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Comparative Sensitivity of *Pseudokirchnetiella subcapitata* vs. *Lemna* sp. to Eight Sulfonylurea Herbicides

M. Mohammad, T. Kishimoto, K. Itoh, K. Suyama, H. Yamamoto

Life and Environmental Science, Shimane University, Matsue 690-8504, Japan

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Aquatic plants play a major role in the environment of stagnant and flowing waters. They produce organic matter and oxygen, and provide food for a variety of aquatic organisms. Herbicides play an important role in agricultural practices, and there are more than 300 herbicides. Because there is considerable potential for the contamination of waterbodies with herbicides through overspray, drift, and runoff, it is important to assess the adverse impacts these chemicals may have on non-target organisms in aquatic ecosystems.

Sulfonylureas (SU) are a group of potent herbicides the use of which has increased dramatically since their discovery about two decades ago (Beyer et al. 1988; Brown 1990; Brown and Cotterman 1994). SU are characterized by broad-spectrum weed control at very low rates, good crop selectivity, very low acute and chronic animal toxicity, and no propensity to bioaccumulate in non-target organisms. All these characteristics make SU ideal candidates for replacing some of the older herbicides in an effort to reduce the quantity of chemicals used (Pimentel et al. 1991). SU acts through inhibition of acetolactate synthase (specific to plants and microorganisms) and thereby blocks the biosynthesis of branched-chain amino acids, leading to the rapid cessation of plant cell division and growth (Brown 1990; Schloss 1994). Much research concerning SU toxicity on weeds and crops has been reported (Beyer et al. 1988; Brown 1990; Dastgheib et al. 1995), however, little is known about the toxicity of SU against aquatic organisms. Fairchild et al. (1997) reported that the floating vascular plant *Lemna minor* showed higher sensitivity against two SU compared with the unicellular algae *Pseudokirchnetiella subcapitata*, whereas similar sensitivity was observed between the organisms for the other types of herbicides. Peterson et al. (1994) rated the hazard of four SU to algae as moderate to potentially low, but very high for duckweed at effective environmental concentrations. The objective of this work was to investigate the relative sensitivity of these two species to the other eight SU to generalize the previously observed tendency, and to clarify the relationship between toxicity and chemical properties of SU.

MATERIALS AND METHODS

The selection of SU herbicides for this study was made on the basis of current

Correspondence to: K. Itoh

major agricultural use in Japan (Table 1). All the chemicals (99.9%, analytical standard) were purchased from Wako Pure Chemical Industries Ltd., Hayashi Pure Chemical Industries, or Kanto Chemical Co. Inc., Japan. Stock solutions (200 or 1000 ppm) were prepared by dissolving with acetone and different concentrations of test solution were prepared by mixing with 20X-APP growth medium for *Lemna* sp. and OECD medium for algae (2002a; 2002b). The final concentration of acetone in the test solution was less than 0.01%. All stock solutions were prepared just before the experiments.

Table 1. Sulfonylurea herbicides used in this study.

Common name	chemical name and CAS number	Log Kow
Bensulfuron-methyl	methyl 2-[[[[[(4,6-dimethoxypyrimidin-2-yl)-amino]carbonyl]amino]sulfonyl]methyl]-benzoate, 83055-99-6	0.62*
Pyrazosulfuron-ethyl	ethyl 5-[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]amino]sulfonyl]-1-methyl-1*H*-pyrazole-4-carboxylate, 93697-74-6	1.3*
Imazosulfuron	2-chloro-*N*-[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]imidazo[1,2-*a*]pyridine-3-sulfonamide, 122548-33-8	0.049*
Cyclosulfamuron	*N*-[[[2-(cyclopropylcarbonyl)phenyl]amino]-sulfonyl]-*N*-(4,6-dimethoxypyrimidin-2-yl)-urea, 136849-15-5	1.41*
Flazasulfuron	*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]-carbonyl]-3-(trifluoromethyl)-2-pyridine-sulfonamide, 104040-78-0	1.08**
Ethoxysulfuron	2-ethoxyphenyl [[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]sulfamate, 126801-58-9	1.01**
Thifensulfuron-methyl	methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin 2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate, 79277-27-3	0.02*
Nicosulfuron	2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]-carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide, 111991-09-4	-1.8*

* Tomlin (2000) ** Uesugi et al. (1997) Values are at pH 7, but unstated for pyrazosulfuron-ethyl and flazasulfuron.

