline. Efforts will be made to get the remaining oil from fields that have had declined output. However, the major challenge will be to study the ecotoxicological effects of the attempts to convert the tar sands into some type of fuel. The Athabasca site will furnish much useful information, but as usual, each site will be ecologically unique in some characteristics, and, as a consequence, each will require somewhat different ecotoxicological methods and procedures. Validation of the ecotoxicological predictions will also require site-specific modifications.

3. Ecological overshoot: **Ecological overshoot** means using resources faster than they can be regenerated. August 22 was Earth Overshoot Day for 2012, marking the date when humanity had exhausted nature's budget for the year (Global Footprint Network, www.footprintnetwork.org). The most obvious solution to the ecological overshoot is to curtail resource extraction and use. However, with global human population increasing by 230,970 persons per day (2012 World Population Data Sheet, Population Reference Bureau), plus adverse economic impacts, this approach is unlikely. The most obvious short-term solution is to restore or rehabilitate damaged ecosystems. Because toxic substances were probably involved in many such situations, ecotoxicological tests will be essential to determine when the site will be suitable for recolonization.

ACKNOWLEDGMENTS

I am indebted to Darla Donald for editorial assistance meeting the requirements of the publisher.

Recognition of our increasing influence on the biosphere since the late eighteenth century has led a growing group of scientists to the weighty proposition that the Holocene epoch has ended and a new epoch begun. Their argument begins by restating that we divide the history of our planet into periods based on profound geological, atmospheric, biological, or climate changes during its evolution. For instance, the present Holocene epoch is agreed to have started as the last ice age ended. The argument continues by pointing out that several human-induced changes have taken place that are similar in impact to many used in the past to distinguish among geological epochs. Steffen et al. (2011) identify four such changes: (1) climate change; (2) significant alteration of elemental cycles such as those of nitrogen,* phosphorus, and sulfur; (3) substantial modification of the terrestrial water cycle; and (4) precipitation of the sixth mass extinction event in the history of life. The name, **Anthropocene**, was proposed for this new epoch by E. F. Stoermer of the University of Michigan, and since then, has been advocated by Nobel Laureate, Paul Crutzen,† in many publications, e.g., Crutzen (2002), Rockström et al. (2009a,b), Steffen et al. (2011), and Zalasiewicz et al. (2010). They propose the late eighteenth century as the beginning of this epoch dominated by human activity (Crutzen, 2002; Zalasiewicz et al. 2010). Suggestive of the value of accepting that a new epoch has emerged, the Anthropocene context is already being used to gauge our impact on the oceans (e.g., Vidas, 2011; Tyrrell, 2011), climate (Kellie-Smith and Cox, 2011), and land resources (Ellis, 2012).

One important theme coming out of initial assessments of the Anthropocene is the comparison of current use of resources to the estimated planetary boundary values. As long as humanity does not exceed these planetary boundary limits, it will be unencumbered in its pursuit of social and economic evolution progress (Figure 1.11). Currently, our activities have exceeded the values for climate change, nitrogen cycling, and species loss. It is disconcerting to read these analyses and realize that boundary values are not yet established for chemical pollutants. How can ecotoxicologists "[respond] to environmental changes with prompt remedial ecological restoration … when an important threshold has been crossed" as John Cairns advocates in Vignette 1.1 if we have no idea if an important threshold has been crossed yet? We remain uncertain whether we are or are not exceeding the biosphere's capacity to cope with our chemical wastes.

* For example, Law (2013) describes recent findings that atmospheric deposition of nitrogen from agricultural fertilizers use has increased so much that its influence can be seen on global evergreen forests.

† Crutzen received the 1995 Nobel Prize in Chemistry with Mario Molina and F. Sherwood Rowland based on their work on atmospheric ozone chemistry and the impact of anthropogenic releases on the formation of the ozone hole.

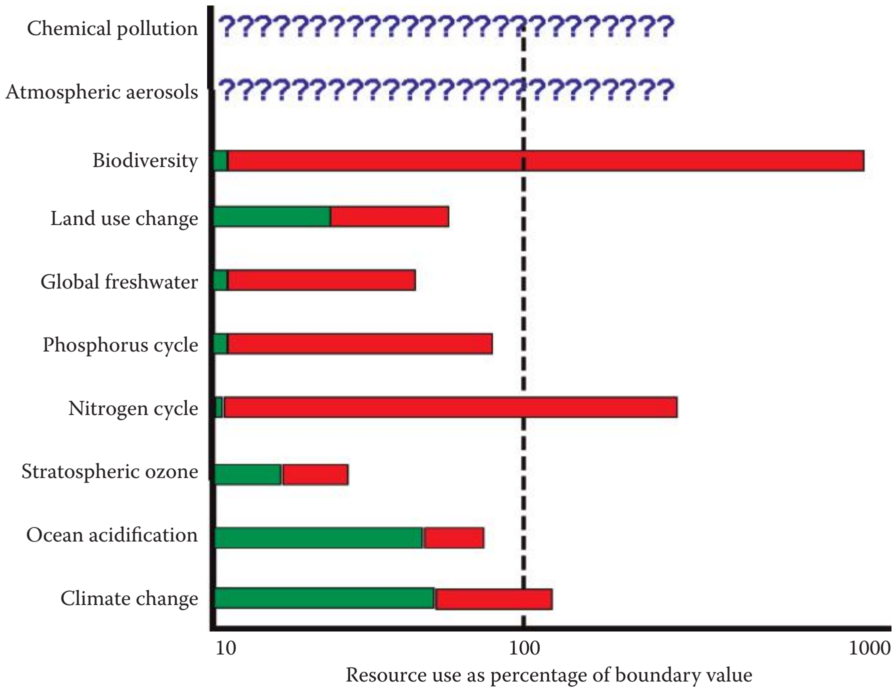

FIGURE 1.11 **Planetary boundary values** and estimates of preindustrial (green) and current values (red). Information extracted from Table S2 of Rockström et al. (2009b). According to these authors, humanity "has the freedom to pursue long-term social and economic development" only if these boundary values are not exceeded. Climate change units: carbon dioxide concentration; Ocean acidification: aragonite saturation ratio; Stratospheric ozone: ozone expressed in Dobson unit; Nitrogen cycle: Metric tonnes removed from the atmosphere for human use annually; Phosphorus cycle: Metric tonnes of flow into oceans annually; Global freshwater use: consumptive use of runoff as km^3 annually; Land use change: percentage of land cover converted to cropland; Biodiversity: number of species lost per million species annually.

1.3 ECOTOXICOLOGY

The subject of this book could have been labeled environmental toxicology or ecotoxicology because the definitions of both terms are rapidly converging. Often, use of the term, environmental toxicology, implies that only effect of environmental contaminants to humans will be discussed. Such an implication would be inappropriate for this book. Some definitions of ecotoxicology seem to exclude discussion of humans except as the source of contaminants (Table 1.1), but the original definition given to ecotoxicology by Truhaut (1977) includes effects to humans. Truhaut's rationale, as paraphrased by Bacci (1996), is quite sound: "human health cannot be protected unless in conjunction with wildlife protection…." Ecotoxicology was selected here with the intention of emphasizing effects to ecological entities without ignoring complementary knowledge associated with effects to humans. Ecotoxicology was chosen after some introspection because a strong and confining conventional ecosystem-or-lower emphasis is implied in many of its definitions. For example, the textbook by Connell (1990) specifies that the scope of ecotoxicology includes "organisms, populations, communities, and ecosystems" (Table 1.1). More and more, this conventional individual → population → community → discrete ecosystem context is necessarily being extended to metacommunity → ecosystem → landscape → ecoregion → continent → hemisphere → biosphere. There is a simple reason of this: the conventional framework has gradually grown insufficient to encompass all germane

Table 1.1 Definitions of Ecotoxicology and Environmental Toxicology

Definition	Reference
Environmental toxicology	
1. The study of the effects of toxic substances occurring in both natural and manmade environments	Duffus (1980)
2. The study of the impacts of pollutants upon the structure and function of ecological systems (from molecular to ecosystem)	Landis and Yu (1995)
Ecotoxicology	
1. The branch of toxicology concerned with the study of toxic effects, caused by natural and synthetic pollutants, to the constituents of ecosystems, animals (including human), vegetable and microbial, in an integrated context	Truhaut (1977)
2. The natural extension from toxicology, the science of poisons on individual organisms, to the ecological effects of pollutants	Moriarty (1983)
3. The science that seeks to predict the impacts of chemicals upon ecosystems	Levin et al. (1989)
4. The study of the fate and effect of toxic agents in ecosystems	Cairns and Mount (1990)
5. The science of toxic substances in the environment and their impact on living organisms	Jørgensen (1990)
6. The study of toxic effects on nonhuman organisms, populations and communities	Suter (1993)
7. The study of the fate and effect of a toxic compound on an ecosystem	Shane (1994)
8. The field of study, which integrates the ecological and toxicological effects of chemical pollutants on populations, communities and ecosystems with the fate (transport, transformation and breakdown) of such pollutants in the environment	Forbes and Forbes (1994)
9. The science of predicting effects of potentially toxic agents on natural Hoffman et al. (1995) ecosystems and nontarget species	Hoffman et al. (1995)
10. The study of the pathways of exposure, uptake and effects of chemical agents on organisms, populations, communities, and ecosystems	Connell (1990)
11. The study of harmful effects of chemicals on ecosystems; the harmful effects of chemicals (toxicology) within the context of ecology	Walker et al. (2001)
12. The study of harmful effects of chemicals upon ecosystems and includes effects on individuals and consequent effects at the levels of population and above	Walker et al. (2012)

subjects. As applied here, **ecotoxicology** is the science of contaminants in the biosphere and their effects on constituents of the biosphere, including humans.

1.4 ECOTOXICOLOGY: A SYNTHETIC SCIENCE

1.4.1 Introduction

Ecotoxicology is a synthetic science in that it draws together insights and methods from many disciplines (Figure 1.12). Questions about effect are posed at the molecular (e.g., enzyme inactivation by a contaminant), to the population (e.g., local extinction), and to the biosphere (e.g., global warming) levels of biological organization. Questions of fate and transport are addressed from the chemical (e.g., dissolved metal speciation), to the habitat (e.g., contaminant accumulation in depositional habitats), and to the biosphere (e.g., global distillation of volatile pesticides) levels of physical scale. Sometimes, this can produce a confusing complex of scales and associated specialties. The key to maintaining conceptual coherency in this complex of interwoven and hierarchical topics was articulated by Caswell (1996), "… processes at one level take their mechanisms from the level below and find their consequences at the level above.… Recognizing this principle makes it clear that there are no truly 'fundamental' explanations, and make it possible to move smoothly up and down the levels of the hierarchical system without falling into the traps of naive reductionism or pseudo-scientific holism." Understanding fates and effects at all levels is essential for effective environmental stewardship (Newman, 1996). Equally important is the integration of our collective understanding of fate and effects at all levels into a coherent and self-consistent body of knowledge (Newman and Clements, 2008).

Although all levels are equally important, they contribute differently to our efforts and understanding (Figure 1.13). Questions dealing with lower levels of the conceptual hierarchy, such as, biochemical effects, tend to be more tractable and have more potential for easy linkage to a specific cause than do effects at higher levels such as the biosphere. Changes in δ-aminolevulinic acid dehydratase (ALAD) activity in red blood cells can be assayed inexpensively and quickly linked to lead exposure of humans or wildlife. The general loss of fish species from Canadian lakes was much more challenging to document and to link to sulfur and nitrogen oxide emissions from a distant city that produced acid precipitation. As a consequence, effects at lower levels of the ecological hierarchy are used more readily in a proactive manner than are those at higher levels. They can indicate the potential for emergence of an adverse ecotoxicological effect, whereas effects at higher levels are useful in documenting or prompting a regulatory reaction to an existing problem. Although highly tractable and sensitive, the ecological relevance of effects at lower levels is much more ambiguous than effects at higher levels of organization. Most reasonable biologists would agree that a 50% reduction in species richness is a clear indication of diminished health of an ecological community. But a 50% increase in metallothionein in adults of an indicator species provides an equivocal indication of the health of species populations contributing to the associated community. Relative

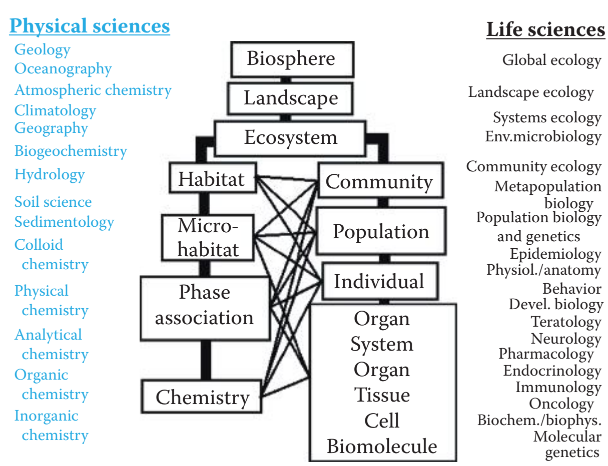

Figure 1.12 Hierarchical organization of topics addressed by ecotoxicology. Disciplines contributing to understanding abiotic interactions are listed on the left side of the diagram and those contributing to understanding biotic interactions are listed on the right. Important interactions, denoted by lines connecting components, occur between biotic and abiotic components.

