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Rapid Tests for Community-Level Risk Assessments in Ecotoxicology

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Synonyms

Screening tests; range-finding tests

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Glossary

Accuracy The closeness of an estimate of a parameter to the real value of this parameter.

Precision The repeatability of an estimate of a parameter.

Up-down test Single-species toxicity test where individual organisms are sequentially exposed (in contrast to concurrently exposed in conventional toxicity tests). The results of individuals exposed previously are used to select exposure concentrations in subsequent tests (with new individuals). This results in the exposure concentration to move up and down between the sequential exposure of individual test organisms.

Abbreviations

LC50 Lethal concentration for 50% of a test population
SPEAR SPEcies At Risk
SSD Species sensitivity distributions

Definition

Rapid tests are toxicity tests which are designed to determine the approximate sensitivity of a range of species sampled from specific communities of interest.

Rapid tests obtain approximate estimates of the sensitivity of many species-chemical combinations in a relatively short period of time. The aim of rapid testing is to collect a statistical sample of species sensitivities from particular communities. This sample is useful for risk and hazard assessment at the community level.

Rapid tests involve single-species tests but with multiple species tested concurrently. These tests use fewer resources, relative to conventional tests per species-chemical combination tested. The term "rapid" does not refer to the duration of exposure to a chemical but rather to time (and other resources) required per species-chemical combination tested. The main savings are in the number of individuals tested per species and in holding multiple species in the same test chamber but housed separately in baskets. Rapid tests involve some loss in precision (or repeatability), relative to conventional tests. However, by being able to quickly and less expensively test many species from specific communities of interest, rapid tests should result in better estimations of risk of chemicals to ecological communities than is possible with conventional tests.

Rapid tests have been developed for aquatic invertebrates, but the principles of obtaining approximate estimates of the sensitivity of a sample of species from specific environments should be extendable to other groups of organisms.

Background

Single-species toxicity testing determines the sensitivity of a species to chemicals. By conducting such tests with the same species with different chemicals, the relative toxicity of the chemicals can be determined. It is, however, well known that there is variation in the results of the identical test involving the same chemical and test species performed multiple times both within and between laboratories (Baird et al. 1989; Moore et al. 2000; Warren-Hicks et al. 2000). This variability confounds comparisons between different tests as it is uncertain whether any apparent difference is due to a real difference in toxicity, natural variation in tolerance, or variation between laboratory conditions. A high level of precision has the benefit of allowing test results to be directly compared without concern for inter-test and interlaboratory variability. In many applications of ecotoxicology, precision of single-species tests is paramount. For example, the toxicity of the various components of an effluent stream may be compared with single-species tests to trace the cause of toxicity. In this case, high precision is required so that comparisons can be made.

Ecotoxicologists have adopted various strategies to reduce inter- and intra-laboratory variation of test results. These strategies included a preference for the use of asexual reproducing species (e.g., daphnids) from which genetically identical individuals could be obtained and the development of standardized test protocols (Baird et al. 1989). These protocols specify how a valid test is conducted and define endpoints and the minimum level of replication, number of treatments, etc.

Increasing precision is desirable; however, it is not cost-free. In general, methods which increase precision are more resource and time intensive than less precise methods. Thus, for a fixed set of resources, the higher the precision of tests, the fewer number species-chemical combinations can be tested. A desire to increase precision also leads to favoring a limited range of test species for which there exist biological and autecological knowledge and which are available in large numbers, inexpensive to obtain, and have low-control mortality. Additionally, as the majority of ecotoxicologists are based in North America and Western Europe, more protocols have been developed for species from these regions than elsewhere in the world. Thus, the species that have been widely tested are in no way a reflection of the community composition of species in nature.

In order to assess hazard and risk to ecological communities, several ecotoxicologists independently began combining ecotoxicological data from multiple species into species sensitivity distributions (SSDs) in the late 1970s and 1980s (see Posthuma et al. 2001). SSDs are cumulative distributions of the sensitivity of different species to a chemical. The sensitivity data are conventionally taken from the ecotoxicological literature and/or global databases such as the US EPA aquatic tox database (http://cfpub.epa.gov/ecotox/) and not from species specifically selected to represent any known community (Forbes and Calow 2002). The data points in SSDs are then treated as samples of a statistical population of species sensitivities from which it is possible to estimate the concentration of the chemical

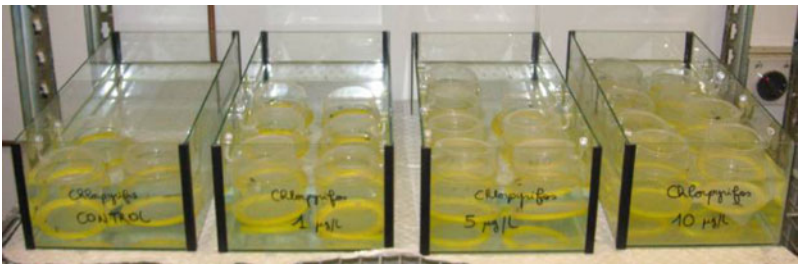
of interest that will protect p% of species. However, such estimation requires several assumptions (Forbes and Calow 2002). Critical in these assumptions is that the sample of species from which there exists sensitivity data is (a) sufficiently large and (b) is drawn from a random sample of species from the communities for which statistical inference is to take place.

Under the SSD paradigm, the precise testing of a few species – appropriate for comparing the results of single species – is highly questionable (Kefford et al. 2005a). This is due to the fact that for SSDs, the relevant variability is that between species, whereas that within species variation is ignored. Indeed, when the same species is tested on the same chemical but at different temperatures, pH, salinity, feeding regime, competitive pressure, UV radiation, etc., toxicity is affected. These (and many other) factors vary widely in natural environments. What is the point of determining the precise sensitivity of species under one standard set of conditions, when these conditions vary widely in nature?

For SSDs, the relevant level of replication is the species, whereas the variation in sensitivity within species is ignored. Rapid toxicity tests (Kefford et al. 2003, 2005a) are thus based on the logic that for a fixed budget and time, more species can be tested if some *shortcuts* to conventional methods are used. For an SSD, species can be thought of as the primary level of replication, with replication of individuals and treatments considered as being of secondary importance. While there may be some loss of precision, the accuracy of the SSD to represent the relative sensitivity of real communities can be maximized by sampling from real communities and increasing the number of replicate species at the expense of reducing the number of replicate individual organisms and/or treatments.

How to Conduct Rapid Toxicity Tests?

Rapid toxicity tests have been used with freshwater (Kefford et al. 2003, 2005b, 2006; Dunlop et al. 2008) and marine (Kefford unpublished information) invertebrates. Rapid toxicity testing involves (a) the collection of a sample of a number of (replicate) species from specific systems of interest and (b) testing these species using a method which aims only to determine the approximate sensitivity of each species. The species are selected for testing with the aim of covering a representative sample of species from specific communities. It uses a number of "shortcuts" including limited replication (of individuals and treatments), limited pretest acclimation, testing multiple species in the same test vessel concurrently but housed to prevent physical interspecific interaction in baskets (Fig. 1), and accepting right- (e.g., $>$ 5 mg/L) and interval-censored (e.g., 5–10 mg/L) estimates of sensitivity. The (species level) data collected from rapid tests may be in conventional terms inferior, but by sampling (a) the sensitivity of more species and (b) a collection of species from real communities, rapid toxicity tests will result in SSDs representative for real communities. Rapid testing meets several assumptions required by SSDs



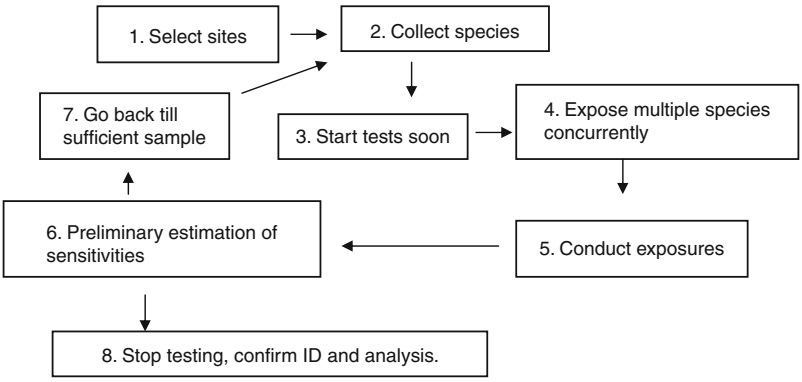
Rapid Tests for Community-Level Risk Assessments in Ecotoxicology, Fig. 1 A typical setup of a rapid test. Each tank contains a different treatment, in which there are *baskets* that house multiple species in the same test solution but prevent them from physically interacting. In this case, up to eight baskets can be placed in each tank (Photo taken by Alizée Rouane)

(Forbes and Calow 2002). Thus, SSDs which use a wider range of data from rapid toxicity tests should provide better estimates of the hazard and risks to communities than do SSDs which only use fewer data that can be generated from conventional tests.

Figure 2 and the following notes set out the general principles by which these tests have been conducted. Further details are given in the documents cited above.

