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Chapter 7

Toxicity of Agrochemicals on Freshwater Invertebrates — A Short Review

Daniel Robles-Vargas

Additional information is available at the end of the chapter

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Abstract

The increase of worldwide population and the need to control pests are some of the factors that have led to the application of agrochemicals on agricultural areas to protect and increase crop production. Nevertheless, these substances are of environmental concern since they can reach water reservoirs and act on non-target organisms. Therefore, different aquatic species have been tested to evaluate their sensitivity to different toxicants, including pesticides, so as to elucidate the secondary effects of these chemicals to estimate "safe levels" in aquatic media. A wide variety of toxicity tests can be found in literature to evaluate the toxicity of xenobiotics in the environment at organismal and sub-organismal levels under different regimes. This chapter focuses on those tests performed with some freshwater invertebrates (cladocerans and rotifers) to study the toxicity of four important classes of pesticides.

Keywords: Toxicity, agrochemicals, bioassays, cladocerans, rotifers

1. Introduction

The need to provide enough food to the growing worldwide population and control pests are some factors that have led to the application of agrochemicals (pesticides) on agricultural areas to protect and increase the crop production [1]. Despite the advantages offered by pesticides, these substances can turn into an environmental concern since they can leave their action point mainly by surface water runoff and reach water reservoirs, which could alter the aquatic environment and pose a threat to human health [2-4]. The majority of these

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chemicals have a synthetic basis, and different categories have been established to classify them depending on their chemical structure. Some of the most representative agrochemicals with great ecological impact are organochlorine hydrocarbons (DDT), organophosphates (parathion and diazinon), carbamates (carbaryl and methiocarb) and pyrethroids (deltamethrin), where the first group is characterized by its stability in the environment after being released. Pollution of freshwater ecosystems with these chemicals is well known and has been reported for several regions worldwide and it represents a problem of consideration for the preservation of the aquatic environment [5-8]. All these pesticides act by altering the organism's nervous system [6].

In this context, scientists have worked to develop and standardize protocols to evaluate the toxic effects of a wide variety of pollutants on certain living organisms known as "sentinel organisms" or "bioindicators" [9]. Bioassays are toxicity studies that can be performed with organisms that represent an important component of ecosystems and are able to respond to xenobiotics, and therefore, bioassays may be used to predict "safe levels" of toxicants in the environment. Among bioindicators, freshwater invertebrates are used frequently due to their importance as primary consumers of algae and herbivores representing a key link in trophic webs [10-12]. Moreover, some aspects like a) abundance, b) wide distribution, c) maintenance and easy culture in the laboratory, d) genetic stability and e) sensitivity are considered to select test organisms [13, 14].

Standard toxicity tests are usually performed with a single species to assess the toxicity in water samples and different endpoints can be evaluated, such as motility, reproduction and enzymatic inhibition. The endpoint "motility", usually corresponds to a short-term (acute) toxicity assay and represents the concentration of chemicals that reduces the motility to 50% of the animals after 24 or 48 h exposure and the result is expressed as EC_{50}. This assay also can be interpreted as the lethal concentration for 50 percent of individuals (LC_{50}). For the long-term (chronic) tests, behavioral changes (grazing and filtration rates, phototaxis and survival) and reproduction assays can be conducted. The reproduction assay evaluates the effects on reproduction typically after 21 days of exposure and is represented by EC_{50}. This parameter estimates the concentration that inhibits 50% of reproductive effort [15-19].

Within the chronic test category, ecotoxicologists have used another approach to evaluate toxicity known as "sub-lethal effects tests" by estimating variations in biochemical or physiological components (biomarkers). Their importance is based on their capability to indicate damage to the organism following exposure to concentrations of contaminants that are not acutely toxic. Some examples are the enzymatic inhibition and genomic responses (genotoxicity) that indicate disturbances occurring at the sub-organismal level [20-22].

Measurement of different endpoints can provide valuable toxicological information to derive water quality criteria for the safe release of compounds into aquatic bodies [13, 23]. This chapter focuses mainly on those studies performed with freshwater invertebrates that are representative for comparison purposes according to their availability in literature.

