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Toxicity of 21 Herbicides to the Green Alga *Scenedesmus quadricauda*

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The pollution of aquatic systems by agricultural chemicals has attracted great concerns from the public (Wang and Freemark 1995). A great number of studies have been conducted to determine the harm of these pollutants on living organisms in aquatic systems (Wong 2000). Herbicides are often used in agriculture to reduce or destroy weeds, to avoid competition for nutrients and light between crops and weeds. An undesirable side-effect from the use of these herbicides is that they enter freshwater ecosystems by spray drift, leaching, run-off, or accidental spills. Contamination of surface waters by herbicides has been reported to have direct toxic effects on populations of phytoplankton. Also, when these primary producers are affected, indirect effects on ecosystem functioning and animal populations can also be expected (Van den Brink and Ter Braak 1999). Microalga play an important role in the equilibrium of aquatic ecosystems being the first level of the trophic chain to produce organic matter and oxygen. The rest of aquatic biota is strictly dependent upon the photosynthetic activity of these organisms. Perturbations of phytoplanktonic populations and alterations of their primary production may have severe repercussions on the biotic community (Campanella et al. 2000).

A test organism's sensitivity to toxic substances is a complex issue, as it involves types of toxicants, environmental conditions, test methods, and other factors. Some studies have shown that the sensitivities of plants and other groups of organisms vary widely among toxicants. Sensitivity not only varies among toxicants, but also among taxonomic groups and species within taxa (Boyle 1984). Sensitivity to toxicants between algae and plants is so large that algal toxicity testing should not be used as a surrogate in testing vascular plants (Wang and Freemark 1995). In a comparative phytotoxicity study, Hughes and Erb (1989) examined the relative sensitivity of four species of algae and one species of duckweed to 13 pesticides. They reported that no species could be identified as "always being the most sensitive or always the least sensitive." Some reports have been published about the comparative toxicity of solvents toward various organisms (Tadros et al. 1994). Yet few reports involved the differential response

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of various green algal species to herbicides (Kasai et al. 1993). As to the comparative differential sensitivity of herbicides to green algae, a set of the acute toxicity test has been devised. In the present study, 21 herbicides have been tested to examine their effect on the green alga *Scenedesmus quadricauda* and then compare their differential sensitivity three other green algae, *Scenedesmus obliqnus*, *Chlorella vulgaris* and *Chlorella pyrenoidosa*. This article (part II) deals with 21 herbicides from 10 different chemical classes, with 6 different modes of action—lipid synthesis, photosynthetic process, hormone synthesis and protoporphyrinogen oxidase (Protox), glutamine synthase and EPSP synthase inhibiting herbicides.

Table 1. Selected herbicides, chemical class and mechanism target.

NO.	Herbicides	Formulations	Chemical class	Mechanism target
1	Butachlor	90%TC[a]	Chloroacetamides	Lipid synthesis
2	Metolachlor	50%WP[b]		
3	Mefenacet	95%TC		
4	Acetochlor	80%TC		
5	Atrazine	38%SC[c]	Triazines	Photosynthetic process
6	Simazine	92.2%TC		
7	Ametryn	92%TC		
8	Cyanazine	97.81%TC		
9	Prometryne	77.13%TC		
10	Isoproturon	95%TC	Ureas	
11	Diuron	50%WP		
12	Methabenzth -iazuron	97.8%TC		
13	Chlorotoluron	95%TC		
14	Paraquat	20% SL[d]	Bipyridyliums	
15	Bromoxynil	95%TC	Benzonitriles	
16	Quinclorac	90%TC	Quinoline carboxylic acids	Hormone synthsis
17	Fluroxypyr	20%EC[e]	Pyridnecarboxylic	
18	MCPA	90%TC	Phenoxycarboxylic acids	
19	Oxadiargyl	80%SL	Oxadiazole	Protoporphyrinogn Oxidase(Protox)
20	Glufosinate	13.5%EC	Phosphinic acids	Glutamine synthase
21	Glyphosate	95%TC	Glycines	EPSP synthase

[a]TC (technical product); [b]WP (wettable powder); [c]SC (suspension concentrate); [d]SL (soluble concentrate); [e]EC (emulsible concentrate).

