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Adverse Effects of Herbicides on Freshwater Zooplankton

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1. Introduction

The use of herbicides to control weeds is a part of agricultural management throughout the world. Unfortunately, the indiscriminate use of these herbicides may have impacts on non-target organisms (Sarma et al., 2001; Nwani et al., 2010). The long persistence of many herbicides in freshwater suggests that they are capable of producing adverse effects on freshwater zooplankton. Dalapon persist in water for 2 to 3 days, paraquat and diquat persist more than dalapon, and 2,4-D amine salt persist for 4 to 6 weeks; chlorthiamid breaks down into dichlobenil that stays for three months in water. On the other hand, terbutryne and diuron persist for more than three months in the water. These periods of time in the water show that most herbicides will cause serious adverse effects in the populations of freshwater zooplankton (Newbold, 1975). The herbicide n-chloridazon (n-CLZ) is degraded to desphenyl-chloridazon (DPC). This transformation product is more toxic than n-CLZ, and can last more than 98 days in surface water. Maximum concentrations of 7.4 µg/L DPC have been found in Germany (Buttiglieri et al., 2009). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is one of the most commonly used herbicides found in the rural environments, easily transported and one of the most detected pesticides in streams, rivers, ponds, reservoirs and ground waters (Battaglin et al., 2003; Battaglin et al., 2008). It has a hydrolysis half-life of 30 days and relatively high water solubility (32 mg/L), which aids in its infiltration into ground water. Atrazine concentrations of 20 to 700 µg/L in runoff surface waters have been reported (Nwani et al., 2010). Table 1 show some physicochemical properties of herbicides which are used to determine the toxic effects on freshwater zooplankton, as well as lethal values of some of these herbicides.

Herbicides	CAS Registry number	Molecular formula	Brekdown in water	Mobility in water	Species	LC_{50} mg/l 24h	48 h	96 h	Reference
2,4 –D	94-75-7	$C_8H_6Cl_2O_3$	4 to 6 weeks		*Pteronarcys californica* (I)		1.8		Walker (1971)
					Daphnia pulex (C)		3.2		Walker (1971)
					Simochepalus serrulatus (C)		4.9		Walker (1971)
					Daphnia magna(C)		>100		Newbold (1975)
Dalapon	75-99-0	$C_3H_4Cl_2O_2$	2 to 3 days	very mobile	*Pteronarcys californica* (I)		100		Sanders and Cope (1968)
					Simocephalus serrulatus (C)		16		Walker (1971)
					Daphnia pulex (C)		11		Walker (1971)
					Daphnia magna (C)		6		Newbold (1975)
Dichlobenil	1194-65-6	$Cl_2C_6H_3CN$	2 to 3 months	low	*Hyalella azeteca* (A)		12.5	8.5	Wilson and Bond (1969)
					Callibaetis sp. (I)		15.2	12	Wilson and Bond (1969)
					Limnephilus sp. (I)		23.3	13	Wilson and Bond (1969)
					Enallagma sp. (I)		24.2	20.7	Wilson and Bond (1969)
					Pteronarcys californica (I)		8.4		Cope (1966)
					Daphnia pulex (C)		3.7		Cope (1966)
					Simocephalus serrulatus (C)		5.8		Cope (1966)
					Daphnia magna (C)		3.7		Newbold (1975)
Diquat	2764-72-9	$C_{12}H_{12}N_2$	8 to 11 days	immobile	*Hyalella azeteca* (A)		0.12	0.048	Wilson and Bond (1969)
					Callibaetis sp. (I)		65	33	Wilson and Bond (1969)
					Limnephilus sp. (I)		> 100	> 100	Wilson and Bond (1969)
					Enallagma sp. (I)		> 100	> 100	Wilson and Bond (1969)
					Daphnia magna (C)		7.1		Newbold (1975)
Paraquat	4685-14-7	$C_{12}H_{14}N_2$	7 to 14 days	immobile	*Simocephalus serrulatus* (C)		0.45		Walker (1971)
					Daphnia pulex (C)		0.24		Walker (1971)
					Daphnia magna (C)		3.7		Newbold (1975)
Terbutryn	886-50-0	$C_{10}H_{19}N_5S$			*Daphnia magna* (C)		1.4		Newbold (1975)
Diuron	330-54-1	$C_9H_{10}Cl^2N_2O$	3 months	low	*Daphnia magna* (C)		1.4		Newbold (1975)
Propanil	709-98-8	$C_9H_9Cl_2NO$		low	*Daphnia magna* (C)	43.74	5.01		Villarroel et al. (2003)
					Daphnia magna (C)		0.14		Rohm and Haas (1991)
					Ceriodaphnia dubia (C)		1.65		Moore et al. (1998)
Tebuthiuron	34014-18-1	$C_9H_{16}N_4OS$		very mobile	*Daphnia magna* (C)	44.2			Meyerhoff et al. (1985)

(A) = Amphipoda; (C) = Cladocera; (I) = Insecta.

Table 1. Toxicological properties of some herbicides used to determine lethal and sublethal toxicity.

2. Generalities of the adverse effects of herbicides on freshwater zooplankton

Ecological effects of herbicides in freshwater systems occur direct and indirectly. Indirect effects of herbicides are defined as observed effects on consumer populations in freshwater invertebrates that are not caused by direct toxicity but due to adverse effects on primary producers such as algae and macrophytes (Fairchild, 2011). An herbicide induced death suddenly because cuts off oxygen supply during a period when growth and reproduction by freshwater zooplankton are taking place. Individuals of *Simocephalus vetulus* (Crustacea) may have died in the diquat treated ponds because of lower oxygen supply that benefited *Daphnia longispina* because increased its populations (Brooker & Edwars, 1973).

Fairchild (2011) argues that atrazine did not produce neither direct nor indirect effects on aquatic invertebrates/vertebrates. However a recent review by Rohr and McCoy (2010) concluded that atrazine produces indirect and sublethal effects on fish and amphibians at environmentally relevant concentrations. These effects were observed in reproductive success, sex ratios, gene frequencies, populations, and communities. However, these effects remain uncertain and restricted to few species. Other authors report of many indirect effects of pesticides on freshwater zooplankton obtained through meso- and microcosm experiments (see section 8 of this chapter).

The study of the direct effects of herbicides on freshwater zooplankton results in a complex mixture of data on lethal and sublethal values obtained from standard toxicity tests assessing one species relationship with chemicals of high purity in the lab, to meso- and microcosms experiments, field studies, use of biomarkers, and DNA microarrays. However, aside from environmental health protection agencies reports, the data on the mainstream scientific literature is scarce and restricted to: a) few test species, b) models, and c) small number of herbicides. The result of this diagnosis is a scattered picture with many uncertainties, but also with many opportunities for environmental toxicology research. Perhaps the fact that many authors argue that there are no direct effects of herbicides on freshwater zooplankton at environmental concentrations (Fairchild, 2011) or that herbicides do not represent a threat to aquatic communities (Relyea, 2005; Golombieski et al., 2008) has discourage research in this area. However, these authors failed to consider a series of circumstances that might be consider while analyzing the potential of herbicides for adverse effects:

a. Many herbicides are applied as commercial formulae and the formulae can be more toxic to non-target organism than the active ingredient. That is the case of glyphosate and its different commercial formulae (Domínguez-Cortinas et al., 2008).
b. The safe standards and good application techniques for herbicides are not followed as strictly as they should in developed countries and certainly less so in underdeveloped or poorly developed countries. That means that the theoretical concentrations in which many Quantitative Structure/Activity Relationship (QSAR´s) model for herbicides are based on might not apply in many cases and true environmental concentrations might be underestimated.
c. Relyea & Hoverman (2006) argue that results have shown that some herbicides may interact with a range of different natural stressors and that synergism among herbicides and other pesticides has not been studied at all. Therefore, the interaction between herbicides and the cocktail of toxicants found in many polluted sites throughout the

world has not been analyzed, and therefore, the assumption that some herbicides do not interact with other toxicants at environmentally relevant concentrations to produce direct adverse effects on freshwater zooplankton is just unsustainable (just to put it in ecological terms).

d. Ecotoxicogenomics and the development of new and more sensitive biomarkers that are unveiling effects on freshwater zooplankton (especially on endocrine disruption) at very low environmentally relevant concentrations (see sections 8 and 9 of this chapter) might change the opinion of many researchers on adverse effects of herbicides.
e. The data (at least in the mainstream scientific literature) on potential effects of herbicides on freshwater zooplankton is extremely scarce and restricted to no more than five or six taxonomic groups and less than 30 herbicides.