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2. Freshwater invertebrates as sentinel organisms

2.1. Cladocerans

In general terms, *Daphnia magna* (figure 1) and *Ceriodaphnia dubia* represent the main daphnids (Class Crustacea, order Cladocera, family Daphniidae) used as bioindicators. Moreover, *Daphnia carinata* and *Daphnia galeata* have also been tested and have been included in the chapter. These organisms known as "water fleas" have filter-feeding habits and are ubiquitous species in temperate freshwater bodies. Usually, daphnids reproduce by an asexual reproduction mechanism called "parthenogenesis" [15, 24, 25].



Figure 1. *D. magna* (Female individual. Photograph taken under stereomicroscope (January, 2015) by Doctor Gustavo E. Santos-Medrano, Chemistry department, Universidad Autónoma de Aguascalientes)

2.1.1. Daphnids as bioindicators

2.1.1.1. Daphnia magna

Sánchez *et al*. (1999) [26] performed a two-generation reproduction test (chronic assay: 21-day life study) to assess the effects of diazinon at different concentrations ranging from 0.05 to 1.0 ng/L. This agrochemical is an organophosphorus pesticide used to fight leaf-eating insects. The parameters evaluated in the parental organisms (F_o) as in the offspring (first and third brood) were size, survival and fecundity. Between the main findings, a remarkable decrease in longevity, number of individuals per female, brood size and number of broods per female in F_o was noted as the pesticide concentration was increased. These parameters did not decrease dramatically in the offspring as compared with control and individuals showed a higher reproduction rate than their parental mothers. Moreover, fecundity and growth from first and third brood did not recover completely. A 24h LC_{50} of 0.86 μg/L was obtained.

Bettinetti *et al*. (2013) [27] monitored reproduction (21-day assay) and survival as toxicity endpoints in daphnids exposed to pp′-DDE (pp′- Dichlorodiphenyl – dichloroethylene), a more stable metabolite of the organochloride p,p′-DDT (dichlorodiphenyl-trichloroethane) through diet and to different concentrations in water. For the treatment with contaminated

algae, a decrease of 83.3% was registered in neonates production for mothers exposed to the highest dose (795.6 pp´DDE ng/per organism) at day 21 and were shorter in length compared to control group. A reduction in grazing activity (i.e. ingestion activity) was also observed in mothers with 24 ng/mg (dry weight basis). Exposure to pp´-DDE in water showed that the maximum metabolite concentration used (6 μg/L) increased the mother's long-term mortality by 50% and reduced the fecundity of the surviving mothers by 32.4%. A 48h IC_{50} (concentration at which 50% of the individuals presented immobilization) of 5.08 μg/L was estimated.

Another modality of toxicity test corresponds to the behavioral response under toxicant exposure. Martins *et al*. (2007) [28] evaluated the phototactic behavior "phototaxis" in *D. magna*, which consists of individuals' movement towards or away from a source light as a possible outcome of different natural phenomena (finding prey, reproduction) and stress (predator avoidance, photochemical damage) [29-31]. In this study, strong positive phototactism (movement toward a light source) clones were used and were exposed to 11 chemicals commonly found in freshwater environments, including pesticides. It was observed that the fungicide "Thiram" (carbamate) reduced markedly the phototactic behavior and was detected within 0.25 h of exposure at a concentration of 9.38 μg/L. Furthermore, carbamate was detected at a lower concentration than the 48h LC_{50} (210 μg/L) reported for the bioindicator.

Toxic effects of pesticides also have been evaluated considering food availability. Pereira and Golcalves (2007) [32] evaluated acute and chronic toxicity of methomyl (carbamate) to different daphnid species, including *Daphnia magna*, under different food level regimes. *D. magna* showed the greater resistance to the toxicant and presented a 48h EC_{50} (concentration at which 50% of the individuals presented immobilization) of 24.17 μg/L. For the chronic toxicity tests, a decrease in reproduction was observed as methomyl concentrations were increased and sensitivity was greater at low food levels.