Fronds of *Lemna* sp. were collected from the pond in front of Shinjiko Nature Museum, Hirata, Shimane prefecture, Japan. After collection, steps were taken to eliminate the contaminating organisms. A sample of plant materials was taken and the roots were cut off. The fronds were then shaken vigorously in clean water, followed by immersion in a 0.5% (v/v) sodium hypochlorite solution for 1 minute. The fronds were then rinsed with sterile water and placed on agar medium containing 1% saccharose to confirm the sterility. Visibly contamination-free fronds were then transferred to the same agar, and cultured for eight weeks. Sufficient colonies were transferred aseptically from the stock culture into fresh sterile medium and cultured for 10 days under the test condition before starting the test. *P. subcapitata* ATCC 22662 was used in this study. Culture stocks were maintained in the OECD medium and subcultured once a month.

Both *Lemna* sp. and *P. subcapitata* were tested according to the draft OECD guidelines for the testing of chemicals (2002a; 2002b), using the same lighting and temperature conditions (12:12 light:dark cycle, cool white fluorescent lighting at 85 $\mu E^{-2} s^{-1}$, 24 $\pm$ 2 °C). For *Lemna* sp., tests were conducted for a 7 day exposure period under static conditions using 9 fronds in each 100 mL test beaker containing 50mL growth medium. The beakers were covered by transparent wrapping paper with some pores for aeration. Frond numbers were counted at the third, fifth and seventh days of the test period. Inhibition of growth was estimated on the basis of frond number and area. For algae, tests were conducted for a 3 day exposure period under static conditions using 10,000 cells/mL in each 100 mL Erlenmeyer flask containing 30 mL growth medium. Inhibition of growth was estimated by microscopic cell counting on the first three days of the test period.

A range-finding test was conducted at 0, 1, 10, 100 and 1000 ppb concentrations for each chemical to determine test solution concentrations for a definitive test. The concentration resulting in 50% growth inhibition (EC50) was determined from the definitive test, in which six concentrations (factor ratio = 2) covered the range causing 0-90% growth inhibition. Each concentration was tested in triplicate and independent experiments were repeated two times for both species. Toxicity data were expressed as EC50 determined by Ecotox-Statics 2.4 (The Japanese Society of Environmental Toxicology).

RESULTS AND DISCUSSION

Growth of *Lemna* sp. and *P. subcapitata* decreased with increasing concentration of all herbicides tested. Fronds in the control medium remained green and healthy throughout testing. For *P. subcapitata*, cells enlarged were by about two times on herbicide application. EC50s of the eight SU for the two species are summarized in Table 2. Based on the EC50 values, the two test species responded very differently to each SU, ranging from 0.91 to 14.5 ppb for *Lemna* sp. and from 0.27 to >1000 ppb for *P. subcapitata*. The most toxic and least toxic herbicide were cyclosulfamuron and nicosulfuron, respectively, for both organisms. Five of eight SU (bensulfonyl-methyl, imazosulfuron, ethoxysulfuron, thifensulfuron-methyl and nicosulfuron) showed stronger toxicity to *Lemna* sp. than *P. subcapitata*. One SU, pyrazosulfonyl-ethyl, was more toxic to *P. subcapitata* than *Lemna* sp. Cyclosulfamuron and flazasulfuron had similar toxicity to both species.