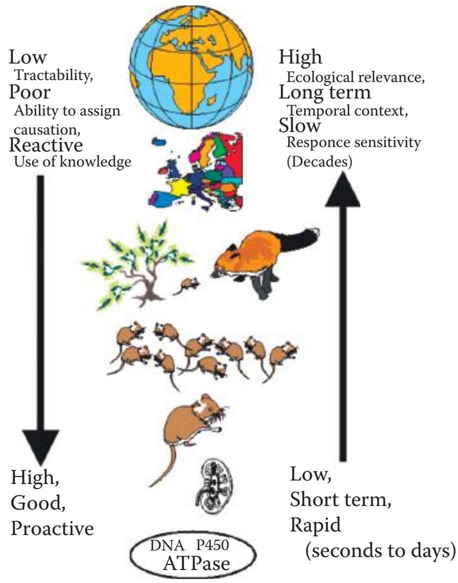

Figure 1.13 Hierarchical organization of topics in ecotoxicology relative to ecological relevance, general tractability, ability to assign causation, general use of knowledge, temporal context of consequence, and temporal sensitivity of response.

to those at higher levels of biological organization, effects at lower levels tend to occur more rapidly after the stressor appears and to disappear more quickly after it is removed. Considering all of these points, it is clear that information from all relevant levels of biological organization should be used together as described by Newman (2001, 2013) and Newman and Clements (2008).

1.4.2 Science, Technology, and Practice

There is a general lack of unanimity in ecotoxicology that leads to much confusion: there seems often to be no single voice indicating how one should conduct oneself. The main objective of this section is to describe how the outwardly inconsistent activities of ecotoxicologists come together to address three intermeshing and equally important goals.

Ecotoxicology has diverse goals (scientific, technological, and practical) in addition to diverse contributing disciplines (Slobodkin and Dykhuizen, 1991; Newman, 1995). The diversity of subjects prompts most ecotoxicologists to specialize in particular areas and to look only peripherally at other information. Subsets of ecotoxicologists must block out of consideration major portions of our collective knowledge structure to move effectively toward their respective and more focused goals. For example, distinct, but overlapping, subsets of information and methodologies are used by the scientist, analyst, and regulator. Whereas a scientist might relay on the conventional hypothetico-deductive method during laboratory experimentation, this approach would be an impediment to a regulator who might use a weight-of-evidence or expert opinion approach instead to expeditiously assess the need for remediation at a particular site despite high uncertainty. The use of a standard method with a suboptimal detection limit might hinder achievement of a scientific goal such as accurately quantifying rates at which a microcontaminant moves between ecosystem components, yet be a necessary compromise for achieving the regulatory goal of generating a consistent water quality database from which decisions and actions can be derived.

Slobodkin and Dykhuizen (1991) observed that much confusion is generated if the distinct intentions and approaches are not understood and respected by the professionals in any applied science. Difficulties arise if methods of one ecotoxicologist are judged unacceptable by another without recognition of differing goals. Because the goals of ecotoxicologists are tightly intermeshed and boundaries are not easily drawn, there is sometimes the appearance of inconsistency or confusion in the field. Again, the purpose of this section is to dispel some of this apparent inconsistency by delineating these three goals or sets of intentions (scientific, technological, and practical), and generally describing the means by which they are pursued. One extremely important aspect of practical ecotoxicology, environmental law, cannot be discussed adequately in the available space. As a partial remedy that provides a general impression of existing legislation in different countries, short appendices covering key Australian, Central American, Chinese, Indian, European Union, South African, and U.S. laws and regulations are offered to the reader by guest authors.

1.4.2.1 Scientific Goal

The goal of any science is to organize knowledge based on explanatory principles (Nagel, 1961). It follows that the scientific goal of ecotoxicology is to organize knowledge, based on explanatory principles, about contaminants* in the biosphere and their effects. The approaches used to reach this scientific goal are well established. But they are worth reviewing because they are taught informally and consequently, many unthoughtful opinions exist regarding the conduct of science. The discussion below is condensed from Newman (1995, 1996, 2001) who reviewed the works of Sir Karl Popper, and extraordinary articles by Platt (1964) and Chamberlin (1897) from the context of ecotoxicology. It is modified at the end by extending these features based on materials in Newman and Clements (2008) and Newman (2013).

In the early history of science, untested or weakly tested theories were used to explain specific phenomena (Chamberlin, 1897). A question was presented to an acknowledged expert and explanation given based on some prevailing or ruling theory. This was enough to fit the phenomena or observation into the existing knowledge structure. Facts gradually accumulate around the ruling theory, fostering a sense of consistency that enhances belief. Such uncritical acceptance of an explanation based on a ruling theory (**precipitate explanation**) is considered unacceptable in modern science. In fact, the first of Descartes' four rules of proper scientific reasoning is the following:

> *… never to accept anything as true that I did not know to be evidently so: that is to say, carefully to avoid precipitancy and prejudice.*
>
> **Descartes (1637)** (Translated by Sutcliffe [1968])

Credibility based solely on conventional wisdom is no longer an acceptable approach to science. The foundation premise of science is now *nullius addictus judicare in verba magestri*, that is, "inquiry is not obliged to the word of any authority." Unfortunately, precipitate explanation can reappear periodically in scientific disciplines, especially applied sciences such as ecotoxicology. Consequently, it is important to recognize precipitate explanation in any field and avoid it in your own work.

Modern sciences have replaced the ruling theory with the working hypothesis. The **working hypothesis** is never accepted as true and only serves to enhance the development of facts and their relations by functioning as the focus of the falsification process (Chamberlin, 1897). One decides to conditionally accept the working hypothesis based on current evidence. Experiments and less-structured experiences are used to test the working hypothesis. The falsification process

* Terms such as pollutant, contaminant, xenobiotic, and stressor have specific and distinct connotations. A **pollutant** is "a substance that occurs in the environment at least in part as a result of man's activities, and which has a deleterious effect on living organisms" (Moriarty, 1983). A **contaminant** is "a substance released by man's activities" (Moriarty, 1983). There is no implied adverse effect for a contaminant although one might exist. A **xenobiotic** is "a foreign chemical or material not produced in nature and not normally considered a constitutive component of a specified biological system. [It is] usually applied to manufactured chemicals" (Rand and Petrocelli, 1985). A **stressor** is that which produces a stress. **Stress** "at any level of ecological organization is a response to or effect of a recent, disorganizing or detrimental factor" (Newman, 1995). As will be discussed, the terms have slightly different legal definitions too.

is often conducted using the null hypothesis–based statistics developed by Ronald A. Fisher in the 1920s for the Popperian falsification of hypotheses. The working hypothesis approach still has a proclivity toward precipitate explanation because a central theory or hypothesis tends to be given favored status during testing. Chamberlin (1897) suggested application of the **method of multiple working hypotheses** to reduce this tendency. The method of multiple working hypotheses reduces precipitate explanation and subjectivity by considering all plausible hypotheses simultaneously so that equal amounts of effort and attention are provided to each. In fields where multiple causes or interactions are common, it also lessens the tendency to stop after "the cause" has been discovered.

In any modern science, a hypothesis is never assumed to be true regardless of the approach used. But it can gain enhanced status after repeated survival of rigorous testing. Status is not legitimately enhanced unless tests also have high powers to falsify. Unfortunately, consistent application of weak testing can lead to the progressive dominance of an idea by repetition alone. Weak testing is occasionally used to promote an idea or approach; consequently, members of any science such as ecotoxicology must be able to recognize false paradigms that emerge from weak testing and to avoid weak testing in their own work. Further, tests involving imprecise or biased measurement should be avoided because they frequently generate false conclusions and foster confusion.

Gradually, observational and experimental methods produce a framework of explanatory principles or paradigms about which facts are organized. These **paradigms** (generally accepted concepts in a healthy science that withstood rigorous testing and, as a result, hold enhanced status as causal explanations) are learned by members of a discipline and define the major context for inquiry in the field. They act as nuclei around which ancillary concepts are formulated and as a framework for further testing and enrichment of fact. Unlike ruling theories, these paradigms remain subject to future scrutiny, revision, rejection, or replacement. They are explanations that are currently believed to be the most accurate and useful reflections of truth, but they are not absolute truths. This is an important distinction to keep in mind. For example, the paradigm of matter conservation (matter cannot be created nor destroyed) was an adequate explanation of phenomena until Einstein demonstrated its conditional nature. It was then incorporated into a more inclusive paradigm (relativity theory) with the qualification that relativistic mass (mass + mass equivalent of energy) is constant in the universe, but mass may be converted to energy and vice versa ($\Delta E = \Delta m (c^2)$). In a field with mixed goals such as ecotoxicology, the conditional status of scientific explanations or paradigms is sometimes forgotten.

Two general and interdependent types of behavior occur in any science: normal and innovative science (Kuhn, 1970). **Normal science** works within the framework of established paradigms, and increases the amount and accuracy of our knowledge within that framework. The contribution of normal science is the incremental enhancement of facts and articulation of ideas with which paradigms can be reaffirmed, revised, or replaced by new paradigms (Kuhn, 1970). Most scientific effort is normal science and the collective work of ecotoxicologists is no exception. In contrast, **innovative science** questions existing paradigms and formulates new paradigms. Innovative science is completely dependent on normal science and can only occur after the incremental enrichment of knowledge brought about by normal science has uncovered inconsistencies between facts and an established paradigm. The opposite is also true. Normal science without enough innovative science results in stagnation and dubious inferences.

Although normal science tends to be more important in a young field, an excessive preoccupation with details ("tyranny of the particular" [Medawar, 1967]) or measurement (*idola quantitatus* [Medawar, 1982]) can slow the maturation and progress of a science. Conversely, insistence on rigorous hypothesis testing before the accumulation of enough facts and establishment of accurate measurement techniques can lead to premature rejection of a hypothesis that might otherwise be accepted. Both normal and innovative science must be balanced in a healthy science. In ecotoxicology, many areas still require more normal science before innovative science can be applied effectively. In many other areas of this maturing science, the tyranny of the particular and *idola* *quantitatus* exist at the expense of much needed innovative science (Newman, 1996). A balance between normal and innovative science is essential to effectively achieve the scientific goal of ecotoxicology. The long-term benefit of such a healthy balance will be optimal efficiency and effectiveness in environmental stewardship.

1.4.2.2 Technological Goal

The technical goal of ecotoxicology is to develop and then apply tools and methods to acquire a better understanding of contaminant fate and effects in the biosphere. Often, some activities in technology are indistinguishable from normal science; however, their goals are distinct. Relative to plainly scientific endeavors, the benefits to society of technology are more immediate but slightly less global. Although analytical instrumentation is an obvious component of ecotoxicological technology, other components include standard procedures and approaches, and computational methods. Many of these technologies can also become pertinent to the practical goals of ecotoxicology *when used to address specific problems*. Consequently, the distinction between technology and practice is also based on context.

The development of analytical instrumentation able to detect and quantify low concentrations of contaminants in complex environmental matrices has been essential to the growth of knowledge. For example, the number of commercial atomic absorption spectrophotometers (AAS) increased exponentially in the 1950s and 1960s, making possible the rapid measurement of trace element contamination in diverse environmental materials (Price, 1972). Flameless AAS methods lowered detection limits for most elements and enhanced analytical capabilities even further. Now, a wide range of atomic emission, atomic absorption, atomic fluorescence, and mass spectrometric techniques are available for the study of elemental contaminants at levels ranging from $mg{\cdot}g^{-1}$ to $pg{\cdot}kg^{-1}$ concentrations. Gas chromatography (GC) techniques allowed study of the more volatile organic contaminants. Techniques including GC coupled with a mass spectrometer (GC–MS), more effective columns for separation, and improved detectors have all enhanced our understanding of fate and effects of organic contaminants. For organic compounds less amenable to GC-related techniques, innovations such as advanced separation columns and high-pressure pumps have quickly improved high-pressure liquid chromatographic (HPLC) methods. Overarching all of these advances have been computer-enhanced sample processing, analytical control, and signal processing. These, and a myriad of instrumental techniques, have appeared in the last few decades and allowed rapid advancement of scientific ecotoxicology.

