Invertebrates in Testing of Environmental Chemicals: Are They Alternatives?

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An enlarged interpretation of alternatives in toxicology testing includes the replacement of one animal species with another, preferably a nonmammalian species. This paper reviews the potential of invertebrates in testing environmental chemicals and provides evidence of their usefulness in alternative testing methodologies. The first part of this review addresses the use of invertebrates in laboratory toxicology testing. Problems in extrapolating results obtained in invertebrates to those obtained from vertebrates are noted, suggesting that invertebrates can essentially be used in addition to rather than as replacements for vertebrates in laboratory toxicity tests. However, evaluation of the ecologic impact of environmental chemicals must include defining end points that may frequently differ from those classically used in biomedical research. In this context, alternative approaches using invertebrates may be more pertinent. The second part of the review therefore focuses on the use of invertebrates *in situ* to assess the environmental impact of pollutants. Advantages of invertebrates in ecotoxicologic investigation are presented for their usefulness for seeking mechanistic links between effects occurring at the individual level and consequences for higher levels of biologic organization (e.g., population and community). In the end, it is considered that replacement of vertebrates by invertebrates in ecotoxicity testing is likely to become a reality when basic knowledge of metabolic, physiologic, and developmental patterns in the latter will be sufficient to assess the effect of a given chemical through end points that could be different between invertebrates and vertebrates. — *Environ Health Perspect* 106(Suppl 2):593–611 *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/593-611lagadic/abstract.html*

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Introduction

According to the classical definition given by Russell and Burch (*1*), the term alternative refers to any technique that replaces the use of animals, reduces the need for animals in a particular test, or refines an existing technique to reduce the amount of suffering endured by the animal (*1–3*). An enlarged interpretation of alternative includes the replacement of one animal species with another, particularly if the substituting species is nonmammalian (*3–5*). As such, invertebrates usually raise less societal concern than mammals, birds, or even lower vertebrates such as fish. In situations where microorganisms, cultured cells and tissues, and other *in vitro* methods are unsuitable replacements for animals, invertebrate species have received particular attention. The use of the horseshoe crab (*Limulus polyphemus*) instead of the rabbit for pyrogenicity testing constitutes perhaps the best example of such an alternative using invertebrates, as it has totally replaced the classical rabbit test. This test, based on the use of a lysate of *L. polyphemus* amoebocytes, is simpler, more rapid, and more sensitive than the corresponding vertebrate test (*6*).

During past decades, the development of alternatives to vertebrate testing techniques has focused essentially on biomedical applications, namely toxicologic screening and drug metabolism (*2,3*). In this particular area, invertebrates have played only a minor role (*7*), although they can now be used to replace vertebrates in some particular testing protocols (*3*). Ecologic toxicity testing must define end points that may differ from those classically used in biomedical research. In this context, alternative approaches using invertebrates may be facilitated. For the assessment of effects of environmental chemicals on animals, the greatest homology between species tends to occur at the most fundamental, suborganismal levels of organization, and less so at the level of the organism. In turn, ecologic consequences of environmental contamination are likely to be better evaluated from investigations at individual, population, and community levels. In any of those situations, invertebrates have actual or potential uses. In particular, invertebrate species seem to be useful animal models to link, in a mechanistic way, suborganismal effects of environmental chemicals to changes at population and community levels.

This paper reviews advantages and disadvantages for the use of invertebrates in testing environmental chemicals. The first part of this review addresses the use of invertebrates in laboratory toxicology testing. Problems in extrapolating results obtained in invertebrates to those obtained from vertebrates are noted, especially regarding effects on basic physiologic and biologic processes. The second part focuses on the use of invertebrates *in situ* to assess the environmental impact of pollutants. Advantages of invertebrates in ecotoxicologic investigation are presented for their usefulness for seeking mechanistic links between effects occurring at the individual level and consequences for higher levels of biological organization (e.g., population and community). In laboratory tests or field studies, the actual or potential value of invertebrates as alternatives is evaluated from their ability to replace, complete, or prevent the use of vertebrates.

Invertebrates in Laboratory Toxicology Testing

Invertebrates are being used extensively in laboratory tests for evaluating the toxicity of chemicals. The development of bioassays using invertebrates has been stimulated by both biologic and toxicologic characteristics of these organisms. Biological aspects are related mainly to their maintenance in

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Abbreviations used: CYP, cytochrome P450; HDTA, hydra developmental toxicity assay; SLRLT, sex-linked recessive lethal test; SMART, somatic mutation and recombination assay; TBT, tributyltin.

controlled conditions. Toxicologic interests result primarily from the specificity of invertebrate responses to specific chemical classes. However, target site specificity and associated specific toxicologic responses may limit extrapolation of effects to vertebrates.

Advantages in Using Invertebrates for Risk Assessment

Risk assessment of environmental chemicals includes hazard identification, dose-response assessment, exposure assessment, and risk characterization (*8,9*). Invertebrates have been used for decades in acute and chronic toxicity tests for hazard identification. Various characteristics of invertebrates account for their universal use in toxicity tests.

Maintenance and Handling. Many invertebrate species can be cultured easily under laboratory conditions because they are of small size and display high fecundity and short life span. These characteristics make the simultaneous breeding of various species easier than for vertebrates. Handling of animals is also easy and the number of replicates for each tested concentration or dose may increase, thus improving the statistical significance of test results without a significant increase in testing cost.

The short life span of numerous invertebrate species provides an opportunity to save time and money. Indeed, a short time in invertebrates refers to a few days (from 2 to 4 days), whereas short-term tests in rodents require 7 to 14 days to be completed. The amount of time that laboratory personnel spend observing animals and recording observations is therefore reduced. These factors, and the lower cost of buying and/or breeding invertebrates, yield significant reduction in the overall cost of toxicity testing.

Since the beginning of the 1980s, four tests using individual specimens obtained from cryptobiotic or dormant eggs (cysts) have been proposed—two on marine (*10,11*) and two on freshwater (*12,13*) invertebrates. Of primary interest with these cyst-based toxicity tests is that they eliminate the need for stock culturing of test species. Animals can be hatched synchronously; the young individuals originate from genetically defined stocks and are in the same physiologic condition. Therefore, uncertainty about test animal availability is eliminated, the costs of testing are greatly lowered, and the potential for standardization and precision is significantly enhanced (*14*).

Genetics. Some invertebrate species are parthenogenetic, thereby reducing genetic variability. This is one of the main arguments that led to the widespread use of cladocerans (especially *Daphnia magna* and *Ceriodaphnia dubia*) in toxicity testing (*15,16*). However, genetic heterogeneity between clones of *D. magna* issued from a unique parental clone has been reported by Baird et al. (*17*), who attributed this heterogeneity to mutations.