Sensitivity between same species has been tested by Toumi *et al*. (2013) [33], who studied the toxic effects of the pyrethroid "deltamethrin" on two daphnid strains provided from different laboratories. The results of acute toxicity tests (immobilization) revealed a 48h EC_{50} of 0.32 and 0.63 μg/L, for strain 1 and 2, respectively. In terms of chronic toxicity (survival and reproduction), deltamethrin induced significant effects and embryo deformities were found, which, according to the authors, gives certain evidence that this agrochemical could have endocrine disruptive effects. Nevertheless, it is worth mentioning that differences in sensitivity for the two strains were observed.

2.1.1.2. Ceriodaphnia dubia

Shen *et al*., (2012) [34] have reported some toxicological data using *C. dubia* after conducting acute and chronic assays for two pyrethroids: deltamethrin and α-cypermethrin. Results showed lethal toxicity on the cladoceran and 48h LC_{50} for immobilization of 0.06 and 0.84 μg/L were recorded for each agrochemical, respectively. For the 8-day chronic assays, survival and reproduction endpoints were evaluated, thus obtaining an EC_{50} of 116 and 34.7 ng/L for deltamethrin and 209 and 97.8 ng/L for α-cypermethrin, respectively.

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Metabolic activation of pesticides via cytochrome P450 (a protein superfamily involved in the metabolism of xenobiotics and endogenous compounds) has been tested by El-Merhibi *et al*. (2004) [35] by studying the toxicity of the organophosphorus chlorpyrifos in the presence or absence of the inhibitor/inducer of cytochrome P450 "piperonyl butoxide (PBO)". Among the main findings, a 48h LC_{50} for mortality was estimated at 0.05 μg/L in the absence of PBO. Moreover, a reduction in acute toxicity was evident in the presence of piperonyl butoxide in response to increasing concentrations of this chemical.

2.1.1.3. Daphnia carinata

The Australian native species *D. carinata* has been tested by Cáceres *et al*. (2007) [36] to evaluate the acute toxicity of chlorpyrifos and its metabolite 3,5,6-trichloropyridinol (TCP) separately and in combination tests using a cladoceran media and river water. A greater toxic effect was observed in the cladoceran media by TCP (48 LC_{50}: 0.20 μg/L) but showed no toxicity in natural water, whereas the parental compound exhibited a 48h LC_{50} for river water and cladoceran media of 0.3 and 0.24 μg/L, respectively. The absence of toxicity by the metabolite in river water was attributed to the microbial activity that led to its degradation. The toxicity assessment using both chemicals at a concentration of 0.12 μg/L did not affect the survival of the species in natural water but reflected an additive effect and caused 72% mortality in the artificial media. According to the researchers, this finding suggests that joint toxicity could behave in a different manner when chemicals are in the environment and differences between the media composition should be considered.

Acute and chronic toxicity of chlorpyrifos was evaluated by Zalizniak and Nugegoda (2006) [37] using three successive daphnid generations. For the lethal toxicity, a 48h LC_{50} was estimated for parent generation (0.5 μg/L). In long-term toxicity assays (21-day survival), fecundity, time to the first brood and female size were monitored. The number of offspring per female in parent individuals was significantly reduced. The main endpoints altered in the first generation were survival and fecundity, whereas the time to the first brood and an indication of hormesis (response stimulation and inhibition at low and high concentrations, respectively) were evident in the second generation. Moreover, the lowest concentration tested (0.005 μg/L) yielded the lowest number of offspring per female. For the third generation, daphnids showed a remarkable sensitivity at low concentrations of chlorpyrifos (0.025 μg/L).

