Chapter 4

Rotifers as Models in Toxicity Screening of Chemicals and Environmental Samples

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Abstract

An important objective of aquatic ecotoxicology is to determine the effects of toxic compounds in organisms that play a central role in aquatic communities where rotifers have a large impact on several important ecological processes. The contribution of the rotifers to secondary production in many aquatic communities is substantial as they are often the larger fraction of zooplankton biomass at certain times of the year. In addition to the importance of their ecological roles in aquatic communities, the rotifers are attractive organisms for ecotoxicological studies by its short life cycles and rapid reproduction, their small size, and little volumes needed for culture and toxicity assays. The main end points used in ecotoxicological studies are mortality, reproduction, behavior, and biomarkers. Such parameters are included in international regulations from all over the world, where different species are used to evaluate the effect of environmental samples or chemical compounds. The high diversity of rotifers is an important issue because it can modify their relative susceptibility to toxicants. Thus, more studies are needed to know the relations and mechanisms involved in clonal variation, sensitivity, and development, which can be all assessed by state-of-the-art procedures.

Keywords: aquatic toxicology, ecotoxicology, metal toxicity, acute toxicity, endocrine disruption

1. Introduction

The analytical equipment can identify and quantify a chemical substance but not its toxicity in the organisms or the environment, which can be evaluated only in life organisms [1]. Toxicity testing in water samples assesses the concentration and exposure time of the chemical substances that produce an adverse effect in aquatic organisms, generating useful data for risk assessment. A toxicity test can be accepted by the scientific community if (a) it is capable to predict adverse effects for a variety of compounds in different organisms, (b) it must be replicable with statistical-based analysis, (c) its data must include adverse effects in a range of concentrations in real exposure times, (d) it must be useful to evaluate a risk, (e) it is economic and easy to perform, and (f) it is sensitive and realistic [2].

A model organism for a toxicity test must be abundant, native, or representative of the ecosystem that would be impacted, with ecological and economic importance; there must be a good knowledge of its basic biology that helps interpretation of data and be available for routine maintenance in the laboratory [2].

Toxicity tests have been developed for rotifers to assess many and diverse end points like (a) mortality, (b) reproduction, (c) production of amictic females, (d) cyst production, (e) probability of extinction, (f) behavior, (g) ingestion rates, (h) swimming activity, (i) *in vivo* enzymatic activity, and (j) genetic expression, among others [3–5].

Most of the toxicity tests in rotifers are lethal tests that rely in mortality as the end point to calculate the LC50 value typically at 24 or 48 h without feeding [5]. These tests once standardized are the base to develop monitoring protocols to assess water quality [4, 5]. On the other hand, chronic toxicity tests assess sublethal parameters like behavioral, physiological, or reproductive alterations as the first responses to toxic substances [5, 6], which show high sensitivity in shorter periods of time. The rotifer species *Brachionus calyciflorus* and *Brachionus plicatilis* are among the species most frequently used for both lethal and chronic tests [7, 8].

1.1. Phylum Rotifera

Rotifers are aquatic or semiaquatic microscopic invertebrates with nearly 1850 species; they are unsegmented, pseudocoelomate, and bilaterally symmetric [4, 9, 10]. Nowadays, two classes are recognized: Pararotatoria (with the single order Seisonacea) and Eurotatoria, with two subclasses, Bdelloidea and Monogononta. The size of rotifers ranges from 50 to 2000 μm in length. Most of the species are free swimmers, but some species are fixed to some substratum [4]. Their morphology is of saccate type that is cylindrical with three easily recognizable regions: corona, trunk, and foot (Figure 1) [11].

1.2. Ecological relevance

Rotifers are cosmopolitans. They inhabit aquatic environments of the three types: marine, freshwater, and estuarine [9]. Most rotifers are freshwater, littoral with a few species truly planktonic. The few species that comprise the order Seisonacea and nearly 100 species of the subclass Monogononta are exclusively marine [4]. Species of the subclass Bdelloidea are found in freshwater ecosystems, lakes, temporary pools, interstitial water, soil, moss, and lichens [4, 12]. Rotifers are important in freshwater environments due to having one of the highest reproductive rate among metazoans, thus obtaining high population densities in short times, being dominant in many zooplanktonic communities. They act as links between the microbial community and the higher trophic levels. Rotifers colonize habitats quickly and convert

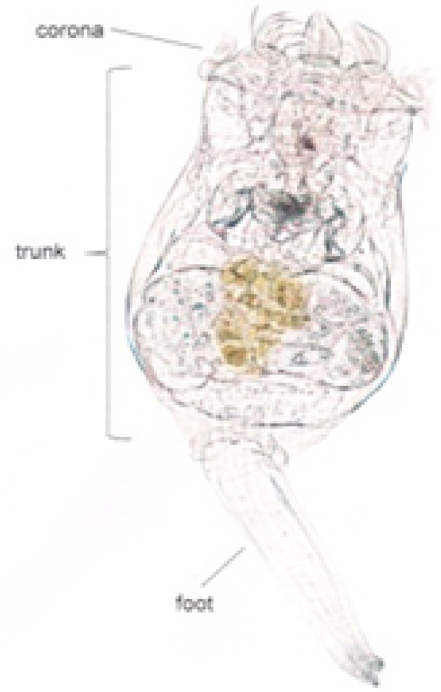


Figure 1. Diagram of the freshwater rotifer *Brachionuscalyciflorus* (Monogononta) [11].

primary production (algae and cyanobacteria) in a usable form for secondary consumers, making energy available for the next trophic levels. In interstitial water from swampy soils, they contribute to nutrient recycling [4]. Some bdelloids and Monogononta are abundant in wastewater treatment plants as part of activated sludge, in filtration systems, or residual lagoons feeding on the bacterial biomass [2, 13]. The population dynamics of rotifers is well characterized both in field as in the laboratory; for this reason, they are useful to investigate ecological and demographic principles. Besides, they are frequently used to assess aquatic toxicity from a population point of view. The intrinsic growth rate (r) is an end point commonly used and has proved to be highly sensitive [5, 14].

1.3. Culture

Rotifers can be obtained directly from a natural aquatic system or from laboratory cultures. Rotifers can be hatched from cysts, and today there are toxicity kits of two species: *B. calyciflorus* and *B. plicatilis* (Rotoxkits). The culture of rotifers has been developed for freshwater, estuarine, and marine organisms taken directly from the natural environment after a previous filtration, sterilization, and neutralization are performed. There is also synthetic hard like EPA medium [15] or synthetic marine water (Instant Ocean) that can be adjusted to the desired salinity. In freshwater species, usually the pH level is maintained in the physiological range (6.5–8) and temperature oscillates between 20 and 30°C. Marine species are maintained at salinities of 10 to 20 psu depending on a particular strain [16]. Rotifers are mainly filter feeders on microalgae, bacteria, or detritus; a few species are predatory. The supply of fresh and optimal food is the main problem for culturing rotifers. However, numerous rotifer species have been kept in the laboratory routinely [4]. Among the main marine microalgae used to feed rotifers are as follows: *Nannochloropsis* sp., *Chaetoceros* sp., *Dunaliella* sp., *Pyramimonas*, *Isochrysis* sp., and *Tetraselmis* sp.; for freshwater: *Nannochloris* sp., *Nannochloropsis* sp., and *Chlorella* sp. [16]. Culture media have to be renewed 2–3 times a week, and the supply of food must be frequent. Some species are maintained on diluted suspensions of commercial fish food, grain extracts or infusions, manure, and soil [4]. Among the most cultured species are found those of the genus *Brachionus*: *B. plicatilis*, *B. rotundiformis*, and *B. calyciflorus* [17, 18]. Some species of the genus *Lecane* can be easily cultured [19]. The economic importance of these rotifer species that are mass cultured are mainly based in their use as live feed for fish larvae and crustaceans in aquaculture [18].

1.4. Reproduction

The life cycles of rotifers are shorter than many other animals [14]. The Bdelloidea are reproduced by exclusive parthenogenesis and males are unknown. In the subclass Monogononta, the life cycle is haplodiploid with cyclic parthenogenesis as the dominant phase (amictic females), whereas the sexual reproduction (mixis) involves mictic diploid females that produce haploid eggs that if fertilized produce diploid embryos or cysts (in old literature they are called "resting eggs"), which go through diapause before hatching into amictic females that go back to the asexual phase of the cycle (Figure 2). Mixis is triggered by specific environmental stimuli like high population density or photoperiod [18, 20]. Parthenogenesis eliminates the high cost of producing males resulting in a rapid growth population [18], which allows for the high production of clonal individuals that can be used for aquaculture or toxicity tests.