MATERIALS AND METHODS

Tested herbicides with chemical classes and mode of action (Retzinger 1997) are shown in Table 1, were dissolved in $\leqslant$ 0.05% acetone or in distilled water.

The toxicity tests were carried out with the freshwater unicellular green alga *S. quadricauda* obtained from the Institute of Wuhan Hydrobiology, the Chinese Academic of Science. The alga was kept on agar slants at approximately 4℃. The medium for the algal growth inhibition test was prepared using HB-4 medium and was sterilized at 121℃, 1.05 kg cm^{-2} for 30 min; described in detail in Ma (2003).

Cells of *S. quadricauda* were propagated in advance. Fifteen mL aliquots of the HB-4 medium containing single algal cells (initial spectrophotometric data was OD_{680nm}=0.05) were distributed to sterile 50 mL Erlenmeyer flasks. The media of *S. quadricauda* were then treated with various herbicide concentrations in a previous test to develop on adequate range of toxicity for each herbicide. This range of concentrations was then used for EC_{50} determination (Moreno-Garrido et al. 2000). Cell counts were correlated with absorbance over time for 96 hr on a Shimadzu UV-2401PC spectrophotometer at 680 nm wavelength and growth of algal cells was calculated indirectly using spectrophotometric data. Each herbicide concentration was tested in triplicate. Appropriate control systems containing no herbicide were included in each experiment. Control and treated cultures were grown under the same conditions. In each experiment, percent inhibition values, relative to growth in control systems, were calculated using spectrophotometric data (Ma et al. 2001). EC_{50} values were calculated using linear regression analysis of transformed pesticide concentration as natural logarithm data versus percent inhibition (Ma et al. 2001). For detailed experimental methods see Ma (2003).

RESULTS AND DISCUSSION

Acute toxicity of 21 herbicides to the green alga *S. quadricauda* is shown in Table 2. The 96 h EC_{50} values of lipid synthesis inhibitor such as butachlor, metolachlor, mefenacet and acetochlor varied around 0.2-4.4 mg/L (10^{-6}-10^{-7} M). Auxin herbicides such as fluroxypyr, quinclorac and MCPA varied from 52-214 mg/L (10^{-4} M), Auxin herbicides stimulate ethylene biosynthesis by inducing the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase. In susceptible dicots, increased levels of ethylene trigger an accumulation of abscisic acid (ABA), in susceptible grasses, the levels of tissue cyanide (HCN), a co-product formed during ethylene biosynthesis, increased. These increases in ethylene, ABA, and HCN cause epinasty of leaves, growth retardation, and senescence (Moreland 1999). The acute toxicity of auxin herbicides against *S. quadricauda* was lower than others. The same results have also been obtained