Herbicides can produce bioaccumulation and biomagnification, but the data is buried in different reports and few scientific articles, that a review is greatly needed. For instance, some herbicides like benfluralin, bensulide, dacthal, ethalfluralin, oxadiazon, pendimethalin, triallate, and trifluralin have the potential to accumulate in sediments and aquatic biota (USGS, 1999).

Lethal effects of a few herbicides have been determined so far in only the following freshwater zooplankton groups: amphipods, cladocerans, copepods, malacostracans, and rotifers. The information on herbicide toxicity on freshwater zooplankton is limited and mainly focused on studies of population dynamics and effects on the biodiversity of the community. Sublethal effects of herbicides on freshwater zooplankton species have focused on demographic parameters (mainly life tables and determination of "r" values), of three groups: amphipods, cladocerans, and rotifers.

Herbicides may affect the population dynamics of freshwater zooplankton by controlling individual survival and reproduction, and by altering the sex ratio. Herbicides might also produce the following effects at the community and ecosystem levels: a) induction of dominance by small species, b) an increase of species richness and diversity, and c) elongation of the food chain and reduction of energy transfer efficiency from primary producers to top predators (Hanazato, 2001).

Biomarkers used so far to study effect of herbicides on freshwater zooplankton correspond to: a) enzyme inhibition, b) mRNA expression levels, c) gen induction, and d) grazing rate inhibition.

3. Mechanism of action of herbicides related to adverse effects on freshwater zooplankton

Herbicides represent a broad variety of chemical classes of compounds, which acts over diverse sites of metabolic functions and energy transfer in plant cells (Duke, 1990). Only a few herbicides classes have a known molecular site of action, moreover, the molecular site of action and the mechanism of several important herbicide classes is still unknown (Duke, 1990). Among known mechanisms of action of herbicides, there are herbicides that inhibit photosynthesis, those that inhibit pigments and those that inhibit seedling growth (Duke, 1990; Prostko & Baughman, 1999; Gunsolus & Curran, 1999). An undesirable side-effect of herbicides is that they may enter freshwater ecosystems by spray drift, leaching, run-off, and/or accidental spills (Cuppen et al., 1997). Surface water contaminations by herbicides

have been reported to have direct toxic effects on phytoplankton, epiphyton, and macrophytes. Furthermore, herbicides have indirect effects over zooplankton and animal populations (Relyea, 2005, 2009; Cuppen et al., 1997), affecting all trophic chains in freshwater reservoirs. Several studies show that herbicides selectively decreased primary producers, leading to a bottom-up reduction in the abundance of consumers due to food limitation (Fleeger et al., 2003). Contaminant-induced changes in behavior, competition and predation/grazing rate can alter species abundances or community composition, and enhance, mask or spuriously indicate direct contaminant effects (Fleeger et al., 2003). Thus, the impacts that herbicides exerts on freshwater communities are one of the main concerns about the use of these chemical compounds. The mechanisms of action of herbicides are classified according to site or specific biochemical process that is affected and are summarized in Table 2; these mechanisms have been described in plants. Below are some examples of the adverse effects of some herbicides according to their mechanism of action in freshwater zooplankton.

3.1 Amino acid synthesis inhibitors

One of the most important herbicides in this category is glyphosate because is extensively used in the aquatic environment. Martin et al. (2003), determined the acute toxicity of technical-grade glyphosate acid, isopropylamine (IPA) salt of glyphosate, Roundup and its surfactant polyoxyethylene amine (POEA) in Microtox® bacterium (*Vibrio fischeri*), microalgae (*Selenastrum capricornutum* and *Skeletonema costatum*), protozoa (*Tetrahymena pyriformis* and *Euplotes vannus*) and crustaceans (*Ceriodaphnia dubia* and *Acartia tonsa*); generally the toxicity order of the chemicals was: POEA > Roundup® > glyphosate acid > IPA salt of glyphosate, while the toxicity of glyphosate acid was mainly due to its high acidity. In *Ceriodaphnia dubia* the LC50 = 147 mg/L to glyphosate acid and for *Acartia tonsa* was LC50 = 35.3 mg/L. Glyphosate produced adverse effects on the embryonic development on time (3 and 8 mg/L), duration of juvenile and reproductive periods, average lifespan, net reproductive rate (8.0 and 10.50 mg/L), and the intrinsic population increasing rate on the freshwater rotifer *Brachionus calyciflorus* (Chu et al., 2005).

Meyerhoff et al. (1985) observed a lower length in *D. magna* exposed to the herbicide tebuthiuron than in blank control animals when the cladocerans were exposed to 44.2 mg/L herbicide. Hanazato (1998) indicated that the neonatal body size determines the size at maturation. The reduced growth rate of neonates due to the chemicals will result in a smaller size at maturation and thus a smaller adult size, leading to smaller clutch sizes.

3.2 Cell-membrane disrupters

The way in which terbutryn exerts its toxicity to rotifers is not clear. The survival curves for all *Brachionus* sp. cultures fed with terbutryn-exposed microalgae showed a drastic mortality showed that population density decreased as terbutryn concentration increased in the microalgal cells. In fact, this species of rotifer did not survive beyond four days when fed with microalgae exposed to 500 nM terbutryn. Percentage of reproductive females in rotifer populations fed with terbutryn-exposed microalgae decreased significantly as herbicide concentration increased (Rioboo et al., 2007). Interestingly the highest concentration of herbicide tested is no toxic to the algae *Chlorella vulgaris* viability, at least after 24 h of exposure (González-Barreiro et al., 2006).

Mechanism of action	Herbicide Family Chemistry	Affected site or biochemical process
Amino Acid Synthesis Inhibitors	Sulfonylureas	Inhibition of Acetolactate synthase enzyme (ALS)
	Imidazolinone	Inhibition of 5-enolpyruvyl shikimate 3-phosphate synthase (EPSP)
	Triazolopyrimidine	
	Pyrimimidinylthiobenzoate	
	Sulfonyl amino carbonyl triazolinones	
Cell-Membrane Disrupters	Bipyridliums	Inhibition of protoporphyrinogen oxidase (PPO oxidase)
	Diphenylethers	Electron acceptors, formation of reactive oxygen species (ROS)
Growth Regulators	Triazolinone	Alteration of hormonal balance
	Oxadiazoles	
	Arsenical	
	Phenoxi acids	
	Benzoic acids	
	Pyridine acids	
	Quinilonecarboxylic	
Lipid Synthesis Inhibitors	Aryloxyphenoxyproprionates	Inhibition of Aceytl Coenzyme-A carboxylase (ACCase)
	Cyclohexanediones	

Table 2. Mechanism of action of herbicides (Plimmer et al. 2005).

The herbicide molinate was tested in *Daphnia magna*, and the reproduction was significantly reduced when molinate concentration was increased in the medium, but only this effects was higher in the parental daphnids (F0) than the F1-1st and F1-3rd offspring, seem to be adapted to the herbicide molinate, showing more longevity and reproduction than their parental (Sánchez et al., 2004). Similar result were found by Julli & Krassoi (1995) who observed a significant decreased in total young per female in three broods of *Moina australiensis* when exposed to molinate.

Paraquat was toxic to almost all compartments of the plankton community including zooplankton like: rotifers (*Brachionus calyciflorus*, *Lecane* sp., *Conochiloide* sp., *Asplanchna* sp., and *Hexarthra* sp.), copepods (*Thermocyclops decipiens*, *Mesocyclops* sp.) and cladocerans (*Diaphanosoma excisum*), leading to a reduction in biomass, numbers, and overall trophic functioning, in fact *Thamnocephalus decipiens* exhibited dose-dependent sensitivity to paraquat (Leboulanger et al., 2011). Paraquat may induce peroxidation processes in non-target animal species. Furthermore, paraquat may interfere with the cellular transport of polyamines. Cochón et al. (2007), investigate some aspects related to paraquat-induction of oxidative stress (lipoperoxidation, enzymatic activities of catalase and superoxide dismutase) and also the levels of polyamines (putrescine, spermidine and spermine) in two species of freshwater invertebrates, the oligochaete *Lumbriculus variegatus* and the gastropod *Biomphalaria glabrata*. In *L. variegatus* did not induce membrane lipoperoxidation and only a transient decrease in CAT activity was observed. After 48 h of exposure, an increase of lipoperoxidation and a decrease of SOD activity were registered in the snails. It could be hypothesized that the higher resistance of *L. variegatus oligochaetes* could be due in part to a lower ability to activate the paraquat and also to a protective role of polyamines.