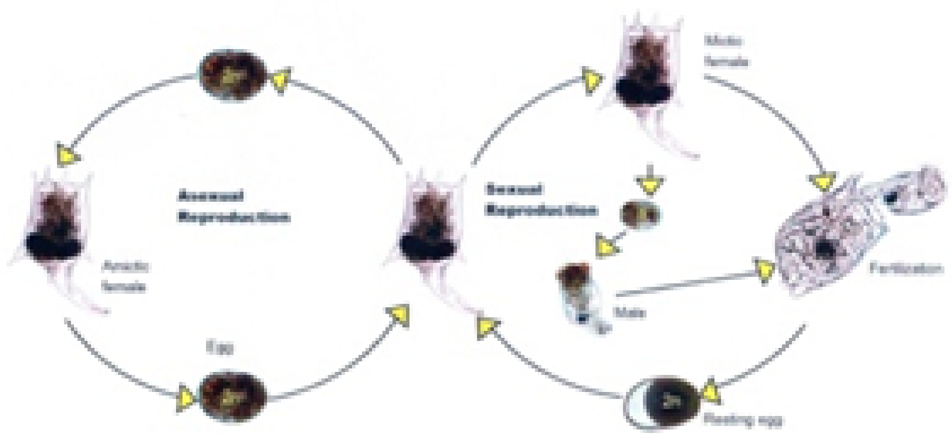


Figure 2. Reproductive cycle of *Brachionus calyciflorus*. Modified from Alvarado-Flores [21].

1.5. Cysts

Once the eggs are produced, they fall into the bottom and are deposited in the sediments [18]. The cysts are diploid and have a thick cover and can be viable for many years in dormancy. Cysts are very resistant to harsh environmental conditions like draught and freezing. Cysts can be dispersed through wind, water, or migratory animals. Under favorable conditions in a specific habitat, the cysts respond to a specific environmental clue (photoperiod, temperature, and salinity) and starts hatching producing an amictic diploid female [4]. Cysts can be stored for great periods of time without losing viability having rotifers in the desired period of time [22]. The use of rotifers hatched from cysts to develop toxicity tests was introduced by Snell and Persoone [23]. Nowadays, it is possible to obtain cysts from marine (*B. plicatilis*) and freshwater (*B. calyciflorus*) species in the market.

1.6. Rotifers as sentinels or bioindicators

Aquatic invertebrates are attractive model organisms in aquatic toxicology due to their short generation time compared with fishes besides their small size require small test volumes [14, 24]. Their small size, the fact that they are filter feeders, and the permeability of the integument made rotifers quite susceptible to chemical and physical changes [19, 25]. Due to the importance of rotifers in the aquatic trophic webs, they are useful as sentinel species to indicate toxicant exposure in affected ecosystems [4].

The knowledge of the (a) basic biology of rotifers, (b) sensitivity to contaminants at the physiological and demographic levels, (c) cosmopolitan distribution, (d) great ecological relevance, (e) high growth rate, (f) availability of neonates, (g) high ingestion rates, (h) ease of culture and handling, (i) transparency, (j) short life cycles, (k) ease to obtain clonal individuals, and (l) cyst production makes rotifers useful tools for assessment of aquatic ecosystems [4, 5, 8, 17, 18, 22, 24, 26].

2. Rotifers as model organism among invertebrates

Invertebrates are the most widely distributed organisms on the Earth and consist of a large and very diverse group, consisting in more than 30 phyla, several of which include more than 1000 different species [27]. The largest phylum within invertebrates is the Arthropoda, with more than one million species, in which insects and crustaceans are the two largest groups [28]. Aquatic crustaceans is a larger group in comparison to the aquatic insects, which a few of them have aquatic larvae [29]. Consequently, crustaceans are the most numerous and ecologically important group of invertebrates in marine and freshwater ecosystems, playing an important role in regulatory toxicity testing, whether in field or laboratory conditions [30, 31]. In addition, these methods are cost-effective because of the facility to obtain amictic eggs or in some cases resting eggs, which have been used to produce commercial kits for toxicity evaluation [32].

Current international protocols include several invertebrates' taxa for toxicity assessment (Table 1), in which cladocerans are the most used organisms and have more approved protocols for both acute (lethal) and (sub)chronic (sublethal) water toxicity assays than other taxa. The genus *Daphnia* is the most studied taxon within cladocerans. For the acute toxicity test, three replicates for at least five toxicant or effluent concentrations are required, every one of them with 100 mL, 48 h for exposure, without feeding [33]. Moreover, the chronic toxicity assay needs almost the same conditions but with feeding and daily medium renewal for 21 days [34]. In comparison, the *Brachionus* or other rotifer bioassays need only 1 mL per replicate [23], and the results can be obtained within 2 days for acute toxicity evaluation, and in 5 days for the chronic ones. Hence, rotifers tests involve similar conditions to the cladocerans, but when time is a matter of concern, the former is the best option to know the toxicity of chemicals or field samples.

Rotifers are even more versatile than daphnids. In April 2010, after the Macondo oil spill in the Gulf of Mexico, the Environmental Protection Agency of the United States (USEPA) in order to assess the impact of petroleum and oil dispersants on the aquatic biota recommended using *B. plicatilis*, a species complex that included *B manjavacas*, *B. plicatilis sensu stricto*, and *B. rotundiformis* among other that can exist [35]. This cost-effective test can be replicated hundreds of times in very short periods of time when necessary [36].

The Organization for the Economic Cooperation and Development (OECD) encourages developing test protocols that include crustaceans [37]. In this concern, the copepod *Eucyclops serrulatus* has been proposed as a model organism, with acute toxicity evaluations in 96 h [38] and chronic toxicity assessment in about 60 days [39]. These periods differ due to intrinsic characteristics of the species; copepods last longer to reach sexual maturity after hatching and require mating to obtain eggs for assays, while rotifer eggs are produced by parthenogenesis [40–42]. Nevertheless, with regard to the importance of copepods in aquatic ecosystems, there is not yet any approved protocol for toxicity evaluation as in rotifers [43, 44].

The cnidarian *Hydra attenuta* is another model organism included in standard methods [45]. This one is particularly interesting since lethal and sublethal end points are assessed in the same assay by quantifying the number of death animals and abnormalities found in the survivor hydras during 96 h for acute procedures, which are performed in 12-well microplates, whit a test volume of 4 mL [46]. Clubbed tentacles appearance is the first sign of abnormalities due to toxicants exposure. From this point, abnormalities follow shortening tentacles and the tulip stage (all tentacles have disappeared), and finally the disintegration of animal bodies occurs. Therefore, lethal effects should be registered from the tulip stage [47].

It is worth to compare with some rotifers in which it is also possible to determine sublethal effects in the same time period. Alvarado-Flores et al. [48] found abnormal body shapes in *B. calyciflorus* after vinclozolin exposure, demonstrating this pesticide also acts as an endocrine disruptor. As result, both models can be used for sublethal toxicity screening in short periods of time, but the difference lies on the apparent ease to determine such effects, while in *Hydra attenuata* are very obvious. In *B. calyciflorus*, more than one thousand organisms were analyzed to find statistically abnormalities in this rotifer species.

Bivalves in ecotoxicology procedures refer the use of species such as *Lampsilis siliquoidea*, *Corbicula fluminea*, *Leptodea fragilis*, *Ligumia subrostrata*, and *Megalonaias nervosa*, among others

[49, 50]. In the scientific literature, most of the bivalve specimens used in toxicological research and evaluations have been collected from environmental samples or commercial producers [51–54]. Glochida are dissected from adult bivalves and need a fish host to develop into juveniles [55–57]. Both immature stages, glochida and juveniles, are used for water quality assessment or chemicals released into aquatic systems [58]. In contrast, rotifer experimental organisms are produced in laboratory-controlled conditions, and animals are ready to use a few hours after hatching instead for several weeks to have juvenile unionids. Hence, rotifer bioassays are less time consuming than bivalves protocols.

In freshwater sediment, toxicity screening several protocols are currently carried out by international regulation agencies (Table 1). Nevertheless, rotifers are not included as test species, but it has been proved that the genus *Lecane* is suitable for those evaluations, representing a cost-effective and high reliable alternative to the standardized methods.