In some cases, the genetic variability of several strains of the same species is known. This is the case, for example, in insect species for which strains resistant or sensitive to selected pesticides have been isolated (*18–22*). Therefore, the simultaneous use of these strains in toxicity assessment would provide the opportunity to take into account this genetic variability and even to analyze the genetic origin and transmission patterns of interstrain differences in susceptibility.

Ecologic Specificity. Invertebrates occupy key positions in the food webs of aquatic and terrestrial ecosystems, and some species or groups of species (e.g., daphnids) are present throughout a wide range of habitats. The hazards of environmental chemicals, therefore, can be evaluated on a large panel of species with specific ecologic characteristics. Even more accurate ecologic information can be obtained from standardized tests by including environmental matrices in the test systems (*23*). For example, assessment of the hazard of sediment or soil-bound molecules can be performed using filter-feeding, deposit-feeding, or soil-dwelling invertebrates (*24–31*). Standardized testing procedures and recommendations using invertebrates have thus been proposed for evaluating the toxicity of contaminated soils or sediments (*32–41*).

Comparative Sensitivity of Mammals and Invertebrates to Chemicals

Attempts have been made to compare invertebrate responses to toxicants with those of mammals. A good correlation has been established between the median efficient concentration of various chemicals to *D. magna* and the oral median lethal dose of the same compounds in rats. Although the relation between the two sets of data was nonlinear, it suggests that more toxic chemicals could be identified using the *D. magna* toxicity test as a screening test (*3*).

The acute toxic effects of the 50 priority chemicals of the Multicentre Evaluation of *In Vitro* Cytotoxicity program were evaluated using three cyst-based tests (*Artemia salina, Streptocephalus proboscideus,* and *Brachionus calyciflorus*), the *D. magna* test, and the Microtox test (Microbics Corp., Carlsbad, CA), along with an evaluation of various physical properties of these compounds (*42*). Statistical analysis of experimental data demonstrated that *in vitro* tests and rodent tests were better predictors of human toxicity expressed as human acute oral lethal dose compared with physicochemical parameters. Statistical analysis of the data using multivariate partial least square regression showed that the use of a battery of invertebrate toxicity tests was a promising screening tool to predict human acute toxicity.

Earthworms (especially *Eisenia foetida*) are also frequently used in the evaluation of short-term toxicity of environmental chemicals. For *E. foetida* acute toxicity tests, Neuhauser et al. (*43,44*) established a toxicity rating scheme similar to the scheme based on lethality in rodents (Table 1). Roberts and Dorough (*45*) and Neuhauser et al. (*46*) have shown that toxicity rating using earthworm tests gives approximately the same results as the rodent system.

Alternative Tests Using Invertebrates

A survey of scientific literature published between 1992 and spring 1996 reveals that the use of invertebrate alternatives is marginal and mainly concerns developmental toxicity and genotoxicity testing (Table 2).

Developmental Toxicity Testing. The use of invertebrates was the first alternative to classical tests in developmental toxicity (Table 3). An *in vitro* teratogen assay has been developed that uses *Drosophila* embryo cell cultures (*47*). End points selected in

Table 1. Comparison of rat and earthworm toxicity rating systems.

Rating	Designation	Rat LD_{50}, mg/kg	*Eisenia foetida* LC_{50}, µg/cm^2
1	Supertoxic	<5	<1.0
2	Extremely toxic	5–50	1.0–10
3	Very toxic	50–500	10–100
4	Moderately toxic	500–5000	100–1000
5	Relatively nontoxic	>5000	>1000

Abbreviations: LC_{50}, median lethal concentration; LD_{50}, median lethal dose. Data from Neuhauser et al. (*43,44*).

Table 2. Relative frequency of various types of alternatives to live vertebrates in scientific publications.[a]

	Document (journal article or report) publication date					
	1990 (*n*=80)	1991 (*n*=209)	1992 (*n*=563)	1993 (*n*=582)	1994 (*n*=493)	1995 (*n*=405)
Vertebrates						
Higher vertebrates, cell lines	12.5	8.7	9.7	10.8	8.9	8.4
Higher vertebrates, cell cultures	35.0	31.4	31.9	25.4	22.2	34.7
Higher vertebrates, embryos, organs, organ slices	15.0	14.0	14.5	12.9	11.5	10.8
Artificial organs	0	1.4	2.7	2.1	4.3	0.8
Lower vertebrates, cell lines	0	0	0	1.4	0.5	0.3
Lower vertebrates, cell cultures	0	1.0	0.9	0.9	0.7	1.0
Lower vertebrates, embryos, organs, organ slices	2.5	1.9	0.7	0.9	0.2	0.3
Invertebrates	6.25	1.0	0.7	0.5	1.4	1.0
Plants	1.25	0	0.4	1.0	0.2	1.0
Bacteria, fungi, protozoans	5.0	3.9	2.5	3.6	2.2	3.2
Methods	8.75	15.9	18.0	22.5	27.8	14.2
Reviews	11.25	11.1	11.5	9.1	9.1	12.4
Structure–activity relationships	0	4.3	0.5	1.0	1.2	1.6
Miscellaneous	2.5	5.4	6.0	7.9	9.8	10.3

n, total number of references identified for the year noted. [a]Data from the National Library of Medicine/U.S. National Institutes of Health (*354*).

Table 3. Examples of the use of alternative developmental toxicity tests based on invertebrates.

Test organism	Model or criteria	Reference
Dugesia dorotocephala (Planaria)	Regeneration	(*57,58*)
Hydra attenuata (Cnidaria)	Embryonic development	(*51–54,59–61*)
Dictyostelium discoideum (Mollusca)	Embryonic development	(*62*)
Sea urchins (Echinodermata)	Whole organism development	(*63*)
Drosophila melanogaster (Insecta)	Intact and embryonic cells; morphology	(*47–50*)
Acheta domesticus (Insecta)	Embryonic development	(*64*)
Artemia salina (Crustacea)	Disruption of elongation; DNA and proteins levels in nauplia larvae	(*65*) (*66*)

this system to assess the teratogenic potential of any agent (physical or chemical) involve detection of interference with normal muscle and/or neuron differentiation, induction of heat shock (stress) proteins, and inhibition of normal neurotransmitter levels (*48*). Various experiments have shown that this assay can be used as a teratogen screen and in mechanistic studies of abnormal development, gene involvement in teratogenic resistance, and possible role(s) of heat shock proteins in preventing birth defects (*47–50*).

Two of the proposed developmental toxicity prescreen assay systems based on invertebrates use the coelenterate *Hydra attenuata*. One method uses intact body segments of the adult hydra (regeneration assay) and the other uses artificial embryos consisting of reaggregated dissociated terminally differentiated and pluripotent cells of adult hydra (*51,52*). The regeneration assay (using body segments) appeared ineffective for prescreening chemicals for selective developmental toxicity hazard potential, whereas the use of the artificial embryo in the hydra developmental toxicity assay (HDTA) agreed with published vertebrate studies (*52–55*). The HDTA is based on the adult/developmental ratio that expresses the relationship of toxic doses in the adult and offspring (*53*). Although it will probably not replace animal testing, this test has potential in prioritizing the conduction of required mammalian tests (*56*).