Table 2. Dose response relationship of herbicides to *S. quadricauda.*

NO.	Regression equation[a]	Significance level	Coefficient correlation	EC_{50} (mg/L)	EC_{50} (mol/L)
1	P=103.32+34.71lnC	0.01	0.97	0.2	6.9×10^{-7}
2	P=54.08+10.86lnC	0.01	0.95	0.6	2.4×10^{-6}
3	P=94.51+28.91lnC	0.01	0.98	0.2	7.1×10^{-7}
4	P=26.10+16.29lnC	0.09	0.90	4.3	1.6×10^{-6}
5	P=90.43+12.70lnC	0.02	0.97	4.1×10^{-2}	1.9×10^{-7}
6	P=106.90+30.35lnC	0.01	0.98	0.15	7.6×10^{-7}
7	P=91.82+22.61lnC	0.02	0.97	0.15	6.9×10^{-7}
8	P=91.69+27.18lnC	0.02	0.97	0.2	8.9×10^{-7}
9	P=102.15+11.27lnC	0.05	0.94	9.7×10^{-3}	4.0×10^{-8}
10	P=173.37+32.28lnC	0.05	0.94	2.1×10^{-2}	1.0×10^{-7}
11	P=265.44+36.45lnC	0.04	0.95	2.7×10^{-3}	1.1×10^{-8}
12	P=120.15+ 20.58 lnC	0.09	0.90	3.3×10^{-2}	1.5×10^{-7}
13	P=164.80+28.86lnC	0.01	0.98	1.8×10^{-2}	8.8×10^{-9}
14	P=766.94+53.06 lnC	0.04	0.95	1.3×10^{-6}	7.2×10^{-12}
15	P=13.40+27.46lnC	0.08	0.91	3.7	1.2×10^{-5}
16	P=-234.52+53.05lnC	0.06	0.93	213.3	8.8×10^{-4}
17	P=-69.36+30.18lnC	0.01	0.98	52.1	1.4×10^{-4}
18	P=-129.29+40.24lnC	0.01	0.94	86.1	4.2×10^{-4}
19	P=-64.02+38.70lnC	0.01	0.98	19.0	5.5×10^{-5}
20	P=-126.05+45.85lnC	0.02	0.92	46.5	2.3×10^{-4}
21	P=-127.49+41.70 lnC	0.01	0.98	70.5	4.1×10^{-4}

[a] P (percent inhibition); C (herbicide concentration)

using *S. obliqnus*, *C. pyrenoidosa* and *C. vulgaris* as a test organism (Ma and Liang 2001; Ma et al. 2001). The EC_{50} values of the protoporphyrinogen oxidase (Protox) inhibitors oxadiargyl were 19 mg/L (10^{-5} M). Protox inhibitors lead to the accumulation of substrate, protoporphyrinogen, which is readily oxidized to proto IX by oxidative enzymes. Proto IX is an effective photosensitizer and in the light it transfers absorbed energy to molecular oxygen to form singlet oxygen. The singlet oxygen peroxidizes lipids leading to the destruction of cellular membranes (Moreland 1999).

The EC_{50} values of the glutamine synthase inhibitor--glufosinate were 46 mg/L (10^{-4}M). The EC_{50} values of the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSP Synthase) inhibitor--glyphosate were 70 mg/L (10^{-4}M). It causes the concentration of glyoxylate to elevate which inhibits RuBP carboxylase, the first enzyme involved in carbon fixation.

Table 3. Differential sensitivity of the green algae to herbicides.

NO.	[a]Ratio of *SQ / SO*	[b]Orders	Ratio of *SQ /CP*	[b]Orders	Ratio of *SQ /CV*	[b]Orders
1	3.1×10^{-2}	--	6.0×10^{-2}	--	2.5×10^{-2}	--
2	3.5×10^{-2}	--	5.4×10^{-2}	--	3.6×10^{-2}	--
3	0.4×10^{-2}	---	23.8	++	[c]Δ	[c]Δ
4	0.1	-	0.6	-	0.1	-
5	0.3	-	0.2	-	9.9×10^{-2}	--
6	0.5	-	1.8	+	7.0×10^{-2}	--
7	13.1	++	526.6	+++	[c]Δ	[c]Δ
8	1.5	+	0.9	-	1.6	+
9	5.9	+	0.8	-	0.1	-
10	0.9	-	4.4	+	1.0	
11	0.2	-	2.0	+	0.6	-
12	9.6×10^{-2}	--	1.8×10^{-2}	--	[c]Δ	[c]Δ
13	0.2	-	1.3×10^{-2}	--	0.7	-
14	5.9×10^{-5}	----	1.4×10^{-2}	--	7.0×10^{-3}	---
15	7.1×10^{-2}	--	0.8	-	4.3×10^{-2}	--
16	32.6	++	168.4	+++	7.2	+
17	1.9	+	17.1	++	1.3	+
18	2.4	+	3.9	+	0.6	-
19	[c]Δ	[c]Δ	3.5	+	0.3	-
20	6.6	+	16.5	++	45.4	++
21	1.2	+	19.9	++	15.2	++