	Class	**Order**	**Family**	**Species**
			Freshwater	
Cladocera	Branchiopoda	Diplostraca	Daphniidae	*Daphnia magna* [62-65]
				D. pulex [66-68]
				D. similis [69]
				D. carinata [70-71]
				Ceriodaphnia dubia [62, 64, 66, [72]
Copepoda	Branchiopoda	Anostraca	Thamnocephalidae	*Thamnocephalus platyurus* [73-76]
	Maxillopoda	Calanoida	Cyclopidae	*Eurytemora affinis* [77-80]
				Eucyclops serrulatus [31, 81-83]
Rotifera	Monogononta	Ploima	Brachionidae	*Brachionus calyciflorus* [43, 84]
				B. rubens [23, 85-87]
				B. patulus [88-90]
			Lecanidae	*Lecane quadridentata* [91-93]
				L. hamata and L. luna [94,95]
				Euchlanis dilatata [96, 97]
			Asplanchnidae	*Asplanchna brightwelli* [98-101]
Cnidaria	Hydrozoa	Anthoathecata	Hydridae	*Hydra attenuata* [45]
				H. vulgaris [102-104]
Mollusca	Bivalvia	Unionoida	Unionidae	*Elliptio complanata* [58]
				Lampsilis siliquoidea [58]
		Veneroida	Corbiculidae	*Corbicula fluminea* [105-108]

	Class	Order	Family	Species
			Freshwater sediments	
Crustaceans	Malacostraca	Amphipoda	Hyalellidae	*Hyallela azteca* [109, 112]
Annelida	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus variegatus* [113-115]
				Stylodrilus heringianus [116, 117]
Rotifera	Bdelloidea		Philodinidae	*Philodina roseola* [118]
Arthopoda	Insecta	Diptera	Chironomidae	*Chironomus dilutus* [109, 110, 119-121]
				C. riparius [109, 110, 119-121]
			Seawater	
Rotifera	Monogononta	Ploima	Brachionidae	*Brachionus plicatilis* [43]
Echinodermata	Echinoidea	Arbacioida	Arbaciidae	*Arbacia punctulata* [122]
Arthropoda	Malacostraca	Mysida	Mysidae	*Mysidopsis bahia* [123]
Copepoda	Maxillopoda	Calanoida	Acartiidae	*Acartia tonsa* [44, 124]
		Harpacticoida	Miraciidae	*Amphiascus tenuiremis* [44]
Molluscs	Bivalvia	Mytiloida	Mytilidae	*Mytilus edulis* [125, 126]
		Ostreoida	Osteridae	*Crassostrea gigas* [127]
			Seawater sediments	
Annelida	Polychaeta	Capitellida	Arenicolidae	*Arenicola marina* [128]
Artropoda	Malocostraca	Amphipoda	Corophiidae	*Leptocheirus plumulosus[110, 129, 130]*

Note: Numbers in square brackets indicate reference number

Table 1. Toxicity evaluations protocols using different invertebrates for freshwater and freshwater sediments, seawater, and seawater sediments.

Differently to the protocols mentioned above, all those for seawater and seawater sediments are found in standardized probes approved by agencies such as USEPA, ASTM, and the OECD (Table 1). As previously described, *B. plicatilis* is a marine rotifer used in seawater toxicology evaluation, an although it is not included in the USEPA guidelines, the agency recommended its used based on the ASTM method [43]. For sediments, the prime option is the polychaetes such as *Arenicola marina*, mainly collected from beach sand in different locations around the world. The protocol involves an exposure time of about 28 days using artificial sediment and field sediments samples [59–61]. Moreover, *B. plicatilis* has been exposed to interstitial water from littoral ecosystems; although it is sensitive to bioavailable toxicants in pore water, it does not respond in the same manner that those organisms that live in the sediment.

Afterward, the use of rotifers in all the above-mentioned matrices is possible due to some advantages, such as their relative small size, their simple organization, their short life cycles that permit multigenerational studies in very short time periods, their reproduction through parthenogenesis, and their genetic homogeneity that leads to almost identical offspring [22]. Despite of these characteristics, there is still a need to continue researching the effect of toxicants and to improve protocols for rotifers in (eco)toxicology to better understand their physiology and how external factors alter rotifers normal responses, since studies with rotifers are not as numerous as other taxa (Figure 3).

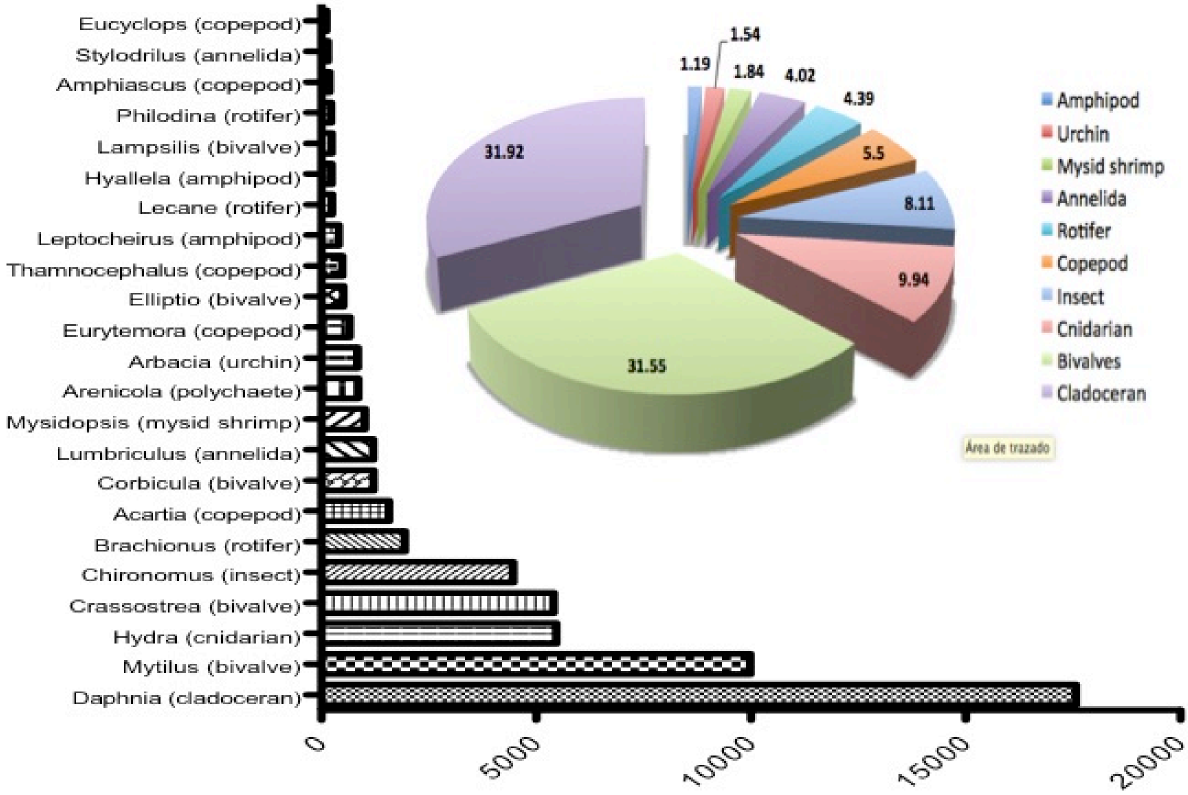


Figure 3. Number of publications for the groups described in the text. The databases consulted were Elsevier (www.sciencedirect.com), Springer (www.link.springer.com), and Wiley Online Library (http://onlinelibrary.wiley.com). Query terms were the genera name and the word "toxicity". Pie chart data represent percentages based on published material.

3. Rotifer sensitivity

The toxicity of a wide variety of chemicals including organic compounds, metals, and pharmaceuticals that can be found in water reservoirs as a result of human activity has been tested by performing toxicity tests using rotifers as bioindicators as these organisms play an important ecological role in aquatic environments [3, 131]. Rotifers of the genus *Brachionus* can be found between the most commonly used tests organisms [132]; moreover, several rotifers are included for comparison purposes.