Genotoxicity Testing. A number of genotoxicity tests based on *D. melanogaster* have been proposed. These tests are of two types, detecting either somatic (somatic mutation and recombination assay [SMART]) or germinal (sex-linked recessive lethal test [SLRLT]) mutations (*67,68*). SLRLT is the best-validated *Drosophila* assay (*69,70*), but SMART protocols are less time consuming and provide the opportunity to detect a broad range of genetic alterations (*71–75*) using well-known genetic markers (eye color, wing cells, hairs, etc.). However, results frequently depend on the *Drosophila* strain used (*76,77*), especially for compounds that require metabolic activation (*78*). Moreover, results of SLRLT and SMART are sometimes different (*68*). The polytene chromosomes of some insect species (chironomids, *Drosophila*) appear to be promising tools to assess the genotoxic effects of environmental contaminants (*79–82*).

Recently, the micronucleus test has been performed on marine mollusks to evaluate genotoxic effects of pollutants released in the marine environment (*83*). According to Burgeot et al. (*83*), the absence of precise criteria for micronuclei identification in mollusks and possible artifacts due to viral infection are handicaps for application of the micronuclei assay in the marine environment. Another limitation of this assay is the requirement of expensive equipment for observation.

Mussels and earthworms have recently been used to detect DNA single-strand breaks caused by contaminants of marine water and soil, respectively. For this purpose, the comet assay (*84*) has been adapted to isolated cells; coelomyocytes in earthworms (*85*), and digestive gland cells in mussels (*86*).

Pharmacologic Models. Selected organs, tissues, or cells of some invertebrates are being used extensively to elucidate mechanisms involved in drug and environmental chemical toxicity. Among these, nervous structures are frequently used to investigate the effects of neuroactive substances such as insecticides. For example, the study of pyrethroid mode of action was performed using various experimental models including squid giant axon (*87–92*), crayfish giant axon and stretch receptor organ (*93–98*), snail neurones (*99,100*), cockroach nerve cord and giant axon (*88,101–107*), and isolated insect neurons (*108–111*).

Problems in Extrapolating from Invertebrates to Vertebrates

Extrapolation of responses to environmental chemicals from invertebrates to vertebrates (including humans) presents several problems.

Developmental Toxicity. Despite their value in the screening of new chemicals, developmental toxicity tests on invertebrates will not replace mammalian developmental assays. Asexual or parthenogenetic invertebrates are unsuitable for the evaluation of toxicant effects on gametogenesis. Moreover, it is unlikely that the development of one invertebrate species could be used to accurately model vertebrate development, as extrapolation from mammals to

humans is already difficult. This statement may appear to contradict recent advances in developmental biology that have shown that the genetic elements of development are highly conserved, even between invertebrates (e.g., *Drosophila*) and humans (*112–116*). However, most developmental toxicants seem to act at the level of cytoplasmic processes that appear to be as variable between species as the genetic sequences are conserved (*117*). To consider that the development of embryos of all vertebrates and most invertebrates follows the same program of blastula formation and development of ecto-, meso-, and endoderm may thus appear to be an oversimplified view of the apparent unity among animals (*4*). So many differences exist between vertebrates and invertebrates from fecundation to embryogenesis that exact homologies cannot be established. Indeed, in addition to differences in egg structure, composition, and evolution, the most important difference in embryogenesis is probably related to the position of the central nervous system: the nerve cord is in a ventral position in invertebrates but in a dorsal position in vertebrates. Such embryonic differences can be considered an impediment to the substitution of vertebrates by invertebrates in developmental testing (*56*).

Carcinogenicity. On the basis of present knowledge, it seems unrealistic to propose an alternative use of invertebrates for carcinogenicity testing. Indeed, even when tumorlike lesions have been reported in wild invertebrates, mainly shellfish (*118,119*), a clear link with mutagen and carcinogen concentration in tissues has rarely been established. Moreover, the exact succession of events leading to tumor initiation and development is not precisely known, and there is no evidence that these invertebrates may constitute a valuable model of mammal carcinogenesis. So the use of rodent carcinogenicity bioassays should still constitute the best approach to evaluate carcinogenic potential of chemicals for humans (*120*) because of the considerable molecular and cellular similarities in carcinogenic processes among mammals, including rodents and humans (*121–123*), even if some unexplained differences remain (*124*). Furthermore, no invertebrate alternative can be proposed to detect carcinogens that are not mutagens (nongenotoxic carcinogens) (*125*).

Pharmacokinetics and Pharmacodynamics. Observable effects of a chemical in an organism classically result from various events ranging from the processes that control the access and concentration of biologically active compounds (either the parent compound or a mixture of the parent compound and one or more of its metabolites) at sites of action to the series of biophysical, biochemical, cellular, and physiologic changes that result from the interaction between the biologically active compounds and their sites of action (*126–130*). Between-species differences in response to exposure may clearly result from changes in one or more of these phenomena. The concentration of biologically active compounds at sites of action may vary between species and between clones or strains within the same species. These variations may result from qualitative and quantitative differences in penetration, distribution, and metabolism of the toxicant (*131–136*).

Some routes of entry are typical of vertebrates (e.g., lung and skin) and their importance in the penetration of chemicals cannot be assessed using invertebrates. For example, the cuticle of arthropods and the skin of vertebrates are very different in structure and relative permeability to toxicants. Lung, cardiovascular, and kidney lesions cannot be identified in invertebrates that do not have such organ structures. Liver and gallbladder are also specific structures of vertebrates, even if functionally analogous organs may be found in some invertebrates (e.g., digestive gland in mollusks, hepatopancreas in crustaceans, etc.).

Interaction of a particular compound with putative sites of action may be radically different from one species to another because of interspecies variability in site sensitivity and distribution; specific cellular or molecular targets can be less abundant or even absent in some animal models. Moreover, responses resulting from interactions between toxicants and sites of action may vary according to molecular structures and/or metabolic pathways specific to different biologic models. For example, pyrethroid pesticides are much more toxic for arthropods than for mammals because of enhanced metabolism in the latter and differences in target structure and conformation (*137–140*). Molecular interactions between toxicants and sites of action may result in different external expressions of individual toxicity in vertebrates and invertebrates. Thus, some organochlorine pesticides (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene, dieldrin) significantly alter eggshell thickness in some bird species through interaction with calcium metabolism in the shell gland (*141*), whereas calcareous shell formation anomalies have rarely been reported in invertebrates except for some bivalve mollusks (*142*).