Again, procedures and protocols are also important components of environmental technology. Pertinent procedures vary widely. As an example, they may include such activities as the mapping of **ecoregions**—relatively homogeneous regions in ecosystems or associations between biota and their environment—as a practical means for defining sensitivity of U.S. waters and lands to contaminants (Omernik, 1987; Hughes and Larsen, 1988). These naturally similar regions of the country are grouped for development of a common study or management strategy. Another important example is a crucial technology created through seminal papers such as the series defining the generation and analysis of aquatic toxicity data (e.g., Sprague, 1969, 1970; Buikema et al. 1982; Cherry and Cairns, 1982; Herricks and Cairns, 1982). The establishment of a procedural paradigm for ecological risk assessment (e.g., Environmental Protection Agency [EPA], 1991a) constitutes a technological advance as well as a contribution to ecotoxicology's practical goals. General methods for **biomonitoring** (use of organisms to monitor contamination and to imply possible effects to biota or sources of toxicants to humans) (e.g., Goldberg, 1986; Phillips, 1977) and applying **biomarkers** (cellular, tissue, body fluid, physiological, or biochemical changes in extant individuals that are used quantitatively during biomonitoring to imply presence of significant pollutants or as early warning systems for imminent effects) (e.g., McCarthy and Shugart, 1990) are also important technologies developed in the last several decades. Most biomonitoring programs are only possible now because of the advances in analytical instrumentation described above.

Experimental design schemes, statistical methods, and computer technologies are also important here. Valuable descriptions of experimental designs and statistical methods are provided by professional organizations (e.g., American Public Health Association, 1981) and government agencies (e.g., EPA 1985a, 1988a, 1989a,b), facilitating effective data acquisition to enhance our understanding of contaminant fate and effects. These often have easily implemented computer programs associated with them (e.g., EPA, 1985a, 1988a, 1989b). Other computer programs have been developed by EPA to enhance scientific progress. An example is the MINTEQA2 program (EPA, 1991b), which predicts speciation and phase association of inorganic toxicants such as transition metals. Numerous programs for statistical analysis of toxic effects data are available from EPA (e.g., EPA, 1985a, 1988a, 1989b) and commercial sources. Geographic information system (GIS) technologies discussed in later chapters have been developed to study nonpoint source contamination over large areas such as watersheds (e.g., Adamus and Bergman, 1995).

Some technology-related approaches are difficult to understand if an inappropriate context is forced upon them. Some are focused primarily on supporting scientific goals while others are designed to support the practical goals of ecotoxicology. Unfortunately, the complex blending of goals in ecotoxicology makes this a common source of confusion. The designed use of any technology must be kept clearly in mind to avoid confusion and generation of misinformation. For example, standard or operational definitions (e.g., acute vs. chronic effect, sublethal vs. lethal exposure) may have dubious scientific value relative to predicting the actual impact of toxicants in an ecological context. The 96-hour duration of the conventional acute toxicity test was selected because it fits conveniently into the workweek, not because it has any particular scientific underpinnings. The operational distinction made between acute and chronic exposures is also partially arbitrary. An acute "sublethal" exposure could eventually produce a fatal cancer. Regardless, when appropriately and thoughtfully applied, such standard definitions and associated tests are invaluable in applying our technology to various scientific subjects, such as to determining if the free metal ion is the most toxic species of a metal by using standard acute toxicity endpoints.

Qualities valued in technologies are effectiveness (including cost effectiveness), precision, accuracy, appropriate sensitivity, consistency, clarity of outcome, and ease of application. As discussed below, several of these qualities are also important in practical ecotoxicology.

1.4.2.3 Practical Goal

The practice of ecotoxicology has as its goal the application of available scientific knowledge and technologies to document or solve specific problems. During the process, some scientific knowledge will be marginalized to expeditiously resolve the problem at hand. What might seem from a rigid scientific vantage to be an incomplete depiction of reality is, in fact, the most expeditious means of defining and resolving the problem. Many technologies are relevant to practical ecotoxicology; however, the goal of their application is also to solve or document a particular environmental situation. Techniques appropriate for the practical ecotoxicologist may be general such as methods for determining contaminant leaching from wastes (e.g., Anonymous, 1990) or the application of microarray techniques to infer the cause of a particular adverse effect. Predictive software such as the QUAL2E program (EPA, 1987a), which estimates stream water quality under specific discharge scenarios, may also be important tools in achieving practical goals. Other tools include specific steps to take during implementation of a method, e.g., biomarker-based biomonitoring on U.S. Department of Energy sites (McCarthy et al. 1991). They might involve guidelines for the practice of risk assessment on hazardous waste sites (EPA, 1989c) or for waste basin closure. In each of these instances, the goal is not to understand the ecotoxicological phenomena more completely or to develop a technology to better study a system. The goal is to address and resolve a specific problem. Indeed, attempts to conduct scientific work in such efforts or expend resources only to develop a novel technology could delay progress toward the practical goal—solving the immediate problem and removing potentially harmful pollutants.

Practical tools may also include criteria and standards for regulation of specific discharges or water bodies. For example, water quality **criteria** are estimated concentrations of toxicants based on current scientific knowledge that, if not exceeded, are considered protective for organisms or a defined use of a water body (or some other environmental media). Criteria are developed for individual contaminants, e.g., aluminum (EPA, 1988b), cadmium (EPA, 1985b), copper (EPA, 1985c), lead (EPA, 1985d), and zinc (EPA, 1985d) using a standard approach (i.e., EPA, 1985e). On the basis of scientific knowledge, they are used to recommend toxicant concentrations not to be exceeded as a result of discharges into waters. Although the example here is that for water, criteria are also defined for other media such as air (see U.S. Clean Air Act in the Appendix) and sediments (Shea, 1988; Di Toro et al. 1991).

On the basis of the criteria and the specified use of a water body, water quality standards may be set for contaminants. **Standards** are legal limits permitted by each U.S. state for a specific water body and thought to be sufficient to protect that water body. Both criteria and standards are designed with the intent to avoid specific problems, but they are based partially on existing scientific knowledge. Consequently, a healthy growth of scientific knowledge in ecotoxicology is essential to improving our progress toward the practical goals of ecotoxicology. Indeed, criteria and standards (EPA, 1983) are revised periodically to accommodate new knowledge.

Effectiveness, precision, accuracy, sensitivity, consistency, clarity, and ease of application are valued in practical ecotoxicology as well as in technical ecotoxicology as discussed above. Also important to practical ecotoxicology are the following: unambiguous results, safety, and clear documentation of progress during application.

1.5 SUMMARY

At the close of the World War II, the dilution paradigm failed with clearly unacceptable consequences to human health and ecological integrity. The influence of humans on the biosphere has grown so extensive that scientists now propose that we have entered into a new geological epoch, the Anthropocene. Expertise in ecotoxicology is now critical to our well-being in the Anthropocene. Ecotoxicology, the science of contaminants in the biosphere and their effects on constituents of the biosphere including humans, has emerged to provide such expertise.

Ecotoxicologists have overlapping yet distinct scientific, technological, and practical goals that must be understood and respected. Our current knowledge available for achieving these goals (Figure 1.14, upper panel) requires further expansion and more integration (Newman and Clements, 2008). Although the knowledge applied to these goals overlaps, there remain many instances of inappropriate or inadequate integration. For example, present regulations remain biased toward single species tests done in the laboratory, yet our scientific knowledge clearly indicates that results from multiple species tests are at least as valuable to understanding risk. Recognizing the continual need for reintegration of knowledge, lawmakers have wisely incorporated periodic review and revision into major legislation and associated regulations. Further, new or improved technologies are continually drawn into our scientific efforts, e.g., new molecular technologies applied to assay genetic damage and identify causes of adverse effects. The scientific foundations of the field should also expand and come into balance with technology and practice. This is done most effectively using the methods described in this chapter and explained more fully by Newman and Clements (2008) and Newman (2013). Passé and flawed behaviors such as precipitate explanation, overdominance of normal science, the tyranny of the particular, and *idola quantitatus* should be avoided as impediments to scientific progress. An understanding and respect for the different goals of ecotoxicologists must also prevail to appropriately apply science and technology to practical problems of environmental stewardship.

Ecotoxicology is rapidly becoming a mature discipline and, hopefully, will soon achieve an effective balance of knowledge to best address its scientific, technical, and practical goals (Figure 1.14, lower panel). Scientific understanding must expand in all directions, especially toward

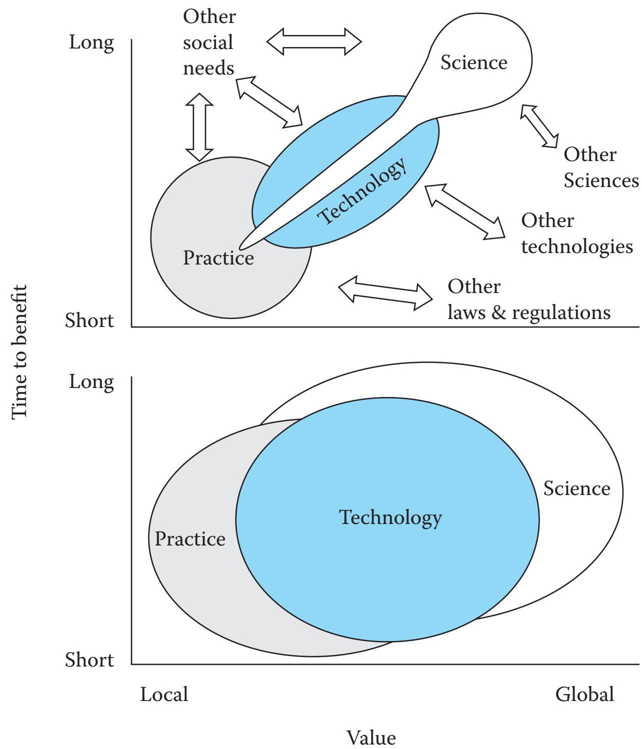

Figure 1.14 Present (top panel) and ideal (bottom panel) balance among scientific, technological, and practical components of ecotoxicology. The relative amount of effort in each is reflected by area on the plots of scale of value (local to global) and time to realize (short to long term) benefit from these components of the field.

more global and long-term phenomena. Technology and practice must do the same. The sound laws presently implemented in most countries should, or are rapidly evolving, to accommodate the continual improvements to technology and science.

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Fourth Edition

PRINCIPLES OF ECOTOXICOLOGY

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Introduction

The term *ecotoxicology* was introduced by Truhaut in 1969 and was derived from the words *ecology* and *toxicology.* The introduction of the new term reflected a growing concern about the effects of environmental chemicals on species other than humans. It identified an area of study concerned with the harmful effects of chemicals (toxicology) within the context of ecology. Until now environmental toxicology focused mainly on the harmful effects of environmental chemicals on humans, e.g., the effects of smoke on urban communities. However, environmental toxicology in its widest sense encompasses the effects of chemicals on ecosystems as well. Thus ecotoxicology is a discipline within the wider field of environmental toxicology (see Calow, 1994). In the present text, it is defined as the study of harmful effects of chemicals upon ecosystems and includes effects on individuals and consequent effects at the levels of population and above.

Despite this definition, much early work described as ecotoxicology related little to ecology or toxicology. It was concerned with the detection and determination of chemicals in samples of animals and plants. Seldom could the analytical results be related to effects on individual organisms, let alone effects on populations or communities. Analytical techniques such as gas chromatography, thin layer chromatography, and atomic absorption facilitated the detection of very low concentrations of chemicals in biota. Establishing the biological significance of the results was a more difficult matter! One of the main themes of this text is the problem of progressing from the measurement of concentrations of environmental chemicals to establishing their effects at the levels of the individual, the population, and the community.