2.1.1.4. Daphnia galeata

Some researchers have studied the effects of chlorpyrifos using *Daphnia galeata* as bioindicator. van Wijngaarden *et al*. (1993) [38] monitored immobility and mortality as acute toxicity endpoints and a 48h EC_{50} of 0.3 μg/L was recorded. van den Brink *et al*. (1995) [39] evaluated chronic toxicity (24 days) simulating an indoor microcosm, including different zooplankton (*D. galeata*), phytoplankton and macro-invertebrate species under low concentration exposure (0.1 μg/L). Daphnid population reduced to zero within the first week of the test, nevertheless, stress factors like predation could have influenced this finding. Moreover, the agrochemical affected zooplankton species, which resulted also in community alterations.

2.1.1.5. Biomarkers in Daphnia magna

In relation to agrochemical toxicity assessment using biomarkers, Guilhermino *et al*. (1996) [40] proposed inhibition of the enzyme acetylcholinesterase (AChE) as an acute toxicity indicator. This enzyme involved in neural transmission has been monitored under carbamates and organophosphates exposure. Liu *et al*. (2012) [41], conducted both an acute and a sub-lethal study (21 days) with chlorpyrifos. In acute bioassays, 48h EC_{50} for immobilization was (7.12 μg/L). Moreover, in the chronic test, a recovery on AChE activity was noted after the second day of exposure, this probably because *D. magna* developed adaptive mechanism(s) to mitigate stress.

A genotoxicity study was conducted by Pereira *et al*. (2010) [42] to analyze gene transcription after acute exposure (48 hours) of third to fifth brood juvenile daphnids to the insecticide methomyl at a concentration of 10.5 μg/L. It was found that the agrochemical was able to induce several genes and affect neuronal transmission. mRNAs of a digestive enzyme (α-amylase) and diverse lipoproteins were up-regulated, which according to the authors followed the need of carbohydrate breakdown for energy production and was an effort to maintain homeostasis under toxicant stress. mRNAs of genes involved in defense mechanisms (galactose-binding C-type lectins, cystatins and ferritins) were also up-regulated. As mentioned by the authors, lectins and cystatins play an important role in the general immune response as they participate in hemolymph coagulation and ferritin expression might indicate oxidative stress. Even when stress responses were evident, a strong evidence for expression responses related exclusively to genes associated with the pesticide target site was not found.

An assay to elucidate toxicity mechanisms of carbamates using a biomarker (AChE) was implemented by Jeon *et al*. (2013) [43] by exposing *Daphnia magna* to carbaryl (carbamate). Results were compared to the USEPA ECOTOX database for the organophosphorus irreversible AChE inhibitor "diazinon". For the carbamate, an IC_{50} for *in vitro* AChE activity and a 48h EC_{50} value for immobilization were obtained (0.56 μM and 63 nM, respectively). The latest parameter was greater than that proposed by USEPA ECOTOX for diazinon (3.0 nM). Low toxicity was observed by carbaryl acting through a reversible inhibition mechanism. This finding was attributed to the instability of the AChE-carbaryl complex where a lower hydrophobicity of this chemical is possible. A declining energy reserve was also identified since lipid and glycogen reservoirs decreased with an increase of protein content.

In another study, Toumi *et al*. (2015) [44] estimated AChE activity in 3 *Daphnia magna* strains (strains 1 and 3 already identified; clonal identification of strain 2 remains unknown) after deltamethrin exposure, a pyrethroid insecticide, reporting a significant decrease on the enzyme activity in 2 strains. Variation in sensitivity between strains was observed and different 48h IC_{50} (concentration at which 50% of AChE was inhibited) and 48h EC_{50} values were obtained for immobilization endpoint. Thus, the strain 1 registered the lower EC_{50} (0.32 μg/L) and strain 3 the lower IC_{50} (0.016 μg/L). For strain 2, an EC_{50} of 0.63 μg/L and IC_{50} of 0.018 μg/L were registered. Lowest Observed Effect Concentration (LOEC) for each strain was also estimated (Strain1: 80.6 ng/L; Strains 2 and 3: 20.1 ng/L). According to the authors, the interclonal variability observed in these results can lead to propose AChE as a biomarker of susceptibility (the response is specific for each strain) for deltamethrin exposure in D. magna.