Detoxication and Recovery. Qualitative and quantitative interspecific variations in detoxication enzymes account for significant differences in species susceptibility to chemicals, even though some general mechanisms have been conserved through evolution. This variability is already considerable within vertebrates (*143,144*). Cytochrome P450(CYP)-dependent detoxication of chemicals provides a good example of variations among vertebrate and invertebrate species. Molecular forms of CYP classically involved in xenobiotic metabolism in vertebrates such as CYP1A have not been clearly identified in invertebrates (*145*). Catalytic activity toward substrates metabolized by CYP1A is widely expressed in invertebrates (*146–148*). Conversely, isoforms such as CYP6 (*149–159*), CYP9 (*160,161*), and CYP18 (*161*) are more specific for insects. Consequently, many chemicals that induce CYP1A1 in vertebrates elicit responses from other cytochrome forms, especially CYP6A and CYP4 in insects (*151,152,154,156, 159,162–165*).

Significant variability in elimination pathways of chemicals also exists. For example, arthropods may eliminate externally adsorbed toxic compounds such as heavy metals through molting. The anatomy and morphology of internal organs may also significantly affect the distribution and fate of chemicals, e.g., the invertebrate circulatory system is frequently an open system, whereas that of vertebrates is always closed. Urinary and biliary excretion of xenobiotics certainly plays a more significant role in the clearance of toxicants in vertebrates than in invertebrates.

The efficiency of recovery and of homeostatic mechanisms are of particular interest when extrapolating between animal species (*166,167*). Invertebrates are generally more tolerant than vertebrates (especially homeotherm vertebrates) because of lower energy requirements. Sometimes they can also react to exposure through passive protection mechanisms such as quiescence and dormancy. They can also deeply modify their metabolic pathways (shifting to anaerobic metabolism, for example) in response to stress. On the other hand, basal metabolism of vertebrates (especially mammals) is often much more elevated than those of invertebrates, thus enhancing the rapid distribution of toxicants within their body.

Use of Invertebrates for the Evaluation of Ecologic Impact of Chemicals

For decades, invertebrates have been used largely for the monitoring of chemicals in the environment. Their advantages and disadvantages as bioindicators of environmental quality have been extensively reviewed (*168–175*) and will not be further discussed in this paper. Biologic, ecologic, and toxicologic characteristics render invertebrates useful to detect pollutants in specific habitats, mainly through their bioaccumulation potential, and to evaluate individual effects of exposure (diagnostic indicators). In addition, assumptions have been made as to how invertebrates would allow prediction of effects at population and community levels and could therefore be used as early-warning indicators of deterioration or restoration of ecosystem structure and function. In any of these situations, a few examples exist that illustrate the extent to which invertebrate species can successfully be used as alternative animal models to vertebrates.

Biologic and Ecologic Characteristics of Invertebrates for *in Situ* Evaluation of Chemicals

Advantages of invertebrates that have the potential to be used for the evaluation of environmental chemicals are mainly those of sentinel animals (*176–178*). Invertebrate species are the most numerous in the animal kingdom. Many of them, such as mussels, meet the essential characteristics of sentinel animals but for others, specific characteristics may limit their use as sentinels. As compared to vertebrates, the main advantage of invertebrates as sentinel animals for *in situ* evaluation of pollutants is unquestionably their ability to colonize every compartment of the biosphere, developing various strategies for resource exploitation. Hence, by their position at different levels of food webs, invertebrates play functional key roles in ecosystems. The diversity in ecologic characteristics of invertebrates provides the opportunity to evaluate environmental chemical impact on a large panel of species that differ, for example, by their mobility (mobile vs sessile), their food sources and feeding habits (predators, deposit feeders, herbivores, etc.), or their life history.

Distribution of Invertebrate Populations. Though widely distributed as a taxonomic group, only a few invertebrate species (mainly marine species) are common to different continents. This is also true for most vertebrate species. In invertebrates, however, weaker interspecific barriers and the existence of subspecies and strains that often can inbreed may result in a taxonomic continuum throughout large geographic areas but, as discussed below, may further complicate identification of the organisms used for *in situ* (eco)toxicologic investigations.

Most invertebrate species usually occur as large populations, often larger than any vertebrate population, with patchy local distribution. They can therefore be abundant at a particular location where sampling is facilitated. Such large invertebrate populations are sometimes heterogenous in age and/or developmental stage, as several generations may cohabitate at the same time in the same place. For some of those populations that have identifiable generations, changes in population structure can reflect effects of pollutants. Age or generation criteria can be related simply to the relative size of individuals within the population. More reliable indicators of age, such as lipofuscin in crustaceans (*179,180*) or annual growth marks in sedentary or fixed mussels (*181*), can be used to assess a sort of historical impact of chemicals. For other species, however, adults do not survive their offspring, and populations are more homogenous in individual size and age.

Habitat. Invertebrates can be found in a wide range of habitats, even in those where vertebrates are absent. In this case, they frequently represent the only way to biomonitor pollutants and evaluate effects on animal biologic systems. In habitats where vertebrates and invertebrates coexist, the latter usually have more intimate contact with the substrate. For example, soil vertebrates such as moles or shrews do not feed directly on the substrate, whereas earthworms ingest an enormous quantity of soil from which they extract their food and which can also be contaminated by soil pollutants. Thus, bioconcentration factors for cadmium, lead, and zinc in moles are closely related to metal concentrations in earthworms but not in soils (*182*). Similarly, information on the contamination levels of aquatic sediments are likely to be more reliable when directly obtained from burrowing benthic invertebrates than from the bottom-feeding fish that eat them (*30,183*). Another advantage of many invertebrates for habitat-specific indication of chemical contamination and effects is their limited ability to move large distances. Seasonal migrations of a number of vertebrates such as fish or birds impose a limit to the interpretation of the levels and origins of chemical contamination and significance of effects (*184,185*). In water or soil and sediments, invertebrates cannot escape exposure to accidental discharges of pollutants as vertebrates can. Sedentary behavior should probably be considered in a more restricted sense for invertebrates. Fixed, sessile, aquatic organisms indeed reflect an extreme form of sedentary behavior that represents a unique feature of some invertebrates such as cnidaria, barnacles, or certain bivalve mollusks. Programs of *in situ* monitoring of coastal waters have appropriately been based on this specific characteristic. For example, the Mussel Watch is using the common mussel *Mytilus edulis* as a sentinel species (*186–192*). Prosobranch gastropods such as *Nucella lapillus* or *Littorina littorea*, which are merely sedentary animals, are sensitive to tributyltin (TBT) that induces imposex (i.e., the development of male characteristics in female snails), and have been used as bioindicators to investigate the environmental impact of TBT (*193–201*).