A test organism with high sensitivity to detect adverse effects of xenobiotics is always desirable; nevertheless, differences in sensitivity among species can always be found. Generally, these are due to the biological characteristics of test organisms, the type of chemical, and its mode of action; thus, one species might be very sensitive to one toxic but not that sensitive to another compound [133]. This section focuses on the sensitivity of different rotifers, all of them from freshwater ecosystems, with exception of the marine species *B. plicatilis* and *Brachionus koreanus*, when testing the toxicity of pesticides, organic compounds, and metals attending their toxicological importance and the availability of literature.

Some examples of these variations on sensitivity are shown in Table 2. In relation to pesticides, malathion (organophosphorus) displays the lower toxicity and has a toxicity range from 33.72 to 45.5 mg/L, where the lower toxicity level is reported to *B. calyciflorus* and the higher to *B. plicatilis* [134, 135].

Lindane, an organochloride pesticide, and methyl parathion (organophosphorus) show moderate toxicity and have wider toxicity ranges in comparison to malathion. For the organochloride, toxicity range is given by *B. koreanus* and *B. plicatilis* (14 and 35.89 mg/L, respectively). In the case of methyl parathion, which shows higher acute toxicity, a range from 0.607 mg/L for *Euchlanis dilatata* to 29.19 mg/L for *B. calyciflorus* was found. Another organophosphorus of ecotoxicological relevance, chlorpyrifos, tends to affect significantly aquatic invertebrates due the LC50 values reported for *B. koreanus* (3.9 mg/L) and *B. calyciflorus* (11.85 mg/L) [96, 134, 136, 137].

The organochlorides endosulfan and pentachlorophenol (PCP) stand as highly toxic agrochemicals. For endosulfan, *B. koreanus* and *B. plicatilis* correspond to the reference organisms to establish the range because of their 24-h LC50 values found in literature [135, 136]. For PCP, the lowest and highest toxicity levels were noted for *B. calyciflorus* [138, 139].

On literature, little information is available about the toxicity of other organic compounds such as benzene, toluene, xylene, and hexane. Nevertheless, Ferrando and Andreu-Moliner [7] and Pérez-Legaspi et al. [95] have obtained some toxicological data (Table 2). For xylene, hexane, and toluene, *B. calyciflorus* and *B. plicatilis* are the reference organisms, where the first was most sensitive for the three chemicals [7, 95]. For benzene, rotifers from the genus *Lecane* resulted being more sensitive, but also a wide toxicity range is registered from 6.97 for *Lecane luna* to 3762 mg/L for *Lecane hamata* [95].

The lowest LC50 values, and thus, more toxic metals, were reported for zinc, copper, and mercury. Besides, a relatively not wide toxicity range can be observed for these three chemicals (Table 3). The most sensitive test organisms in these cases were *Lecane quadridentata*, *B. plicatilis*, and *B. calyciflorus*, respectively, while the more resistant were *Brachionus havanaensis* (Zn), *P. acuticornis* (Cu), and *L. hamata* (Hg) [93, 95, 135, 140, 143, 144].

Moreover, it is important to mention that there are physical factors that can alter the sensitivity of a test organism when performing a toxicity test. One example is given by Preston et al. [138],

Pollutant	Acute toxicity range in terms of 24h-LC50 (mg/L)
Pesticides	
Malathion	33.72[134] - 45.5[135]
Lindane	14[136] - 35.89[137]
Methyl parathion	*0.607[96] - 29.19[134]
Chlorpyrifos	3.9[136] – 11.85[137]
Endosulfan	4[136] - 6.6[135]
PCP	0.21[138] - 2.16[139]
Another organic compounds	
Xylene	252.7[7] - 495.9[7]
Hexane	68.3[7] - 154.3[7]
	Acute toxicity range in terms of 24 and 48h-LC[50](mg/L)
Benzene	6.97[95] – 3,762[95]
Toluene	113.3[7] - 552.6[7]

Note: Numbers in square brackets indicate reference number

*48h LC_{50} value

In the case of inorganic toxicants (Table 3), lead appears as the "less" toxic and with the wider toxicity range from 0.035 for *E. dilatata* to 56.2 mg/L for *Philodina acuticornis* [97, 140]. For cadmium, *E. dilatata* shows a great sensitivity with a 48-h LC50 of 0.014 mg/L, while *B. plicatilis* is more resistant (39 mg/L) [97, 141]. The most sensitive rotifer to chromium was *Lecane luna* (48-h LC50 of 3.26 mg/L) [95], and the highest 24-h LC50 of 17.4 mg/L was registered for *B. calyciflorus* [142].

Table 2. Toxicity range for some pesticides and organic compounds to rotifers used in bioassays.

Metal	Acute toxicity range in terms of 24 and 48h-LC[50] (mg/L)
Lead	0.035[97] - 56.2[140]
Cadmium	0.014[97] – 39[141]
Chromium	3.26[95] - 17.4[142]
Zinc	0.123[93] - 2.271[143]
Copper	0.01[135] - 1.9[140]
Mercury	0.06[144]- 1.37[95]

Note: Numbers in square brackets indicate reference number

Table 3. Toxicity range for different metals to rotifers used in bioassays.

where a decrease in LC50 values for PCP and mercury using *B. calyciflorus* as bioindicator was recorded as UV-B exposure time was increased.

Other physical condition that might modify the sensitivity of a test organism is desiccation. Robles-Vargas and Snell [145] found a remarkable resistance of *Philodina sp*. emerged from desiccation to PCP, chlorpyrifos, and mercury in comparison to continuously hydrated rotifers when reproductive tests were implemented [145].

According to the information reviewed and in terms of acute toxicity, rotifers from the family Brachionidae tend to show a great sensitivity to different chemicals, including organic and inorganic compounds. Invertebrates from the family Euchlanidae and Lecanidae appear to be sensitive mainly to inorganic toxicants. Furthermore, rotifers belonging to the family Philodinidae exhibit a notorious resistance to chemicals.

4. Rotifer database for screening toxicants

Every chemical product that can reach ecosystems and, as a consequence, the human being needs to be characterized to avoid noxious effects on all organisms exposed. At first instance, toxicological studies were mainly anthropocentrically focused, but that point of view would not have been enough to protect our own existence at all if affecting organisms in different ecosystems would have continued. It is known that rotifers play an important role as secondary producers and in some freshwater ecosystems represent the larger fraction of biomass of zooplankton [146]. Therefore, these organisms are nowadays considered as model organisms for toxicological screening.

Currently, one can find in the scientific literature reports of almost every class of chemical compound used or produced by man, investigating their toxicological properties. These papers are relatively abundant in several websites specialized in gathering such kind of information, which is available to everyone who has access to the published material. For scientist, collecting this material is a daily activity that guarantees their studies to follow the right direction by not working in exactly the same topic and manner, which would represent the duplicity of information but at higher cost because of multiple double efforts and resources investment. Nonetheless, it must be pointed out that differences in susceptibility to toxicants can be found among clones or strains, which is very interesting to assess by multiclonal analyses [22, 147]. However, when it is not possible to access multiple clones or strains, previous reports (published material) from databases become more relevant as a source of comparison to improve the comprehension of the observed results.

The USEPA, in an attempt to ease the search for environmental toxicological data, has created the ECOTOX database, hosted in their website [150]. It provides a user-friendly interface and allows discrimination among taxonomic and chemical groups, aquatic or terrestrial environment, tests results or conditions, and type (year) of publication. Although it does not give access to the cited references, it returns a list of publications to be consulted elsewhere. In the ECOTOX results table, the data shown are species name, exposure type, chemical, media type, end points, bioconcentration factor, effects, and statistics, among others (Figure 4). A complete list of all abbreviations used in the ECOTOX database can be directly downloaded for the same host website.

Figure 4. Print screen of ECOTOX database results. Query terms were *Brachionus* and chromium, selected from the options displayed in the database [150].

Nowadays, toxicity assays with rotifers include very diverse toxicants, and a relatively high number of test have been assessed the effect of inorganic (metals and metalloids) and very versatile organic compounds like pesticides, solvents, colorants, detergents, and emergent toxicants, in which health and care products and pharmaceuticals form part of it (Table 4). Some of these toxicants are effective at concentrations as low as some nanograms per liter, altering rotifers population dynamics by different mechanisms like endocrine disruption.