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Barata *et al*. (2004) [45] also assessed inhibition of AChE and carboxylesterase (CbE) after pulse exposures of *D. magna* to three pesticides in the presence and absence of triphenyl phosphate and 2-(O-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide (CBDP) CbE inhibitors. A 24h LC_{50} of 1.28, 12.38 and 762.93 nM was estimated for chlorpyrifos, malathion and carbofuran (carbamate), respectively. Both enzymes in the treatments with the carbamate were inhibited in less than 2 hours as this toxicant does not need to be activated by cytochrome P450 enzymes. Moreover, results showed higher sensitivity of CbE (lower concentration inhibition) against organophosphorus pesticides, which according to the authors was due to a higher enzyme affinity to the oxons formed (malaoxon and chlorpyrifos-oxon). It was suggested that this finding could be involved in conferring protection to AChE by sequestering available pesticides. Besides, the two CbE inhibitors increased mortality response as more agrochemicals availability was present for AChE. β-esterase activity in individuals exposed to carbofuran reached an activity similar to control level in less than 12h, whereas in daphnids exposed to organophosphates both enzymes resumed their activity between 24 and 96h to achieve 50% and almost complete recovery levels, respectively.

Some digestive enzymes have been used as biomarkers. De Coen *et al*. (1998) [46] monitored ingestion activity using fluorescent labeled latex microbeads and the digestive enzymes "esterase, trypsin and β-galactosidase" under the exposure of pentachlorophenol (PCP) and lindane, both organochloride pesticides. Considerable reduction in esterase (greater than 50%) and ingestion (90min EC_{50}: 0.3 mg/L) activities were induced by lindane. Moreover, a drastic decrease in ingestion activity was noticed compared to digestive enzyme inhibition. According to the authors, this was probably due to an energy-saving strategy, since ingestion demands more activity, and therefore, daphnids could maintain a certain food assimilation efficiency. 24h EC_{50} values for immobilization were estimated (PCP: 0.7 mg/L; Lindane: 1.8 mg/L).

Another aspect of interest in ecotoxicology is that some pesticide metabolites can exhibit more toxicity than their parental compounds as it has been reported previously by Belden and Lydy (2000) [47]. Guilhermino *et al*. (1996) [48] tested AChE activity with *D. magna* under the organophosphorus pesticide "parathion" as parental chemical and its metabolite "paraoxon" and observed such phenomena as the EC_{50} values from *in vivo* AChE inhibition and acute toxicity (48h EC_{50}) for parathion (2.4 and 2.2 μg/L, respectively) were higher than those obtained for paraoxon: 0.2 μg/L for both *in vivo* AChE inhibition and acute assay.

2.2. Rotifers

Within the phylum Rotifera, rotifers of the genus *Brachionus* have been widely used as test organisms due their cosmopolitan distribution, ease of maintenance in the laboratory, rapid reproduction and availability of resting eggs (widely used in ecotoxicology) as these microscopic freshwater invertebrates play an important link in food webs by making biomass available for higher trophic levels [49-51]. For comparison purposes, *Brachionus calyciflorus* (figure 2), *Brachionus patulus* and one rotifer from the genus *Lecane* were included.



Figure 2. *B. calyciflorus* (Female individual. Photograph taken under optical microscope (January, 2015) by Doctor Gustavo E. Santos-Medrano, Chemistry department, Universidad Autónoma de Aguascalientes)

2.2.1. Brachionus calyciflorus

Fernández-Casalderrey *et al*. (1992) [52] studied the chronic effects of diazinon on *B. calyciflorus*. A 24h LC_{50} of 29.22 mg/L was estimated. Low concentrations of the agrochemical (range: 5–19 mg/L) were capable to reduce survival, fertility, life expectancy and reproduction with a dose-response relationship.