[a] SQ, SO, CP and CV stand for *S. quadricauda*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*; their EC_{50} data see Ma et al.(2001; 2002).
[b] order denotes EC_{50} ratio; +, ++ and+++ stand for 1-10×, 10-99× and 100-999 × in separately; -,--, --- and ----stand for 0.1-1.0×, 0.01-0.1×, 0.001-0.01× and <0.001× in separately.
[c] Δ denotes no data.

The EC_{50} values of the photosynthesis-inhibiting herbicides were the lowest among the tested herbicides. The EC_{50} values of bromoxynil was 10^{0} mg/L, simazine, ametryn and cyanazine were 10^{-1} mg/L, atrazine, methabenzthiazuron, isoproturon and chlorotoluron were 10^{-2} mg/L, prometryne and diuron 10^{-3} mg/L and paraquat were 10^{-6} mg/L. Their molar concentration varied around 10^{-5} to 10^{-12} M and in general their molar concentration was 10^{-7} to 10^{-8} M. Acute toxicity of this type of herbicide against *S. quadricauda* was the highest of all tested herbicides. The same results have also been obtained using *S. obliqnus*, *C. pyrenoidosa* and *C. vulgaris* as a tested organism (Ma and Liang 2001; Ma et al. 2001).

Comparing the acute toxicity of 21 herbicides with different primary modes of action to the green alga *S. quadricauda*, the order from high to low was: the photosynthesis-inhibiting herbicides > lipid synthesis inhibitor > protox inhibiting herbicides > glutamine synthase inhibiting herbicides > EPSP synthase inhibiting herbicides > auxin herbicides, similar results have also been obtained using *S. obliqnus*, *C. pyrenoidosa* and *C. vulgaris* as test organisms (Ma and Liang 2001; Ma et al. 2001).

Wide variations occurred in response to the tested herbicides among the four individual species of green algae (Table 3). Compared with *S. obliqnus*, *S. quadricauda* was more sensitive to the 11 herbicides— butachlor, metolachlor, mefenacet, acetochlor, atrazine, simazine, diuron, methabenzthiazuron, chlorotoluron, paraquat and bromoxynil, and less sensitive to other 4 herbicides— ametryn, prometryne, MCPA and glufosinate. As to the rest 5 herbicides, *S. quadricauda*'s sensitivity was close to that of *S. obliqnus*.

In comparison with *C. pyrenoidosa*, *S. quadricauda* was less sensitive to the 10 herbicides—mefenacet, ametryn, isoproturon, diuron, quinclorac, MCPA, fluroxypyr, oxadiargyl, glufosinate and glyphosate,and more sensitive to other 6 herbicides— butachlor, metolachlor, atrazine, methabenzthiazuron, chlorotoluron and paraquat. Yet, for both of them, similar sensitivity to the rest 5 herbicides.

Likewise, in contrast to *C. vulgaris*, *S. quadricauda* was more sensitive to the 9 herbicides— butachlor, metolachlor, acetochlor, atrazine, simazine, prometryne, paraquat, bromoxynil and oxadiargyl, and less sensitive to 3 other herbicides— quinclorac, glufosinate and glyphosate. And the rest 6 similar.