New disciplines frequently present problems of terminology, and ecotoxicology is no exception. Several important ecotoxicology terms are used inconsistently in the literature. Their use in the present text will now be explained. Both pollutants and environmental contaminants are chemicals that exist at levels judged to be above those that would normally occur in any component of the environment. This immediately raises the question of what is to be considered normal. For most man-made organic chemicals such as pesticides, the situation is simple—any detectable level is abnormal because the compounds did not exist in the environment until humans released them. Conversely, chemicals such as metals, sulfur dioxide, nitrogen oxides, polycyclic aromatic hydrocarbons (PAHs), and methyl mercury occur naturally and existed in the environment before humans appeared. The variations in concentrations of these chemicals from place to place and from time to time make it difficult to judge their normal ranges.

The distinction sometimes made between pollutants and contaminants raises further difficulties. In the wider literature, the term pollutant implies that the chemical in question causes environmental harm. A contaminant is a chemical present at levels above those that might be judged normal and may or may not have caused environmental damage. In this text, examples of environmental chemicals have been chosen that are widely regarded as pollutants. They have been shown to have caused environmental harm or clearly exhibit the potential to do so at environmentally realistic levels.

By contrast, the contaminant term implies that a chemical is not necessarily harmful at environmentally realistic levels. The difficulties with this distinction are threefold. The first is the general toxicological principle that toxicity is related to dose (Chapter 5). Thus a compound may answer to the description of pollutant in one situation but not in another—a problem mentioned earlier. The second issue is the lack of general agreement about what constitutes environmental harm or damage. Some scientists regard deleterious biochemical changes in an individual organism as harmful; others reserve the term for declines in populations. Third, the effects of measured levels of chemicals in living organisms or in their environments are seldom known, but the term pollutant is applied to them. Judgment of this issue is made more difficult by the possibility of potentiation of toxicity when organisms are exposed to mixtures of environmental chemicals. To minimize these terminology, problems of "pollutant" will refer to environmental chemicals that exceed normal background levels and can cause harm. It would be attractive to reserve the term for particular chemicals in situations in which they have been shown to cause harm, but because problems of measurement, this usage would be too restrictive. Harm encompasses the biochemical or physiological changes that adversely affect individual organisms' birth, growth, or mortality rates. Such changes would necessarily produce population declines if other processes (e.g., density dependence) did not compensate (Chapter 12).

Whether a contaminant is a pollutant therefore depends on its level in the environment, on the organism considered, and whether the organism is harmed. Thus a compound may answer to the description of pollutant for one organism but not for another. Because of the problems in demonstrating harmful effects in the field, the terms pollutant and contaminant will, to a large extent, be used synonymously because it can seldom be said that contaminants have no potential to cause environmental harm in any situation. We will use environmental chemical to describe any chemical that occurs in the environment without judging whether it should be regarded as a pollutant or as a contaminant.

Another word that has been used inconsistently in the literature is the term biomarker. Here, biomarkers are defined as biological responses to environmental chemicals at the individual level or below, demonstrating departure from normal status. Biomarker responses may be at the molecular, cellular, or whole-organism level. Some workers would regard population responses (changes in number or gene frequency) as biomarkers. However, as the latter tend to be much longer term than the former, it may be unwise to use the same term for both. In the present text, the term biomarker will be restricted to biological responses at the level of the whole organism or below. An important thing to emphasize about biomarkers is that they represent measurements of effects, which can be related to the presence of particular levels of environmental chemicals; they provide a means of interpreting environmental levels of pollutants in biological terms.

Finally, the organic pollutants considered in this text are examples of xenobiotics (foreign compounds). They play no part in the normal biochemistry of living organisms. Xenobiotics will be discussed in Chapter 5.

Although ecotoxicology is a relatively new discipline, it is worth emphasizing that chemical warfare has been waged in the natural environment since early in the evolutionary history of Planet Earth. Both plants and animals produce chemical weapons. Plants produce metabolites that are toxic to animals that graze upon them. In turn, animals developed enzyme systems (e.g., forms of cytochrome P450) that can metabolize and thereby detoxify such compounds. This phenomenon has been called a co-evolutionary arms race (Harborne, 1993).

It is worth recalling that a number of naturally occurring compounds exert insecticidal actions and this property has been harnessed commercially by marketing these compounds as insecticides—or using them as models for the development of novel insecticides. Examples include natural pyrethrins and nicotine, both of which have been used as insecticides and as models for novel commercially developed insecticides. Conversely, the detoxifying enzymes present in insects can protect against commercially developed insecticides. Indeed, the continued and indiscriminate use of insecticides leads to the emergence of resistant strains of insects via a process that might be described as unnatural selection. Whichever term is used, the study of the molecular basis of the development of resistance provides a superb example of the operation of the principle of natural selection—a phenomenon of great interest to Darwinians.

These considerations extend beyond the responses to chemical weapons produced by animals and plants during the so-called co-evolutionary arms race. Other naturally occurring chemicals including methyl mercury, methyl arsenical compounds, polycyclic aromatic hydrocarbons, and certain heavy metals exert selective pressures on living organisms. These chemicals originate from the weathering of rocks, natural events such as volcanic activity, and the metabolic actions of microorganisms. Thus, ecotoxicology can be viewed against the background of the effects of a wide range of naturally occurring toxic compounds acting on living organisms during the course of evolution.

Approach and Organization of This Book

An exciting feature of ecotoxicology is that it represents a molecules-to-ecosystems approach that relates to the genes-to-physiologies approach originally identified by Clarke (1975) and extensively developed in North America in the 1980s (Feder et al., 1987). Moreover, it analyzes experimental manipulations on the largest of scales (although the experiments were not designed as such). Thus metal pollution, acid rain, and the applications of pesticides have affected whole ecosystems, sometimes with dramatic consequences for their populations. In ecotoxicology, the ecosystem response is studied at all levels. Initially (Figure 0.1), the molecular structures of pollutants and their properties and environmental fate are considered (Section I of this book).

Ecophysiologists generally analyze the impact of pollutants on an organism's growth rates, birth rates, and death rates; indeed, as explained above, pollutants can adversely affect these vital rates. This makes it desirable to understand how adverse effects on vital rates have implications for populations (Chapters 12 and 13). Consequently it is, in principle, possible to evaluate pollutants quantitatively in terms of their population effects. This emphasis on vital rates as crucial intervening variables, linking physiological effects to population effects, is a particular feature of this book. The approach is continued in Chapter 13 to consider whether and how quickly resistant genes increase in populations. The rate at which resistant genes increase is measured by the population growth rate of the population of resistant genes. The population growth rate of resistant genes is a measure of their Darwinian fitness. Although this is not the conventional population-genetic measure of fitness, it is particularly useful in ecotoxicology because it (alone) shows explicitly how the fitness of resistant genes depends on the effects those genes have on their carriers. To summarize, the approach taken in this book allows linkage to be made between the different levels of organization shown in Figure 1, from molecules to physiologies to populations, right through to ecosystems. This is the underlying basis for the biomarker strategy, which seeks to measure sequences of responses to pollutants from the molecular level to the level of ecosystems (Chapters 10, 15, and 16). The use of biomarkers in biomonitoring is described in Chapter 11. These four chapters are placed at the end of their respective sections of the book. They represent the practical realization of theoretical aspects described in earlier chapters.

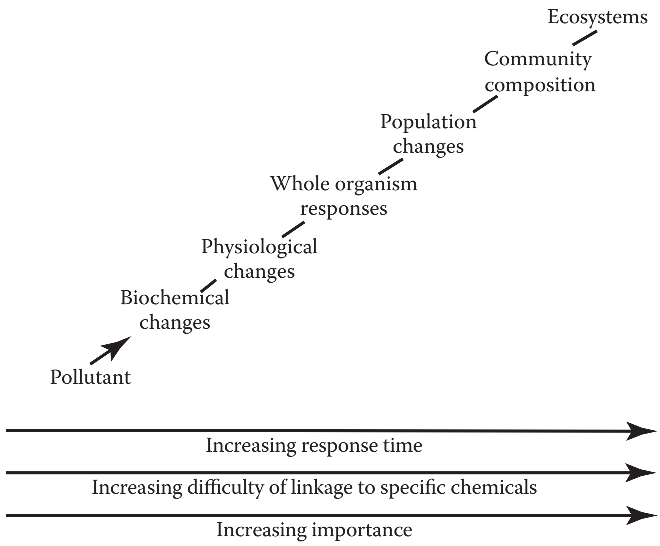

FIGURE 0.1
Schematic relationship of linkages between responses at different organizational levels.

The text is divided into three parts. Section I describes major classes of organic and inorganic pollutants, their entry into the environment, and their movement, storage, and transformation within the environment. Thus, this part bears a certain resemblance to toxicokinetics in classic toxicology which deals with the uptake, distribution, metabolism, and excretion of xenobiotics by living organisms (Chapter 5). The difference is in complexity. Ecotoxicology deals with movements of pollutants in air, water, soils, and sediments and through food chains, with chemical transformation and biotransformation.

Section II deals with the effects of pollutants on living organisms and resembles toxicodynamics in classic toxicology. The difference is again one of complexity. Toxicodynamics focuses on interactions of xenobiotics and their sites of action; ecotoxicology covers a wide range of effects on individual organisms at differing organizational levels (molecular, cellular, and whole animal). Toxicity data obtained in laboratories are used for the purposes of risk assessment. Effects of pollutants are studied in the laboratory, an approach that can lead to the development of biomarker assays (Chapter 10). The use of biomarker assays in biomonitoring is discussed in Chapter 11. The chapter also considers effects at the population level, thereby looking ahead to the final part of the text.

Section III addresses the questions of greatest interest to ecologists. What effects do pollutants have at the levels of population, community, and whole ecosystem? This takes the discussion into the disciplines of population biology and population genetics. Classic toxicology is concerned with chemical toxicity to individuals. Ecotoxicologists are interested in effects at the level of population community and whole ecosystem. Effects at the population level may be changes in numbers of individuals (Chapter 12), changes in gene frequency (as in resistance; Chapter 13), or changes in ecosystem function (e.g., soil nitifrication; Chapter 14). Effects may be sublethal (e.g., on physiology or behavior) rather than lethal. They may be indirect (e.g., decline in predator numbers because of direct chemical toxicity may lead to an increase in numbers of its prey). It is often difficult to establish effects of pollutants on natural populations. However, the development of appropriate biomarker assays can help resolve this problem.

Applications and Conclusions

The principal areas of application of ecotoxicology are the biomonitoring of environmental pollution (including the use of bioassays and biomarkers), the investigation of pollution problems, the conducting of field trials (particularly of pesticides), the study of the development of resistance and, finally, risk assessments of environmental chemicals—an area of growing importance that receives particular emphasis in this fourth edition of *Principles of Ecotoxicology*.

Risk assessment is required to establish whether novel chemicals are safe to use. In particular there is need to show they will not harm populations of non-target organisms. Current practice is largely based on laboratory testing of chemicals on a small number of organisms chosen in part for practicality (e.g., they complete their life cycles quickly in laboratory conditions) and in part as being representative of other organisms with similar lifestyles. These tests have yielded much valuable data and we refer to the results of such tests at appropriate places in the text. We do not describe in detail the methods currently in use in risk assessment, which vary to some extent between States. Our aim instead has been to provide a basis for understanding what happens to chemicals in the real world, where they go and how they ultimately degrade, and how they effect the individuals and populations that encounter them. Thus we hope our book will provide a solid foundation for all those contemplating a career in the important and interesting field of risk assessment.

A companion volume to this book covers the mechanistic aspects of ecotoxicology in greater depth and detail than this text. It is titled *Organic Pollutants; An Ecotoxicological Perspective*, 2nd Edition by C. H. Walker (2009). In the chapters that deal with individual groups of pollutants, it is structured in a similar way to the present text as shown in the following table

Divisions in This Text	Corresponding Sections in Chapters 5–12 of Organic Pollutants
Section I: Pollutants and their fates in ecosystems	Chemical properties, metabolism and environmental fate
Section II: Effects of pollutants on individual organisms	Toxicity of pollutants(s)
Section III: Effects of pollutants on populations and communities	Ecological effects

We hope that our book illustrates the truly interdisciplinary character of ecotoxicology. The study of the harmful effects of chemicals on ecosystems draws on the knowledge and skills of ecologists, physiologists, biochemists, toxicologists, chemists, meteorologists, soil scientists, and others. It is nevertheless a discipline with a distinct character. In addition to the important applied aspects that address current public concerns, it has firm roots in basic science. Chemical warfare is nearly as old as life itself and the evolution of detoxification mechanisms by animals to avoid the toxic effects of xenobiotics produced by plants is paralleled by the recent development of resistance by pests to pesticides made by humans.