This researcher group also evaluated chronic toxicity of the organophosphorus methylparathion by feeding the rotifers with *Nannochloris oculata* and *Chlorela pyrenoidosa*. A 24h LC_{50} of 29.19 mg/L was estimated. Life expectancy and reproduction rate were reduced with increasing concentrations of the pesticide. Chronic exposure at 5 mg/L could lead to rotifer population extinction. Moreover, the decline was greater on those individuals fed with *Chlorela* (larger in size than *Nannochloris*) [53].

Ke *et al*. (2009) [54] studied the effects of three pesticides including chlorpyrifos on population growth and sexual reproduction. The results showed an induction on the mictic rate (sexual reproduction) since resting egg production was increased at concentrations between 0.1 and 100 μg/L. A similar response was found for the population growth as this parameter was increased but only at pesticide concentrations from 0.01 to 100 μg/L. Mortality was observed only at the highest treatment concentration (1000 μg/L) after 24 h exposure.

In another study, ingestion rate was proposed as a sub-lethal stress indicator by Juchelka and Snell (1994) [55] for this species. *B. calyciflorus* was exposed to different dilutions of PCP, diazinon and chlorpyrifos under short exposure scheme (30 min) and fluorescence intensity was measured to estimate the number of microspheres ingested in 5 min after exposure treatments. 24h LC_{50}, ingestion (NOEC: No observed effect concentration) and reproductive rate (48h NOEC) values of 1.2, 0.13 and 0.11 mg/L, 31, 20 and 8 mg/L and 12, 0.25 and 0.23 mg/L were estimated for PCP, diazinon and chlorpyrifos, respectively.

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2.2.2. Brachionus patulus

In [56], the effects of different sub-lethal concentrations of DDT under high and low food levels on *Brachionus patulus* were assessed. Survival and fertility were higher for the high food level. Authors hypothesized that this finding might be the result of a healthier status as the individuals were better fed, thus acquiring more toxicant resistance. Moreover, a dose-response relationship was observed for all endpoints tested in both food regimes.

2.2.3. Lecane quadridentata

Lecane quadridentata (figure 3) is found in Mexican waters (Lake Chapala) and has been used by several authors to assess different toxicity endpoints against several toxicants including metals, organic compounds and pesticides [57-60].



Figure 3. *Lecane quadridentata* (Female individual. Photograph taken under optical microscope (January, 2015) by Doctor Gustavo E. Santos-Medrano, Chemistry department, Universidad Autónoma de Aguascalientes)

In [61], a study was conducted to perform three toxicity tests "lethal (48h mortality), sub-lethal (inhibition of AChE activity) and chronic (5 day inhibition of the instantaneous growth rate) assays" using carbaryl and methylparathion. The carbaryl pesticide exhibited the higher chronic toxicity (EC50 2.22 mg/L) but greater lethal and sub-lethal toxicity was registered with the organophosphorus pesticide (9.4 mg/L for both bioassays). Moreover, the growth rate was more sensitive in comparison to the esterase activity and was proposed by the authors as a biomarker to assess the toxicity of anticholinesterase pesticides.

3. Relevance of sensitivity of cladocerans and rotifers against pesticides

As mentioned earlier, freshwater invertebrates tend to be sensitive against pollutants and are key factors to maintain freshwater ecosystem quality, thus, preserving these organisms in their habitat is important to guarantee the entire water reservoir health. In the present chapter,

different studies to assess the toxicity of four main classes of pesticides on freshwater invertebrates were reviewed.

According to the available data on literature, *Daphnia magna* has been widely used in toxicity assays with agrochemicals, nevertheless, it is not always the most sensitive species. In table 1, where additional references for comparison purposes were included (Additional References: [62- 69]), inter- and intraspecies differences on sensitivity are shown. For example, *Ceriodaphnia dubia* exhibited the greatest sensitivity in acute and chronic toxicity against lindane in comparison to *D. magna* and *Brachionus calyciflorus* and showed the lowest 24h LC50 for PCP. Besides, this cladoceran showed the lowest 48h LC50 for the pyrethroids deltamethrin and cypermethrin and the organophosphorus pesticide diazinon. In some other cases (carbaryl and methylparathion), *D. magna* was more sensitive than the rest of the bioindicators. For the genus *Brachionus*, a marked tolerance was observed for the four agrochemicals considered. These differences could be attributed to the toxicant interaction with the test organism, differences in toxicity patterns of each agrochemical and to certain conditions used in the bioassay: type of assay, test duration, presence or absence of food and chemical concentration.