A comparison of the relative sensitivity of *Lemna* sp. and *P. subcapitata* to SU

Table 2. EC50 of sulfonylurea herbicides for *Lemna* sp. and *P. subcapitata.*

Sulfonylurea herbicide	EC50 (ppb) *P. subcapitata*	EC50 (ppb) *Lemna* sp.
Bensulfuron-methyl	11.8	2.23
Pyrazosulfuron-ethyl	0.96	3.49
Imazosulfuron	206	1.46
Cyclosulfamuron	0.27	0.91
Flazasulfuron	1.29	1.66
Ethoxysulfuron	2.84	1.72
Thifensulfuron-methyl	359	7.16
Nicosulfuron	>1000	14.5

is presented in Fig 1. Data points that fall on the dotted line indicate equal sensitivity of the two species. The collective data indicate that *Lemna* sp. is more sensitive to most of the chemicals tested in this study as mentioned above. The points that fall near the solid line indicate that the EC50 of the five SU are correlated ($\log y = 3.54 \log x - 0.41$, $R^2 = 0.976$, where x and y are EC50 for *Lemna* sp. and *P. subcapitata*, respectively) between the two species tested. When all SU tested are included, the equation becomes poorly correlated ($\log y = 2.42 \log x - 0.06$, $R^2 = 0.513$).

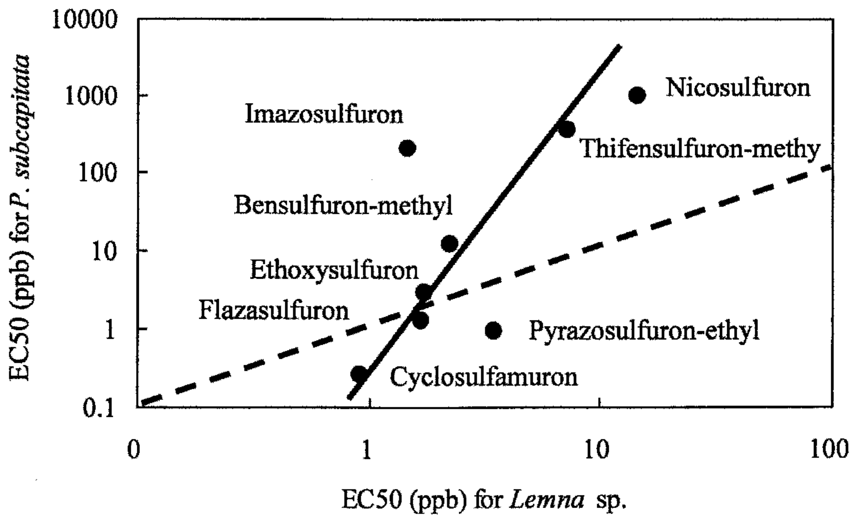

Figure 1. Comparison of relative sensitivity of *Pseudokirchnetiella subcapitata* and *Lemna* sp. to 8 sulfonylurea herbicides. The dotted line indicates equivalent sensitivity of the two species. EC50's of 5 herbicides near the solid line are correlated. EC50 of nicosulfuron for *P. subcapitata* was >1000 ppb.