Prospects

To date, the motivation for rapid toxicity testing has been to generate toxicity data for a range of species from which to construct SSDs which are representative of specific communities. Another application of rapid toxicity tests is the development of trait-based biomonitoring indices to detect the effect of chemical contaminants, such as the Species At Risk (SPEAR) biomonitoring indicators (Liess and Von der Ohe 2005; see the "► Species at Risk (SPEAR) Biomonitoring Indicators" entry in this encyclopedia). Trait-based indictors use biological attributes of species (e.g., body size and respiration type), rather than their taxonomy, and are a promising tool for detecting the effect of specific stressors (Menezes et al. 2010). Schäfer et al. (2011) argue that for stressors that act on a physiological level (e.g., chemical toxicants), these physiological traits (e.g., physiological sensitivity measured using laboratory tests) are likely to be critical. Studies of the effect of salinity (Schäfer et al. 2011), hydrocarbons and surfactants (Beketov and Liess 2008), and pesticides (Liess et al. 2008; Schäfer et al. 2011) on stream invertebrates have all found that physiological sensitivity was a key trait for selectively detecting effects of the toxicant. An obstacle for biotic indices incorporating physiological sensitivity is the general lack of species sensitivity data and the time-consuming task of collecting them. Using rapid toxicity tests, however, such data could be collected at rates which are higher than are generally acknowledged (Schäfer et al. 2011). The approximate nature of rapid toxicity test results is not likely to be critical for biotic



Rapid Tests for Community-Level Risk Assessments in Ecotoxicology, Fig. 2 Steps by which rapid tests are conducted. The numbers refer to notes that expand upon the steps below: *1*. Select sites to cover the range of ecological communities where statistical inference on the effect of toxicants is desired. *2*. Collect a variety of species from these sites (Fig. 3) and transport them to the laboratory where you are going to conduct the test. Extended transport of organisms can sometimes cause significant mortality. As much as practical, the transport period should be minimized and the organisms protected from stressful temperature, dissolved oxygen, and rough carrying. For some taxa, you will get high abundances (e.g., > 50), but many will be rarer, including only finding one individual per taxon (Ellingsen and Gray 2002). Collect all taxa you find regardless of how many individuals you find. *3*. Start exposure as soon as possible/practical. Discard any dead or suspect individuals. Do not leave your organisms to acclimate to the laboratory conditions, except for the temperature of the water they are in which should be left to adjust to the test temperature. The condition of the organisms during testing is thus as similar as practical to their condition in nature. *4*. Expose multiple species concurrently (Fig. 1). Use a regression design and do not replicate treatments (Warne and Van Dam 2008). For abundant taxa, place multiple individuals in a control and a number of different concentrations (of the toxicant of interest). For rare taxa, this will not be possible – as for some species only a few individuals will be found. For such taxa, use the up-down method (Sunderam et al. 2004) as modified by Kefford et al. (2003). The up-down test involves the sequential exposure of test individuals rather than concurrent exposure as in conventional toxicity tests. This way, LC50 values can be estimated by exposing about six individuals with a similar level of precision as can be estimated with 60 individuals exposed in conventional tests (Sunderam et al. 2004). The modification to the up-down test is that not always one individual is sequentially exposed; instead, all individuals collected on each collection trip are exposed to one or more (typically 2–3) concentrations, but as with the standard up-down test, the sensitivity is determined over multiple sequential exposures. *5*. Conduct exposures and record mortality and survival. Preserve (and label) all individuals, either on death or at the end of the test, for later identification. *6*. At the completion of the test, make preliminary estimates of sensitivity of each taxon to design the concentrations used with them in future testing. *7*. Go back to step 2, until a sufficient sample of species sensitivity is obtained. *8*. Stop testing, confirm identifications (ID) of the taxa tested, and analyze the data to determine the sensitivity of each taxon tested. Each taxon should be identified to the lowest level practical, which will not always be the species level. Some taxa will be collected on multiple collection trips and from different sites; thus, the sensitivity estimates of these taxa will include considerations of spatial and temporal variation in sensitivity

Rapid Tests for Community-Level Risk Assessments in Ecotoxicology, Fig. 3 Sweep netting to collect marine invertebrates near Casey, Antarctica, for rapid toxicity tests with metals (Photo taken by Paul Goldsworthy). Sorting stream (freshwater) invertebrates in Queensland, Australia, for rapid toxicity tests with salinity (Photo taken by Ben Kefford)

indices. This is because some indices use binary grouping of physiological sensitivity – sensitive or tolerant (Liess and Von der Ohe 2005) – while other trait-based approaches use fuzzy classification to deal with the uncertainty of trait data (e.g., Piscart et al. 2006).

Experimental tests are not necessarily the only source of information on species sensitivity. For example, Hickey et al. (2008) have combined rapid toxicity test results and expert opinions calibrated with Bayesian statistics (Grist et al. 2006). Other potential information on species sensitivity includes statistically derived estimates (Dwyer et al. 1992; Morton et al. 2008) and field estimates of sensitivity (Leung et al. 2005). There is much potential to combine the results of rapid toxicity tests with other sources of information to improve SSDs models.

Rapid toxicity tests have been conducted only on aquatic invertebrates. Although different methods would be involved, it should be highly feasible to design approximate tests for other organisms which allow for rapid toxicity testing of a sample of species from specific communities.

Conclusions

Rapid toxicity tests are a new method which sample the sensitivity of replicate species from specific communities of interest and enable SSDs to be produced which should better reflect the species sensitivities in real ecological communities. Rapid toxicity tests should also be useful for developing trait-based biotic indicators.

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Cross-References

- ▶ Active Biomonitoring
- ▶ Benthic Community Ecotoxicology
- ▶ In Situ Bioassays in Ecotoxicology
- ▶ POCIS Passive Samplers in Combination with Bioassay-Directed Chemical Analyses
- ▶ Species at Risk (SPEAR) Biomonitoring Indicators
- ▶ Test Batteries in Ecotoxicology

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REACH Legislation in Ecotoxicology

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Article Outline

Abbreviations
Definition
Historical Background
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Abbreviations

50-53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
CMRs	Carcinogenic, mutagenic, reprotoxic (reproductive toxicity)
CSA	Chemical safety assessment
CSR	Chemical safety report
ECHA	European chemicals agency
ERA	Environmental risk assessment
EUSES	European union system for the evaluation of substances
IUCLID	International uniform chemical information database
N	Dangerous for the environment
PBT	Persistent, bioaccumulative, toxic
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
QSAR	Quantitative activity relationship
REACH	Registration, evaluation, authorisation and restriction of chemicals
SIEF	Substance information exchange forum
TDG	Technical guidance documents
vPvB	Very persistent, very bioaccumulative

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Definition

REACH, the acronym for **R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emicals, is a European Community Regulation which addresses the production and use of chemical substances and their potential impacts on both human health and the environment. Ecotoxicology is concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems: animals (including humans), plants, and microorganisms, in an integral context (Truhaut 1977). REACH legislation in ecotoxicology aims at evaluating and managing the toxic effects of substances on ecosystems.

Historical Background

Over the last century, the role of chemicals in everyday life has constantly augmented all around the world. As a consequence, the production and the release of chemicals in the environment have also increased. Starting from the early 1960s, concerns about human health and the environment began to arise, and the first EC legislative frameworks to control chemicals were elaborated.

A major chemical industry accident, which occurred in Seveso (Italy) in 1976, accelerated the process: a vapor cloud containing tetrachlorodibenzo-para-dioxin (TCDD better known as dioxin) was released from a reactor of pesticides and herbicides manufacturing plant. TCDD is lethal to man at microgram doses; after the accident where kilograms of the chemical were released, more than 2,000 people were treated for dioxin poisoning. More than 3,300 animals, mainly poultry and rabbit, were found dead within days after the accident, and over 80,000 were killed to prevent TCDD from entering the food chain. After this accident, the first EU directive, "Council Directive 82/501/ECC on major-accident hazards of certain industrial activities (OJ No L 230 of 5 August 1982)," known as Seveso Directive, was adopted in 1982. In 1996, after two major accidents, one in Bhopal, India, 1984, and the second in Basel, Switzerland, 1986, the Seveso Directive was amended twice to broaden its scope.

Initially, only harm due to the intrinsic chemical properties was considered; various directives and regulations were developed to deal with specific classes of chemicals. Subsequently, the legislation dealt with pollution, mainly in water and air. The next step took legislation from hazard assessment, i.e., the determination of the dangerousness of chemicals, to risk management (Van Leeuwen and Vermeire 2007). At the beginning, risk management consisted in the prevention or the reduction of emissions of a chemical to protect workers and consumers only. Later, protection of the environment was also included. Thus, public authorities were at first responsible for undertaking risk assessments of substances, not the manufacturers, importers, or users.

Over the years, as a consequence of the increasing awareness of chemical hazard, some 40 pieces of legislation were developed within Europe. However, differences

among countries, lack of data on both the (eco)toxicity and use of chemicals, and inappropriate allocation of responsibilities on public authorities encouraged the development of regulation (EC) No. 1907/2006 of the European Parliament and of the Council of December 18, 2006, concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), commonly known as REACH regulation. It is described as the most complex legislation in the European Union's history, has taken 7 years to pass (1999–2006), and has entered into force on June 1, 2007.

The main purposes of REACH are to ensure a high level of protection of human health and the environment, to ensure free movement of substances within EU countries, and to stimulate competitiveness and innovation.