Mode of Resource Exploitation. By their modes of feeding, invertebrates can be contaminated by specific routes that do not exist for vertebrates. For example, assessment of the hazard of sediment- or soil-bound molecules can be performed using filter-feeding, deposit-feeding, or soil-dwelling invertebrates. Filter feeding is one of the main reasons for the use of marine and freshwater mussels for biomonitoring waterborne pollutants (*171,188–190*). Among other uses, the mussel *Mytilus galloprovincialis* has proved useful in genotoxic risk assessment surveys in the marine environment (*202*). The mode of contamination of earthworms has raised the idea that coelomyocytes may be a suitable cell model to assess the genotoxic potential of soil chemicals using the comet assay (*85*). Several benthic invertebrates such as annelids or bivalve mollusks extract their food from sediment or soils by specialized organs or behavior. A bioindication system based on communities of nematodes that inhabit soils and aquatic sediments has recently been proposed (*23*). This approach, which has the advantage of being ataxonomic, combines different feeding types of the nematodes and their ability to colonize and persist in varying habitats.

Oligochaete or insect species that live in the sediment and extract their food from water may permit investigation of the behavior and effects of pollutants at the water and sediment interface and evaluation of the toxicity of sediments (*24–31*).

Position in Food Webs. Invertebrates occupy key positions at every level of food webs in aquatic and terrestrial ecosystems. A number of invertebrate species are herbivorous; they can feed on every part or form of plants and algae (leaves, stems, roots, buds, flowers, pollen, nectar, seeds, etc.). They can therefore be contaminated by chemicals that are stored in specific plant tissues. This also accounts for natural toxicants (phytotoxins), and has been proposed as an explanation of the predisposition of many invertebrate species, especially insects, to tolerate a wide array of toxic molecules and to rapidly develop resistance to manmade chemicals (*203*).

Deposit-feeding invertebrates play a key role in the initiation of recycling animal and plant organic material. Arthropods probably provide the largest number of saprotrophic species in decaying plant material; they also play an important role as scavengers.

Predatory behavior is widespread among invertebrates. For example, dragonfly or dytiscids larvae are active predators that are at the upper level of food webs in small bodies of water (*204,205*). These invertebrates can be used to integrate the effects of environmental chemicals on the entire biocenosis, playing the same role as vertebrates in other ecosystems (*206*).

Invertebrates represent an important and sometimes unique food source for many vertebrates. They play an important role in the contamination of predatory vertebrates through biomagnification processes (*30,183*).

Life History. Life stage differences in sensitivity to pollutants indicate that a thorough knowledge of the life history of a species is necessary for a reliable assessment of effects at both individual and population levels (*207*). Juvenile stages of animals, both vertebrates and invertebrates, are far more sensitive to a large variety of pollutants. However, the eggs or larval stages of some invertebrate species are sometimes more resistant than adults because of particular structures such as chorionous membranes or gelatinous mucus. Molting specifically appears as a critical step of the arthropod life cycle and gives rise to sensitive parameters to assess toxic effects of chemicals on individual growth. Toxicants can act either by delaying the molt or by disrupting the molting process, thus sometimes increasing mortality and/or inducing body deformities (*208,209*). Duration of the intermolt period, mortality rate, and frequency of body part abnormalities can be used as individual and population criteria to assess the effects of pollutants. For example, chironomid deformities have been used extensively in a survey of the water quality of the Great Lakes (*210,211*) and other North American lakes and rivers (*212–220*).

For many invertebrate species, generation time is limited to a particular seasonal period. For example, most insects are abundant in spring and summer but populations dramatically decrease in autumn and are virtually absent in winter. This also occurs in most vertebrate species, but population density usually remains sufficient to allow sampling throughout the year. Thus, the effects of seasonal increases in soluble copper could not be assessed through the abundance and distribution of immature stages of any single ephemeroptera species in a small mountain stream, suggesting that heavy metal monitoring would require a series of different species with asynchronous life cycles (*207*). However, some species of aquatic and soil invertebrates do not undergo important population decline at a particular season. For example, mussels, and to some extent earthworms, remain available for continuous *in situ* monitoring of pollutants even though seasonal sensitivity should be taken into account when potential effects must be evaluated.

Toxicologic and Ecotoxicologic Responses of Invertebrates to Chemicals in the Environment

Toxicologic and ecotoxicologic responses of invertebrates certainly have a key role in the overall impact assessment of environmental chemicals. For decades, they commonly have been used in *in situ* case studies as diagnostic indicators of pollutant fate and impact at individual, population, and/or community levels (*170,221–224*). In a number of those situations, invertebrate species cannot be considered alternatives according to the definition of Russell and Burch (*1*) because their use was not directed to replace vertebrates. Invertebrates were used primarily as true sentinel organisms on the basis of abundance, sampling facility, and wide spectrum of ecologic characteristics and sensitivity to chemicals. The use of invertebrates as bioindicators or bioaccumulators was therefore validated before that of vertebrates. More recent investigations have clearly highlighted the interest in using invertebrates to link individual responses with changes in populations or communities, as such correlations will be a great value for rapid, early warning assessment of the environmental impact of chemicals (Figure 1). The use of invertebrates in such a strategy may prevent adverse effects in vertebrates.

Preliminary Methodologic Considerations. INVERTEBRATE SIZE. With the exception of large marine species such as deep sea squid, invertebrates are smaller than most mammals and even the majority of vertebrates. Although reduced body size is an advantage for the use of invertebrates in laboratory testing, it may complicate field sampling and investigations that require a certain amount of tissue.

Sampling methods for invertebrates have greatly improved with increased knowledge of their biology and ecology. Standardized procedures now exist that reduce sampling bias, increase power–cost efficiency, and allow interlaboratory comparisons for sound assessment of chemicals distributed worldwide (*225,226*).

Though reduced biomass is sometimes counterbalanced by individual abundance, this often necessitates pooling several individuals to obtain sound information. Biochemical parameters used as biomarkers offer typical examples of such a strategy. All biomarkers do not suffer similar experimental constraint. Thus, histologic and physiologic biomarkers are classically used in single invertebrate organisms (*227,228*). In contrast, enzyme activities, metabolism products, energy-yielding substrates, or hormones are usually measured in pools of individuals (*229*). This increases the reliability of measurements but in turn may result in smoothing out interindividual variability. However, increased variability of biomarker responses is a feature that has been commonly observed in pollutant-exposed organisms and may constitute in itself a signal of exposure (*230–232*).

Efforts have been made to reduce the number of individual invertebrates used for measurement of toxicologic effects. These have been facilitated by recent and continuous improvements in biochemical and molecular biology techniques. Thus, single-animal tests are now available for field-sampled invertebrates (*233–238*).