Toxic response to chemicals may be quite variable between the two species. Fletcher (1990) found that vascular plants were more sensitive than algae to 16 herbicides, less sensitive to 10 herbicides, and equally sensitive to 7 herbicides. Mecoprop is more toxic to *Lemna minor* than to *Scenedesmus subspicatus* (Kirby and Sheahan 1994). In contrast, algae appeared more sensitive to fungicides than duckweed (Verdisson et al. 2001). Information on SU toxicity to algae and aquatic plants is quite limited, however previous studies (Peterson et al. 1994; Fairchild et al. 1997) and our study indicate that most SU exhibited stronger toxicity to duckweed than algae. In addition, it should be noted that there was a wider range of SU EC50 values for *P. subcapitata* than *Lemna* sp. As the expected environmental concentrations of SU were reported as 3-20 ppb (Peterson et al. 1994), differences in toxicity of SU against the algae should be considered for risk assessment. In the case of other green algae, *Chlorella vulgaris*, the range of EC50 for seven SU was from 0.30 to 67.3 ppm, which was less toxic and not as wide as that for *P. subcapitata* (Ma et al. 2002). At 20 ppb, for example, *Lemna* sp. would be critically damaged by most SU, whereas *P. subcapitata* would be affected by five SU used in this study, but no effect would be expected for three SU. *C. vulgaris* would not be affected at these concentrations by many SU. SU danger to green algae would be rather low as it is a less sensitive species. These differences in toxicity might be due to differential metabolic abilities of the species, but the details are not fully understood.

A highly significant correlation was observed between toxicity and Log Kow for *P. subcapitata* as shown in Fig. 2 ($\log y = -2.08x + 2.48$, $R^2 = 0.987$, where x and y are Log Kow and EC50, respectively). Less water soluble SU have stronger toxicity.

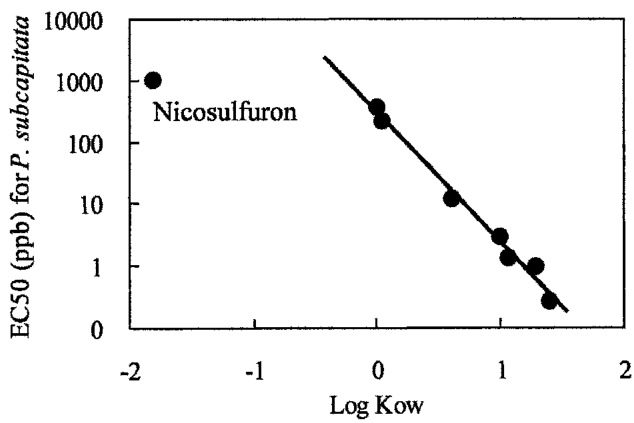

Figure 2. Relationship between Log Kow and EC50 of 8 sulfonylurea herbicides for *Pseudokirchnetiella subcapitata*. EC50 of nicosulfuron was >1000 ppb.

The same tendency was observed for *Lemna* sp. when pyrazosulfuron-ethyl and imazosulfuron were removed due to poor correlation of toxicity between the two species used in this study (Fig. 3). The equation was: log y = - 0.367 x + 0.606, R^2 = 0.907, where x and y are Log Kow and EC50 of the six SU, respectively. When all SU tested are included, the correlation is weaker (log y = - 0.300 log x + 0.574, R^2 = 0.630).

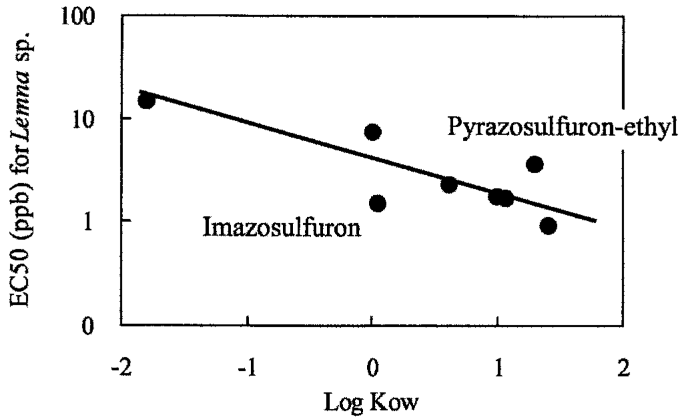

Figure 3. Relationship between Log Kow and EC50 of 8 sulfonylurea herbicides for *Lemna* sp.