Needs

The former EC legislative framework for chemical substances stemmed from many different directives and regulations that developed and evolved over time. Different rules were set for "existing" and "new" chemicals, the latter having to be tested before being placed on the market. A large number of substances were manufactured and placed on the marked for decades, sometimes in substantial amounts without information on the risk they posed to human health and the environment. Therefore, there was a lack of publicly available information on "existing" substances necessary for their assessment and control. In addition, information had only to be provided by manufacturers and importers, but not by users. Hence, information on use and exposure arising from downstream applications were scarce as well. Furthermore, public authorities were responsible for undertaking risk assessments of substances rather than industry which had better knowledge of its chemicals and their specific use. Moreover, "new" chemicals had to be notified and tested starting with volumes of 10 kg per year, thereby hindering innovation by discouraging invention of new substances. These reasons triggered the need for a new unique European policy on chemicals and their safe use.

Main Features

The aims of REACH are to guarantee a high level of human health and environmental protection from the risks posed by chemicals, to promote alternative test methods and the free circulation of substances within the European market, to encourage innovation, and to enhance competitiveness of the European Union chemical industry.

REACH attempts to attain these goals by creating a single system for all chemicals, replacing all previous ones, by closing the knowledge gap for more than 30,000 existing substances and providing information on both their acute and long-term effects, and by inciting use and development of safer substances.

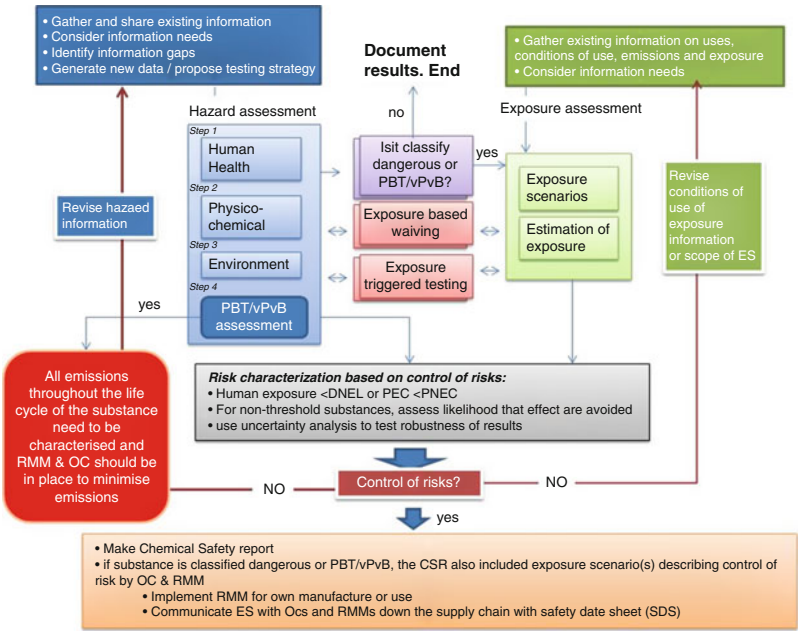
Registration is mandatory for all substances imported or manufactured above 1 t per year, unless they are exempted in Article 2 of the regulation. These exemptions concern chemicals which are considered covered by another legislation equivalent to REACH, e.g., pharmaceuticals, biocides, pesticides, or for which the application of REACH regulation will not lead to improved management of the substance. However, the final REACH text does not, in all cases, establish effective exemptions for these classes of substances. For example, Article 15(2) in REACH declares that "active substances manufactured or imported for use in biocidal products only [. . .] shall be regarded as being registered." Thus, if a chemical is used in biocidal products only, it is considered as registered, not exempted from registration, but if the same chemical is used for both biocidal and non-biocidal products, then it has to be registered.

However, a list of substances exempted from registration is present in REACH Annex IV. To be included in this list, it is necessary that "sufficient information is known about the substances that they are considered to cause minimum risk because of their intrinsic properties."

The most relevant characteristic of REACH legislation with respect to ecotoxicology is the so-called reverse principle. Before REACH, governmental agencies had to prove that a chemical was dangerous for the environment and thus to restrict its use. Often, concerns about the dangers of a substance were raised only after strong evidence of damage became visible. Moreover, since the risk assessment process takes time, the dangerous compound could remain in use without (or with limited) restrictions for a long time even after the first concerns appeared. Last but not least, the costs of risk assessment of a substance were borne by the governmental agency and not by the companies earning profits from its commercialization. With REACH entering into force, the responsibility and the costs of risk assessment are moved from government to industry.

Now, before a substance can be commercialized in Europe, the importer or producer has to prove that its use does not present a risk for the environment. The legislation includes not only new chemicals but also existing ones: their use must be proven safe, or they will be banned from the EU. If alternatives for a dangerous chemical are not possible and if the economical interest outweighs its concerns, a special license can be obtained from the authorities. In this case, the chemical can still be employed but under strict management conditions.

All companies manufacturing or importing chemical substances in the European Union in quantities of one ton or more are required by REACH to register them. As a result, 143,000 chemicals on the European market have been preregistered. Depending on the chemical annual tonnage and its intrinsic properties, a complete registration dossier has to be submitted by November 2010, June 2013, or June 2018 (ECHA guidance documents). For all these chemicals, ecotoxicity is evaluated using a methodology similar to that described in the European Technical Guidance Documents, ex. TDG 2003. In short, for each substance, ecotoxicity is evaluated in six compartments: sewage treatment plant (i.e., toxicity toward microorganisms),



REACH Legislation in Ecotoxicology, Fig. 1 Required steps to verify if risks connected with the use of a chemical are controlled

freshwater, freshwater sediment, marine water, marine sediment, and terrestrial compartment. The number and type of data, i.e., short- or long-term toxicity, is directly linked to the tonnage of the substance marketed or imported to the European Union as it gives an indication of exposure potential. Therefore, the higher the tonnage, the higher the amount of information required. For each compartment, a predicted no effect concentration, or PNEC, a concentration which causes no adverse effect to the environment, is estimated for each compartment. If the PNEC is higher than the predicted environmental concentration (PEC), the concentration one expects to find in the environment, the substance is accepted as environmentally compatible. In general, PNECs are estimated on the basis of assays performed on at least three species. As a consequence, a significant amount of data may be necessary to fulfill the requirements. To limit the number of experiments and since every substance can only be registered once, REACH legislation strongly encourages registrants to share all existing data. This is a second important REACH feature. For this purpose, the IUCLID-5 database has been developed and allows to collect, store, maintain, and exchange relevant data on chemical substances. For the first time, the results of all experiments conducted in industrial laboratories are no longer kept confidential but become available within the boundaries of the registration process. Furthermore, industries refusing to share data must justify their action and can be sanctioned if their reasoning proves inadequate. In addition, if some

endpoints or information are missing, the integrated testing strategy will guide registrants through various alternatives and possibilities, before considering experimental testing. For instance, nonexperimental data derived from QSAR and expert systems can be used to fill data gaps. Read-across and grouping of chemicals having the same structure and properties are also possible.

All data can thus contribute to the general knowledge of a substance, allowing a better understanding of its properties and ecotoxicity. Once all information is gathered, it is necessary to assess if the risks connected to its use are controlled. Generally, it is not possible to register and therefore import/manufacture/use a chemical in the EU if its risks are not controlled. An iterative process guides the registrant in the process of assessing and controlling the risks (Fig. 1). Its final result is reported in the Chemical Safety Report which may be required as part of the registration dossier.

Cross-References

- ▶ Compliance and Enforcement Toxicity Testing
- ▶ Environmental Research Needs (in Ecotoxicology) in Relation to Public Policies
- ▶ Impacts of Land Contaminants on Aquatic Ecosystems
- ▶ Science-Policy Linkages in Ecotoxicology
- ▶ Water Quality Guidelines for the Protection of Aquatic Ecosystems

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Rotifers in Ecotoxicology

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Article Outline

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Glossary

Acanthocephala Parasitic worms characterized by a retractable proboscis with spines and hooks that it uses to be securely attached at their hosts. They have complex life cycle and are considered a sister taxon of Rotifera.

Amictic Adjective that indicates the absence of mixing or exchange. In rotifers, it indicates females with asexual reproduction or a diploid egg which have not been fertilized.

Bdelloids Class of rotifers with trophi ramate and wormlike body. Approximately 360 species with obligate parthenogenetic reproduction and paired ovaries. They live on surfaces (plants or stones) or on the sediments.

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Biomarker Measurements of alterations in biological components, structures, behaviors, or biochemical, physiological, and genetic processes, resulting from sublethal exposure to xenobiotics in an organism.

Carbamate Organic compounds derived from carbamic acid, widely used as insecticides. Their inhibition of acetylcholinesterase is relatively reversible. They have a low environmental persistence.

Corona Ciliated anterior region used in locomotion and food gathering.

Dimorphism Differences or changes in the external appearance of males and females of the same specie (size, shape, color, behavior).

Diploid Organism having two sets of chromosomes, usually one from each parent

Endpoint It is a response which is measured in a living organism during an ecotoxicological test, and it registers a potential adverse effect on individual and population levels.

Fungicide Chemical compounds used to kill or inhibit fungi or fungal spores.

Haploid Organism having only one complete set of chromosomes.

Herbicide Substance used to eliminate unwanted plants.