3.3 Growth regulators

Sarma et al. (2001) reported that the herbicide 2,4-Dichlorophenoxy acetic acid had a negative influence on the population growth of *Brachionus patulus* when the rotifers were directly exposed via water and food. Interestingly, Relyea (2005) reported 2,4-Dichlorophenoxy acetic acid had no effect on zooplankton. But exists LC_{50} = 363 and 389 mg/L values (96 h) for the *Daphnia magna* (Johnson & Finley, 1980; Verschueren, 1983, respectively). Boyle (1980) determinate the effects on 2,4-D herbicide applied two concentration 5 and 10 kg/ha, and quantifier the planktonic invertebrates (number per liter of water) rotifers and crustaceans: with a concentration of 5 kg/ha of 2,4-D, found 320 rotifers species and 40 of crustaceans, and found 207 rotifers species and 34 crustaceans with 10.0 kg/ha.

3.4 Lipid synthesis inhibitors

Metazachlor is a frequently used herbicide with high concentrations in surface waters and effects on zooplankton caused by changes in habitat structure in species such as *Keratella quadrata*, *Lecane* spp, *Brachionus calyciflorus*, *Polyathra dolicoptera* and *Bosmia longirostris*. For species such as *K. quadrata*, *Alonella excisa*, *Acropercus harpae*, *Chydorus sphaericus* and some ostracods species with negative weights indicated a decrease in abundance after metazachlor application. In contrast, species like *P. dolichoptera* or *Ceriodaphnia quadrangula* increased in abundance in the treatments as compared to the controls as indicated by the positive weight (Mohr et al., 2008). Direct toxic effects of metazachlor were not expected

since this group is generally unable to synthesize fatty acids and therefore membrane functions will not be disrupted directly. EC_{50} value of 22.3 mg/L (48h) was found for *Daphnia magna* (FAO, 1999).

Another lipid synthesis inhibitors herbicide is norflurazon and is a bleaching, preemergence. Horvat et al. (2005) found that the toxicity of norflurazon caused mortality in *Polycelis feline,* and morphological and histological changes in treated animals compared to corresponding controls. The most prominent histological changes were damage of the outer mucous layer, lack of rhabdites, damage to epidermis and extensive damage to parenchyma cells.

3.5 Pigment inhibitors

Pigments inhibitors affecting plant cell by preventing the formation of photosynthetic pigments (chlorophyll and carotenoids) localized in leaf tissues, trough interfere both the chlorophyll and terpenoid synthesis pathway, inhibiting their synthesis (Duke, 1990; Prostko & Baughman, 1999; Gunsolus & Curran, 1999). This condition cause rapid photobleaching of green tissue of leafs, due the Photosystem I (PS I) reduce a chemical group of the structure of these herbicides to a radical that reduce molecular oxygen to superoxide radical. This reaction repeats continuously to form large amounts of superoxide radical; producing lipids peroxidation and photobleaching (Duke, 1990), giving to affected plants a white or translucent appearance. Because this effect, pigment inhibitors are often called "bleaching herbicides" or "photobleachers" (Prostko & Baughman, 1999). This herbicide class includes isoxazolidinones (i.e. clomazone), pyridazinones (i.e. norflurazon), fluridone, difunone, amitrole and *m*-phenoxybenzamides (Duke, 1990).

This type of herbicides has not direct effects on freshwater zooplankton, but can have indirect negative effects on them. The mechanism of action of these herbicides is targeted to photosynthetic organisms (plants), in the case of freshwater communities, the phytoplankton are the organisms that suffers direct negative effects, which affect them drastically reducing their population. However, the reduction of phytoplankton population may cause indirect negative effects on the zooplankton due a reduction of feed availability for zooplankton, reducing their abundance and/or inducing changes in the taxa composition of zooplankton (Relyea, 2005, 2009).

3.6 Photosynthesis inhibitors

Herbicides that inhibit photosynthesis are the most common type. These herbicides disrupt the vital process of photosynthesis that allows plants to convert the solar light energy into glucose. This type of herbicides binds to the quinone-binding protein (D1 protein) of photosynthetic electron transport, blocking the electron transport. Photosynthesis inhibitors herbicides include triazines (i.e. atrazine), phenylureas (i.e. linuron), uracils, nitriles and benzothidiazoles (Duke, 1990; Gunsolus & Curran, 1999; Prostko & Baughman, 1999). Diuron blocks photosynthetic electron transfer in plants and algae, it might also affect freshwater zooplankton (Leboulanger et al., 2011).

Photosynthesis inhibitors have not direct effects on freshwater zooplankton, but can have indirect effects on them. These herbicides affect mainly to phytoplankton that suffers direct toxic effects, which entails to reducing their population. Thus, the reduction of food supply, modifications of both reproduction and feeding behavior of zooplankton may cause indirect

effects on the zooplankton, resulting in decrease of the abundance of some taxa (indirect negative effect), increase of some taxa (indirect positive effect), both decrease of diversity and changes in species composition of zooplankton (Solomon et al., 1996; Cuppen et al., 1997; Hanazato, 1995; Relyea, 2005, 2009; Chang et al., 2008).

Chang et al. (2008) studied the effects of application of simetryn (20 and 80 µg/L), a methylthiotriazine herbicide, and the fungicide iprobenfos (100 and 600 µg/L), on zooplankton community composed by rotifers and cladocerans. They applied four treatments (low and high concentrations of both pesticides), and their results showed that the herbicide have less apparent direct impact on zooplankton abundance within a short period; however, they observed that the diversity and species composition changed with simetryn application, suggesting that the structure of zooplankton can be altered by the herbicide application (Chang et al., 2008).

The mode of action of atrazine is blocking electron transport in photosystem II leading to chlorophyll destruction and blocking photosynthesis (Nwani et al., 2011). Dodson et al. (1999), found that atrazine have effects on male production of *Daphnia*, changing the sex ratio, which exerts a control of *Daphnia* population dynamics.

Cuppen et al. (1997) studied the effects of a chronic application of linuron (at concentrations of 0.5, 5, 15, 50 and 150 µg/L during 28 days) on freshwater microcosms, which included phytoplankton, zooplankton and macroinvertebrates. They observed that the direct negative effect of linuron on several algae (cryptophytes, diatoms) and the positive effect on green algae *Chlamydomonas* resulted in a decrease of several Rotatoria and an increase in Copepoda, and to a lesser extent, Cladocera.

3.7 Seedling growth inhibitors

This type of herbicides includes dinitroanilines (i.e. trifluralin), acetanilides (i.e. acetochlor) and thiocarbamates (i.e. EPTC). The seedling growth inhibitors are divided into two groups: a) root inhibitors; and b) shoot inhibitors. The first group binding to tubulin protein and disrupt the cell division, which inhibit the root elongation and lateral root generation. About second group, little is known about their mechanism of action, but is believe that disrupt protein synthesis and waken cell wall (Duke, 1990; Prostko & Baughman, 1999).

These herbicides may impact indirectly on freshwater zooplankton, due the direct negative effects on phytoplankton, which may be sensitive to disruption of their cell division process, limiting the growth and multiplication of phytoplankton, reducing the feed availability for zooplankton, decreasing their reproduction rate and their population (Fleeger et al., 2003; Relyea, 2009).

Relyea (2009) examined the effect of acetochlor and metolachlor on zooplankton at low concentrations (6-16 p.p.b.); he encountered that there was no clear indication of any indirect effects from the addition of these herbicides to zooplankton, and in one zooplankton taxon (*Ceriodaphnia*) the mixture of five herbicides (acetochlor, metolachlor, glyphosate, atrazine and 2,4-D) added at concentrations of 6-16 p.p.b. caused an increase in abundance. The few studies about acetochlor and other herbicides (atrazine and 2,4-D) suggest that low concentrations of these herbicides have not effect in cladoceran survival, or may cause an increase of their population due to high reproduction rate in cladocerans (Relyea, 2009).