INVERTEBRATE TAXONOMY. As mentioned, the precise taxonomic position of many invertebrate species remains unclear. This may complicate intraspecies comparisons of the response to pollutants between animals sampled in distinct sites and also between field-collected and laboratory organisms. For example, the common and globally distributed marine mussel *M. edulis*, which is used extensively in environmental monitoring, is a complex of three

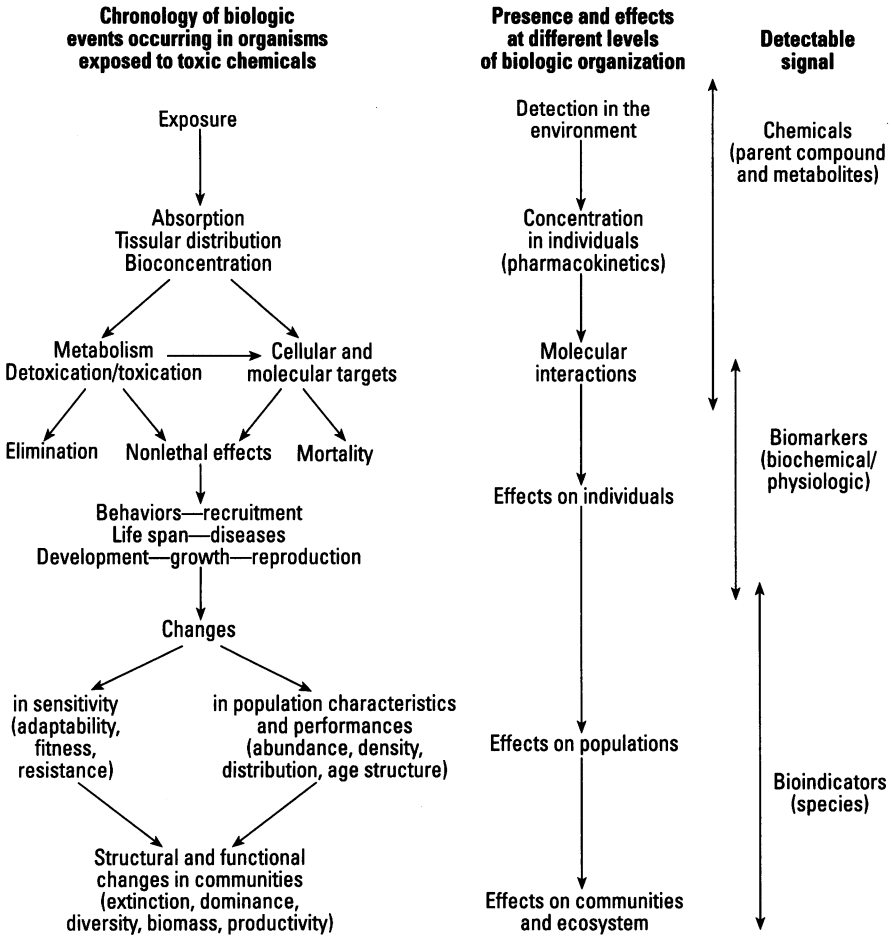

Figure 1. Diagram of potential effects and related measurable signals induced by environmental chemicals at different levels of biologic organization.

species (*239*). This also raises the problem of increasing cost and time for ecotoxicologic investigations on invertebrates. Ataxonomic approaches have been proposed that are based on individual size, biomass, or functional feeding groups (*240,241*). When applied to soil and sediment nematodes, freshwater macroinvertebrates, and lake plankton, such approaches have been considered highly relevant at the ecosystem level (*23,242*). However, contradictory results have also been reported that may be attributed to ecosystem characteristics, species adaptation, or problems with the scale and resolution at which data were collected or analyzed (*243*). In many cases, effects on the biota and biologic processes can be assessed at taxonomic levels higher than species (*244–246*). For example, quantitative and/or qualitative changes in chironomid subfamilies are sufficient to detect impacts of chemicals on aquatic ecosystems (*247–250*). As stated by Ferraro and Cole (*251,252*) and Morrisey (*253*), an appropriate strategy therefore would be to identify organisms at the lowest taxonomic level required to reveal differences among places and/or times.

INBREEDING. In the field, several subspecies or strains of invertebrate species often cohabitate in the same location. Inbreeding occurs naturally in animals that belong to different subspecies and strains, but also within species described as taxonomically distinct. Individual characteristics are therefore likely to vary from one individual to another within natural populations. Such defined physiotypes may display differential susceptibility to chemical exposure, and the relative proportions of each physiotype in the sample would determine reaction of the entire population (*254*). Sampling should therefore be able to image the variability of natural invertebrate populations. Local conditions may also interfere with the ability of individuals to undergo chemical stress, and should also be taken into account for reliable interpopulation comparisons. Differential susceptibility can also result from genetic differences among individuals (*207,232*). Changes in genetic characteristics have been investigated extensively in relation to the development of insecticide resistance in insects and mites (*255,256*). Change in gene frequency is a common mechanism of resistance in aphids (*257–259*) and mosquitoes (*260–264*). Because of their reproductive strategy and extremely fast generation time, such resistance mechanisms are more likely to develop in insects than in vertebrates. Increased tolerance and acquired resistance may thus impede the use of invertebrate populations for long-term monitoring of chronic discharge of chemicals in the environment (*265–267*).

Use of Individual Invertebrates for Impact Assessment of Environmental Chemicals. MEASURED PARAMETERS. Indices based on presence/absence and abundance of invertebrate species have been developed largely through animal bioindication approaches and address responses at the community level (*169,268–270*). However, they only give an instantaneous picture of the state of an ecosystem based on changes in species diversity and richness as ecologic conditions change. In particular, such nonspecific macroscopic parameters fail to reveal contamination of individuals and subsequent biochemical and/or physiologic changes that may affect maintenance, growth, and reproduction. Individual contamination and biochemical and physiologic changes can be assessed through chemical analysis and biomarker measurements, respectively. From this point of view, invertebrates do not differ from vertebrates, and it can reasonably be stated that any of those measures can be conducted equally in individuals of both groups.

Analytical procedures that reveal contamination of animals by environmental chemicals are very similar, whatever the species, developmental stage, or tissue considered. Parameters derived from chemical analysis, such as the bioconcentration factor or half-life, can be compared between vertebrates and invertebrates, and intercalibration experiments can be performed for selected chemicals that could prevent the use of vertebrate species for further monitoring of those or related products.

Most of the biochemical and physiologic parameters—the so-called biomarkers—that have been identified in vertebrates can potentially be measured in invertebrates, the only exceptions being biomolecules, structures, or processes that have no equivalents in the latter (e.g., thyroid hormones, endoskeletal structures, kidney or lung

functions, etc.). However, essential biologic molecules and processes are highly conserved throughout the animal kingdom. For example, no immunoglobulins have been found in invertebrates, but functionally analogous proteins, mostly agglutinins, have been identified (*271*). Another typical example concerns steroid hormones; despite the vast evolutionary distance between arthropods and vertebrates, vertebrate-type steroids and peptide hormonelike substances have been identified in insects (*272*), and the molecular mechanisms by which steroids act to regulate genes appear to be conserved (*273*). So methodologic and functional homologies allow common interpretations of direct individual effects of exposure. This is also true when the molecular basis of invertebrate responses are not fully understood. For example, none of the molecular mechanisms of biotransformation enzyme induction by chlorinated pollutants reported to occur in vertebrates have been identified so far in invertebrates in which those enzymes are also inducible. The aryl hydrocarbon receptor that mediates CYP4501A induction in vertebrates may even be lacking in many species of invertebrates (*274,275*). Another example concerns the correspondence that can be tentatively established between eggshell thinning in birds (widely used to assess environmental impact of chemicals) (*141,276*) and shell thickening in oysters (*Crassostrea gigas*) exposed to TBT (*277–282*). Advances in the knowledge of similarities in the molecular basis of toxicant action tend to show that the mechanisms of chemical toxicity are largely identical in humans and animals (*3*). This suggests that highly sensitive molecular techniques could be used in either animal species. However, the expression and significance of molecular and biochemical parameters at the individual level may differ when measured in invertebrates or vertebrates.