Illoricate Organisms with a thin and flexible integument (e.g., families Conochilidae, Notommatidae, Proalidae).

Loricate Organisms in which extensive regions of the integument are thicken and rigid (e.g., families Brachionidae and Lecanidae).

Mastax Pharynx of rotifers with powerful muscular wall that contains tiny, calcified, jawlike structures called trophi.

Mictic Adjective that indicates mixing or exchange. In rotifers, it indicates a haploid egg produced by sexual reproduction. Eggs with fertilization produce cysts and amictic females. Unfertilized eggs produce males.

Monogonont Class of rotifers found mostly in freshwater, but also in soil and marine environments. 1,600 species with sexual or asexual reproduction, a single ovary, and trophi with many forms, except fulcrate or ramate. There are species from free-swimming to sessile.

NB Sources of these definitions are from Dodson (2005), Wallace et al. (2006), and Manahan (2003) (see References section of this entry).

Organochlorine Organic compound containing at least one covalently bonded chlorine atom. The organochlorine insecticides enjoyed wide use in agriculture insect control and malaria control programs. Their acute toxicity is moderate, but chronic exposure may be associated with biomagnification and adverse health effects, particularly in the reproductive system.

Organophosphate Esters of phosphoric acid. Type of synthesized insecticides with low persistence and bioaccumulation, but high neurotoxicity. These compounds inhibit acetylcholinesterase which producing accumulation of acetylcholine in cholinergic synapsis. Accumulation of acetylcholine results in continued stimulation of acetylcholine receptors, which can cause numerous effects related to excessive nerve response.

Parthenogenesis Type of asexual reproduction where one or a few diploid eggs are produced and are genetically identical to the mother. Thus, the offspring of a single adult constitute a genetic clone.

Pesticide Substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.

Pseudocoelomate Organisms possessing a "pseudocoel" (false cavity) which is a fully functional body cavity (it contains muscles, nerves, and digestive, reproductive, and protonephridial organs). A body cavity is any fluid-filled space in a multicellular organism.

Trophi Hard parts (jaws) of mastax that articulate in a specific spatial arrangement. It has taxonomic importance for characterizing families, genera, and often species. There are nine types of trophi based on size and shape of functional units (uncus, ramus, manubrium, fulcrum, alula).

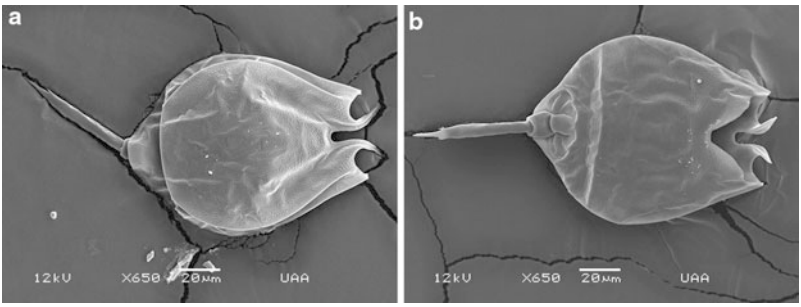
Definition

Rotifers (Rotifera), commonly named wheel animals, refer to a taxonomic group of small aquatic invertebrates employed in aquatic ecotoxicology to measure adverse effects of chemical contaminants and complex environmental samples under both laboratory and field conditions.

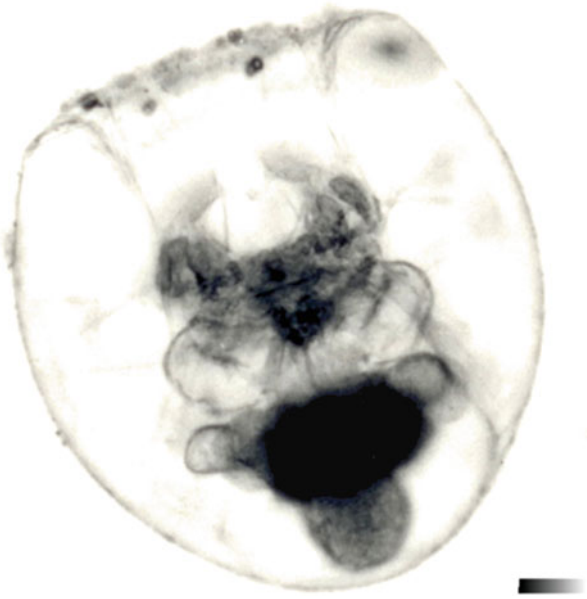
Marked contributions to the field of ecotoxicology provided by studies conducted with rotifers are reported herein. Emphasis is placed on the acute sensitivity of rotifers to three groups of toxicants: metals, organic compounds, and pesticides. The main characteristics of Rotifera, species used for ecotoxicological studies, endpoints, and various other aspects, are also featured. Research prospects for rotifers in ecotoxicology will profit from studies seeking to better understand the relationship between phylogenic distance and species sensitivity to toxicants. Appraising more species of rotifers to determine their sensitivity to a wider variety of toxicants is also to be encouraged. Lastly, recent breakthroughs in environmental genomics suggest that this field of expertise could gain when applied to environmental investigations undertaken with rotifers.

Overview

The phylum Rotifera refers to a taxonomic group of aquatic pseudocoelomate invertebrates characterized by the presence of a ciliate corona and a strong muscular mandible called mastax, formed by a jawlike structure called trophi, and a body wall that might be thickened, in the case of loricate taxa, or not, in the case of illoricates (Wallace et al. 2006). Figure 1 shows *Lecane quadridentata,* a loricate rotifer, and Fig. 2 shows *Asplanchna brightwellii,* an illoricate rotifer.



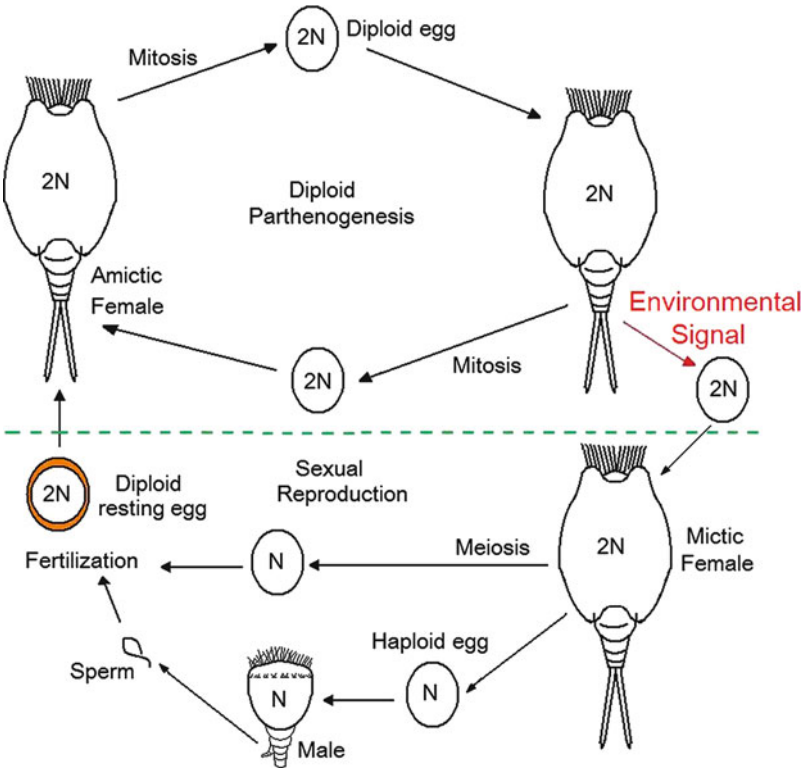
Rotifers in Ecotoxicology, Fig. 1 *Lecane quadridentata* (**a**) Dorsal view. (**b**) Ventral view (Photographs are courtesy of Araceli Adabache and Marcelo Silva-Briano)



Rotifers in Ecotoxicology, Fig. 2 *Asplanchna brightwellii* from Lake Chapala, Mexico. The black bar (*bottom right*) represents a length of 50 µm (Photograph taken by first author)

Taxonomy and Systematics

Although they were considered a phylum for many years (Wallace et al. 2006), the current view is that rotifers do not represent a monophyletic taxon and that they belong to the Syndermata, a phylum that includes the typical rotifers (Monogononta, Bdelloidea, and Seisonaceae), and the acanthocephalans (Min and Park 2009). Rotifers of the Monogononta (by far the most diverse class) reproduce mostly through parthenogenesis by amictic females when the environmental conditions are stable. However, several stimuli such as high population density, chemical compounds, salinity, changes in temperature, and quality or quantity of food can



Rotifers in Ecotoxicology, Fig. 3 Life cycle of a typical monogonont rotifer (Photograph from 3rd author)

induce sexual reproduction with a remarkable dimorphism between females and males in many species (Fig. 3). Sexual reproduction is carried out by mictic (diploid) females that produce haploid eggs. The unfertilized haploid eggs produce males that have short life spans, which fertilize the haploid eggs in the interior of the mictic females producing diploid resting eggs (or cysts). Following a period of latency, and when environmental conditions become favorable, the cysts give origin to amictic females that initiate the parthenogenetic cycle again (Dodson 2005; Wallace et al. 2006). Rotifers of the class Bdelloidea are completely asexual, and no male has ever been found in this class. The class Seisonaceae is currently composed of two parasitic species of obligate sexual reproduction (Dodson 2005).