Species	Chemical compound or metal	Species	Chemical compound or metal
Bc	1,1,1-trichloroethane [150]	Bc	Lauryl alcohol [150]
	1,3,5-Trinitrobenzene [150]	Ab, Ai, Bc, Ed Lh, Ll, Lq, Pa	Lead [91, 94, 97]
	1-Dodecanesulfonic acid, Sodium salt [150]	Ap, Bc,Br, Kq	Lindane [150]
	1-Octanol [150]		Linuron [150]
Bp	2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) [151]	Bc	Lithium [157]
Bc, Kc	2,3,4,6-Tetrachlorophenol [150]		m- and p-Xylene [150]
Ba, Ka,Kc	2,4,5-T [150]		Malathion [150]
Bc	2,4,6-trichlorophenol [150]	Br	Malathion [150]
	2,4,6-Trinitrotoluene [150]	Pa	Malathion [150]

Species	Chemical compound or metal	Species	Chemical compound or metal
	2,4-Dichloro aniline [150]	Bc	Malathion [152]
	2,4-Dichlorophenoxyacetic [150]	Bc	Malathion [153]
	2,4-dinitrochlorobenzene [150]	Bc, Li, Lq	Manganese [150], [166], [169]
	2,4-xylenol [150]	Bc	MARLON A390 [150]
	2,5-Dichloro aniline [150]	Bc, Bp, Ed, Lh, Ll, Lq, Pa	Mercury [91, 94, 97]
	2- [2- [2-(Dodecyloxy)ethoxy]ethoxy]ethanol, Hydrogen sulfate, Sodium salt [150]	Bc	Metallic silver [150]
	3,4-dichloro aniline [150]	Av	Methacrylamide [171]
	Acetaminophen [150]		Methacrylic acid [150]
Bc, Lh, Ll, Lq	Acetone [94, [150]]	Bc	Methanol [150]
Bc	Acetylsalicylic acid [152, 153]		Methoprene [150]
Av	Acrylamide [154]	As, Ba, Bc, Bp, Ed	Methyl parathion [97, 150]
	Acrylic acid [150]		Molinate [150]
Bc	Aldrin [155]		N,N,N-Trimethyl-1-octanamonium chloride [150]
	Alkyl* sodium benzene sulfonate [150]	Bc	N,N-Dimethyl-1-dodecanamine [150]
	Alkyl* trimethyl ammonium chloride [150]		N,N-Dimethyl-1-octanamine [150]
Ab, Bc, Li, Lq	Aluminum [93, 150, 156]		Naphthalenol [150]
Bc	Amitriptyline [152, 153, 157]		Naproxen [150]
Bc, Br	Ammonia [150]		n-Hexane [150]
Bc, Pa	Ammonium chloride [150]	Bc, Pa	Nickel [150]
Bc	Amphetamine sulfate [153]		Nicotine [152, 153]
Bc	Aroclor 1260 [150]		Nitric acid, Cadmium salt [150]
Bc, Pp, Pr,	Arsenic [150, 158>]	Bc	Nonyl phenol [150]
Bk	Atenolol [159]		Ofloxacin [161, 165]
Bc, Kq	Atrazine [150]		Orphenadrine [152, 153, 157]
Bc	Atropine [152, 153, 157]		Oxytetracycline [150]
Bc	Barium nitrate [150]	Bc, Bk	Oxytetracycline [159, 165]
Bc, Lh, Ll, Lq	Benzene [94, 150]	Bc	Paracetamol [152, 153]
Bc	Bezafibrate [160]		Para-chlorophenol [150]
Ab, Ai, Bc, Bh, Bp, Br, Bu, Ed, Lh, Ll, Lq, Pa	Cadmium [94, 143, 150, 160]	Bc	Para-dichlorobenzene [150]

Species	Chemical compound or metal	Species	Chemical compound or metal
Bc	Caffeine [150]		P-chloroaniline [150]
Pa	Calcium hypochlorite [150]	Ag, Bc, Br, Kq, Pa,	Pentachlorophenol PCP [150]
Bc, Bk	Carbamazepine [159, 161, 162]	Bc	Phenobarbital [152, 153, 157]
Ba, Hm, Mq, Pa	Carbaryl [150]	Bc, Br, Pa	Phenol [150]
Eb, Kq	Carbendazim [150]	Br	p-Nitrophenol [150]
	Carbofuran [150]	Bp	polybrominated diphenyl ethers [173]
Bc	Carbon tetrachloride [150]	Bc	Potassium chloride [150]
	Cetyl trimethyl ammonium chloride [150]	Bc, Pa	Potassium cyanide [150]
	Chloramphenicol [152, 153, 157]	Bc, Pa	Potassium dichromate [150]
Pa	Chlorine [150]		Prednisolone [174]
Bu	Chlornitrofen [150]		Prednisone [175]
	Chloroacetic acid [150]		Prococene [150]
Bc	Chloroform [150]		Propranolol [152, 153, 157, 176]
	Chloroquine [152, 153]	Bc	Quinidine [152]
Ab,Ba,Bb, Bc Bq, Bu, Eb, Ft, Hi, Hm, Kq, Mq, Pq, Tp	Chlorpyrifos [150,163]		Ranitidine [177]
Bc, Bp, Lh, Ll, Lq	Chromium [94, 150, 163]		Selenium [150]
Bc	Clarithromycin [165]	Pa	Silver nitrate [150]
Pa	Cobalt chloride [150]	Br	Simetryn [150]
Ab, Bc, Bp, Br, Lh, Ll, Lq, Li, Pa, Pp	Copper [94, 150, 158, 164, 166, 167]	Bp	SLS [178]
Ab, Bc, Bp	DDT, p,p' [150, 169]	Bc	Sodium bromide [150]
	Diazepam [152]	Bc	Sodium chloride [150]
	Diazinon [150]	Pr	Sodium chromate [150]
Bc	Diclofenac [161, 162]	Bc	Sodium dodecylbenzene sulfonate [150]
	Dicofol [150]	Pa	Sodium fluoride [150]
	Digoxin [152]	Bc	Sodium hypochlorite [150]
Pa	Dimethoate [150]	Bc	Sodium laurate [150]
Bc	Dinitro cresol [150]	Bc, Br	Sodium lauryl sulfate [150]

Species	Chemical compound or metal	Species	Chemical compound or metal
	Diphenylhydantoin [152]		Sodium oxalate [150]
	Endosulfan [150]		Sodium selenate [150]
	Endothall [150]		Sodium tetradecyl sulfate [150]
	Erythromycin [165]		Sulfamethoxazole [159, 161, 165]
	Estradiol [150]	Bc	Testosterone [168]
	Ethinylestradiol [94, 168]		Thallium(I) sulfate [150]
Lh, Ll, Lq	Ethyl acetate [94, 168]		Theophylline [152, 153]
	Ethyl alcohol [150]		ThiobencarB[150]
	Ethyl methacrylate [150]		Thioridazine [153]
Bc	Ethylene glycol [150]	Li	Tin [166]
	Fenitrothion [150]	Lh, Ll, Lq	Titanium [94]
	Fenofibrate [160]	Bc, Lh, Ll, Lq	Toluene [94, 150]
Bc, Pa	Ferrous sulfate [150]		Tributyl phosphate [150]
Ab	Iron [156]	Bc	Tributylstannane [150]
	Flutamide [150]		Tributyltin chloride [150]
Bc	Furosemide [170]	Bc, Kq. Pa	Trichlorfon [150]
	Gemfibrozil [160]	Bk	Trimethoprime [159]
Bc, Lq	Glyphosate [150]	Bc	Verapamil [152, 153]
Bc	Hexachlorophene [150]	Lh, Ll,Lq	Vinyl acetate [94]
	Hydroquinone [150]	Bc	Warfarin [152]
Ab, Li, Lq	Iron [96, 156, 166]		Xylene [150]
Bc	Isoniazid [152, 153]	Ab, Bc, Bh Li, Lq, Pa	Zinc [93, 143, 150, 156, 166]
	Isopropyl alcohol [150]		

Note: superscripts indicate reference number

Notes: Species are abbreviated with the first letters of their genus and species: Ad = *Adineta vaga*, Ab = *Asplanchna brightwellii*, Ag = *Asplanchna girodi*, Ai = *Asplanchna intermedia*, As = *Asplanchna sieboldi*, Ba = *Brachionus angularis*, Bb = *Brachionus bidentata*, Bc = *Brachionus calyciflorus*, Bh = *Brachionus havanaensis*, Bk = *Brachionus koreanus*, Bp = *Brachionus patulus*, Bp = *Brachionus plicatilis*, Bq = *Brachionus quadridentatus*, Br = *Brachionus Rubens*, Bu = *Brachionus urceolaris*, Eb = *Epiphanes brachionus*, Ed = *Euchlanis dilatata*, Ft = *Filinia terminalis*, Hi = *Hexarthra intermedia*, Hm = *Hexarthra mira*, Ka = *Keratella americana*, Kc = *Keratella cochlearis*, Kq = *Keratella quadrata*, Lh = *Lecane hamata*, Li = *Lecane inermis*, Ll = *Lecane luna*, Lq = *Lecane quadridentata*, Mq = *Monostyla quadridentata*, Pa = *Philodina acuticornis*, Pr = *Philodina roséola*, Pp = *Plationus patulus*, Pq = *Platyias quadricornis*, Tp = *Testudinella patina.*