SIGNIFICANCE OF PARAMETERS. Depending on the invertebrate model, parameters derived from chemical analysis may indicate contamination of particular compartments of ecosystems. As discussed above, the large variety of habitats, modes of resource exploitation, behaviors, and functional roles in trophic webs appear to be advantages for using invertebrates to follow the distribution and storage of chemicals in every part of the environment. For one given model species, the environmental significance of chemicals found in field-sampled individuals closely depends on those biologic and ecologic characteristics. Specificity of habitat and trophic position of invertebrate species confers a spatial and functional discrimination power to the indication of chemical contamination of ecosystems.

Toxicologic significance of biomarkers in invertebrates depends on metabolic and physiologic specificities. A fundamental principle of toxicology is that adverse effects caused by chemicals are generally the same in higher animals and in humans (*3*). Also, most metabolic processes are very similar in vertebrates and invertebrates. However, discrepancies exist in the structural and functional expression at the individual level. Such discrepancies certainly are more important between vertebrates and invertebrates than within vertebrates. For example, dysfunction of CYP monooxygenases can affect the metabolism of ecdysteroids and other hormones involved in the molting process in arthropods (*283–290*). Obviously, the same processes, e.g., changes in CYP activity, do not result in similar effects in vertebrates. Such typical individual expression of changes at molecular and biochemical levels that do not exist in vertebrates can easily be identified in invertebrates.

Advantages of Invertebrates for the Assessment of Impact at Population and/or Community Levels. Biochemical measurements are useful in monitoring for effects before they reach population or community levels (*291,292*). However, predicting population or community effects from individual responses to pollutant exposure is difficult to achieve in the natural environment. The complexity of ecosystems requires identification of key species that play critical roles in various communities, the keystone species concept (*293*), and assessment of their responses to the main pollutant classes (*207*). As mentioned above, invertebrate species play key roles in a variety of ecosystems. In a number of situations they even assume ecologic functions that cannot be fulfilled by vertebrates. Invertebrate species have therefore received particular attention in attempts to correlate biochemical responses of individual invertebrates with changes at population and community levels.

Population end points usually have less diagnostic value for particular chemicals than organism-level end points, but they support more accurate predictive assessment of field changes and hence have better ecosystem-level significance (*294–297*).

Generic population end points are listed below [Suter and Donker (*295*) and Suter (*296*)].

Assessment end points:

- No kills: incidents in which large numbers of organisms are killed are generally recognized as undesirable.
- No significant reductions in productivity: this end point is most appropriate to populations of resource species that are harvested.
- No significant reductions in abundance: this end point is more appropriate to nonresource species; it is easier to measure than productivity and is more apparent to nonconsumptive users such as bird watchers and hikers.
- No significant reduction in population range: this end point is useful for regional scale assessments in which range reductions are readily related to the geographic distribution of habitats, disturbances, and contaminant sources.
- No extinctions: this is particularly relevant to assessment of rare or endangered species.
- No significant loss of genetic diversity: this end point is associated with the ability of a population to resist and recover from perturbations.
- No significant loss of population quality: quality is a rather broad term that includes contamination of a population of human food species and high frequencies of tumors, lesions, and deformities that make organisms repellent.

Measurement end points:

- Age-specific mortality
- Fecundity
- Growth

Identical population end points can be identified in vertebrates and invertebrates; the main advantage of the latter is that most of those end points are reached more rapidly because of enhanced growth and reproduction rates. As compared to vertebrates, life-cycle processes of many invertebrate species are accelerated, so that potential adverse effects of environmental changes may become apparent more quickly. Invertebrate population changes are therefore of high value for predictive assessment of the impact of environmental stress, including chemical exposure. Some vertebrate species also have high growth and reproduction rates. For example, rodents and ovoviviparous fish such as Poeciliidae have several generations per year (*298,299*); the number of litters per reproductive season depends mainly on environmental conditions, especially food availability. Among invertebrates, fast reproduction is classically observed in insects such as aphids, mosquitoes, and chironomids, and in cladoceran crustaceans

such as waterfleas, which are able to produce a new generation every 2 or 3 weeks.

To link, in a mechanistic way, individual responses assessed through biomarker measurements to changes at population and community levels is probably one of the most important initial steps for the definition of early-warning indicators of the environmental impact of chemicals (*300,301*). Invertebrate species are particularly suitable for such investigations, as organismal changes can rapidly affect the population (*302–304*). This is not the case for fish or mammal species traditionally used in biomonitoring programs. Recent studies on aquatic invertebrates have provided evidence to support the existence of causal links between organismal responses and changes at population or community levels.

An increasing number of environmental chemicals affect endocrine control of reproduction in animals. First recognized during the 40-year-long investigation of reproductive failure in birds exposed to chlorinated pesticides (*276*), effects on sexual differentiation and reproduction have now been identified in several species of fish, amphibians, reptiles, and rodents (*305–310*) for many different chemicals (pesticides, industrial effluents, and wastes) that also may threaten human health (*311,312*). These endocrine-disrupting chemicals may often affect individual reproductive capacity without affecting survival and growth, as measured from subchronic testing (*313*). In addition, they may elicit effects on the developing embryos that are not manifested until the mature organism enters its reproductive stage (*313*). Animal species with short life cycles may prove to be useful models to provide a more holistic hazard assessment for endocrine-disruptor chemicals (*312*). Some species of aquatic invertebrates have been identified as target animals for endocrine-disrupting pollutants. Comparative studies on the effects of chemicals on reproductive capacity and steroid metabolism in *D. magna* have shown that short-term exposure to toxicants that impair reproduction also affects steroid metabolism (*313–315*). Changes in steroid metabolism, which may result from dysfunction of biotransformation enzymes, can therefore be considered an early-warning indicator of reproductive toxicity. This is further supported by extensive investigations of the effects of TBT on the reproductive system and some other metabolic processes of many marine mollusk species (*316,317*). TBT, an organotin compound used for its antifouling properties in paints on boats, induces the development of male sexual characteristics in female mollusks. This phenomenon, known as imposex, was first identified in *Nassarius obsoletus* (*318*) and has been reported in many other marine organisms with TBT exposure (*193–201,316,317, 319,320*). The dogwhelk *N. lapillus* is highly susceptible to TBT and is widely used for investigating its effects at subcellular, individual, and population levels (*197*). Imposex in female dogwhelks may result from accumulation of testosterone as a consequence of the inhibition of CYP-dependent aromatase, which converts testosterone to 17β-estradiol (*197,321–323*). Because the use of TBT in antifouling paints has been restricted since 1982 in France and since 1987 throughout Europe, North America, Australia, and Japan (*316,317*), the degree of imposex in some dogwhelk populations has decreased (*324*), but the process of recovery of all affected populations and communities is likely to be slow (*197,316,317*). This case study demonstrates that reproductive toxicity can conceivably be predicted from measurements of suborganismal or individual parameters that are potent early warning indicators of pollution by endocrine-disrupting chemicals. The opportunity to detect effects relevant to endocrine disruption from acute and chronic reproduction tests in aquatic and terrestrial invertebrates has recently been considered in ecologic test guidelines for industrial chemicals and pesticides (*312*).