Rotifers Used in Ecotoxicology

Rotifers have many characteristics that favor their use as test models for ecotoxicological studies: ease of culture, exponential growth, small size, and sensitivity

(Dahms et al. 2011). Many species have been cultured. The importance and relevance of rotifers as members of the zooplankton, and as primary and secondary consumers in many aquatic trophic webs, is well documented (Wallace et al. 2006). In some species of the genus *Brachionus,* production of cysts (that can be stored dry at room temperature) is possible. The ability to produce cysts has allowed the development of toxicity kits, called Rotoxkits, employed for acute/chronic marine and freshwater toxicity testing (http://www.microbiotests.be). Cyst production is an outstanding characteristic that has enabled the development of several toxicity protocols using rotifers. In fact, toxicological protocols using eggs obtained by parthenogenesis have been reported for the production of clonal cultures of rotifers. However, the scale of egg production must be optimized to obtain sufficient amounts of DNA material for the undertaking of molecular toxicology studies.

Unfortunately, the database of toxicants investigated with rotifer species is small in comparison with data for other model organisms like *Daphnia magna*, *Drosophila melanogaster,* or vertebrate species (US Environmental Protection Agency 1997), and the taxonomic status of the two rotifer species most used in ecotoxicology (*Brachionus calyciflorus* and *Brachionus plicatilis*: sees Table 1–3) is doubtful as they belong to species complexes, in contrast with cladocerans where species status is better established.

Protocols for acute testing using rotifers are straightforward and consist in the hatching of neonates from cysts 18 or 28 h before start of test (Snell and Janssen 1995) or from parthenogenetic eggs 24 h before start of test (Pérez-Legaspi and Rico-Martínez 2001). Neonates are then transferred to synthetic fresh- or saltwater medium with the correspondent toxicant or control concentration and incubated at 25 °C for 24 or 48 h typically. After the incubation period, the number of dead rotifers is counted, and the LC50 values are calculated (in general using probit analysis of commercial or freely available software).

Modern toxicity studies using rotifers as model organisms started as early as 1964 (Cairns et al. 1978). However, a significant increase in contributions using rotifers for ecotoxicological studies started in the 1990s (Snell and Janssen 1995). Only three genera and seven species of rotifers were reported by Snell and Janssen (1995) for single-species acute or sublethal toxicity testing: five monogononts (*Brachionus calyciflorus*, *Brachionus patulus*, *B. plicatilis*, *Brachionus rubens*, *Dicranophorus forcipatus*) and two bdelloids (*Philodina acuticornis* and *Philodina roseola*). Globally, nine other genera comprising 28 species now comprise additional taxa available to produce measurement endpoints (e.g., LC50 or EC50 values) with well-defined protocols. These additional taxa include the following: *Adineta vaga* (Orstan 1992); *Anuraeopsis fissa* (Sarma et al. 2007)*; Asplanchna brightwellii* (Enesco et al. 1989)*; Asplanchna girodi* (McDaniel and Snell 1999); *Asplanchna intermedia* (Smith et al. 1988); *Asplanchna sieboldi* (Sarma et al. 1998); *Brachionus angularis* (Gama-Flores et al. 2004)*; Brachionus caudatus* (Daam et al. 2010)*; Brachionus havanaensis* (Juárez-Franco et al. 2007)*; B. patulus* (Sarma et al. 2006), called *Plationus patulus* by McDaniel and Snell (1999);

Rotifers in Ecotoxicology, Table 1 Rotifer sensitivity to metals as indicated by various (sub) lethal measurement endpoints (i.e., LCx, ECx, and/or NOEC values). *F* Freshwater species, *M* Marine or estuarine species

Species	Metal	Endpoint	Sensitivity range (mg/L)	References
Anuraeopsis fissa (F)	Zn	LC50 24 h	0.31	Sarma et al. (2007)
Asplanchna brightwellii(F)	Cu	LC50 24 h	0.045	Enesco et al. (1989)
Brachionus calyciflorus (F)	Ag	LC50 24 h	0.007	Snell et al. (1991b)
	Al	LC50 24 h	3.0	Snell et al. (1991b)
	Cd	LC50 24 h	0.18–1.3	Sarma et al. (2006)
				Snell et al. (1991b)
	Cr	LC50 24 h	8.3	Snell and Moffat (1992)
	Cu	LC50 24 h	0.026	Snell et al. (1991b)
				Kegley et al. (2010)
	Cu	LC50 24 h	0.026–0.031	Gill and Epple (1992)
	Hg	LC50 24 h	0.108–0.60	Kegley et al. (2010)
				Burbank and Snell (1994)
	Mn	LC50 24 h	38.7	Kegley et al. (2010)
	Ni	LC50 24 h	4.0	Snell et al. (1991b)
	Pb	LC50 24 h	4.0	Janssen et al. (1994)
	Zn	LC50 24 h	1.30 – 1.65	Snell et al. (1991b)
				Nelson and Roline (1998)
	Pb	EC20 48 h	0.125	Grosell et al. (2006)
	Cd	NOEC 24 h	0.010	Kotila and Hilsenhoff (1978)
	Hg	NOEC 24 h	0.005–0.02	Kegley et al. (2010)
Brachionus havanaensis (F)	Cd	LC50 24 h	0.41	Juárez-Franco et al. (2007)
	Zn	LC50 24 h	2.27	”
Brachionus macracanthus (F)	Cd	LC50 24 h	0.19	Nandini et al. (2007)
Brachionus patulus (F)	Cd	LC50 24 h	0.09–0.5	Sarma et al. (2006)
				Hawryshyn and Mackay (1979)
	Cu	LC50 24 h	0.20	Hawryshyn and Mackay (1979)
	Pb	LC50 24 h	6.15	García-García et al. (2007)
Brachionus plicatilis (M)	Ag	LC50 24 h	0.120	U.S.EPA (1997)
	Cd	LC50 24 h	39.0	Snell et al. (1991a)
	Cu	LC50 24 h	0.120	Snell and Persoone (1989a)
	Ga	LC50 24 h	11.48	Onikura et al. (2008)
	In	LC50 24 h	24.42	Onikura et al. (2008)
	Pb	LC50 24 h	4.0	Snell et al. (1991a)
	Tl	LC50 24 h	100.0	Onikura et al. (2008)
Brachionus rubens (F)	Cd	LC50 24 h	0.810	Snell and Persoone (1989b)
	Zn	LC50 24 h	0.55	Sarma et al. (2007)

(*continued*)

Rotifers in Ecotoxicology, Table 1 (continued)

Species	Metal	Endpoint	Sensitivity range (mg/L)	References
Lecane hamata (F)	Cd	LC50 48 h	0.23	Pérez-Legaspi and Rico-Martínez (2001)
	Cr	LC50 48 h	4.41	”
	Cu	LC50 48 h	0.23	”
	Hg	LC50 48 h	1.37	”
	Pb	LC50 48 h	0.68	”
	Ti	LC50 48 h	15.6	”
Lecane luna (F)	Cd	LC50 48 h	0.35	Pérez-Legaspi and Rico-Martínez (2001)
	Cr	LC50 48 h	3.26	”
	Cu	LC50 48 h	0.060	”
	Hg	LC50 48 h	0.450	”
	Pb	LC50 48 h	0.140	”
	Ti	LC50 48 h	43.0	”
Lecane quadridentata (F)	Al	LC50 48 h	0.157	Torres-Guzmán et al. (2010)
	Cd	LC50 48 h	0.28	Pérez-Legaspi and Rico-Martínez (2001)
	Cr	LC50 48 h	4.50	”
	Cu	LC50 48 h	0.33	”
	Fe	LC50 48 h	0.53	Torres-Guzmán et al. (2010)
	Hg	LC50 48 h	0.40	Pérez-Legaspi and Rico-Martínez (2001)
	Mn	LC50 48 h	38.6	Mejía-Saavedra et al. (2005)
	Pb	LC50 48 h	3.70	Pérez-Legaspi and Rico-Martínez (2001)
	Zn	LC50 48 h	0.123	Torres-Guzmán et al. (2010)
Philodina acuticornis (F)	Ag	EC50 24 h	15.7	Buikema et al. (1974)
	Cd	EC50 24 h	0.10	”
	Co	EC50 24 h	59.00	”
	Cu	LC50 24 h	0.14–1.00	Cairns et al. (1978)
	Hg	EC50 24 h	2.0	Buikema et al. (1974)
	Ni	EC50 24 h	4.1	”
	Pb	EC50 24 h	47.4–150	Buikema et al. (1974)
				Kegley et al. (2010)
	Zn	EC50 24 h	2.40	Buikema et al. (1974)

Brachionus macracanthus (Nandini et al. 2007); *Brachionus rotundiformis* (Araujo et al. 2001); *Brachionus urceolaris* (Hatakeyama 1986); *Brachionus urceus; Euchlanis dilatata* (McDaniel and Snell 1999), *Euchlanis* sp. (Daam et al. 2010); *Filinia longiseta* (Qin and Dong 2004); *Keratella americana* (Vancil 1976); *Keratella cochlearis* (Liber and Solomon 1994); *Keratella quadrata*