3.8 Other kind of herbicides whose mechanism is unknown

Only two other molecular sites of action of herbicides are known. One is the herbicide asulam, which inhibits folate synthesis by inhibiting dihydropteroate synthase, although there may also be a second site of herbicide action associated with cell division. In another hand, the herbicide dichlobenil inhibits cellulose synthesis, but its molecular site of action is unknown. Photoaffinity labeling of cotton fiber proteins with a photoaffinity dichlobenil analogue resulted in specific labeling of an uncharacterized 18 kD protein (Duke, 1990). Amoung the seedling growth inhibitors, the group that inhibits plant shoot elongation have a mode of action almost unknown until today, is believe that this inhibitors disrupt protein synthesis and waken cell wall (Duke, 1990; Prostko & Baughman, 1999). In another hand, is too believed that these inhibitors could have multiple sites of action (Gunsolus & Curran, 1999).

4. Lethal effects of herbicides on freshwater zooplankton

The information on herbicide toxicity on freshwater zooplankton is limited and mainly focused on studies of population dynamics and effects on the biodiversity of the community. Some authors claim that herbicides apparently do not pose a threat to the aquatic communities, or have a lesser adverse effect than other pesticides (Golombieski et al., 2008). Relyea (2005) argue that glyphosate and 2,4-D, have no significant adverse effect on zooplankton biodiversity. Perhaps lethal effects are not so evident. However, symetrin can cause shifts in species composition, diversity and dominance of freshwater zooplankton (Hanazato, 2001; Chang et al., 2008). Therefore, it is convenient to consider data on lethal toxicity to determine the most sensitive species which might enable us to predict the direction of indirect effects on a community (Relyea & Hoverman, 2006).

Few if any environmentally relevant concentrations have been shown to have direct effects on zooplankton, fish, or amphibians in the laboratory (Fairchild, 2011). However a recent review by Rohr & McCoy (2010) concluded that atrazine produces indirect and sublethal effects on fish and amphibians at environmentally relevant concentrations. Furthermore, Domínguez-Cortinas et al. (2008) found that both glyphosate and its commercial product Faena® produce lethal toxicity to the freshwater invertebrates *Daphnia magna* and *Lecane quadridentata* at environmental concentrations (the highest concentration of glyphosate in runoff waters, 5.2 mg/L, was found in runoff occurring 1 day after treatment at the highest rate (8.6 Kg/ha of Roundup®)) (Edwards et al., 1980).

Sublethal effects of glyphosate and its formulae could be found at protective values, like the 65 µg/L value published in the Environmental Guide for protecting aquatic life of the Canadian Government (Environment Canada, 1987) for glyphosate. This value is 6.5-fold higher than the esterase inhibition NOEC value for glyphosate and 2-fold higher than the Faena® esterase inhibition NOEC value obtained by Domínguez-Cortinas et al. (2008). On the other hand, the US EPA (1986) has established a value of 700 µg/L of glyphosate for drinking water, which according to Domínguez-Cortinas et al. (2008) esterase inhibition results may represent a risk (LOEC = 62 µg/L, EC50 = 280 µg/L) especially when we consider the ample presence of acetylcholinesterases in the test organisms (Pérez-Legaspi et al., 2011).

Herbicide	Species	Criteria	Endpoint (mg/L)	Reference
Acroleine	*Daphnia magna* (C)	48-h	LC50 = 0.051mg/L	Holcombe et al., 1987
	Pennaeus aztecus (M)	48-h	LC50 = 0.100mg/L	Eisler, 1994
Atrazine	*Daphnia pulex* (C)	3-h	LC50 > 40	Keith et al., 1995
"	"	48-h	EC50 =36 –46.5	"
"	"	48-h	LC50 = 33	"
"	*Daphnia magna* (C)	26-h	LC50 = 3.6	"
"	"	48-h	LC50 = 9.4	"
"	"	48-h	EC50 = 3.6	"
"	"	24h,48h	EC50 > 39	"
"	"	48-h	LC50 = 6.9 MATC = 0.14-0.25	"
"	*Daphnia macrocopa* (C)	3-h	LC50 > 40	"
"	*Ceriodaphnia dubia* (C)	7-d	LC50 = 2.0	"
"	*Daphnia carinata* (C)	48-h	EC50 = 24.6	Phyu et al., 2004
"	*Hyalella azteca* (A)	96-h 21-d	LC50 = 3.0 LC50 = 1.8	Ralston-Hooper et al., 2009
"	*Diporeia* sp (A)	96-h 21-d	LC50 > 3.0 LC50 = 0.24	"
DEA (desethylatrazine)	*Hyalella azteca* (A)	96-h 21-d	LC50 = 5.1 LC50 > 3.0	Ralston-Hooper et al., 2009
"	*Diporeia* sp (A)	96-h 21-d	LC50 > 3.0 LC50 = 0.33	"
DIA (deisopropylatrazine)	*Hyalella azteca* (A)	96-h 21-d	LC50 = 7.2 LC50 > 3.0	Ralston-Hooper et al., 2009
"	*Diporeia* sp (A)	96-h 21-d	LC50 > 3.0 LC50 = 0.3	"
Diuron	*Daphnia pulex* (C)	96-h 7-d	LC50 = 17.9 LC50 = 7.1	Nebeker and Schuytema, 1998
"	*Hyalella azteca* (A)	96-h	LC50 = 19.4	"
"	"	10-d	LC50 = 18.4	"
Glyphosate	*Daphnia magna* (C)	48-h	NOEC = 120 LOEC = 140 LC50 = 146	Domínguez-Cortinas et al., 2008
"	*Lecane quadridentata* (R)	48-h	NOEC = 120 LOEC = 140 LC50 = 150	"
Glyphosate < 74 % (Faena ®)	*Daphnia magna* (C)	48-h	NOEC = 3.3 LOEC = 6.5 LC50 = 7.9	Domínguez-Cortinas et al., 2008
"	*Lecane quadridentata* (R)	48-h	NOEC = 9.8 LOEC = 13.0 LC50 = 13.1	"
Glyphosate (IPA)	*Ceriodaphnia dubia* (C)	48-h	LC50 = 415.0	Tsui and Chu, 2003
Glyphosate (POEA)	*Daphnia pulex* (C)	96-h	EC50 = 2.0	Servizi et al., 1987
Glyphosate 48 % (RON-DO®)	*Daphnia magna* (C)	24-h 48-h	EC50 = 95.96 EC50 = 61.72	Alberdi et al., 1996
"	*Daphnia spinulata* (C)	24-h 48-h	EC50 = 94.87 EC50 = 66.18	"
Glyphosate (Roundup®)	*Phyllodiaptomus annae* (Co)	48-h	LC50 = 1.06	Ashoka Deepananda et al., 2011

Herbicide	Species	Criteria	Endpoint (mg/L)	Reference
"	*Caridina nilotica* (M)	72-h	LC50 = 107.53	Folmar et al., 1979
		96-h	LC50 = 60.97	
	Daphnia magna(C)	48-h	EC50 = 3.0	Folmar et al., 1979
	Ceriodaphnia dubia (C)	48-h	LC50 = 5.7	Tsui and Chu, 2003
	Daphnia pulex (C)	96-h	EC50 = 8.5	Servizi et al., 1987
	Gammarus pseudolimnaeus (A)	48-h	LC50 = 62.0	Folmar et al., 1979
	Hyalella azteca (A)	48-h	LC50 = 1.5	Tsui and Chu, 2004
Glyphosate (Rodeo®)	*Ceriodaphnia dubia* (C)	48-h	LC50 = 415.0	Tsui and Chu, 2004
	Hyalella azteca (A)	48-h	LC50 = 225.0	Tsui and Chu, 2004
Metribuzin (Sencor®)	*Diaptomus mississippiensis* (Co)	24-h 48-h	LC50 =205.0	Syed et al., 1981
	Eucyclops agilis (Co)		LC50 =150	
Molinate	*Brachionus calyciflorus* (R)	24-h	LC50 = 11.37	Ferrando et al., 1999
"	*Daphnia carinata* (C)	48-h	EC50 = 26.5	Phyu et al., 2004
Paraquat	*Diaptomus mississippiensis*(Co)	24-h 48-h	LC50 =10 LC50 = 5.0	Syed et al., 1981
	Eucyclops agilis (Co)			
"	*Diaphanosoma excisum* (C)	24-h	LOEC = 0.057	Leboulanger et al., 2008
"	*Moina micrura* (C)	24-h	LOEC = 0.577	"
Paraquat 27.6 % (OSAQUAT)	*Daphnia magna* (C)	24-h 48-h	EC50 = 16.47 EC50 = 4.55	Alberdi et al,. 1996
"	*Daphnia spinulata* (C)	24-h 48-h	EC50 = 9.91 EC50 = 2.57	"
Paraquat + metribuzin (1:1) 91% + 9%	*Diaptomus mississippiensis* (Co) *Eucyclops agilis* (Co)	24-h 48-h	LC50 = 29 LC50 = 17	Syed et al., 1981
Pendimethalin 60%	*Daphnia magna* (C)	24-h 48-h	LC50 = 112 LC50 = 53	Kyriakopoulou et al., 2009
S-metolachlor 31.2% + Terbuthilazine 18.8%	*Daphnia magna* (C)	24-h 48-h	LC50 = 20 LC50 = 9.5	Kyriakopoulou et al., 2009
Simazine (Aquazine)	*Daphnia pulex* (C)	48-h	LC50 > 50	Fitzmayer, et al., 1982
Thiobencarb	*Brachionus calyciflorus* (R)	24-h	LC50 = 47.82	Ferrando et al., 1999
2,4-D (2,4-dichlorophenoxyacetic acid)	*Brachionus calyciflorus* (R)	24-h	LC50 = 117	Snell et al., 1991
3,4- DCA (3,4-dichloroaniline)	*Daphnia magna* (C) (adults)	48-h	LC50 = 12	Ferrando and Andreu-Moliner, 1991
"	*Brachionus calyciflorus* (R)	24-h	LC50 = 61.47	"
"	*Daphnia magna,* larva (C)	24-h 48-h 96-h 7-d 14-d 3-w	LC50 = 0.40 LC50 = 0.23 LC50 = 0.16 LC50 = 0.12 LC50 = 0.10 LC50 = 0.10 EC50 = 0.01	Adema and Vink, 1981