Investigations with different green algal species have shown that algae vary greatly in their response to chemicals. Differential sensitivity of the green algae to the compounds could induce species shifts within communities (Boyle 1984; Tadros et al. 1994; Ma 2003). Two extensive databases were used to examine the pattern of species sensitivities to pesticides by Boutin and Rogers (2000). They found that crop species were not consistently more, or less, sensitive to the herbicide tested than non-crop species. The range of species sensitivity increases with an augmentation of numbers of species tested, which suggests that the number of species tested in current guidelines is insufficient. Sensitivity to toxicants is important in determining the suitability of a test for adoption into chemical regulations. Sensitive tests are more likely to yield ECx values that will afford protection to species and communities. Sensitivity not only varies among toxicants, but also among taxonomic groups and species within taxa. Hughes and Erb (1989) examined the relative sensitivity of four species of alga and duckweed to 13 different pesticides, they reported that no species could be indentified as "always being the most sensitive or always the least sensitive" (Wang and Freemark, 1995). The same result has also been obtained in this test and in our previous works using over 50 pesticides as test compounds (Ma and Liang 2001; Ma 2002, Ma et al. 2001; 2002a; b).

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REFERENCES

Boutin C, Rogers CA (2000) Pattern of sensitivity of plant species to various herbicides—an analysis with two databases. Ecotoxicology 9: 255-272

Boyle TP (1984) The effect of environmental contaminants on aquatic alga. In: Shubert LE (ed.) Alga as ecological indicators. Academic press, New York p 237-256

Campanella L, Cubadda F, Sammartino MP, Saoncella A (2000) An algal biosensor for the monitoring of water toxicity in estuarine environments. Water Res 25:69-76

Hughes JS, Erb K (1989) The relative sensitivity of five nontarget aquatic plant species to various pesticides. Presented at 10th annual meeting of the society of environmental toxicology and chemistry, October 28. Toronto, Canada

Kasai F, Takamura N, Hatakeyama S (1993) Effect of simetryn on growth of various freshwater algal taxa. Environ Pollut 79: 77-83

Ma J, Liang W, Xu L, Wang S, Wei Y, Lu J (2001) Acute toxicity of 33 herbicides to the green alga *Chlorella pyrenoidosa*. Bull Environ Contam Toxicol 66:536-541

Ma J, Liang W (2001) Acute toxicity of 12 herbicides to the green alga *Chlorella pyrenoidosa* and *Scenedesmus obliqnus*. Bull Environ Contam Toxicol 67:347-351

Ma J (2002) Differential sensitivity to 30 herbicides among populations of two green alga *Scenedesmus obliqnus* and *Chlorella pyrenoidosa*. Bull Environ Contam Toxicol 68: 275-281

Ma J, Xu L, Wang S, Zheng Y, Jin S, Huang S, Huang Y (2002a). Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. Ecotoxicol Environ Saf 51: 128-132

Ma J, Zheng R, Xu L, Wang S (2002b). Differential sensitivity of two green alga *Scenedesmus obliqnus* and *Chlorella pyrenoidosa* to 12 pesticides. Ecotoxicol Environ Saf 52: 57-61

Ma J, Lin F, Wang S, Xu L (2003) Differential sensitivity of herbicides to green algae: toxicity of 20 herbicides to *Scenedesmus quadricauda*. Bull Environ Contam Toxicol 71 (*in press*)

Moreland, DE (1999) Biochemical mechanisms of action of herbicides and the impact of biotechnology on the development of herbicides. J Pestic Sci

24:299-307
Moreno-Garrido I, Lubian LM Soares AMVM (2000) Influence of cellular density on determination of EC_{50} in microalgal growth inhibition tests. Ecotoxicol Environ Saf 47:112-116
Retzinger, FJ, Smith CM (1997) Classification of herbicides by site of action for weed resistance management strategies. Weed Technol 11: 384-396
Tadros MG, Philips J, Patel H, Pandiripally V (1994) Differential response of green algal species to solvents. Bull Environ Contam Toxicol 52:332-337
Van den Brink PJ, Ter Braak CJF (1999) Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ Toxicol Chem 18:138-148
Wang W, Freemark K (1995) The use of plants for environmental monitoring and assessment. Ecotoxicol Environ Saf 30: 289-301
Wong PK (2000) Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis of *Scenedesmus quadricauda* Berb614. Chemosphere 41:177-182