Rotifers in Ecotoxicology, Table 2 Rotifer sensitivity to organic compounds other than pesticides (with inclusion of some data linked to wastewater studies). *F* Freshwater species, *M* Marine or estuarine species

Species	Substance	LC50 (mg/L)	Duration	References
Brachionus calyciflorus (F)	3,4-Dichloroaniline	62.0	24-h	Snell and Janssen (1995)
	Acetone	51.0	”	”
	Benzene	> 1,000	”	”
	Chlorodinitrobenzene	1.3	”	”
	Chloroform	2.0	”	”
	Dichlorophenoxyacetic acid	117.0	”	”
	Diesel fuel	63.0	”	”
	Fenitrothion	6.7	”	”
	Free ammonia[a]	3.21–4.6	”	”
	Hexane	68.0	”	”
	Phenol	> 150	”	”
	Toluene	113.0	”	”
	Tributyltin	0.19	”	”
	Trichlorfon	47.0	”	”
	Xylene	33.0	”	”
Brachionus plicatilis (M)	3,4-Dichloroaniline	57.5	24-h	Snell and Janssen (1995)
	Acetone	75	”	”
	Amlodipine	0.57	”	DellaGreca et al. (2007)
	Amlodipine A1 (pyridine derivative)[a]	38.69	”	”
	Bezafibrate	60.91	”	Isidori et al. (2007)
	Bezafibrate B1[a, b]	70; 109.32, or no effect reported	”	”
	Chloramines	0.02	”	Snell and Janssen (1995)
	Chlorodinitrobenzene	2.0	”	”
	Chloroform	2.4	”	”
	Dexamethasone and its photoproducts[a]	13.20–48.22	”	DellaGreca et al. (2004)
	Dichlorophenoxyacetic acid	598	”	Snell and Janssen (1995)
	Diesel fuel	345	”	”
	Ethanol	36,840	”	”
	Ethylene glycol	91,319	”	”
	Fenofibrate	64.97	”	Isidori et al. (2007)
	Fenofibrate F1[a]	46.29	”	”

(*continued*)

Rotifers in Ecotoxicology, Table 2 (continued)

Species	Substance	LC50 (mg/L)	Duration	References
	Free ammonia	38	”	Snell and Janssen (1995)
	Free chlorine	0.18	30-min	”
	Free NH_3	17.7	24-h	”
	Furosemide[a]	100, no effect	”	Isidori et al. (2006)
Brachionus plicatilis (M)	Furosemide F1 (dimer 2)[a, b]	120, no effect	24 h	Isidori et al. (2006)
	Gemfibrozil	77.30	”	Isidori et al. (2003)
	Gemfibrozil G1[a, b]	200, no effect	”	Isidori et al. (2003)
	Hexane	156	”	Snell and Janssen (1995)
	Methanol	49,680	”	”
	Municipal solid waste landfills in Southern Italy	all samples with acute toxicity	”	Isidori et al. (2003)
	Phenol	> 400	”	Snell and Janssen (1995)
	Prednisolone and its photoproducts[a]	1.43–35.46	”	DellaGreca et al. (2004)
	Sodium dodecyl sulfate[a]	4.42–5.6	”	Snell and Janssen (1995)
	Sodium lauryl sulfate	40.1	”	”
	Tributyltin	0.3	”	”
	Xylene	496	”	”
	DKW[c]: Dyeing waste	2.9	”	Park et al. (2005)
	DKW: Filtration bed	67.7	”	”
	DKW: Food waste	88.5	”	”
	DKW: Industrial	>100	”	”
	DKW: Industrial waste	37.7	”	”
	DKW: Leather	80.9	”	”
	DKW: Livestock waste	95.7	”	”
	DKW: Mixed	>100	”	”
	DKW: Rural	64	”	”
	DKW: Sewage: urban	74.9	”	”
	DKW: Textile waste	>100	”	”
Brachionus plicatilis hepatotomus (M)	Crude oil[a]	0.23; 0.05	24 h; 48 h	Alayo and Iannacone (2002)
	Diesel 2[a]	1.36; 0.13	”	”
	Diesel 6[a]	3.47; 1.01	”	”

(*continued*)

Rotifers in Ecotoxicology, Table 2 (continued)

Species	Substance	LC50 (mg/L)	Duration	References
Brachionus plicatilis rotundiformis (M)	Crude oil[a]	0.13; 0.04	24 h; 48 h	Alayo and Iannacone (2002)
	Diesel 2[a]	0.65; 0.14	”	”
	Diesel 6[a]	4.20; 0.33	”	”
Brachionus rubens (F)	4-Chloroaniline	100	48 h	Snell and Janssen (1995)
	4-Nitrophenol	6.3	”	”
	Phenol	600	”	”
Lecane hamata (F)	Acetone	7,235	48 h	Pérez-Legaspi and Rico-Martínez (2001)
	Benzene	6,975	”	”
	Ethyl acetate	1,324	”	”
	Toluene	236.7	”	”
	Vinyl acetate	331.8	”	”
Lecane luna (F)	Acetone	6,833	48 h	Pérez-Legaspi and Rico-Martínez (2001)
	Benzene	3,762	”	”
	Ethyl acetate	2,606	”	”
	Toluene	277.4	”	”
	Vinyl acetate	303.4	”	”
Lecane quadridentata (F)	Acetone	5,651	48 h	Pérez-Legaspi and Rico-Martínez (2001)
	Benzene	2,834	”	”
	Ethyl acetate	1,600	”	”
	Toluene	191.4	”	”
	Vinyl acetate	320.1	”	”
Philodina acuticornis (F)	Ammonium chloride	1,140	24 h	Snell and Janssen (1995)
	Phenol	382	”	”
Philodina sp. (F)	Chlorine[a]	0.065–0.13	24 h (at different temperatures)	Cairns et al. (1978)
	Cyanide[a]	0.5–250	”	”
	Phenol[a]	331–371	”	”
	Chlorine[a]	0.047–0.1	48 h (at different temperatures)	”
	Cyanide[a]	20–145	”	”
	Phenol[a]	205–300	”	”

[a]Endpoint values obtained under differing experimental conditions (e.g., temperature, salinity, pH)
[b]Photoproducts
[c]% DKW (% dilution Korean sewage)

Rotifers in Ecotoxicology, Table 3 Rotifer sensitivity to pesticides indicated by LC50 values. *F* Freshwater species, *M* Marine or estuarine species, *OP* Organophosphate, *OC* Organochlorine, *P* Pyrethroid, *C* Carbamate, *Fu* Fungicide, *H* Herbicide

Species	Pesticide	Endpoint	Sensitivity range (mg/L)	References
Brachionus angularis (F)	Methyl parathion (OP)	LC50 24 h	0.636	Gama-Flores et al. (2004)
Brachionus calyciflorus (F)	2,3,4,6-Tetrachlorophenol (OC)	LC50 24 h	2.31– > 16	Liber and Solomon (1994)
	3-4-Dichloroaniline (OC)	LC50 24 h	61.47–62.0	Ferrando and Andreu-Moliner (1991); Snell et al. (1991b)
	Chlorpyrifos (OP)	LC50 24 h	11.85–12.0	Ferrando and Andreu-Moliner (1991); Snell et al. (1991b)
	Cypermethrin (P)	LC50 24 h	0.08	Sánchez-Fortún and Barahona (2005)
	Endosulfan (OC)	LC50 24 h	5.15	Fernández-Casalderrey et al. (1991)
	Fenitrothion (OP)	LC50 24 h	6.7	Snell et al. (1991b); Ferrando and Andreu-Moliner (1991)
	Lindane (OC)	LC50 24 h	22.5	Ferrando and Andreu-Moliner (1991)
	Methyl parathion (OP)	LC50 24 h	29.19	Fernández-Casalderrey et al. (1993)
	Pentachlorophenol (OC)	LC50 24 h	0.74–7.66	Snell et al. (1991b); Snell and Moffat (1992); Liber and Solomon (1994); Preston et al. (2001)
	Permethrin (P)	LC50 24 h	0.22	Sánchez-Fortún and Barahona (2005)
	Phenol (Fu)	LC50 24 h	150	Calleja et al. (1994)
	Resmethrin (P)	LC50 24 h	0.04	Sánchez-Fortún and Barahona (2005)
	Thiophanate-methyl (C)	LC50 24 h	5.02	Xi and Hu (2003)
	Trichlorfon (OP)	LC50 24 h	47–51.94	Ferrando and Andreu-Moliner (1991); Snell et al. (1991b)
Brachionus patulus (F)	Methyl parathion (OP)	LC50 24 h	8.8	Sarma et al. (2001)
Brachionus plicatilis (M)	3-4-Dichloroaniline (OC)	LC50 24 h	57.45	Ferrando and Andreu-Moliner (1991)
	Azinphos-methyl (OP)	LC50 24 h	85	Snell and Persoone (1989a); Guzzella et al. (1997)
	Chlorpyrifos (OP)	LC50 24 h	1.7–10.67	Snell and Persoone (1989a); Ferrando and Andreu-Moliner (1991); Guzzella et al. (1997)
	Cypermethrin (P)	LC50 24 h	0.30	Sánchez-Fortún and Barahona (2005)

(*continued*)

Rotifers in Ecotoxicology, Table 3 (continued)