Mechanistic linkage between effects at different levels of biologic organization has also been achieved using the freshwater amphipod *Gammarus pulex* (*325*). Physiologic energetics assessed through energy allocation to growth and reproduction have been used successfully to predict the concentrations of pollutants that impair growth and reproduction as well as the magnitude of the impairment. Reduced scope for growth correlates with decreased reproductive patterns through reduced offspring size and brood viability (*326,327*). Energy reallocation between maintenance, growth, and reproduction has also been reported for *Asellus aquaticus* (*328*) and *Cambarus robustus* (*329*) exposed to acid effluents containing heavy metals. Changes in physiologic energetics linked to community function for *G. pulex* may be indicative of changes in community structure (*325*). Stress-induced reductions in *G. pulex* feeding rate correlate with reductions in the rate of incorporation of leaf organic material into freshwater food webs (*327*). Field trials further indicated that between-site differences in *G. pulex* feeding rate correlate with differences in community structure, but this correlation did not result from causal relationships between *G. pulex* energetics and community structure (*325*).

For many invertebrate species, correlations have been established between changes at different levels of biologic organization, increasingly supporting their usefulness as early warning indicators of chemical-induced stress in ecosystems.

Conclusion

The interest in alternatives in toxicology has arisen in part because of a concern for animal welfare. In this context, the use of invertebrates raises considerably less societal concern than the use of vertebrates. This is confirmed in legislation, as invertebrates are rarely included in animal welfare improvement laws (bees and ladybugs are only exceptions). Therefore, invertebrates can be used extensively in laboratory testing without any heavy legislative pressure and with only minor emotional consequences (if any) in the public. However, this lack of concern for most invertebrate species results in decreased awareness of effects of chemicals on them. Thus, if evidence shows contamination of an ecosystem, efficient decisions will be made much more rapidly if higher vertebrates (marine mammals, birds, etc.) are threatened, rather than lower vertebrates or invertebrates. Indeed, the vision of one suffering dolphin or rabbit yields much more conscious awareness in the public than thousands of dead insects or crustaceans (except for edible and commercial species). For example, hundreds or thousands of birds can be killed by the same oil spill that may cause the death of several millions of invertebrates or plants, but only the former are shown on television or in newspapers, even though ecosystem productivity mostly arises from lower food web levels of ecosystems.

Yet there is an increasing body of evidence to support the significant role of invertebrates in assessing impact of environmental contaminants on ecosystems. Moreover, large sets of data show that correlations or even causal links can be established between organism-, population-, and perhaps community-level responses using invertebrates. In this particular approach, the use of artificial reconstituted biotopes such as mesocosms for ecosystem scale testing seems to provide the opportunity to significantly improve our knowledge on causal relationships between

responses observed at various levels of biologic organizations (*248,330–332*). Using such systems, the effects of chemicals on individuals, population dynamics, and community structure can easily be followed simultaneously in invertebrates sampled in specific habitats. For such purposes, many calibrated individuals of selected invertebrate species, subspecies, or strains must be maintained under conditions that would be stressful for most vertebrates. In mesocosms or field-scale testing, macroinvertebrates have been widely used for experimental assessment of both risk and impact of chemicals (*249,250,333–351*).

In spite of a global agreement on the usefulness of the integration of laboratory bioassays, ecosystem-level testing, and field investigations (*31,206,331,352,353*), risk assessment of environmental chemicals is still based mainly on laboratory testing procedures. It seems that from a toxicologic point of view, invertebrates can sometimes be used in addition to, but rarely as replacements for, vertebrates. Their use as true alternatives suffers limitations arising from species specificity of individual responses due mainly to differences in metabolism, physiology, and anatomy. Does this really matter when the goal of investigations on invertebrates is to detect pollutants and characterize potential effects on animal biologic systems? To answer this question is far from a straightforward task. Under identical conditions of exposure to a chemical and assuming that similar molecular sites can be targeted, animal responses will depend on many different factors (e.g., individual sensitivity, penetration and tissue distribution of molecules, or metabolic activity). This can be further complicated by environmental factors that have more pronounced effects on poikilotherms (invertebrates and lower vertebrates) than on homeotherms (birds and mammals). Thus, the same chemical may elicit a response in vertebrate species but show no external signs of exposure in invertebrates. Fortunately, because of common molecular mechanisms of toxicologic action, most environmental chemicals affect both vertebrate and invertebrate species. Invertebrates may even exhibit a higher sensitivity to chemicals, especially pesticides, that have no apparent effects on vertebrates. Structural and functional expression of the individual impact of chemicals are often different, but such end points can easily be characterized in invertebrates. For any given chemical, when response in invertebrates could be correlated to that of vertebrates, differences in the individual expression of those effects (individual end points) do not matter, and invertebrates could be substituted for vertebrates in environmental risk and impact assessment. In this context, invertebrates are most useful because some of their reproductive and developmental traits appear as essential conditions for early warning assessment of actual or potential impact at population and/or community level.

Screening tests are the most developed and are likely to remain the major focus of *in vitro* toxicology. However, mechanistic studies probably will become increasingly more important, both in toxicologic evaluation and for risk assessment, because they are not only preferable but necessary to provide a better understanding of chemical–biologic interactions and the consequences of those interactions (*2*). In such a context, invertebrates probably will not replace vertebrates in the assessment of risk of environmental chemicals as long as mechanistic correspondence of the effects of chemicals on critical steps is lacking. In other words, substitution of vertebrates by invertebrates in toxicity testing is likely to become a reality when basic knowledge of metabolic, physiologic, and developmental patterns in the latter will be sufficient to assess the effect of a given chemical through end points that could be different between invertebrates and vertebrates. Comparisons of the effects of chemicals between vertebrates and invertebrates should therefore be promoted. In this process, as already stated by National Research Council (*4*), the necessary verification of experimental results through interspecies cross-reference studies will still require the use of some mammals for establishing and validating invertebrate-based model systems.

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