Ecotoxicology

Effects of Pollutants on the Natural Environment

Colin Walker

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1 Toxicology and Ecotoxicology

SOME DEFINITIONS

Toxicology is concerned with the harmful effects of chemicals upon living organisms. Chemicals that have harmful effects are described as poisons, a term that requires careful definition. This issue was first addressed by Paracelsus (1493), who famously said (in free translation): "All substances are poisons and there is none that is not a poison. It is the dose that makes the poison." In other words, everything can be poisonous if the dose is high enough, but if the dose is sufficiently low, nothing is. This remains essentially true, even though it may be very difficult to administer a dose of some chemicals that is high enough to be harmful. It does, however, argue for caution when using the word *poison*. A substance can only be poisonous when it is given above a certain dose. At lower doses it is not poisonous. This simple principle is often not recognized in popular articles on pollution. Sometimes poisons are reported to exist in food or water at concentrations so low that there is no known risk to the living organisms that encounter them. Thus, the public can be alarmed through disinformation. A little sensation does help to sell newspapers.

There is also the question: What constitutes harm? Simple enough to understand in the case of toxicity tests that use lethality as the endpoint. Here toxicity can be quantified as a median lethal dose (LD_{50}) or median lethal concentration (LC_{50}), for example. But there are many other measures of harm apart from lethality, e.g., neurotoxicity, carcinogenicity, endocrine disruption, etc. Included here are biochemical effects (e.g., inhibition of brain acetylcholinesterase or DNA damage), physiological effects (e.g., disturbance of nerve function), behavioral effects (e.g., responses of animals to stimuli), etc. In human toxicology many indices of harm other than lethality are used in toxicity testing.

The term *ecotoxicology*, first introduced by Truhaut in 1969 (Truhaut 1977), suggests an area of science that brings together ecology and toxicology. It is here defined as "the study of the harmful effects of chemicals upon ecosystems." Ultimately, the greatest concern is about effects at the levels of population, community, and ecosystem. However, an essential part of the subject is the identification of harmful effects at the individual level, which may be translated into these higher-level effects in the field. Important here are the toxic effects of pesticides upon individual pests that lead to the development of resistance at the population level. Ecotoxicity tests that can identify such lower-level effects (e.g., mechanistic biomarkers) have the potential to give forewarning of longer-term change at the population level. Such effects may become evident in field studies, including field trials that are carried out with pesticides or other hazardous environmental chemicals. Mechanistic biomarker tests may be employed in the early stages of environmental risk assessment (Chapters 2 and 19).

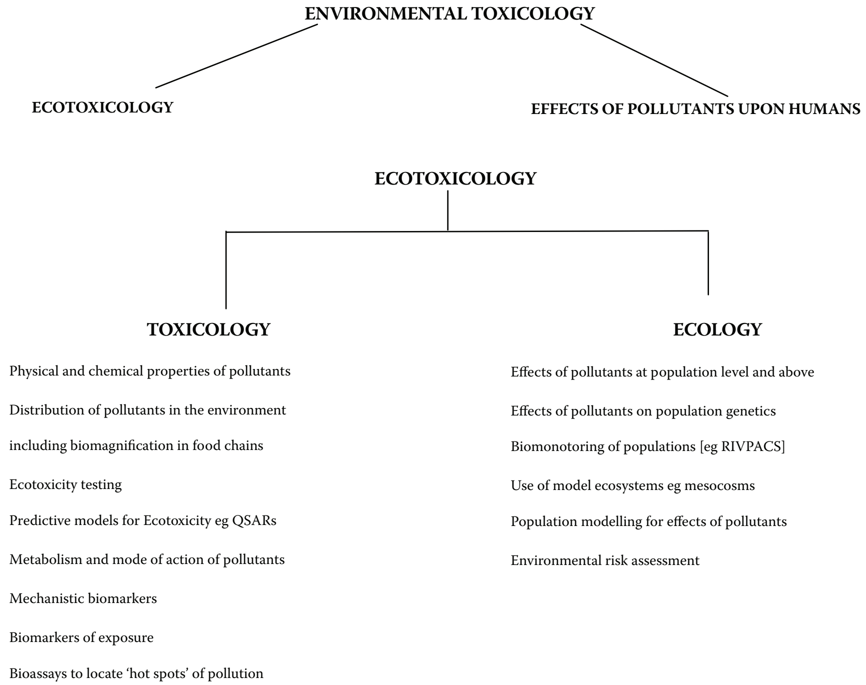


FIGURE 1.1 Relationship of ecotoxicology to other disciplines.

Returning to questions of definition, in human toxicology the effects of chemicals upon individuals are of primary concern, but in ecotoxicology the focus is on effects at the level of population and above. Studying the exposure of humans to hazardous chemicals in the environment does not fall within the definition of ecotoxicology given above and will not be dealt with in this book. Thus, the effects of pesticides upon those who use them in the field, e.g., agricultural workers or pilots involved in aerial spraying, will be regarded as an aspect of human toxicology. The broader term *environmental toxicology* will be taken to include both ecotoxicology and questions about human health hazards arising from exposure to chemicals in the environment (Figure 1.1).

THE DISCIPLINE OF ECOTOXICOLOGY

Aspects of ecology and toxicology that are of importance in ecotoxicology are identified in Figure 1.1. To take toxicological aspects first, the physical and chemical properties of pollutants are critical determinants of their movement, distribution, and persistence. Biochemical and physiological properties (e.g., toxicity, mode of action, and metabolism) determine the effects that pollutants have on living organisms. Metabolism is also critical in determining the fate of pollutants in living organisms and, consequently, whether they undergo biomagnification with movement through food webs. Compounds that are readily biodegradable tend not to undergo biomagnification.

The development of appropriate tests for ecotoxicity is a particularly difficult area. Questions are frequently asked about toxic effects on species that cannot realistically be tested in the laboratory—for reasons of rarity, cost, protection, etc. As a rule, surrogate species are used. These issues will be discussed further in Chapters 2 and 8. Related to this, there is an interest in the development of predictive models for ecotoxicity, which can, in theory, provide a cost-effective alternative to ecotoxicity testing per se (Chapter 19).

An important developmental area is the design of mechanistic biomarker assays. The aim here is to develop user-friendly assays that can provide measures of toxic effect on living organisms in the field after exposure to pollutants (Chapters 2 and 19). Related to this, bioassays have been developed that can be used to identify hot spots of pollution. Included here are cell lines, such as the Calux system, which can detect low levels of coplanar polychlorinated biphenyls (PCBs) and dioxins that interact with the Ah receptor (see Chapter 13).

Turning to ecological aspects, effects at the population level are of primary importance. Population studies in the field and population modeling are relevant here (Chapters 4 and 18). The use of mesocosms and other model ecosystems is an interesting developmental area (Chapters 2 and 4). The use of mechanistic biomarkers, mentioned earlier, can provide vital support for population studies in the field. Effects on the genetic composition of populations are also of great interest, as in the case of development of resistance to pesticides (Chapter 5). Other important ecological topics are biomonitoring for effects of pollutants and environmental risk assessment.

The terms *contaminant* and *pollutant* are used inconsistently in the literature. Throughout this text the broad term *environmental chemical* will be applied to any substance that is found in the environment, without any implication about its abundance or the hazards that it may present. On the other hand, the term *contaminant* will be applied very largely to chemicals that are generated by man and do not normally occur in nature. It will occasionally be applied also to certain naturally occurring substances, when they are found at abnormally high levels, e.g., heavy metals in the vicinity of mine workings or SO_2 gas when it is released at the time of a volcanic eruption. Such environmental chemicals present the same problems to the environment whether they originate from natural or human activity. When detected in the environment they will often originate from both natural and unnatural sources, which are difficult or impossible to distinguish between. It seems logical to simply refer to them as being contaminants regardless of the extent to which they are natural or unnatural in origin, the important point being that they occur at abnormally high levels so far as ecosystems are concerned.

The term *contaminant* does not necessarily indicate that a chemical is hazardous to the environment. The term *pollutant*, however, will be reserved for contaminants for which there is clear evidence of their capacity to cause harm at environmentally realistic concentrations. As a text in ecotoxicology, this book will focus upon situations where contaminants are at sufficiently high levels to cause harm to ecosystems, and may therefore be regarded as pollutants. Chemicals referred to as pollutants in the following text can occur at high enough concentrations to be termed poisons according to the definition of Paracelsus.

The pollution of the environment described here is predominantly the consequence of the activities of man. This is largely self-evident, because the chemicals in question are only produced by man. However, we come back to the question: When can naturally occurring chemicals be regarded as pollutants? Most chemicals that answer to this description are inorganic. They include metals such as cadmium, lead, zinc, copper, nickel, manganese, and mercury and the gases sulfur dioxide, hydrogen sulfide, and nitrogen oxides. Usually these only reach levels that can cause environmental harm because of the activities of man. Thus, lead, cadmium, and mercury have caused pollution problems as a consequence of mining or the activities of the chemical industry. Pollution by sulfur dioxide, which has caused extensive damage to forests in parts of Eastern Europe, has resulted from the burning of brown coal. Further examples will be given in the following text. However, there are, as usual, exceptions to the rule. Pollution of this kind may also arise as the consequence of cataclysmic natural events, such as earth tremors or volcanic action; it is not always due to the activities of man. Disturbances of this kind may expose minerals containing metals to weathering, which can lead to a substantial rise of the concentrations of them in surface waters and soils. Also, volcanic eruptions can cause the release of the gases sulfur dioxide, hydrogen sulfide, and nitrogen dioxide at high enough levels to cause damage to animals and plants. The destructive power of volcanoes was well illustrated—and well recorded—when Mount Vesuvius erupted in 79 AD. The description of this cataclysmic event by the Roman historian Pliny provides the basis for Robert Harris's novel *Pompei* (2003).

Most examples of pollution by naturally occurring substances have been caused by inorganic chemicals, but there are also a few naturally occurring organic chemicals that have been implicated. For example, some heavy metals/metalloids are converted by microorganisms into methyl derivatives. Examples include mercury and arsenic. As will be discussed later, organometallic compounds have different properties from the metals/metalloids from which they are derived—and often they are more hazardous to living organisms. Some of them have been implicated in serious pollution problems. At Minamata Bay in Japan, between the late 1950s and the early 1960s, high levels of methylmercury in the marine environment had toxic effects on fish—and on human beings feeding on the fish. At that time methylmercury was used extensively as a fungicide; environmental residues of methylmercury arose from both release of the manufactured organic form and environmental synthesis from inorganic mercury (Environmental Health Criteria 86 and 101 (WHO 1989)).

Fires due to natural phenomena such as volcanic eruptions or lightning strikes can also lead to pollution. When trees or dry vegetation burn, a diversity of organic compounds is formed. These include products of incomplete combustion, such as polycyclic aromatic hydrocarbons. Among these are some potent carcinogens such as benzo(a)pyrene—one of the compounds in cigarette smoke that is associated with the development of lung cancer by smokers. Thus, with extensive bush or forest fires certain natural organic pollutants may be spread over large areas. Such pollution of the natural world will have occurred long before the appearance of man on the planet.

In all of these examples, whether the consequence of human activity or of natural phenomena, it is likely that the pollutants will have exerted some selective pressure on the populations that are exposed to them. This issue will be discussed further in Chapter 5.

SELECTIVE TOXICITY

As we have seen, toxicity is dependent on dose, and this means that the term *poison* needs to be used with caution. In reality, a chemical is only poisonous if the dose is sufficiently high. It is possible, however, to distinguish between chemicals that have low, medium, high, or very high toxicity to particular organisms, the distinction being based on how high a dose is required to produce a defined toxic effect. This approach has been used in the text *Basic Guide to Pesticides: Their Characteristics and Hazards* by S. Briggs (1992). Here, for example, the following categories are defined for acute (immediate) oral toxicity to animals:

Toxicity Rating	Dose Range
Very high	<50 mg/kg
High	50–500 mg/kg
Medium	500–5000 mg/kg
Low	>5000 mg/kg

These figures illustrate the huge range of toxicity values found for different pesticides across a range of species. It should be remembered when looking at these figures: the higher the lethal dose, the lower the toxicity.