Table 4. Relation of toxicants and rotifers species used in ecotoxicological studies

The available data about toxicity to rotifers show that organic compounds, as a complex group, is the most studied (32.4%) followed by the drugs group (24.58%), which could be due to the complexity of both groups, as they include a huge variety of toxicants. Inorganic compounds among other groups represent 17% out of the total number of tests included in this chapter, which can be split up into inorganic (nonmetallic compounds) and metals (Figure 5). The last one comprised those commonly named heavy metals [148]. Other groups might not be as numerous as the former ones, but this should not be misinterpreted as a lack of interest from the environmental toxicologists, but a challenge and a continuous research to find out how these chemicals are affecting the aquatic ecosystems and their inhabitants like rotifers species.

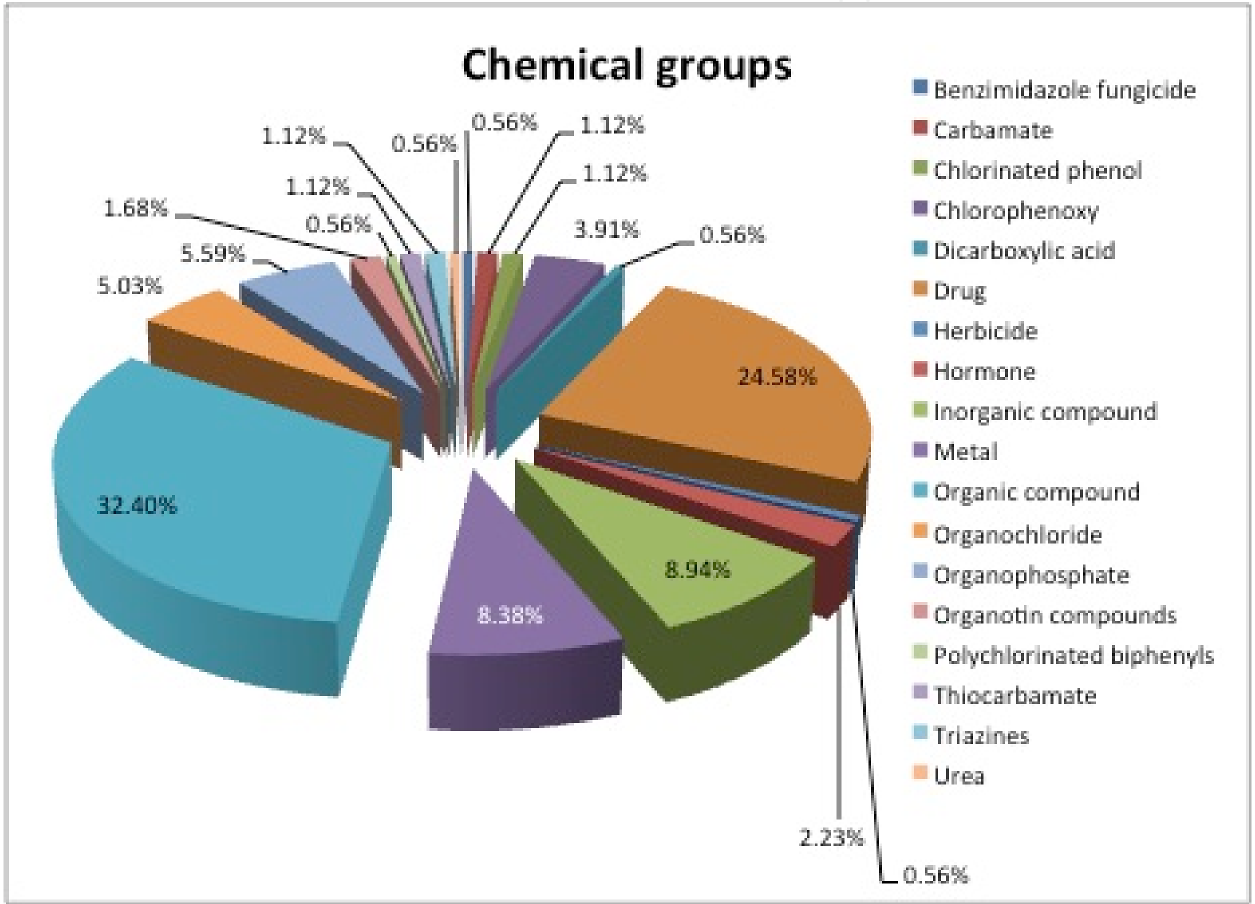
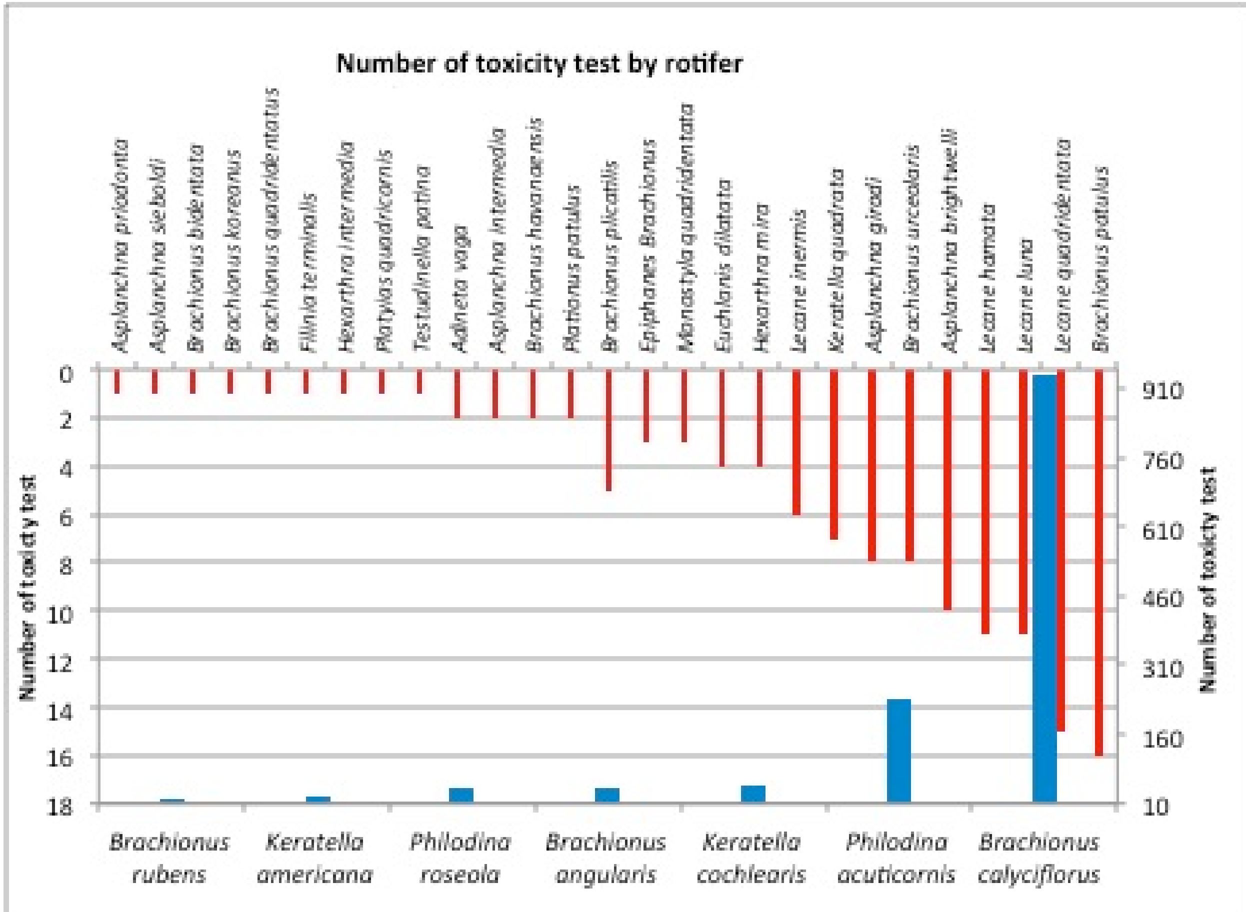


Figure 5. Comparison of toxicity tests between chemical groups conducted with different rotifers species.