Herbicide	Species	Criteria	Endpoint (mg/L)	Reference
"	*Daphnia magna,* adult (C)	48-h	LC50 = 12	"
		96-h	LC50 = 1.0	
		7-d	LC50 < 0.58	
"	*Brachionus calyciflorus* (R)	24-h	LC50 = 62	Snell et al., 1991

Abbreviations. (C) Cladocerans, (R) Rotifers, (Co) Copepods, (A) Amphipod, (M) Malacostracan. LC50 = Median Lethal Concentration, EC50 = Concentration where 50% inhibition occurs, MATC = Maximum Acceptable Toxicant Concentration, LOAEL = Lowest Observed Adverse Effect Level, NOAEL = No Observed Adverse Effect Level, LOEC = Lowest Observed Effect Concentration, NOEC = No observed effect concentration.

Table 3. Lethal toxicity values of herbicides with different species of freshwater zooplankton. Criteria of mortality include different exposure time to herbicide in hours (h), days (d) or weeks (w).

Lethal toxicity tests with freshwater invertebrates are based on standard protocols which are simple, reproducible, and with certain ecological relevance. They are valuable tools to estimate the adverse effect of single chemicals in short periods of exposure (usually 24 and 48 h), with or without food. The most common evaluation parameter is the death or immobility which is represented by the median lethal toxicity (LC50) or the median effect concentration (EC50) (Sarma et al., 2001; Pérez-Legaspi et al., 2011). The cladocerans (*Daphnia* sp., *Ceriodaphnia sp.* and *Moina* sp.) and the rotifer genus *Brachionus*, are among the most used freshwater organisms in toxicity tests (Table 3), mainly due to their great availability, high sensitivity towards many toxicants, ease of handling and culture and high rates of growth and reproduction (Snell & Janssen, 1998; Sancho et al., 2001; Sarma & Nandini, 2006). The amphipod (*Hyalella* sp.) and copepods have also been used (Table 3). Some of these protocols have been recognized by International Standard Organizations (ISO), USEPA, OECD, ASTM, Standard Methods (Snell & Janssen, 1995; Persoone et al., 2009).

Among herbicides, the most studied with freshwater zooplankton are atrazine (Table 3) and glyphosate (Pérez et al., 2011; Table 3). However, the most toxic herbicides are: acroelin (LC50 = 0.051 and 0.100 mg/L), the commercial formula of glyphosate, Faena® for the cladoceran *Daphnia magna* (48h-LC50 = 7.9 mg/L), Roundup® for the copepod *Phyllodiaptomus annae* (48h-LC50 = 1.06 mg/L), and 3,4- DCA (24h-LC50 = 0.40 mg/L) for *D. magna*. On the other hand, glyphosate the active ingredient is less toxic for *D. magna* (48h-LC50 = 146 mg/L) and the freshwater rotifer *Lecane quadridentata* (48h-LC50 = 150 mg/L) than its herbicide formula Roundup®; which suggests that in this particular case the substances present in the commercial formula contribute through synergistic effects to increase the toxicity towards non-target organisms (Domínguez-Cortinas et al., 2008). The 24 and 48 h exposure periods are the most common in the lethal tests, but some tests might last several days. In the case of 3,4-Dichloroaniline (3,4-DCA) the range of *D. magna* LC50 values (0.40 – 0.10 mg/L) decrease as the exposure time increases. Presence of food (microalgae) is a factor that decreases the toxicity of the herbicide as test animals are better fed; they seem to be more resistant (Sarma et al., 2001). In general among freshwater zooplankton the most sensitive model organisms to herbicides are amphipods and crustaceans. However, more toxicity testing with freshwater zooplankton are necessary because data on different species and toxicant are scarce making predictions of herbicide toxicity on zooplankton an

unexplored area, and some herbicides have the potential to alter the dynamics and structure of aquatic communities.

5. Chronic effects of herbicides on freshwater zooplankton

Lethal toxicity data is considered by many environmental health protection agencies in world as reliable and significant, because comes from standard and simplified protocols. However, mortality or immobility is a parameter of lesser sensitivity in estimating adverse effects on freshwater zooplankton. Chronic tests are usually more sensitive because are based on growth, reproduction, physiological, biochemical and genetic characteristics in lower concentrations and longer exposure periods (Table 4). In other words, they assess the first responses (stress, physiological, behavioral and reproductive) to toxicants (Nimmo & McEwen, 1994). Chronic toxicity is usually expressed as the median effective concentration (EC50) or the concentration in which 50% of a specific effect is determined. Many chronic tests rely on life tables that examine demographic parameters (r, Ro, Vx, T and e_o) in freshwater invertebrates. Some chronic tests focus only on growth inhibition arguing that this is an outstanding parameter since involves all steps of a life cycle (embryos, juveniles and adults) during the test period, which makes these tests rapid, sensitive, and relevant ecologically (Snell & Moffat, 1992; Sancho et al., 2001). Besides demographic parameters, tests of chronic effects of herbicides on freshwater zooplankton also involve ingestion rate, enzymatic inhibition and behavioral parameters (Table 4). The most commonly used species belong to cladocerans, rotifers, and one species of amphipod (Table 4). Atrazine is the most studied herbicide regarding chronic effects on freshwater zooplankton; although, studies have been restricted to crustaceans. The most toxic herbicide studied so far is glyphosate, EC50 = 0.28 mg/L, for *in vivo* esterase inhibition in *L. quadridentata*, followed by thiobencarb (EC50 = 0.75 mg/L) for 21 days survival and growth inhibition tests in *D. magna*. The least toxic herbicide is 2,4-D (EC50 = 500 mg/L) for *B. patulus* and EC50 = 128 mg/L, for *B. calyciflorus* (Table 4).

As for lethal tests, the scarcity of data related to chronic effects on freshwater zooplankton becomes a research opportunity to increase the number of taxonomic groups and different herbicides studied, and to diversify the list of chronic parameters as recommended by the American Society for Testing Materials (ASTM) (Sancho et al., 2001). Such an effort would enhance our comprehension of the effects of herbicides in freshwater ecosystems (Hanazato, 2001).