Species	Pesticide	Endpoint	Sensitivity range (mg/L)	References
	Diazinon (OP)	LC50 24 h	26.9–28	Guzzella et al. (1997); Marcial et al. (2005)
	Dimethoate (OP)	LC50 24 h	244	Snell and Persoone (1989a); Guzzella et al. (1997)
	Endosulfan (OC)	LC50 24 h	5.60	Serrano et al. (1986)
	Fenitrothion (OP)	LC50 24 h	8.87–63.7	Ferrando and Andreu-Moliner (1991)
	Fonofos (OP)	LC50 24 h	8.8	Snell and Persoone (1989a); Guzzella et al. (1997)
	Isoprothiolane (OP)	LC50 24 h	64.12	Marcial et al. (2005)
	Lindane (OC)	LC50 24 h	35.89	Marcial et al. (2005)
Brachionus plicatilis (M)	Malathion (OP)	LC50 24 h	59.5–74	Snell and Persoone (1989a); Guzzella et al. (1997)
	Methoprene (OP)	LC50 24 h	31.3	Marcial et al. (2005)
	Methyl parathion (OP)	LC50 24 h	> 67	Guzzella et al. (1997)
	Omethoate (OP)	LC50 24 h	295	Snell and Persoone (1989a); Guzzella et al. (1997)
	Parathion (OP)	LC50 24 h	> 25	Guzzella et al. (1997)
	Pendimethalin 60% (H)	LC50 24 h	132	Kyriakopoulou et al. (2009)
	Pentachlorophenol (OC)	LC50 24 h	1.36	Snell and Persoone (1989a)
	Permethrin (P)	LC50 24 h	0.90	Sánchez-Fortún and Barahona (2005)
	Phenol (Fu)	LC50 24 h	400	Snell et al. (1991a)
	Resmethrin (P)	LC50 24 h	1.28	Sánchez-Fortún and Barahona (2005)
	S-Metolachlor 31.2% + terbuthylazine 18.8% (H)	LC50 24 h	58	Kyriakopoulou et al. (2009)
	Thiophanate-methyl 70% (F)	LC50 24 h	34	Kyriakopoulou et al. (2009)
	Thiram 80% (Fu)	LC50 24 h	0.05	”
	Trichlorfon (OP)	LC50 24 h	274.93	Ferrando and Andreu-Moliner (1991)
Brachionus rubens (F)	Malathion (OP)	LC50 24 h	35.3	Snell and Persoone (1989b)
	NaPCP (OC)	LC50 24 h	0.62	”
	Phenol (Fu)	LC50 24 h	600	Halbach et al. (1983)

(*continued*)

Rotifers in Ecotoxicology, Table 3 (continued)

Species	Pesticide	Endpoint	Sensitivity range (mg/L)	References
Keratella cochlearis (F)	2,3,4,6-Tetrachlorophenol (OC)	LC50 24 h	0.96	Liber and Solomon (1994)
Lecane quadridentata (F)	Carbaryl (C)	LC50 48 h	13.72	Pérez-Legaspi et al. (2010)
	Faena® (H)	LC50 48 h	13.1	Domínguez-Cortinas et al. (2008)
	Glyphosate (H)	LC50 48 h	150	”
	Methyl parathion (OP)	LC50 48 h	9.50	Pérez-Legaspi et al. (2010)
Philodina acuticornis odiosa (F)	Phenol (Fu)	LC50 24 h	142	Hagen et al. (2009)

(Qin and Dong 2004) *Keratella tropica; Lecane closterocerca* (Daam et al. 2010)*; Lecane luna; Lecane hamata* (Pérez-Legaspi and Rico-Martínez 2001)*; L. quadridentata; Lepadella patella* (McDaniel and Snell 1999)*; Philodina acuticornis odiosa* (Hagen et al. 2009)*; and Trichocerca pusilla* (McDaniel and Snell 1999). Assessment endpoints determined in rotifer toxicity studies are varied and have included the following: mortality (with exposure times of 30 min, 24 h, 48 h, or 96 h, as reported in Tables 1, 2, and 3), reproduction inhibition and behavior (see review by Snell and Janssen 1995), enzyme biomarkers (Burbank and Snell 1994; Araujo et al. 2001; Pérez-Legaspi et al. 2002; Pérez-Legaspi and Rico-Martínez 2003; Arias-Almeida and Rico-Martínez 2011), mRNA biomarkers (Cochrane et al. 1994), induction of stress proteins (Wheelock et al. 1999; Kaneko et al. 2002, 2005; Rios-Arana et al. 2005; Suga et al. 2007), and predator–prey interactions (see review by Preston 2003).

Species Sensitivity Distributions

Versteeg et al. (1999) studied species sensitivity distributions in zooplanktonic species for 11 different toxicants among metals, surfactants, and pesticides. Sensitivity distributions were derived from single-species chronic toxicity assays. In all cases, species sensitivities differed by 2–4 orders of magnitude, and the sensitivity of tested species varied considerably among toxicants. Rotifers of the genus *Brachionus* were among the most sensitive to dodecyl sulfate, alkylbenzene sulfonate, and copper, but least sensitive to lindane.

McDaniel and Snell (1999) assessed the sensitivity distributions among nine species of rotifers in response to cadmium and pentachlorophenol (PCP) exposure. Sensitivities differed by two orders of magnitude for both toxicants.

Relative sensitivity among species varied with the toxicant as well as the measurement endpoint (24-h mortality or 30-min in vivo esterase activity). Pérez-Legaspi and Rico-Martínez (2001) compared the sensitivity of 11 different compounds (organics and metals) among three species of the genus *Lecane*: *L. hamata*, *L. luna*, and *L. quadridentata*. The highest interspecies differences in LC50 values (22-fold) were found in the sensitivity to lead.

Studies with Mesocosms and Microcosms

Although mesocosm and microcosm experiments have been performed using rotifers, the latter are often simply included as part of a zooplanktonic assemblage, and little attention is paid to effects that toxicants can have on this particular taxon. There are, however, a few studies that incorporate effects of toxicants to rotifers (Snell and Janssen 1995). In Little Rock Lake, Wisconsin, Gonzalez and Frost (1994) studied the effects of acidification on rotifer species and compared those effects to what they found in laboratory experiments conducted with individual species under different pH regimes. Under food limitation conditions in laboratory experiments, *Keratella cochlearis* displayed reduced survivorship and reproduction, while *Keratella taurocephala* was unaffected. In contrast, acidification of Little Rock Lake resulted in a decrease in food availability for both species. Furthermore, *K. cochlearis* declined in abundance, while *K. taurocephala* increased in abundance due to the reduction of invertebrate predators. Rico-Martínez et al. (1998) studied the natural assemblage of a dam in Mexico that was transferred to a microcosm and then spiked with copper sulfate. *K. cochlearis*, *B. calyciflorus*, and *Platyias quadricornis* were the rotifer species most resistant to copper addition, while *Asplanchna priodonta*, *Lecane bulla*, and *Pompholyx sulcata* were the most sensitive. Addition of copper sulfate drastically reduced zooplankton densities, and recovery of the most resistant species of cladocerans, copepods, and rotifers was only observed after more than 2 weeks. Sugiura (1992) implemented an aquatic microcosm containing a planktonic assemblage that included two rotifers (*Philodina* and *Lepadella*). He added several toxicants in the presence of polypeptone: Cu^{2+}, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), DDT, β-isomer of 1,2,3,4,5,6-hexachlorocyclohexane (β-HCH), and lindane. With a nutrient (polypeptone) at 100–500 ppm and 2,4,5-T at 10–100 ppm, rotifer species were eliminated. No such elimination occurred with copper concentrations up to 0.4 ppm. The population densities were affected by β-HCH at 0.1–3.0 ppm and lindane at 0.01–5.0 ppm in the early stages of the succession, but the population densities became closer to those of the control as the succession advanced. Addition of DDT up to a concentration of 0.5 ppm resulted in small changes in the densities of rotifers.

Koteswari and Ramanibai (2004) investigated the effects of a tannery sewage effluent on a zooplankton assemblage microcosm. They found that the magnitude of changes in the relative abundance of diatoms and rotifers was much greater than that

of green algae, cyanobacteria, copepods, and cladocerans. They also observed that the plankton community response to a toxicant can be nonlinear and that relative abundance and taxonomic composition changes occurred at high concentrations of effluent. Daam et al. (2010) conducted an 8-week microcosm experiment to study the effects of the fungicide carbendazim on a zooplankton assemblage. The genus *Keratella* was the most sensitive among rotifers. Other rotifer taxa (*B. caudatus, B. calyciflorus, L. closterocerca*, *Euchlanis* sp.) were shown to increase in abundance.

Dynamics of Natural Populations

Eutrophication studies conducted with rotifers in the field have been numerous since 1973. These studies found that water-enriching nutrients increased the populations of several species of the genera *Asplanchna*, *Keratella*, and *Trichocerca*. Moreover, *Polyarthra dolichoptera* became scarce due to eutrophication (Wallace et al. 2006). Adverse effects of insecticides and herbicides in rotifers were investigated in experimental ponds, where the most frequently reported response was a change in community structure from dominance by *Daphnia* to dominance by small zooplankters such as rotifers (Hanazato and Kasai 1995; Hanazato 2001). In Canadian ponds, Kreutzweiser et al. (2002) found that 0.70 and 1.75 mg/l of the pesticide azadirachtin produced adverse effects on rotifer communities. Effects of acidification on rotifers that change community structure have also been investigated (Havens 1992; González and Frost 1994). Monteiro et al. (1995) studied metal stress in the Sado River in Portugal. They indicated that *Philodina* sp., and to a lesser degree *Lecane luna*, tolerated high concentrations of Cu, Zn, and Cd.