Any one chemical is often much more toxic to some species than it is to others; in other words, many chemicals show marked *selective toxicity* between different species, sexes, strains, or age groups. For example, most herbicides are much more toxic to plants than they are to animals—and most insecticides are much more toxic to insects than they are to plants. The reasons for these large differences in susceptibility will be discussed in the next section.

Selective toxicity is very important in both human toxicology and ecotoxicology, but for different reasons. In human toxicology the focus of attention is upon a single species—*Homo sapiens*. Species other than man are used as surrogates in toxicity testing. Thus, when carrying out toxicity tests, there is a great interest in finding test species that are most comparable to humans in their toxic response. Unsurprisingly, primates, the surrogates most closely related to humans, are often regarded as the best models. However, this raises serious ethical problems. There is much opposition to the use of primates in toxicity testing by groups promoting animal welfare, and to a very large extent, toxicity testing is performed upon mammals other than primates, e.g., rats, mice, rabbits, or guinea pigs. Here, there is the difficulty of extrapolating results obtained in this way to humans, of translating toxicity values obtained with rats and mice to estimates of toxicity to humans. There is always the question with such toxicity tests: Which laboratory species best represents humans?

In ecotoxicology the situation is quite different. Tests are performed on a few surrogate species that represent a very large number of wild species—many of which are only very distantly related to the wild species seen to be at the highest risk. Very seldom is the test species the same as the species thought to be at most risk in the natural environment.

Common to both ecotoxicology and human toxicology is the same fundamental question: Which surrogate provides the best model for a particular test chemical? In attempting to resolve this question, we need to delve a little more deeply into the mechanisms that are responsible for toxicity. This will be attempted in Chapter 2.

SUMMARY

Ecotoxicology is here defined as the study of the effects of chemicals upon ecosystems. The ultimate concern is on effects of chemicals at the level of population. Effects upon individuals are important if they are translated into effects at the level of population or above. A central question, then, is: When do effects at the individual level become translated into changes at the population level? Changes at the population level may be in population density or in genetic composition. By contrast, human toxicology (medical toxicology) is particularly concerned with effects upon individuals.

In both cases there are legal requirements for toxicity tests using surrogate organisms that will provide data for the process of risk assessment. In medical toxicology these surrogates are vertebrate animals that are regarded as appropriate models for humans. In ecotoxicology a few surrogate species are used to represent a large number of wild species to which they are seldom very closely related.

The term *pollutant* will be reserved for chemicals for which there is clear evidence of an ability to cause harm at environmentally realistic concentrations. A contaminant is not necessarily a pollutant.

There can be large differences between species, sexes, and age groups regarding their susceptibility to the toxic action of any individual chemical. This raises questions about which species are the most suitable ones to use surrogates in toxicity testing. Pesticides, a group of chemicals of considerable interest to ecotoxicologists, have been classified into groups according to their lethal toxicity to animals (Briggs 1992).

FURTHER READING

Briggs, S.A. 1992. *Basic guide to pesticides: Their characteristics and hazards*. Boca Raton, FL: Taylor & Francis. A useful reference book on the toxicity of pesticides.

Walker, C.H., Sibly, R.M., Hopkin, S.P., and Peakall, D.B. 2012. *Principles of ecotoxicology*. 4th ed. Boca Raton, FL: Taylor & Francis/CRC Press. An introductory text in ecotoxicology based on an MSc course, "Ecotoxicology of Natural Populations."

2 Ecotoxicity

INTRODUCTION

Ecotoxicology is primarily concerned with the effects of chemicals expressed at the levels of population, community, or ecosystem. That said, effects at the level of population and above are usually the consequence of effects upon individuals. Thus, lethal and sublethal effects of pollutants on individuals of a species can lead to population decline if they are severe enough. Such effects can be measured upon live animals and plants in the laboratory. This issue will be discussed further in Chapter 4.

In what follows the term *ecotoxicity* will refer to toxic effects that are relevant in ecotoxicology. Both lethal and sublethal effects of chemicals upon individuals are measured in standard *ecotoxicity tests*, and these procedures will be discussed in more detail later in this section. The results obtained using such tests can be utilized in the process of environmental risk assessment, and this will be described in Chapter 16. At the present time most ecotoxicity tests are performed on living animals or plants, but with advances in biochemical toxicology, there is growing interest in the development of in vitro test systems and model systems such as quantitative structure-activity relationships (QSARs) for estimating ecotoxicity.

One complication in ecotoxicology is that harmful effects upon populations in the natural environment are not necessarily direct; they can be indirect as well. There are complex relationships between different populations in nature, and the effect of a chemical on a population of one species may have knock-on effects upon populations of other species that exist in the same ecosystem, even though they themselves are not directly affected by the chemical. For example, a population decline of one species caused by the toxicity of a pesticide may lead to a related decline in another species that feeds upon it. On agricultural land, for example, the intensive use of herbicides can cause a population decline not only of weeds, but also of insects that feed upon weeds. In turn, this decline of insect populations can cause a related decline of grey partridges (*Perdix perdix*)—because of the dependence of their chicks upon a supply of insect food (see Potts 1986, 2000). These and other examples of indirect effects are very important in ecotoxicology and will be returned to later.

This brings us to a fundamental dilemma in ecotoxicology. Effects at the individual level may or may not lead to consequent effects at higher levels of organization. How can we distinguish between effects that lead to population decline and others that do not? This issue will be discussed further in Chapter 4. Ideally, ecotoxicity tests should give forewarning of potential hazards *at the level of population or above*, under field conditions. Unfortunately, statutory ecotoxicity tests cannot be relied upon to do this.

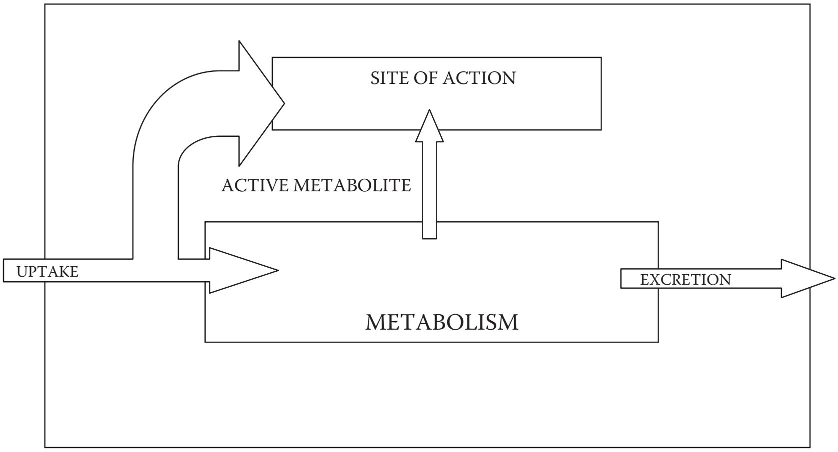


FIGURE 2.1 General model describing the fate of organic pollutants in living organisms.

WHAT DETERMINES ECOTOXICITY?

Why are there large differences in susceptibility to particular chemicals between different groups of organisms? To attempt to answer this question, it is necessary to look a little more closely at the phenomenon of toxicity. Figure 2.1 is a highly simplified representation of the events that occur within a living organism after it is exposed to a chemical that has a significant degree of toxicity. This simple model is based on the processes that occur in animals. However, the picture in plants is essentially similar. Most important, pollutants are fat-soluble (lipophilic) organic compounds, and this model applies particularly to them—although it can, with only minor modification, be applied to other pollutants as well.

When an animal is exposed to a chemical, that chemical may gain entry to the circulatory system and the organs of the body by one or more routes. The most important routes of entry for animals are via the alimentary tract, across the skin or cuticle, through the lungs (terrestrial animals) or the gills (fish). Once inside the animal, it will be carried by the circulatory system to various organs and tissues. Lipophilic pollutants tend to remain in the bodies of terrestrial animals for long periods of time, stored in the fatty tissues (e.g., body fat), unless they are broken down. A very important means of eliminating them, especially in terrestrial animals, is to convert them into water-soluble products that are readily excreted. This important function is carried out by a battery of enzymes that convert them to water-soluble and readily excretable products. Prominent among these are a variety of oxidative enzyme systems that incorporate different forms of cytochrome P450 as their active centers (Box 2.1). These enzyme systems are able to metabolize many fat-soluble molecules that are foreign to the organism in question (xenobiotics). Fish and other aquatic animals are less dependent on this protective system than are terrestrial animals. This is because they can eliminate moderately lipophilic pollutants by diffusion into ambient water (e.g., across the gills)—a route of elimination that is not available to terrestrial animals. These

BOX 2.1 OXIDATIVE ENZYME SYSTEMS OPERATING THROUGH CYTOCHROME P450

The cytochromes P450 are many and varied. They are heme proteins that have the capacity to bind molecular oxygen—rather as hemoglobin does in the blood of vertebrates. Once bound, the oxygen becomes activated by an enzymic process that involves the transfer of electrons. Activated oxygen can then "attack" lipophilic molecules that are bound at a site in the vicinity of the active oxygen. A chemical reaction takes place, transforming the lipophilic molecule into water-soluble products.

The cytochrome P450s are usually located within membranes such as the endoplasmic reticulum of liver cells. Incoming lipophilic pollutants diffuse from the aqueous cytosol into this hydrophobic environment where they can be degraded by these oxidative systems that operate through the cytochromes P450. The products of metabolism (metabolites) are usually water soluble. Consequently, they tend to diffuse out of the lipidic membrane environment into the aqueous cytosol, from whence they are moved out of the cell and are available for excretion. In vertebrates most excretion is via urine or bile.

While this process is usually protective to animals, leading to removal of potentially toxic compounds from the body, it can sometimes misfire. The oxidation of certain carcinogens and organophosphorous insecticides (OPs) causes activation, not detoxication. For examples, see Chapter 10.

***Sources*: Timbrell, J., *Principles of Biochemical Toxicology*, 3rd ed., London: Taylor & Francis, 1999; Walker (2009). Organic Pollutants: An Ecotoxicological Perspective, 2nd ed., Boca Raton: Taylor and Francis.**

detoxifying enzymes tend to be less well represented in fish than they are in terrestrial animals.

Toxic effects are produced when pollutants interact with one or more sites of action within the animal. Many examples of these sites of action will be given in the second part of this text. It is important to emphasize at this early stage that, while many of the toxic effects of pollutants arise because the original molecule interacts with one or more sites of action, in a few, yet critically important, number of cases the molecule that causes the damage is a reactive metabolite. Thus, we can say that the protective metabolism sometimes goes wrong, and the metabolic product causes the damage. This is the case with a number of carcinogens (e.g., benzo(a)pyrene) and some organophosphorous insecticides (e.g., dimethoate and malathion).

PESTICIDES AND OTHER BIOCIDES

Pesticides and other biocides are used with the intention of causing damage to pest species and other organisms that threaten the health or well-being of humans. Because of the potential problems that they present to the environment, pesticides have been subject to more rigorous *ecotoxicity testing* than have most other types of contaminants, as part of the process of *statutory environmental risk assessment*. More ecotoxicity data are usually required about pesticides than for most other industrial chemicals (e.g., pharmaceuticals) that may be released into the environment. On this account, pesticides will be discussed first, before giving attention to other potential contaminants that are not subject to such rigorous testing. With pesticides there are usually clear guidelines as to what uses are permissible—which formulation may be used at which rates and under which circumstances, etc. At least, this is usually the situation in the developed world, where there are normally clear regulations about pesticide use, enforceable by law. In the developing world things are often different. There tend to be far fewer regulations, and those that do exist are not necessarily enforced.

This brings the discussion to a critical issue: Which of the effects that pesticides may have on natural populations are to be seen as acceptable, and which as unacceptable? The control of pests or vectors of disease is clearly both intentional and desirable. On the other hand, the development of resistance in these populations is neither. Resistance appears to be an inevitable outcome of the overuse of pesticides. If the selective pressure of a pesticide is too great over a long period (say ten years of consistent use), the development of resistance in target organisms is to be expected (see Chapter 5). A more controversial question is what effects on nontarget organisms are acceptable. In the first place, harmful effects on populations of beneficial organisms are unacceptable. But there is then the question: Which organisms are to be considered beneficial? Beneficial organisms are taken to include parasites and predators that control pests, also pollinating insects (e.g., bees and hover flies) and soil invertebrates and microorganisms that promote soil fertility. Not included here are the great majority of natural occurring species; these are not usually categorized as beneficial organisms. However, there are also issues about the desirability of biological diversity and the conservation of genes that are potentially beneficial to man, questions that will be dealt with in later chapters.