Several rotifer species have been used to evaluate the effect and toxic mechanisms of the chemical groups aforementioned, where *B. calyciflorus* is the most abundant in the literature, with about 63% out of the total publications (Figure 6). However, there are other species that present advantages over brachionids, such as the littoral genus *Lecane* that might be used to assess the effect of sediment-associated toxicants, or the predator genus *Asplanchna*, used to evaluate toxicants biomagnification among rotifers and other aquatic invertebrates [149]. Therefore, rotifers represent a group of organisms that have not been completely studied and require more attention from toxicologists.



Red bars correspond to left *y*-axis and blue bars to right *y*-axis.

Figure 6. Number of ecotoxicological studies through by different rotifer species.

5. Rotifer tests already used worldwide and in certain regions

The ability to produce cysts has allowed the development of toxicity kits, called Rotoxkits, employed for acute/chronic marine and freshwater toxicity testing [179]. Cyst production is an outstanding characteristic that has enabled the development of several toxicity protocols using rotifers that have been used worldwide [180]. Rotifers are not directly represented in the legislation of several countries (as the cladocerans *Daphnia magna* and *Ceriodaphnia dubia* do). However, toxicity tests with rotifers have been published by official societies [43, 84, 181] Perhaps the most notorious participation of rotifer toxicity tests is when EPA asked BP plc (the former name of the company was British petroleum) to use the acute toxicity test with the euryhaline rotifer *B. plicatilis* to assess the toxicity generated after the 2010´s Gulf of Mexico oil spill [35]. The marine water rotifer toxicity test TK22 was used to analyze thousands of sites in the Gulf of Mexico; toxicity was analyzed for both oil and the oil dispersant used [36].

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5.1. Europe

The Convention for the Protection of the Marine Environment of the North-East Atlantic (the "OSPAR Convention") was established in 1992. The convention started working in 1998. It has been ratified by Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, Netherlands, Norway, Portugal, Sweden, Switzerland, and the United Kingdom and approved by the European Community and Spain. One of their goals was the development of directives for the analysis of toxicity both in marine and freshwater environments. In their 2007 publication "Practical Guidance Document on Whole Effluent Assessment" (OSPAR, 2007), they applied the protocol of determination of the chronic toxicity to *B. calyciflorus* in 48 h [84] and validated its use.

In the Port of Aveiro, Portugal, standardized acute toxicity test using the marine rotifer, *B. plicatilis*, on sediment elutriates revealed higher toxicity levels in cases where the European Union Water Framework Directive suggested that sediment quality was acceptable [183]. Isidori et al. [184] employing *B. plicatilis* in 24-h toxicity tests found that all samples of municipal solid waste landfills in southern Italy expressed acute toxicity.

In Poland, the toxicity of the leachates from sewage sludge were investigated using different toxicological kits: Microtox (*Vibrio fischeri*), microbial assay for toxic risk assessment (ten bacteria and one yeast), Protoxkit F™ (*Tetrahymena thermophila*), Rotoxkit F™ (*B. calyciflorus*), and Daphtoxkit F™ (*D. magna*). Differences were observed in the sensitivity of the test organisms to the presence of sewage sludge in the soil. The highest sensitivity was a characteristic of *B. calyciflorus* [185].

5.2. Oceania

The Resource Management Act 1991 of New Zealand [186] requires local authorities and industry to apply for consent to discharge effluent to water or land. Therefore, the requirement for whole effluent toxicity testing is now being included in these Resource Consents. The Ministry for the Environment (MfE) has encouraged research which evaluates technology used to monitor environmental parameters (e.g., effluent toxicity). Such research was done using the Rotoxkit F™ and Rotoxkit M™ (with *B. calyciflorus* and *B. plicatilis*, respectively). The study concluded that "while very cost effective and with very good precision (repeatability), were not as sensitive as the others, and therefore are not recommended for whole effluent toxicity screening" [187].

5.3. Latin America and the Caribbean

Sarma et al. [188] showed that Mexico City urban wastewater affects instantaneous growth rate of *Brachionus patulus*. Acute 48-h lethal effect measurements generated with *L. quadridentata* on municipal, industrial, and agricultural sites around the city of Aguascalientes, Mexico, indicated that most samples tested were toxic [189]. The municipal drinking water wells of Aguascalientes have also been assessed using acute toxicity tests with *L. quadridentata* [190]. *L. quadridentata* has been used to assess the status of the major wastewater treatment plants in the State of Aguascalientes [191, 192]. An ecotoxicological study of the most important river in the State of Aguascalientes has also been performed [93]. *L. quadridentata* has also been used to assess toxicity in several rivers of the southern Huasteca area of Mexico with high concentrations of manganese (Mn) and the presence of DDT in the sediments and pore water. *L. quadridentata* was highly resistant to DDT and less susceptible to Mn than the cladocerans *D. magna* [165]. José de Paggi and Devercelli [193] 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. *Brachionus* spp. were associated with saline waters in rural areas and *Keratella* spp. (except *Keratella tropica*) with urban water bodies.

5.4. Asia

Many Asian countries have used rotifer toxicity tests for diverse monitoring and scientific purposes. Microcosm studies with rotifers have been used in India to evaluate tannery effluent [194]. *B. plicatilis* has been used to assess the toxicity of the various sewage sludge, one of the major ocean dumped materials in the Yellow Sea of Korea [195].

6. Perspectives for future studies regarding the importance of rotifers as models for toxicity screening of chemicals

Different rotifer species from all around the globe have been used to test the toxicity of a huge number of chemicals, both from freshwater (FW) and marine water (MW) ecosystems (see Rico-Martinez et al., 2013). Such species were initially collected from their natural habitats, a specific biogeographical zone, for their further acclimation to laboratory conditions and use as model organisms in toxicity evaluation protocols.

Nowadays, the rotifer species used as model organisms, due to their representativeness and wide natural distribution, include those of the genus *Brachionus* sp.: *B. calyciflorus* (FW), *B. plicatilis* (MW), *B. manjavacas* (MW), and *B. koreanus* (MW) (Lee et al.; Snell et al.), although some others like *B. rotundiformis* (MW) and *B. ibericus* (MW) could be used as model organisms (Pérez-Legaspi, 2015). Moreover, organisms within the genus *Lecane* sp. have been used for toxicological screening, demonstrating that *L. quadridentata* and other *Lecane* species are good indicators of water quality because of their high susceptibility to toxicants.

Despite all efforts to understand rotifers biology and their susceptibility to contaminants, there is still a need to conduct new studies with rotifers belonging to different habitats (biogeographical zones), climates, and niches. For such studies, researchers should take into consideration topics like clonal cultures obtaining, rotifers identification and classification through morphological and genetic (cytochrome oxidase rDNA, COI) characters, and the production of sexual eggs to preserve them in a resting eggs bank. Such eggs could be also a source for developing toxicity assessments kits, like those of Microbiotests Inc. [179], which mainly uses *Brachionus* sp. resting eggs in very efficient systems for their production.

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Due to their immeasurable dispersion and diversity, annotating correctly the rotifers specimens' origin and recording their chemical backgrounds has become a very important issue because of the variable responses to toxicants observed within the same genus or even within the same species. For example, *Brachionus* sp. VER, isolated from the Gulf of Mexico, was the most tolerant to Macondo oil exposure (LC50 = 19.33%) in comparison to *B. rotundiformis* (LC50 = 11.02%) from Hawaii, *B. plicatilis* ss. (LC50 = 2.47%) from Tokyo, and *B. manjavacas* (LC50 = 5.43%) from Russia [36], which could be due to the presence of cryptic species within the taxon. Recently, researches and students from different countries participated in the workshop "Cryptic Speciation in *B. plicatilis*: A Workshop to Described Species within the Complex," and they estimated that there may be as many as 20 new species for this complex. In addition, the rotifers *B. calyciflorus* and *Lecane bulla* form a part of cryptic species [196, 197]. Thus, genetic and phylogeographic studies should be performed to assess how this species are distributed around the world.