6. Biomarkers assessing adverse effects of herbicides on freshwater zooplankton

The need to rely in parameters more sensitive to estimate adverse effects of toxicants in small concentrations has led to the development of biomarkers. These biomarkers detect small biochemical, cellular, genetic, physiological, morphologic and behavioral variations which can be easily and non-destructively determined in most organisms (Hagger et al., 2006; Walker et al., 2006). These small variations can led to changes in all levels of the biological organization (Hyne & Maher, 2003). These effects are usually more rapid in lower levels of biological organization and can therefore offer more sensitive responses to toxicant exposure inside the populations (Hagger et al., 2006). Therefore, Walker et al. (2006), define a biomarker as any biological response towards an environmental chemical substance

Herbicide	Test organism	Criteria	Endpoint (mg/l)	Reference
Atrazine	*Ceriodaphnia dubia* (C)	4-d	Chronic value = 6.9 NOEC = 5.0 –10 LOEC = 10–20	Keith et al. 1995
"	"	7-d	Chronic value = 3.5 NOEC = 2.5 LOEC = 5.0	"
"	"	7d	NOEC = 5.0	"
"	*Scapholeberis mucronata* (C)	F	1.0	"
"	"	ED 30 – 45-d	1.0	"
Diuron	*Daphnia pulex* (C)	R 7-d	LOAEL = 7.7 NOAEL = 4.0	Nebeker and Schuytema, 1998
"	*Hyalella azteca (A)*	S 10-d	LOAEL = 15.7 NOAEL = 7.9	"
Glyphosate	*Lecane quadridentata* (R)	cFDAam 30-m	NOEC =0.032 LOEC = 0.062 EC50 = 0.28	Domínguez-Cortinas et al. 2008
"	"	PLA2 30-m	NOEC = 5.0 LOEC = 10.0 EC50 = 17.6	"
Glyphosate < 74 % (Faena ®)	*Lecane quadridentata* (R)	cFDAam 30-m	NOEC = 9.8 LOEC =13.0 EC50 = 13.1	Domínguez-Cortinas et al. 2008
"	"	PLA2 30-m	NOEC = 0.4 LOEC = 1.3 EC50 = 4.6	"
Glyphosate (Vision®)	*Simocephalus vetulus*	8-d survivorship and reproduction	0.75 mg/L	Chen et al., 2004
Molinate	*Brachionus calyciflorus* (R)	Ro T r	EC50 = 2.24 EC50 = 5.6 EC50 = 2.7	Ferrando et al. 1999
Paraquat	*Moina micrura* (C)	Population growth rate	not significant effect > 0.022	Leboulanger et al. 2008
Thiobencarb	*Brachionus calyciflorus* (R)	Ro T r	EC50 = 3.4 EC50 = 3.86 EC50 = 3.5 MATC = 3.16 NOEC = 2.0 LOEC = 5	Ferrando et al. 1999
Thiobencarb (S-4-chlorobenzyl diethylthiocarbamate)	*Daphnia magna* (C)	24-h	EC50 = 3.01	Sancho et al. 2001
"	"	R	> 0.30	"
"	"	S, r 21-d	0.75	"
2,4-D (2,4-dichlorophenoxyacetic acid)	*Brachionus calyciflorus* (R)	r 2-d	Chronic value = 70 NOEC= 58 LOEC=83 EC50= 128	Snell and Moffat, 1992

Herbicide	Test organism	Criteria	Endpoint (mg/l)	Reference
"	*Brachionus calyciflorus (R)*	r 2-d	NOEC = 2.5 EC10= 2.38 EC20= 4.91 EC50= 16.8	Radix et al. 1999
2,4-D (technical grade)	*Brachionus patulus* (R)	r	500	Sarma et al., 2001
3,4- DCA (3,4- dichloroaniline)	*Brachionus calyciflorus* (R)	S e_o Ro r Vx T	5.0, 10, 20 > 2.5 ≥ 5.0 > 5.0 2.5 > 5.0	Ferrando et al. 1993

Abbreviations. (C) Cladocerans, (R) Rotifers, (A) Amphipod. LC50 = Median Lethal Concentration, EC50 = Concentration where 50% inhibition occurs, MATC = Maximum Acceptable Toxicant Concentration, LOAEL = Lowest Observed Adverse Effect Level, NOAEL = No Observed Adverse Effect Level, LOEC = Lowest Observed Effect Concentration, NOEC = No observed effect concentration.

Table 4. Chronic toxicity of herbicides assessed to several species of freshwater zooplankton. Criteria consider a decrease or inhibition of the parameter at different exposure time to herbicide in minutes (m), hours (h) or days (d). Parameters: F = Fecundity, ED = Embryonic Development, R = Reproduction, S = Survival, cFDAam = Esterase activity, PLA2 = Phospholipase A2 activity, Ro = Net reproductive rate, T = Generation time, r = Intrinsic rate of population growth, e_o = Life expectancy, and Vx = Reproductive value.

distinct from the normal status of the individual or system health. Biomarkers are classified in three types:

1. Effect biomarkers, which record the exposure of the organism to a toxicant or stressor without being directly related with the specific mechanism of action of the toxicant, and therefore, do not provide information on the level of adverse effect that this change causes (Hagger et al., 2006; Walker et al., 2006).
2. Exposure biomarkers, which provide qualitative and quantitative estimations of exposure to several compounds. These biomarkers are well characterized and associated with the mechanism of action of the toxicant showing the relationship between levels of modification of the biomarker with respect to level of adverse effect (Hagger et al., 2006).
3. Susceptibility biomarker, which provide information of the system´s health and are sensitive to toxicant exposure (Domingues et al., 2010).

There are different types of exposure biomarkers that involve important biological functions and that have been used to assess the adverse effect of many chemical substances. However, use of these biomarkers regarding aquatic invertebrates have been limited due to low availability of biological material, specificity, duration and costs (Hyne & Maher, 2003).

During a risk assessment, it is valuable to consider the range of specificity of the biomarkers. For instance, acethylcholinesterase (AChE) inhibition is consider specific for organophosphate, organochloride, and carbamate pesticides (Walker et al., 2006); and it is necessary to consider enough time to detect the presence of neurotoxic substances in the environment. Besides, AChE inhibition has been assesses in different aquatic invertebrate

species. Therefore, it can be used as a good biomarker for these pesticides. The knowledge of AChE activity and its inhibition by certain herbicides can be used to relate enzymatic activity with the decrease of population densities in the field (Hyne & Maher, 2003). De Coen et al. (2001) demonstrated the relationship between parameters from carbohydrate enzymatic metabolism in *D. magna* and the specific effects of a toxicant suggesting that the activity of the piruvate kinase could potentially be the first warning sign about prolonged effects and to predict quantitative changes in the population.

Records on the use of biomarkers estimating the effect of herbicides on freshwater zooplankton are scarce. Barata et al. (2007) performed *in situ* bioassays with *D. magna*, reporting severe effects on the grazing rate, AchE, catalase, and glutathion S-transferase inhibition associated with the presence of bentazone (487 µg/L), methyl-4-chlorophenoxyacetic acid (8 µg/L), propanil (5 µg/L), molinate (0.8 µg/L), and fenitrothion (0.7 µg/L) in water. Domínguez-Cortinas et al. (2008) found that esterase and phospholipase A2 inhibition are good exposure biomarkers when the freshwater rotifer *L. quadridentata* and the cladoceran *D. magna* are exposed to the herbicide glyphosate and its commercial formula Faena (Table 1 and Table 2).

According to Barata et al. (2007) and Walker et al. (2006), the use of biomarkers is valuable to identify and assess the biological effects whenever toxicants are present in enough concentration to induce a detectable effect. Besides, Hagger et al. (2006), suggest that if the measurement of these effects shows the first responses in lower concentrations than the usual parameters of traditional toxicology, then the sensitivity of biomarker is of great use. It is important to consider that some chronic or sublethal effects can be irreversible and that can take place in ecosystems apparently healthy and where initially they were not detected (Hyne & Maher, 2003). Finally, a biomarker used as an integral parameter has the potential of establishing evidence of adverse effects caused by the presence of chemical substances in a system that can then be related with other levels of biological organization. Therefore, is fundamental to develop more research using biomarkers on freshwater zooplankton that allow to assess the adverse effect of all kind of toxicants (including herbicides), and to use these biomarkers regularly to monitor aquatic ecosystems.

7. Herbicides as endocrine disruptors of freshwater zooplankton species

Although many of the adverse physiological effects of chemicals affecting the neuroendocrine system have been known for over three decades, special attention to this issue only materialized in the early 1990s (Tackas et al., 2002). Given the high volume of use, high level of toxicity to primary producers, and long persistence in the environment, many studies have addressed the capacity of herbicides to disrupt endocrine function at concentrations that commonly occur in surface waters during application periods (Porter et al., 1999). An endocrine disruptor is defined as an exogenous agent that directly interferes with the synthesis, secretion, transport, binding action, or elimination of endogenous hormones and neurohormones, resulting in physiological manifestations of the neuroendocrine, reproductive or immune systems in an intact organism (Tackas et al., 2002).