Miscellaneous Studies Involving Rotifers

A limited number of studies involving rotifers have been reported on nonpoint and point source pollution: municipal solid waste pollution, lotic and lentic systems appraisal, and watershed land use. Park et al. (2005) conducted toxicity testing with *Brachionus plicatilis* to determine LC50 values for Korean wastewaters, reporting acute toxicity from industrial, rural, and urban wastewater (see Table 2). Sarma et al. (2003) showed that Mexico City urban wastewater affects instantaneous growth rate of *Brachionus patulus*. Acute 48-h lethal effect measurements generated with *Lecane quadridentata* on municipal, industrial, and agricultural sites around the city of Aguascalientes, Mexico, indicated that most samples tested were toxic (Santos-Medrano et al. 2007). Isidori et al. (2003) employing *B. plicatilis* in 24-h toxicity tests found that all samples of municipal solid waste landfills in southern Italy expressed acute toxicity.

José de Paggi and Devercelli (2010) examined the influence of watershed land use on microzooplankton around the city of Santa Fe in Argentina. Six rivers and a shallow lake located in rural and urban areas were sampled during 4 weeks.

River microzooplankton abundance and rotifer species assemblages were found to be good indicators of land use. Indeed, species composition was linked to a gradient along conductivity, pH, and chlorophyll a. *Brachionus* spp. were associated with saline waters in rural areas and *Keratella* spp. (except *Keratella tropica*) with urban water bodies.

Bioconcentration and Structure-Activity Studies

Studies aiming to determine bioconcentration factors for chemicals and QSARs (quantitative structure-activity relationships) with rotifers are limited. Bioconcentration factors (BCF) have only been reported with three species of rotifers. BFCs were calculated under laboratory experimental conditions with *Brachionus calyciflorus* for selenium (Dobbs et al. 1996) and PCBs (Joaquim-Justo et al. 1995), as well as for mono-, di-, and tributyltin with *Brachionus plicatilis* (Hong-Wen et al. 2001). A BCF of 49,300 for lead on the predator rotifer *Asplanchna brightwellii* was calculated from data collected in a field study. This was the first report documenting lead biomagnification by a high trophic level organism (Rubio-Franchini and Rico-Martínez 2008). Versteeg et al. (1997) studied effects of surfactants with *B. calyciflorus* by conducting chronic toxicity tests. They found that N-containing amines and quaternary ammonium compounds displayed the greatest toxicity followed by nonionic compounds. Based on their data, they were able to develop a useful parametric QSAR model of prediction.

Endocrine Disruption

Monogonont rotifers are particularly designed for the study of endocrine disruption, because their life cycle sometimes alternates between sexual and asexual generations. Since the pioneering work of Snell and Carmona (1995) showing that sodium pentachlorophenol (PCP), cadmium, chlorpyrifos, and naphthol inhibit sexual reproduction in *Brachionus calyciflorus*, many studies have been dedicated to this topic (see review by Dahms et al. 2011). Readers interested in knowing more about endocrine disruption issues (e.g., contaminants involved and effects on varied biota) are directed to entries of this encyclopedia entitled "▶ New Perspectives in Assessing the Effects of Endocrine-Disrupting Chemicals in Fish," "▶ Estrogenic Endocrine-Disrupting Chemicals," and "▶ Bivalves in Ecotoxicology."

Metals and Inorganic and Organic Compounds

A wide sensitivity range for diverse metals has been reported after performing acute toxicity tests with single species of rotifers (Table 1). More toxicity data

were generated with Cu, Cd, Hg, and Pb than for other metals. For lethal effects, *B. calyciflorus* exposure to Ag (24-h LC50 = 0.0075 mg/L) displayed the most sensitive response (Snell et al. 1991b), while *B. plicatilis* exposed to thallium (24-h LC50 = 100 mg/L) was markedly less sensitive (Onikura et al. 2008). For sublethal effects, Hg toxicity measured with an esterase inhibition endpoint was highest (EC50 = 1×10^{-6} mg/L) for *Lecane luna* (Pérez-Legaspi et al. 2002), while the least sensitive response (EC50 = 59 mg/L) resulted from *Philodina acuticornis* exposure to cobalt (Buikema et al. 1974). For testing with marine rotifers, salt medium concentration decreases the solubility of some metals which in turn decreases the sensitivity of organisms to such metals. This is clearly observed when 24-h LC50 values determined for Ag, Cd, and Cu with *B. plicatilis*, a marine rotifer typically tested at salt concentrations of 15 g/L (Snell and Persoone 1989a; Snell et al. 1991b), are compared with those of *B. calyciflorus*, a freshwater species essentially tested with EPA medium containing 220 mg/L of salts (US EPA 1985). In the case of Pb, however, some endpoint values for freshwater species are similar or higher than those of *B. plicatilis* (see Table 1).

In addition to metals, some other inorganic chemicals such as potassium, sodium, sulfate, and sodium hypochlorite have been investigated using rotifers (Snell and Janssen 1995). A list of nonpesticide organic compounds tested with rotifers is provided in Table 2. Again, salt content in the medium decreases the sensitivity of *B. plicatilis* to certain compounds. For instance, the *B. plicatilis* LC50 values for acetone, chlorodinitrobenzene, chloroform, dichlorophenoxyacetic acid, diesel fuel, hexane, phenol, and tributyltin are 1.2- (chloroform) to 5.5-fold higher (diesel fuel) than the corresponding *B. calyciflorus* LC50 values (Table 2).

Pesticides

Pesticides and their corresponding acute lethality responses determined using rotifer toxicity tests are shown in Table 3. *Brachionus calyciflorus* displayed the highest sensitivity after exposure to resmethrin (24-h LC50 = 0.04 mg/L), while the least sensitive response was generated with *Brachionus plicatilis* exposed to trichlorfon (24-h LC50 = 257 to 293 mg/L). In comparison, the fungicide phenol, included in Table 2, was even less toxic to *Brachionus rubens* (24-h LC50 = 600 mg/L).

Once again, the influence of salinity in raising LC50 values is evident. In fact, almost all *B. plicatilis* LC50 values are 1.3- (fenitrothion) to 32-fold higher (resmethrin) than the corresponding *B. calyciflorus* LC50 values for cypermethrin, fenitrothion, lindane, methyl parathion, permethrin, phenol, resmethrin, and trichlorfon. Only in three cases (chlorpyrifos, endosulfan, and pentachlorophenol) were LC50 values similar. Only in one case (3-4-dichloroaniline) was the *B. plicatilis* LC50 value lower than that of *B. calyciflorus* (see Table 3).

Conclusions and Future Research

The list of rotifer species commonly used in ecotoxicological studies has progressively grown over the years, indicating their increasing popularity and recognition by the scientific community for the role they can play toward hazard and risk assessment of chemicals and complex environmental samples. Several standardized toxicity test methods now include rotifer species of the genus *Brachionus* (Standard Methods 1998; American Society for Testing Materials 1998; ISO 2008). In fact, the US EPA recommended the use of *Brachionus plicatilis* standardized tests to British Petroleum to assess the potential toxicity of the crude oil spill in the Gulf of Mexico and of oil dispersants employed for its remediation (US EPA 2010). Commercial kits called Rotoxkits®, which make use of dormant stages of rotifers (i.e., animals hatched from cysts), are also available with freshwater and marine species for routine and research applications in water toxicity assessment.

Several issues, however, regarding future prospects and use of rotifers in ecotoxicology remain to be addressed. First, an important issue of research would involve elucidating species status using molecular techniques to analyze species complexes. That would contribute to a reliable toxicity database where the sensitivity of each species is correctly assigned, thereby avoiding possible confusion created with sibling and/or cryptic species. Preliminary studies with *Brachionus plicatilis* suggest that there are at least 13 different taxa of this species complex (Suatoni et al. 2006). Second, the number of contaminants thus far appraised with rotifer species to determine their acute (sub) lethal toxicity remains limited, and efforts thus far have focused on conducting such tests within the genus *Brachionus*. As a result, information on the sensitivity of endemic species and those of restricted distribution is lacking. There is unquestionably a need to expand the database and the number of species used. Third, field studies, microcosm/mesocosm experiments conducted with existing and emerging contaminants, as well as wastewater toxicity assessment using rotifers are still quite limited. Increased knowledge concerning effects on ecosystems would clearly result from such endeavors. Lastly, additional gains for ecotoxicology can also be made by searching for new exposure and effect biomarkers in rotifers and by applying genomic techniques to identify up- and downregulated genes crucial for environmental diagnostics. This is an arena still very much in its infancy as far as the Rotifera are concerned (Dahms et al. 2011).

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Cross-References

▶ Biological Test Methods in Ecotoxicology
▶ Microbiotests in Ecotoxicology
▶ Test Batteries in Ecotoxicology

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Suggested Resources

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