In aquatic toxicology, currently there are methods that help elucidating the toxicity mechanisms for different sorts of chemicals. Therefore, they can be listed as follows:

a. Standardized protocols for evaluation of acute, chronic, and sublethal toxicity. These methods are carried out by exposing neonates or resting eggs, for 24 to 48 h or more depending exposure concentrations, periods, and end points to measure. Acute and chronic toxic ratios are still in use despite all new technologies, as they are finally a reference point for further analysis. Mortality or immobility are the common responses observed in acute toxicity tests, but in chronic assays, population parameters are followed during the exposure period, such as the intrinsic rate of population increase (r) obtained from the life table analysis. Another possibility is assessing the hatching percentage, which represent the stability and health of cysts produced during stressful conditions, including abiotic factors like desiccation or the presence of contaminants. Alvarado-Flores et al. [48] evaluated the effect of 1.2 mg/L of the fungicide vinclozolin on the rotifer *B. calyciflorus*. Their findings demonstrated that there was no significant difference between exposed and nonexposed organisms. In addition, the population parameter r of rotifers hatching from VZ-promoted cysts was 1.21 ± 0.063 (mean ± SD), and for rotifers hatching from control cysts was 0.90 ± 0.064.

It has been shown that multigenerational studies should be conducted as these could reproduce what happens in natural conditions when parthenogenetic females are exposed to toxicants and in their offspring is in certain way altered even before hatching [198], a phenomenon called the maternal effect, which could be for good while providing more energetic resources to deal with the stressful conditions in the medium, or negative through inheriting mutations that could bring deleterious effects in consecutive generations.

b. *In vivo* enzyme activity assessment (esterases and phospholipase A2): This method has the potential to assess the adverse effects of contaminants for rotifers. In the rotifer *E. dilatata* (FW), a native species from Mexico, the inhibition of esterases and phospholipase A2 was assessed fluorometrically after *in vivo* exposure (30 min) to sublethal concentrations of metals and pesticides in laboratory conditions. This study concluded that both enzymes are very sensitive to toxicants-induced alterations [96, 97].

c. Stress granules (SGs): Eukaryotes share diverse mechanisms of adaptation and responses to the stress. In this matter, it has been shown in different insects, trypanosomids, yeast, mammal cells, and rotifers, in which they can sequester some proteins and mRNA into granules that protect cellular mRNA. Thus, SGs appear to be useful as biomarkers in rotifers [199].

d. Bioconcentration factors (BCF): According to van der Oost et al. [200], biomarkers of exposure include the quantification of the toxicant or its metabolites. Therefore, BCF are very valuable tools to study exposure to a certain kind of compounds. Moreover, these assessments could help to trace toxicants in discharges to aquatic ecosystems, by monitoring both in laboratory and in natural conditions exposed animals [149, 201]

e. Elemental composition using X-ray analysis on rotifers cuticles. This is an easy method that qualitatively determines different elements of interest, principally inorganic metals [48].

f. Morphological analysis: These are changes induced by toxic exposure; although it could be controversial, it can be carried out by comparing morphological characters through image analysis. Because of rotifers phenotypic plasticity, the comparisons should be carefully performed to avoid misinterpretation of the results. However, is has been demonstrated, in *B. calyciflorus,* that morphological changes occurred after exposure to the fungicide vinclozolin and that abnormal and healthy animals are easily differentiated. However, the percentage of deformities is low, only 0.63% of 2868 organisms. Nevertheless, this is still significantly different to the control groups. Hence, morphometric analysis in rotifers could be a helpful tool to identify unrevealed targets of toxicants, and it might contribute to create a database for such effects and for several rotifers species to further comparisons among them, besides the likely identification process through image analysis [48].

g. Aging in rotifers: because there is a great diversity in aging rates among species, geographical populations, and mutants within species, Smith and Snell [202] designed an experiment to follow rotifers longevity through 84 generations (about 1 year). Their results show that optimal growing conditions (e.g., constant food supply) altered life span and can reduce aging, which could be evolutionary adjustable, with selection working primarily on the length of the reproductive life span. Rotifers are considered good models to investigate the effect not only of toxicant on their life span but also other factors such as caloric restriction and the effect of vitamins. Thus, this represents a new field to incorporate studies with rotifers [203, 204].

h. Hormones: Alvarado-Flores et al. [205] demonstrated the presence of some mammal-like proteins in the rotifer *B. calyciflorus* and concluded that it is necessary to generate more information about catecholaminergic and cholinergic systems in rotifers and the hormones related. Then it will be possible to assess their participation in mechanisms of detoxification and likely be used as toxicity models.

i. Genetic tools in rotifers and their applications

- RNA-seq: With mRNA-seq libraries for obligate parthenogenetic and cyclical parthenogenetic strains of the rotifer *B. calyciflorus*, it has been possible to identify genes specific to both modes of reproduction. Additionally, the studies performed by Hason et al. [206] allowed insights in the reproductive biology of obligated asexual bdelloid rotifers.

- Heat shock proteins (HSPs): The genes for these proteins synthesis are found from bacteria to higher eukaryotes and are related to functions like refolding denatured proteins due to stress that includes heat shock, reason for which they are called HSPs. Smith et al. [207] provided conclusive evidence that *hsp40*, *hsp60*, and *hsp70* are required for rotifer survival following heat stress, but that hsp90 seems not to be essential for survival, at least with their data.

- Metallothionein (Mt): These are low-molecular weight and cysteine-rich proteins present in eukaryotes. They provide potent metal binding and some other functions are being discovered. Their presence in rotifers has been demonstrated as a consequence of chromium exposure [208]

- P-glycoprotein 8P-gp (Pg-p): This protein could be considered as the first line of defense against some chemicals, including pharmaceuticals and endocrine disruptors. This protein has been found and characterized in the rotifer *B. koreanus*. Specimens of this rotifer were exposed to several pharmaceuticals that retarded growth and promoted the overexpression of Pg-p [209].

- Epigenetics: Germ cells can be specified early in embryogenesis by maternal determinants inherited in the cytoplasm of the oocyte or they can be selected later in the embryonic development from undifferentiated precursors by a localized inductive signal that is called epigenesis [210]. Epigenetic processes were found in the ovary of *B. plicatilis*, describing the participation of *vasa* and *nos* genes. As the first description of its kind, it opens the possibilities to explore and perform embryo development within the phylum.

- Cell-penetrating peptides (CPPs): Liu et al. (2013) demonstrated that some little peptides can penetrate cell membranes and delivered their cargoes; then their function (if cargoes have it) can be assessed as the functional HR9-delivered plasmid DNAs and RFP coding sequences that could be actively expressed in rotifers. This method provides a tool not only for genetic material but also for nanoparticles and proteins, which in the future could facilitate studying the effect of chemicals within rotifer cells.

j. Innate immunity in rotifers: In invertebrates, the nomenclature, annotation, and reports of cytokines could be controversial. Nonetheless, there is a continuous and increasing knowledge about cytokine-mediated immune regulation, although adaptive immune responses are likely absent in invertebrates, including rotifers. In this field, Jeong et al. [211] identified three genes of lipopolysaccharide-induced TNF-α factor (LITAF) in *B. koreanus*. The *in silico* analysis showed that these genes could be involved in innate immunity in primitive rotifers. In addition, exposure to lipopolysaccharide caused the overexpression of *LITAF1* and *LITAF2* but depleted glutathione concentration. Thereafter, *LITAF* genes have potential sensitivities to immune stimulator-triggered oxidative stress.

In conclusion, rotifers as models for ecotoxicological tests present several advantages, including a relative short life cycle that allows multigenerational studies and epigenetic research to unveil functions and processes in mictic and amictic rotifers, the simplicity of their body structure that ease the permeability of dyes for *in vivo* examination to quantify toxicant concentrations (e.g., Leadmium green®) or for systems descriptions like both the cholinergic and catecholaminergic systems, and their easy culture conditions and supply of resting eggs from different sources like some commercial brands. Nowadays, there are several protocols that describe the use of rotifers as indicators of water quality and safety as even thousands of probes can be performed in very short periods. Furthermore, every year, new technologies are becoming available to explore in deep detail the effect, the mechanisms, and the targets of toxicants. Hence, rotifer studies cannot be the exception, opening new possibilities to explore and describe more accurately the interaction of toxicants with the aquatic biota.

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