Sometimes the outcome of statutory risk assessment of a new pesticide, based upon ecotoxicity tests performed in the laboratory, raises questions about possible harmful effects that it may have if used in the field. This concern may arise because of lethal or sublethal effects shown toward test animals. It may then be decided that further testing is necessary before the compound can be approved for marketing by a legislative authority (see Chapter 18). Here, one of the options for the manufacturers of the pesticide may be to carry out field trials to establish whether or not the pesticide does have harmful effects when used in the field (see Somerville and Walker 1990). Unfortunately, field trials are expensive and time-consuming and are seldom carried out in practice. All too often the value of the process of risk assessment is limited because of considerations of cost. This issue will be discussed further in Chapters 4 and 18.

INDUSTRIAL CHEMICALS OTHER THAN PESTICIDES

Returning to environmental chemicals other than pesticides and biocides, statutory requirements for ecotoxicity testing are usually less stringent. Most industrial chemicals are not very toxic to living organisms. That said, pharmaceuticals present a special case because they are, by definition, biologically active. They are used as human or veterinary medicines— e.g., beta blockers, analgesics, antipyretics, diuretics, chemotherapeutic agents, contraceptives, etc. When administered to humans, the doses are usually well below those known to have harmful effects. Typically, only very low concentrations find their way into the environment. However, some problems of pollution have arisen with pharmaceuticals. There has been concern, for example, about a component of the contraceptive pill, ethinylestradiol (EE2), which has been shown to cause endocrine disruption in fish. Also, the anti-inflammatory drug diclofenac has caused serious declines in vulture populations in India when administered to cattle. More generally, questions have been asked about the possible collective effects of pharmaceuticals when they are present in complex mixtures in surface waters—albeit at very low concentrations (see Chapter 19).

These issues aside, pharmaceuticals in general are not usually subject to the rigorous ecotoxicity testing that is required for pesticides and biocides—which are intentionally released into the environment at high enough concentrations to have ecotoxicological effects!

PROTOCOLS FOR ECOTOXICITY TESTING

When determining protocols for the ecotoxicity testing of a new pesticide or biocide, its chemical, physical, and biological properties come into consideration. So too do questions about its release into the environment: where and how it will be released, at what rate, and in what form. These factors can all contribute to the ecological risk.

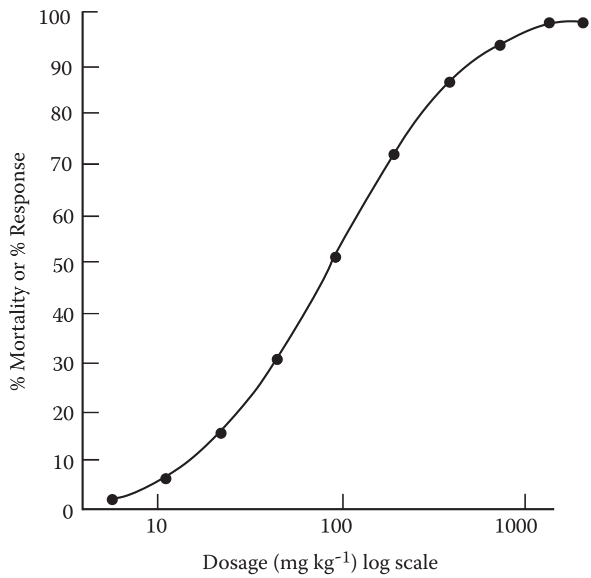
A fundamental issue in ecotoxicity testing is the range of doses to be given to test species. Doses may be given orally or topically to terrestrial animals. Aquatic species (e.g., fish, amphibians, molluscs) are exposed to a range of concentrations in water. Logically, such tests should cover the range of concentrations likely to be encountered in the living environment, including relatively high levels expected when considering worst-case scenarios. Although this approach is generally accepted, complications can arise when there are legislative requirements for median lethal doses or concentrations (LD_{50} or LC_{50}). Countries differ markedly in their requirements for such data. Sometimes doses need to be given that are far above expected environmental concentrations in order to determine a value for median lethal dose or concentration required by statute. Animals are exposed to concentrations that greatly exceed levels that are likely to occur in the environment—in order to obtain values that are not relevant when estimating environmental risk. Both environmental scientists and campaigners for animal welfare are strongly opposed to this, but legislative requirements for this kind of data still exist. This issue will be discussed further in Chapter 18.

Ecotoxicity tests are carried out upon selected indicator species to yield data that can be used for statutory environmental risk assessment. This process will be discussed in more detail in Chapter 18. In an ideal world these tests would be carried out upon those species deemed to be particularly at risk. However, in the real world, this is seldom possible. A limited number of laboratory species are used as surrogates for the species actually at risk in nature. Typically, only a few species of mammals, birds, fish, or beneficial invertebrates are available for routine ecotoxicity testing. This immediately raises uncertainties about differences in susceptibility between the species seen to be at greatest risk *in the field* and the *surrogates for them* that are used in ecotoxicity testing. A further question is at what stage in its life cycle is an animal or plant most susceptible to the toxic action of a chemical; ideally, ecotoxicity tests should be performed at the most susceptible developmental stage. Questions include: Are young animals more susceptible than older ones? Are the nymphs or larvae of insects more or less susceptible than adults? And, are birds particularly susceptible when the embryo is developing within the eggshell? Questions such as these arise when designing ecotoxicity tests. Unfortunately, because of constraints of cost, availability of skilled labor, etc., much testing is standardized, leaving only limited opportunity for a flexible approach to address these issues.

Another important issue in ecotoxicity testing is which endpoint should be used. Commonly the endpoint used has been death—at least, until recently. Tests have been carried out to determine median lethal dose (e.g., LD_{50}) or median lethal concentration (LC_{50}) for animals maintained in the laboratory. However, as will become apparent later in this text, population declines may be caused by sublethal effects. Effects upon reproduction or behavior can occur at pollutant levels well below median lethal doses or concentrations, and these can cause populations to decline. Examples of this have included the decline of populations of birds of prey due to eggshell thinning caused by p,p′-DDE and the decline of certain molluscs due to loss of fertility caused by tributyl tin. Recently there has been evidence suggesting that populations of bees can decline because of the behavioral effects of neonicotinoid insecticides (Henry et al. 2012; Whitehorn et al. 2012). There is growing interest in designing ecotoxicity tests that will measure relevant sublethal effects of pesticides and other pollutants. Included here are in vitro testing methods using cell cultures and tissue preparations. A lethal toxicity test is rather a blunt instrument, and as knowledge of ecotoxicology advances, it is to be hoped that more sophisticated ecotoxicity tests will emerge. Once again, however, there are limitations of resources and cost when it comes to the development of improved ecotoxicity testing methods.

DETERMINATION OF MEDIAN LETHAL DOSE AND MEDIAN LETHAL CONCENTRATION

The lethal toxicity of a chemical to mammals, birds, and other vertebrates is often expressed as a median lethal dose (LD_{50}). In routine toxicity testing a single oral dose is given to individual animals to obtain a measure of acute oral LD_{50}. Groups of animals are given doses of a test chemical over a range of values that centers on a rough estimate of LD_{50} obtained in preliminary testing. The numbers of individuals that die in each group over a fixed period are recorded, and these values, expressed as percentages, are plotted against the doses, thus generating a *dose-response curve*. The graphical presentation of this is shown in the upper section of Figure 2.2. As can be seen, the graph is curvilinear in character. A statistical transformation of data can now be performed. If the percentages of mortality are converted into probit units, this curvilinear response becomes a straight line, as shown in the lower



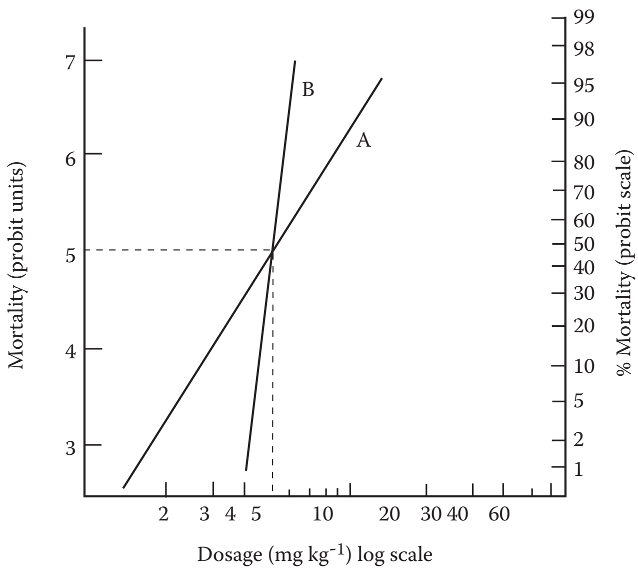


FIGURE 2.2 Determination of LD_{50}. (From Walker, C.H., et al., *Principles of Ecotoxicology*, 4th ed., Boca Raton, FL: Taylor & Francis/CRC, 2012.)

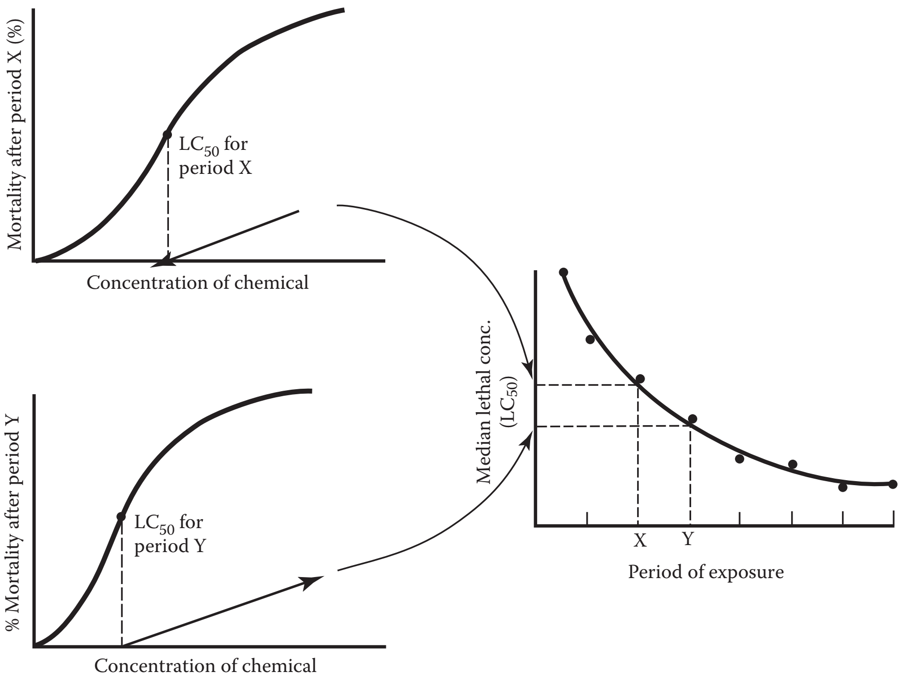


FIGURE 2.3 Determination of LC_{50}. (From Walker, C.H., et al., *Principles of Ecotoxicology*, 4th ed., Boca Raton, FL: Taylor & Francis/CRC, 2012.)

section of Figure 2.2. It is now possible to read from the graph an estimate of LD_{50}, the dose that will lethally poison 50% of the test animals. A statistical transformation of this kind can give a more reliable estimate of values at the extremities of this dose-response curve. More information on this approach can be found in Finney's *Probit Analysis* (1964).

With aquatic organisms such as fish and aquatic invertebrates, lethal toxicity is often expressed as the lethal concentration in the ambient medium, water. Here, there is a different situation from that just described for acute oral toxicity testing which is often based upon the administration of a single dose of a chemical to a test animal. Aquatic organisms are continuously exposed to chemicals present in ambient water, and percentage of mortality will increase over time as the tissue concentrations rise. This is illustrated in Figure 2.3.

After a series of preliminary tests, a range of concentrations of chemicals in water are selected for further testing. Groups of test organisms are then exposed to different concentrations of the test chemical over a period of time, and mortalities are recorded at regular intervals. From these plots estimations of LC_{50} can be made for different periods of exposure to the chemical. In general, the longer the exposure period, the lower the LC_{50}. Important to emphasize here is that the lower the LC_{50} value, the higher the toxicity. With data of this kind, a period of exposure can be chosen for routine testing. Typically, median lethal concentrations for toxicity to fish are measured over a period of ninety-six hours.