Aquatic toxicity studies have shown that cladoceran fecundity and survival endpoints are not affected at atrazine concentrations below 100 µg/L (Takacs et al., 2002). However, Dodson et al. (1999) revealed that chronic exposure of *Daphnia pulicaria* to very low

concentrations (0.5 μg/L) of atrazine induced a shift in the population sex ratio due to increased male production, indicating sex ratio is a very sensitive, ecologically-relevant endpoint. Males were produced in stress situations, in response to environmental signals such as shortening day length, reductions in food supply and pheromones produced in crowded populations (Dodson et al., 1999).

Villarroel et al. (2003) compared acute toxicity, reproductive and growth, and feeding activity alterations in *D. magna* exposed to several concentrations of propanil herbicide in a 21-days study. Some parameters analyzed were affected by herbicide: Survivorship did not decrease with increasing concentration of propanil, except with higher concentration (0.55 mg/L); number of neonates born, brood size and number of broods per female as well as the intrinsic rate of growth (r) decreased as the concentrations of propanil increased in the medium. EC50 values indicated that reproductive parameters, like the number of young per female (0.21 mg/L) and brood size (0.26 mg/L) were the most sensitive endpoints in response to propanil exposure. The filtration and ingestion rates were reduced significantly after 5-h exposure to this herbicide; this would be related with lose of coordination and paralysis caused for toxic effects of herbicide on nervous system of *D. magna* (Villarroel et al., 2003).

Other studies have shown that uptake of herbicides can directly affect survival, population growth, reproduction and feeding of rotifers. Riobbo et al. (2007) found that the *Brachionus* sp. population density decreased when females were fed with *Chlorella vulgaris* cells previously exposed to different concentrations of terbutryn, with a maximum survival of 4-days with 500 nM terbutryn in the medium. Terbutryn accumulated in *C. vulgaris* provoked a decrease in the feeding rate of *Brachionus* cultures, and a 66% reduction of the number of eggs per reproductive female compared to controls.

These results suggest that endocrine effects on zooplankton are caused by direct or indirect exposure to herbicides, where population growth rate and sex ratio can be the more sensitive parameters.

8. Field studies, mesocosms, and microcosms, involving herbicides and freshwater zooplankton

Among non-target organisms affected by herbicides in freshwater bodies, plankton and its components (bacterio-, phyto-, and zooplankton) are known to respond on short timescales to low levels of pollutants (Daam et al., 2009), mainly owing to their intrinsic sensitivity and high population turnover (Relyea, 2005). Secondary effects of herbicides on these organisms are difficult to predict since they depend on interactions between species, herbicides and the original structure of the ecosystem (Wendt-Rasch et al., 2003). For aquatic ecosystems, toxicity testing ranges from standard tests under laboratory conditions to field studies, including microcosm and mesocosm experiments (Caquet et al., 2000). These studies in enclosures are valuable tools that can help to understand how herbicides exposure may affect ecosystems as a whole, and be an aid in the assessment of the various risk scenarios resulting from the use of these chemicals (Wendt-Rasch et al., 2003).

Most of the information on the ecotoxicity of herbicides in aquatic communities is related to individual o combined effects of exposure to these chemicals at the ecosystem level (Thompson, 2006). Wendt-Rasch et al. (2003) reported no significant effects on copepod nauplii

and rotifers from exposure during 14 days to metsulfuron methyl (0, 1, 5, 20 μg/L) in 24 enclosures of 80 L (height: 0.65 m, diameter: 0.4 m) in water bodies adjacent to agricultural fields. Metsulfuron methyl is a sulfonylurea herbicide that affects the synthesis of essential amino acids in plants, and hence inhibits cell division. It is highly water-soluble and has a low sorption coefficient (Tomlin, 1997). However, herbicide exposure had a significant effect on the conductivity, pH and total nitrogen in the enclosures (Wendt-Rasch et al., 2003).

Plankton communities from a tropical freshwater reservoir in Mozambique were monitored for 5 days after exposure to nominal concentrations of diuron (2.2 and 11 μg/L) and paraquat (10 and 40.5 μg/L), commonly used in the tropics for agriculture and disease vector control. Diuron blocks photosynthetic electron transfer in plants and algae, and paraquat generates superoxide O_2^- that affects all cellular components (Leboulanger et al., 2011). In general, zooplankton was slightly sensitive to diuron, and very sensitive to paraquat. Nauplii or cyclopidae copepodites and adults did not differ in microcosms inoculated with diuron relative to the controls. However, the adult stages of the copepod *Diaphanosoma excisum* were slightly reduced in high concentration compared with the control. A reduction in rotifer biomass was also noticed with a below significance level ($p = 0.072$). Low concentration of paraquat caused a significant reduction in *Thermocyclops decipiens* copepodite biomass relative to controls, whereas high treatments reduced the carbon biomass in all groups of zooplankton, mainly the cladocera and copepod nauplii (Leboulanger et al., 2011).

In PVC tanks of 150 L with water from the Paraná River, Gagneten (2002) evaluated the effects of paraquat (0.1, 0.2, 0.4 and 0.8 ml/L) on zooplankton community for 35 days of exposure. Contrary to what was observed with the species richness dominated by rotifers (55%), cladocerans (18%), and copepods (15%), paraquat negatively affected the zooplankton density, especially in higher concentrations. The chemical effect of the herbicide was higher on rotifers *Anuraeopsis*, *Lecane*, *Phylodina* and *Conochilus*; on the cladoceran *Ceriodaphnia*; on copepods *Eucyclops* and *Notodiaptomus*, and on thecamoebians *Arcella* and *Cucurbitella*. Dissolved oxygen, pH and water hardness did not vary significantly between controls and treatments during the experimental period. According to Pratt and Barreiro (1998), it is necessary to consider species composition, inter- and intraspecific interactions and environmental factors, such as physicochemical parameters, when analyzing the impact of herbicides on aquatic communities. This interaction between herbicides and biological and environmental factors may reduce or increase the impact of pollution on aquatic ecosystems (Gagneten, 2002).

Interactions of herbicides with others environmental stressors have also been studied. Chen et al. (2004, 2008) examined effects of interactions among pH (5.5 and 7.5), two levels of food concentrations, and the formulated products Vision® (glyphosate: 0.75 and 1.50 mg acid equivalent/L) and Release® (triclopyr) on cladoceran *Simocephalus vetulus*. Herbicide treatments resulted in significant decreases in survival, reproductive rate, and development time for *S. vetulus* at levels 5–10× below predicted worst case environmental concentrations (2.6 mg/L). High pH increased the toxic effects of the herbicide on all response variables even though it improved reproductive rate of *S. vetulus* over pH 5.5 in the absence of herbicide. Stress due to low food also interacted with pH 5.5 to diminish *S. vetulus* survival. These results support the general postulate that multiple stress interactions may exacerbate chemical effects on aquatic biota in natural systems.

Atrazine is a selective herbicide with long residual activity used on crops such as corn, sorghum, sugarcane, conifers, forestry and lawn care applications (Solomon et al., 1996). Degradation rates in water are highly variable. The DT50 in water has been estimated to range from 3-90 d or more and in sediment the range was 15-35 d (Huber, 1993). Several invertebrate community studies have been conducted with atrazine in field situations using mesocosms or whole ponds. The population density of cladocerans in ponds treated at 20 µg/L was lower than that in control ponds even one year after contamination. The most sensitive effect concentration for invertebrates in outdoor enclosures was 0.1 µg/L in which herbivorous zooplankton were reduced in abundance (Tackas et al., 2002).

Indirect effects on zooplankton were reported by Jüttner et al. (1995) during a 6 week mesocosms study. Total numbers of the cladoceran *Daphnia longispina* declined in all 7 enclosures following treatment with atrazine. This was accompanied by reduced egg ratios between day 3 and day 21. In both cases, effect concentration was 318 µg/L. Likewise, effect concentration on reduction in the density of copepod nauplii, *Synchaeta* sp. and *Polyarthra* sp was from 68, 132, and 318 µg/L atrazine, respectively. Van den Brink et al. (1995) detected only slight reductions in primary productivity over 7 weeks in multispecies microcosms exposed to 5 µg/L atrazine, and observed no significant effects on cyclopoid and cladoceran species or on the amphipod *Gammarus* and the rotifer *Keratella*.