In this context, to obtain a more accurate toxicity estimation of water samples polluted with agrochemicals, different endpoints should be evaluated. For example, Pérez-Legaspi *et al*. (2010) included [61] acute and chronic toxicity assays and a biomarker to evaluate the toxicity of methylparathion and carbaryl on *Lecane quadridentata,* where the chronic test showed the higher sensitivity for both xenobiotics. In addition, the use of standardized protocols together with an additional test (using a different test organism) could help to obtain a more reliable conclusion about the actual condition of the freshwater system under evaluation. Also, mesocosm studies could help to estimate the effects of pesticides at the community level and predict long-term effects.

Additionally, when evaluating toxicity in water samples, some other considerations become important. In natural water bodies, it is likely that aquatic organisms are exposed to different agrochemicals and for longer periods, thus, phenomena such as synergism between pesticides and bioaccumulation (accumulation of substances in an organism) are possible and can aggravate the ecological impact by an increase in their toxicity as the pollutants could move through food chains and reach final consumers including the human being. When considering these two phenomena in ecotoxicological studies a more realistic and representative result can be obtained.

In relation to the water quality criteria for aquatic life protection (table 1), some values seem to be appropriate to protect aquatic organisms (lindane, PCP, DDE), however, in some cases there is no criteria available (methylparathion) and for other agrochemicals the recommended concentration could be not that protective, such as those proposed for malathion, chlorpyrifos, diazinon, cypermethrin, deltamethrin, carbaryl and DDE, as these values are close to the toxicity values registered for some endpoints. Pesticide toxicity on freshwater organisms is evident and the need for continuous generation of ecotoxicological data to protect aquatic life and human health still remains.

Agrochemical		Species	Endpoint				
			Acute toxicity		Chronic toxicity	Sub-lethal toxicity (biomarkers)	Water quality criteria for aquatic life protection
			24h LC_{50} (mortality) (mg/L)	48h LC_{50} (immobilization) (mg/L)	Reproduction (EC_{50}) (mg/L)	*In vivo* 48h IC_{50} (AChE) (mg/L)	(mg/L)
Organochloride pesticides	Lindane	*Dm*	1.8[p]	1.7[w]	0.34[w] (16 d)	-	2x10^{-4}[**]
		Cd	-	0.045[w] (7 day)	0.013[w] (7 day)	-	
		Bc	22.5[v]	-	-	-	
	PCP (sodium salt)	*Dm*	0.7[p]	0.35[w]	-	-	5x10^{-4}[*] (long term) 0.019[+] at pH 7.8 (short term) 0.015[+] at pH 7.8 (long term)
		Dc	-	1.3[w]	-	-	
		Cd	0.149[w]	-	-	-	
		Bc	1.2[c]	1.2[w]	0.27[w] (2 day)	-	
	DDE	*Dm*	-	5.08x10^{-3} [d]	-	-	3x10^{-5} [**]
Carbamates	Thiram	*Dm*	-	0.21[e]	-	-	2x10^{-4} [**]
	Methomyl			0.024[f]			3.5x10^{-3}[**]
	Carbaryl	*Dc*	0.1[w]	0.035[w]	-	-	3.3x10-3* (short term) 2x10-4* (long term)
		Cd	-	0.012[w]	8.6x10^{-3}[w] (7 day)	-	
		Dm	-	0.012[q]	-	-	
		Lq	-	13.72[r]	2.2[r] (5 day)	17.19[r](45 min)	
Pyrethroids	Deltamethrin	*Dm* (S1)	9.4x10^{-3} [g]	3.2x10^{-4} [g,i]	-	5.8x10^{-5} [i]	1x10^{-7}[**]
		Dm (S2)	8.86x10^{-3} [g]	6.3x10^{-4} [g,i]	-	1.8x10^{-5} [i]	
		Dm (S3)	-	8.8x10^{-4}[i]	-	1.6x10^{-5} [i]	
		Cd	8.4x10^{-4} [h]	6x10^{-5} [h]	3.47x10^{-5} [h] (8 day)	-	
	Cypermethrin	*Cd*	2.5x10^{-3}[h]	8.4x10^{-4}[h]	9.78x10^{-5} [h] (8 day)	-	1x10^{-6}[++] (short term) 2x10^{-6}[++] (long term)
		Dm	2x10^{-3}[w]	89[w]	-		
Organophosphorus pesticides	Diazinon	*Dm*	8.6x10^{-4} [a]	1.1x10^{-3}[w]	2x10^{-4}[w] (21 day)	-	1x10^{-5}[**] 1.7x10^{-4}[+]
		Dc	8x10^{-4}[w] (32h)	-	-	-	
		Cd	-	2.5x10^{-4}[w]	-	-	
		Bc	29.22[b] 31[c]	- 31[w]	11[w] (2 day)	-	
	Chlorpyrifos	*Cd*	1.7x10^{-4}[w]	5x10^{-5} [j]	-	-	2x10^{-5}[*] (short term) 2x10^{-6}[*] (long term)
		Dc	-	3x10^{-4} [k]	-	-	
				5x10^{-4} [l]	-	-	
		Dg	-	3x10^{-4} [m]	-	-	
		Dm	4.48x10^{-4} [o]	7.12x10^{-3} [n]	-	5.2 x10^{-3} [o]	1x10^{-5}[**]