BOX 2.2 POTENTIATION AND SYNERGISM

In the natural environment organisms are often exposed to mixtures of environmental chemicals. There is then the question: What is the toxic effect of a mixture of compounds upon an organism? Very often the toxicity of a mixture approximates to the summation of the toxicities of the individual compounds. However, this is not always the case. Taking the example of just two compounds to which an animal is exposed at the same time, if the toxicity significantly exceeds additivity, there is said to be *potentiation*; if, however, the toxicity is significantly less than additive, there is said to be *antagonism*. From a toxicological point of view, significant levels of potentiation in mixtures can be a cause of concern.

A particular case of potentiation is termed *synergism*. Here, a mixture of two compounds is substantially greater than additive—but one of the two compounds in the mixture, the *synergist*, would not be toxic on its own at the dose in question. Frequently a synergist has the effect of inhibiting the metabolism of the other, "toxic" compound.

Synergists of this kind have sometimes been used to enhance the effectiveness of pesticides, e.g., the addition of the synergist piperonyl butoxide to natural pyrethrin insecticide. However, this is not normally practiced today because of unforeseeable risks. A synergist such as piperonyl butoxide may increase the toxicity of chemicals in the environment other than pesticides.

That said, piperonyl butoxide and related inhibitors can be useful in the identification of mechanisms of resistance to insecticides. Frequently, oxidative detoxication by P450-based monooxygenase is a resistance mechanism developed toward pyrethroids and certain other insecticides. Where this is the case resistance will be substantially decreased if this synergist is applied together with the insecticide. Inhibition of the resistance mechanism increases toxicity.

An important point to emphasize is that, up to this point, discussion has been about the toxicity of individual compounds. In nature, however, the situation is more complicated than this, and organisms are commonly exposed to mixtures—and this raises the issue: What is the toxicity of a mixture? This issue is discussed further in Box 2.2.

ECOTOXICITY TESTING THAT USES SUBLETHAL ENDPOINTS

The foregoing two sections have described ecotoxicity testing procedures that utilize lethality as the endpoint. In principle, the same testing protocols can work for sublethal endpoints, many of which have or can be used in ecotoxicity testing. As will become apparent later in this book, sublethal effects, e.g., on reproduction or behavior, may cause populations to decline in the natural environment. Sublethal effects that have been used as endpoints in ecotoxicity testing upon animals have included reductions in reproductive success, the thinning of avian eggshells, changes

BOX 2.3 BIOMARKERS

Biomarkers are here defined as "biological responses to environmental chemicals, at the individual level or below, that demonstrate a departure from normal status." Thus, biochemical, physiological, morphological, and behavioral effects will all be considered as biomarkers. Biological responses at higher levels, e.g., of populations, communities, and ecosystems, are not covered by this definition.

The most valuable biomarkers provide some measure of the progress of a mechanism of toxicity—and will here be termed *mechanistic biomarkers*. Other biomarkers indicate only the presence of a chemical, and do not measure toxic manifestations. These will be termed *biomarkers of exposure*.

Mechanistic biomarkers include inhibition of brain acetyl cholinesterase by organophosphorous insecticides (Chapter 10), eggshell thinning caused by p,p'-DD*E* (Chapter 9), and imposex in bivalve molluscs caused by tributyl tin (Chapters 11 and 15).

Biomarkers of exposure include the production of vitellogenin when assay systems are exposed to estrogens and the induction of certain enzymes (Chapter 15).

Biomarker assays can be used in field studies to give evidence of population declines that are caused by pesticides and other pollutants (see discussion in Chapters 18 and 19).

Sources: **Peakall, D.B., *Animal Biomarkers as Pollution Indicators*, London: Chapman & Hall, 1992; Peakall, D.B., and Shugart, L.R., eds., *Biomarkers: Research and Application in the Field of Environmental Health*, Berlin: Springer Verlag, 1993; Walker, C.H., et al., *Principles of Ecotoxicology*, 4th ed., Boca Raton, FL: Taylor & Francis/ CRC Press. 2012.**

in behavior, neurotoxic effects, and endocrine disturbances. The irony is that these effects can present a greater threat to natural populations than do lethal ones.

Sublethal effects of chemicals upon living organisms can be measured using what have been termed *biomarker assays*. These are described in Box 2.3. Biomarker assays can be used to measure responses of animals and plants using testing procedures that employ sublethal doses of chemicals. From such tests values can be determined for doses corresponding to no effect level, 50% effect, etc.

BIOASSAYS FOR MEASURING TOXICITY

Both cellular systems and genetically manipulated microorganisms have been utilized in the development of bioassays that measure the toxicity of environmental samples. Such bioassays can be used to detect the presence of toxic materials in samples of water, soil, sediment, sewage sludge, air, or material from landfill sites

TABLE 2.1
Some Bioassay Systems

Name of Bioassay	Organism	Principle of Operation	Types of Pollutants Detected	Reference
Microtox	*Vibrio fischerii* A bacterium	Pollutants reduce bioluminescence	Diverse	Persoone et al. 2000
Ames test	Strains of the bacterium *Salmonella typhimurium*	Mutation of microorganism leads to loss of histidine dependence	Various mutagens	Maron and Ames 1983
Calux	Rodent hepatoma cells	Induction of cytochrome P450 1A leads to light emission	Polyhalogenated aromatic hydrocarbons (PHAHs), e.g., dioxin	Garrison et al. 1996
Fish hepatocyte 1	Rainbow trout hepatocytes	Release of the protein vitellogenin	Estrogens	Sumpter and Jopling 1995
Fish hepatocyte 2	Rainbow trout hepatocytes	Induction of cytochrome P450 1A	PHAH, e.g., dioxin	Pesonen et al. 1992

(Lynch and Wiseman 1998). They seldom, if ever, respond only to the toxicity of one particular compound, but they may indicate the presence of a certain type of compound—e.g., an endocrine disruptor or an inducer of a particular enzyme.

They are particularly useful for environmental monitoring—for checking water quality near sewage outfalls or identifying hot spots of pollution in lakes, inland seas, mining areas, and industrial sites. They can measure toxicity caused by mixtures of pollutants rather than individual ones. They can be relatively inexpensive and easy for the nonexpert to use. Thus, with the inevitable constraint of cost, they can identify pollution problems that would remain undetected by more expensive measures such as chemical analysis. Some individual bioassay systems are identified in Table 2.1.

The Microtox assay is nonspecific and responds to a wide range of pollutants. The test organism is the bacterium *Vibrio fischerii*, which emits bioluminescence. A variety of pollutants can have an adverse effect on the bacterium, and this leads to a reduction in bioluminescence. The degree of reduction of bioluminescence provides a measure of toxic effect. It is sensitive to a range of pollutants, but its value is limited because it lacks specificity.

The Ames test has been widely used because it is able to detect mutagens. Apart from its usefulness in environmental monitoring, it has been used to test for the mutagenic properties of industrial chemicals. The organisms used in the Ames test are histidine-dependent strains of the bacterium *Salmonella typhimurium*, i.e., strains of the organism that have a requirement for the presence of the amino acid histidine in the medium in which they grow. When these strains are exposed to certain mutagens, they undergo mutation and the mutant forms do not have histidine dependency; i.e., they will grow in media that do not contain histidine. The number of mutant cells produced after treatment with the chemical can be quantified—and the extent to which mutant forms of the bacterium are generated after treatment with a chemical gives a measure of its mutagenicity.

A valuable feature of the Ames test is that it includes a metabolic activating system containing oxidative enzymes extracted from mammalian liver. Many potent carcinogens, including some of the aromatic hydrocarbons in cigarette smoke, are not mutagens in themselves—but they are converted into highly reactive and unstable metabolites within mammals—and it is these metabolites that damage DNA and initiate cancer. The oxidative enzyme system can form these reactive metabolites within the test system. So, the Ames test not only detects substances that are mutagenic in themselves, but also detects mutagenic metabolites that are generated within the test system.

The Calux system utilizes hepatoma cells of rodents. By a process of genetic engineering a reporter gene for the enzyme *luciferase* has been incorporated into these cells. When certain polyhalogenated aromatic hydrocarbons (PHAHs), such as dioxins or coplanar polychlorinated biphenyls (PCBs), enter them they interact with a binding site called the Ah receptor, which is present in the cytosol (cell fluid). After this the Ah receptor, together with the bound PHAH, passes to the nucleus, where they cause the reporter gene to send a message to the enzyme luciferase, which is also incorporated into this type of cell. When the enzyme receives this message, it emits light. The quantity of light emitted is proportional to the quantity of PHAH that has initiated this sequence of events. In this way the quantity of certain PHAHs in environmental samples can be measured. The use of this system will be discussed further in Chapter 13.

Fish hepatocyte preparations have been used for environmental monitoring in two distinct ways. First, for the detection of estrogens. Estrogens interact with an estrogen receptor—and this leads to the release of the protein vitellogenin. The quantity of vitellogenin released can be measured and is proportional to the quantity of the estrogen present. The second use is for measuring the *induction* of the enzyme cytochrome P450 1A1 by planar molecules such as coplanar PCBs and PHAHs (see mention of Ah receptor above). Induction involves an increase in the quantity of the enzyme, which can be quantified by radio immunoassay. More will be said about induction later in this chapter.

MODEL ECOSYSTEMS

Another approach to ecotoxicity testing is to measure the effects of chemicals upon model ecosystems. These include microcosms and mesocosms. Mesocosms are usually aquatic systems and include ponds, simulated streams, and enclosures within lakes, estuaries, or coastal waters. Both of these types of model systems permit adequate replication when testing the effects of chemicals on the composition of communities, so that detailed statistical analyses can be performed using adequate controls (Caquet et al. 2000; Walker et al. 2012, chap. 14). Thus, they have an advantage over large-scale field trials where replication is difficult, if not impossible. They also have the advantage that the tests can use ecological endpoints as well as toxicological ones. The main disadvantage is that they are model systems that can be difficult to relate to the real world.

ETHICAL ISSUES

Toxicity tests have raised issues of ethical concern. In particular, there has been much criticism of testing methods that cause suffering to vertebrate animals (Walker 2006). The protesters have ranged from responsible organizations like the European Centre for the Validation of Alternative Methods (ECVAM), Interagency Coordinating Committee Validation for Alternative Methods (ICCVAM), and Fund for the Replacement of Animals in Medical Experiments (FRAME) to militant animal rights organizations, members of which have caused damage and intimidated staff at testing laboratories. There is common ground between these responsible organizations and ecotoxicologists who seek more scientific testing methods. As argued earlier, lethal tests are of limited value in ecotoxicology, and there is a case for replacing them with tests that work to critical sublethal endpoints without causing unnecessary suffering. With advancements in certain areas of biochemical toxicology, it should become easier to extrapolate from the results of in vitro tests to expected outcomes in vivo. The development of sophisticated in vitro ecotoxicity tests could satisfy the aims of both ecotoxicologists and those concerned about animal welfare—it could also, in the long term, be more cost-effective than standard ecotoxicity testing methods using live animals.

SUMMARY

The term *ecotoxicity* here refers to toxic effects that are relevant within the field of ecotoxicology. Ecotoxicity tests are performed on free-living species such as earthworms, beetles, freshwater shrimps, molluscs, trout, locusts, and many others. They are also performed upon laboratory species such as rats, mice, feral pigeons, and Japanese quail, which are used as surrogates for free-living species seen to be at risk from environmental chemicals such as pesticides.

Ecotoxicity testing of some kind is often a necessary preliminary to statutory risk assessment of environmental chemicals, i.e., chemicals produced commercially that will be released into the environment. Risk assessment is intended to establish whether it is environmentally safe to do this. The statutory requirements for ecotoxicity testing and accompanying risk assessment vary considerably between countries. Generally speaking, they are more stringent—and more rigorously enforced by developed countries than by developing ones. In general, there are more stringent requirements for the testing of pesticides and biocides than for the general run of industrial chemicals. Occasionally, if risk assessment of a new pesticide or biocide is inconclusive, a field trial may be carried out to establish the environmental safety, or otherwise, of the product.

Ecotoxicity tests performed on animals may work to either the lethal endpoint or sublethal ones. In recent years more attention has been given to sublethal effects than was formerly the case; this has come with the recognition that sublethal effects, e.g., on reproduction or behavior, may cause population declines. There continues to be interest in the development of biomarker assays that can measure the progress of toxic effects.

Bioassays that utilize cellular preparations are useful for biological monitoring and identifying hot spots in the environment that are highly polluted.

FURTHER READING

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