Lozano et al. (1992) studied the temporal variation in abundance (% of control) of zooplankton following a single dose of esfenvalerate in 5 different concentrations (0.01, 0.08, 0.2, 1.0, 5.0 µg/L). Mesocosms were shallow (0.5 - 1.1 m depth), had sediment and macrophytes and ranged between 25 – 1100 m^3 in volume. Dose-response curves showed that the initial impact on abundance and the subsequent recovery were dependent on the concentration: decreasing in Cladocera and Copepoda, and increasing in phytoplankton and Rotifera. Perschbacher et al. (2002) and Perschbacher and Ludwig (2004) tested the adverse impacts of common aerially applied herbicides for rice on phytoplankton, zooplankton, and water quality in 12 mesocosms (500 L, 0.7 m depth). Clomazone (0.6 kg active ingredient/ha), thiobencarb (3.4), pendamethalin (1.1), quinclorac (0.6), halosulfuron (0.07), bensulfuron methyl (0.07), triclopyr (0.4), 2,4-D-amine (1.7), and molinate (5.6) produced no measurable effects on plankton or water quality. Propanil (4.5) and diuron (1.4) significantly reduced oxygen production by 75% after their application and stimulated chlorophyll *a*, too. It was assumed to be related to compensatory action by the algae for photosynthesis inhibition. The increase in chlorophyll *a* concentration suggests an increase in food availability for zooplankton and is ultimately believed to have been responsible for the observed increase in numbers of rotifers and copepods, but not cladocerans (Perschbacher et al., 2002).

Marcial and Hagiwara (2008) determined acute toxicity of the mefenacet herbicide on the copepod *Tigriopus japonicus*, the cladoceran *Diaphanosoma celebensis* and the rotifer *Brachionus plicatilis*. Compound exposure was carried out in 6-well polystyrene plates, and mortality was evaluated after 24 h. Although species showed different sensitivities to herbicide, a dose-response relationship was consistent in all cases. *B. plicatilis* was particularly resistant to mefenacet, while *T. japonicus* and *D. celebensis* are comparatively sensitive.

Mohr et al. (2008) monitored for 140 days the effects of metazachlor (5, 20, 80, 200, and 500 µg/L) on stream and pond communities. In this study, metazachlor strongly affected

mesocosms communities at all concentrations. Direct negative effects were most prominent for chlorophytes whereas diatoms and cryptophytes seemed insensitive. The effects on zooplankton were caused by changes in habitat structure due to the strong decline of macrophytes. The slow degradation of metazachlor combined with the absence of recovery in both chlorophytes and macrophytes was likely to cause long-lasting effects on aquatic ecosystems.

Jenkins and Buikema Jr. (2009) studied effects of simazine (0.1, 0.5 and 1.0 mg/L) on zooplankton and physical-chemical parameters in *in situ* microcosms for 21 days. Herbicide induced decreases in dissolved oxygen and pH, but induced increases in nitrate and ammonia levels compared to control microcosms. Rotifers dominated the zooplankton and were differentially affected by simazine. The dominant species, *Kellicottia bostomensis*, exhibited a positive response to simazine, as did *Keratella cochlearis*, due to lesser mortality in higher concentrations of simazine. *Polyarthra vulgaris* was unaffected, but *Synchaeta pectinata* was impaired by simazine at day 21.

These micro- mesocosms studies indicate that decrease in zooplankton density in the treated ponds probably was not caused by direct toxic effects of the herbicides, but to indirect effects resulting from reduced algal productivity, a change in the food source or a change in the competition for a food source.

9. Molecular genetics, DNA and protein microarrays, environmental genomics relating herbicides and freshwater zooplankton

The integration of genomic-based tools and ecotoxicology is a promising approach that may provide a broad view of how living systems respond to a given stressor (Neumann & Galvez, 2002; Robbens et al., 2007; Snape et al., 2004).

Transcription profiling using microarrays is one of the most prominent genome-wide technologies within ecotoxicogenomics since it provides an overview of changes in gene expression linked to chemical exposure (Pereira et al., 2010). Very recently, cDNA microarray-related techniques have been successfully used to address transcriptional responses of *D. magna* to different environmental toxicants, including pharmaceuticals, heavy-metals, pesticides and PAHs (Connon et al., 2008; Heckmann et al., 2008; Soetaert et al., 2006, 2007; Watanabe et al., 2007).

The evaluation of herbicides genotoxicity has been an important research line, to investigate the alterations in the molecular pathway in the organism. The most important organism for this test is *Daphnia magna*. Table 5 shows some alterations and DNA damages caused for some herbicides.

The effects of herbicides on freshwater zooplankton has been studied on molecular pathways and DNA, for example Pereira et al. (2010), to understanding the genomic responses of *D. magna* to chemical challenges, exposed to the herbicide propanil to compare phenotypic effects with changes in mRNA expression level. Propanil highly promoted synthesis of innate immunity response systems (more details in Table 3) and elicited specific up-regulation of gene transcription within neuronal pathways, including dopa decarboxylase and syntaxin 6. Atrazine induced hemoglobin genes (dhb1, dhb2 and dhb3) in *D. magna* through the hormonal pathways. This hypothesis was tested by modeling the

combined effects of atrazine and the terpenoid hormone mimic pyriproxyfen on hemoglobin mRNA levels assuming the same mechanism of action (concentration addition model) and alternatively, assuming different mechanisms of action (response addition model) (Rider & Leblanc, 2006).

Herbicide	DNA alterations	Reference
Terbutryn	Cytogenetic damage Primary DNA damage	Moretti et al., 2000
Atrazine	Mutagenic and genotoxic potencial	Kaya et al., 2000
		Pino et al., 1988
	DNA damage	Clements et al., 1997 Tennant et al., 2001
	Expression of haemoglobin genes	Rider and LeBlanc, 2006
Propanil	Promoted transcriptions genes of: Haemoglobin synthesis Neuronal pathways Up-regulated genes specifically related to defense mechanisms	Pereira et al., 2010

Up-regulated genes specifically related to defense mechanisms

Table 5. DNA alterations by herbicides.

10. Conclusions and future research

The study of the adverse effects of herbicides on freshwater zooplankton is an unexplored field. Studies in Quantitative Structure/Activity Relationship (QSAR´s) are scarce or missing (at least from mainstream scientific literature). Ecotoxicogenomics studies are scarce and restricted to few herbicides and one species: *Daphnia magna*. Regarding biomarkers applied to herbicide exposure the small set of data available suggest that the potential of herbicides for producing adverse effects on freshwater zooplankton can be high, and warrants future research. Presently, atrazine and glyphosate are the two herbicides of great regulatory concern because of their widespread use, common detection in water having relatively long persistence in freshwater. Lethal toxicity in amphibians has been demonstrated (Reylea, 2005). Still, some authors pose serious doubts about the results suggesting direct and indirect effects of herbicides on invertebrates, amphibians and fish exposed to environmentally relevant concentrations (Fairchild, 2011). These doubts have to be clarified using well designed experiments that include effects on endocrine and immune function. Mesocosms studies will help identify and characterize the mechanisms that modify the sensitivity of zooplankton by exposure to herbicides. Compared to laboratory experiments, mixtures of herbicides combined with physical and chemical factors at the natural environment, could identify physiological, biochemical and behavioral changes more significant on zooplankton communities, mainly rotifers and copepods for which information reported is scarce. However, this chapter already includes recent data on lethal tests that suggest that at least for brief periods of time, some herbicides at environmentally

relevant concentrations can produce mortality, and other relevant sublethal effects in freshwater zooplankton (for example, reduction in rate population growth).

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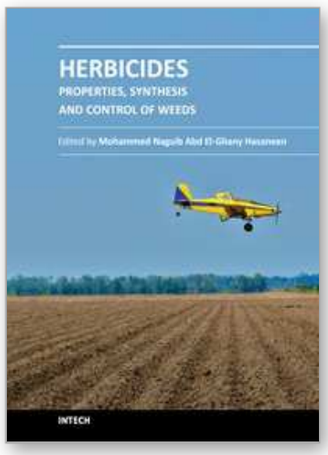

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This book is divided into two sections namely: synthesis and properties of herbicides and herbicidal control of weeds. Chapters 1 to 11 deal with the study of different synthetic pathways of certain herbicides and the physical and chemical properties of other synthesized herbicides. The other 14 chapters (12-25) discussed the different methods by which each herbicide controls specific weed population. The overall purpose of the book, is to show properties and characterization of herbicides, the physical and chemical properties of selected types of herbicides, and the influence of certain herbicides on soil physical and chemical properties on microflora. In addition, an evaluation of the degree of contamination of either soils and/or crops by herbicides is discussed alongside an investigation into the performance and photochemistry of herbicides and the fate of excess herbicides in soils and field crops.

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