In a previous study, the uptake of non-ionized chemicals by plant roots was reported to be greater when their lipophilicity increased within a Log Kow range from - 0.57 to 4.6 (Briggs et al. 1982). This could be attributed to the lipophilic nature of the plasma membrane, which makes chemicals with lower Kow easier to diffuse across the membrane into the cytoplasm. Stronger toxicity of SU with lower Kow seems to be explained by the hydrophobic property. Water solubility of SU is pH dependent, in which they are less soluble in lower pH. Therefore, actual environmental acidity influences the toxicity of SU, and should be considered in risk assessment. Fahl et al. (1995) reported that toxicity and bioconcentration of SU were enhanced by lowering pH in *Chlorella fusca.*

REFERENCES

Beyer EM, Duffy MJ, Hay JV, Schlueter DD (1988) Sulfonylurea herbicides. In: Kearney PC, Kaufman DD (eds) Herbicides: Chemistry, Degradation, and Mode of Action, vol 3. Marcel Dekker, New York, p 117

Briggs GG, Bromilow RH, Evans AA (1982) Relationships between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. Pestic Sci 13: 495-504

Brown HM (1990) Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic Sci 29: 263-281

Brown HM, Cotterman JC (1994) Recent advances in sulfonylurea herbicides. In: Stetter J (ed) Chemistry of Plant Protection, vol 10. Springer-Verlag, Berlin, p 47

Dastgheib F, Andrews M, Morton JD, Barnes MF (1995) Mode of action of chlorsulfuron in a sensitive wheat (*Triticum awstivum*) cultivar: primary and secondary effect on nitrogen assimilation. Ann Appl Biol 127: 125-135

Fahl GM, Kreft L, Altenburger R, Faust M, Boedeker W, Grimme LH (1995) pH-dependent sorption, bioconcentration and algal toxicity of sulfonylurea herbicides. Aquat Toxicol 31: 175-187

Fairchild JF, Ruessler DS, Haverland PS, Carlson AR (1997) Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. Arch Environ Contam Toxicol 32: 353-357

Fletcher JS (1990) Use of algae versus vascular plants to test for chemical toxicity. In: Wang W, Gorsuch JW, Lower WR (eds) Plants for Toxicity Assessment, ASTM STP 1091, American Society for Testing and Materials, Philadelphia, p 14

Kirby MF, Sheahan DA (1994) Effects of atrazine, isoproturon and mecoprop on the macrophyte *Lemna minor* and the algae *Scenedesmus subspicatus*. Bull Environ Contam Toxicol 53: 120-126

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

OECD (2002a) OECD guidelines for the testing of chemicals, revised proposal for a new guideline 221, *Lemna* sp. growth inhibition test. OECD, Paris, France

OECD (2002b) OECD guidelines for the testing of chemicals, proposal for updating guideline 201, freshwater alga and cyanobacteria, growth inhibition test. OECD, Paris, France

Peterson HG, Boutin C, Martin PA, Freemark KE, Ruecker NJ, Moody MJ (1994) Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations. Aquat Toxicol 28: 275-292

Pimentel D, McLaughlin L, Zepp A, Lakitan B, Kraus T, Kleinman P, Vancini F, Roach WJ, Graap E, Keeton WS, Selig G (1991) Environmental and economic effects of reducing pesticide use: a substantial reduction in pesticides might increase food costs only slightly. BioSci 41: 402-409

Schloss JV (1994) Recent advances in understanding the mechanism and inhibition of acetolactate synthase. In: Stetter J (ed) Chemistry of Plant Protection, vol 10. Springer-Verlag, Berlin, p 3

Tomlin CDS (ed) (2000) The Pesticide Manual, 12th edition. British Crop Protection Council, UK.

Uesugi Y, Ueji M, Koshioka M (ed) (1997) Pesticide Data Book, 3rd edition. Soft Science Publications, Tokyo, Japan.

Verdisson S, Couderchet M, Vernet G (2001) Effects of procymidone, fludioxonifl and pyrimethanil on two non-target aquatic plants. Chemosphere 44: 467-474