Agrochemical	Species	Endpoint				Water quality criteria for aquatic life protection (mg/L)
		Acute toxicity		Chronic toxicity	Sub-lethal toxicity (biomarkers)	
		24h LC_{50} (mortality) (mg/L)	48h LC_{50} (immobilization) (mg/L)	Reproduction (EC_{50}) (mg/L)	*In vivo* 48h IC_{50} (AChE) (mg/L)	
	Bc	12[c]	12[w]	0.36[w] (2 day)		$8.3x10^{-5+}$
Methylparathion	*Bc*	29.19[s]	-	-	-	--
	Bp	10.6[w]	-	-	-	
	Lq	-	9.49[r]	6.6[r] (5 day)	9.4[r](45 min)	
	Dm	$3.1x10^{-7}$ [t]	$12x10^{-3w}$	-	-	
	Cd		$2.6x10^{-3w}$	-	-	
Malathion	*Dm*	$4.08x10^{-3}$ [o]	$1.6x10^{-3w}$	$3.6x10^{-4w}$ (16 day)	5.2 $x10^{-3}$ [o]	$5x10^{-5**}$
	Dc	-	0.013[w]	-	-	
	Cd	$3.18x10^{-3w}$	$1.14x10^{-3w}$	-	-	
	Bc	33.72[u]		-	-	

Dm: Daphnia magna; Dg: Daphnia galeata; Dc: Daphnia carinata; Cd: Ceriodaphnia dubia; Bc: Brachionus calyciflorus; Bp: Brachionus patulus; Lq: Lecane quadridentata

S1: strain 1; S2: strain 2; S3: strain 3

References for toxicological data:[a]: [26], [b]: [52], [c]: [55], [d]: [27], [e]: [28], [f]: [32], [g]: [33], [h]: [34], [i]: [44], [j]: [35], [k]: [36], [l]: [37], [m]: [38], [n]: [41], [o]: [45], [p]: [46], [q]: [43], [r]: [61], [s]: [53], [t]: [62], [u]: [63], [v]: [64], [w]: [65]

References for water quality criteria:*: [66], **: [67], [+] [68], [++][69]

Table 1. Toxicological data for rotifers and cladocerans.

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Author details

Daniel Robles-Vargas

Address all correspondence to: danrvargas@gmail.com

Universidad Autónoma de Aguascalientes, Aguascalientes, México

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