Bull. Environ. Contam. Toxicol. (1997) 58:807-813

Bulletin of Environmental Contamination and Toxicology

Effecs of Chlorsulfuron on Growth of Three Freshwater Species of Phytoplankton

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Received: 20 May 1996/Accepted: 4 February 1997

Chlorsulfuron is a sulfonylurea herbicide used for preemergence or postemergence weed control in cereal grains. They provide weed control at levels as low as 2 to 75 g/ha, reducing the amount of chemicals applied to the field by a factor of 100-1000 compared with conventional herbicides, such as alachlor or atrazine (Beyer *et al.* 1988). Sulfonylurea herbicides act specifically by inhibiting the enzyme acetolactate synthase in both plants and microorganisms (LaRossa and Falco 1984; Hartnett *et al.* 1987).

The persistence of chlorsulfuron in soil is mostly influenced by both its rate of chemical (Palm *et al,* 1980; Joshi *et al* 1985) and microbial degradation (Joshi *et al.* 1985) and loss through leaching (Nilsson 1983). Half-lives ranging from 2 weeks (Fredrickson and Shea 1986) to 7.5 months (Thirunarayanan *et al.* 1985). In buffered aqueous solutions, chlorsulfuron is stable for at least 4 weeks at pH 7 and pH 9 and has a half-live of 1 week at pH 4 in acidic aqueous solution (Slates and Watson 1988). Aquatic environment contamination by chlorsulfuron has been reported by Nilsen (1989) and Bechmann *et al.* (1990) and any data concerning its effects on aquatic organisms are very helpful for environmental risk assessment of this herbicide.

There are number of studies dealing with mechanisms and measurements of the toxic effects of chlorsulfuron and other herbicidal sulfonylureas to higher plants. Bioassay procedures based on the growth of maize *(Zea mays* L.), sunflower *(Helianthus annuus* L.), lentil *(Lens esculenta* M.), sorghum *(Sorghum bicolor* L.), sugarbeet *(Beta vulgaris* L.) and other crop plants have been used to study the phytotoxicity and persistence of the chlorsulfuron as affected by concentration and soil type (Sweetser *et al.* 1982; Ray 1982; Nilsson 1983; Nalewaja 1985; Fredrickson and Shea 1986; Kotoula-Syka *et al.* 1993). Data on the algal toxicities of sulfonylureas are not so numerous, but studies of several freshwater species indicate that growth inhibition occurs at approximately 0.36 ppm (Hartnett *et al.* 1987; Grossmann *et al.* 1992).

This study has been carried out to understand the effects of chlorsulfuron on three species of phytoplankton representative of Mediterranean wetlands (two chlorophyceas and one cyanobacteria) isolated from Lake Albufera (Valencia, Spain) in pure cultures.

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MATERIALS AND METHODS

The green algae *Scenedesmus acutus* (Meyens) (Chlorophyceae) and *Chlorella saccharophila* (Krüger) Migula (Chlorophyceae) and the cyanobacteria *Pseuadana - baena galeata* (Böcher) (Cyanophyceae) were used as test organisms. *Scenedesmus* and *Chlorella* were isolated from samples collected at Albufera lake in Valencia (Spain). *Pseudanabaena* was obtained from Dr. Romo (Valencia University). The green algae were grown in a medium recommended by the OECD (1984). *P. galeata* was grown in the medium of Romo and Becares (1992). The stock cultures were maintained in a liquid medium at a temperature of 22 ± 2 °C and a light intensity of 1100 lux on a 12-hr light-dark cycle.

Chlorsulfuron(2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] - benzenesulfonamide, 95% analytical standard) was obtained from “Riedel de Haën”. Chlorsulfuron is a white crystalline solid with melting point 174-178 °C, vapor pressure 4.6 x 10^{-6} mmHg at 25 °C and density 1.52 g/cm^3. Chlorsulfuron volubility in water is very dependent on pH; at 25 °C, the volubility is 0.3 g/1 at pH 5 and 28 g/1 at pH 7 (Slates and Watson 1988).

The inhibition test was carried out following the standard operating procedure outlined in the OECD (1984). The organisms were exposed to concentrations of chlorsulfuron which ranged from 0.07 to 184.7 ppm, for 96 hours. The response to chlorsulfuron was measured by the turbidity at 750 nm wavelength using a spectrophotometer (Beckman DU[R]-70; Ordög and Kuivasniemi 1989) at 24, 48, 72 and 96 hours after the start of the test. Analyses of significant differences were performed using ANOVA and Student Newman-Keuls multiple range test (Reish and Oshida 1987). E_bC_{50} (0-96 hours) values with 95% confidence limits were determined by probit analysis (Abou-Setta *et al*. 1986). In this study, E_bC_{50} is the concentration of chlorsulfuron, derived by the method of calculation “comparison of areas under growth curves”, which results in a 50% growth reduction relative to the control values, at 96 hours (OECD 1984). Further details of test protocols are provided in Sabater and Carrasco (1996).

At the beginning of the assays, herbicide concentrations in test solutions were determined by HPLC and the results were analyzed statistically. These values were used for calculating toxicities of chlorsulfuron. The test cultures samples (25 ml) were acidified with HC1 (1 N) and extracted with three 20 ml portions of dichloromethane by shaking. The combined extracts were evaporated to dryness and the residue was redissolved in 10 ml of acetonitrile:NH_3 0.024 M (30:70, v/v) and analyzed by HPLC with absorbance detection at 254 nm. The column was a 15 cm C_{18} reverse phase (µ-Bondapack) and the mobile isocratic phase was acetonitrile:water (pH 3) (30:70, v/v). Flow rate and injection volume were 1 ml/min and 20 µl, respectively. Retention time for chlorsulfuron was approximately 15 min (Slates and Watson 1988). The extraction recoveries, calculated by comparison with injected standard solutions, were greater than 95%.

RESULTS AND DISCUSSION

The NOEC values (96 hours) or the highest concentrations tested without significant effects (P< 0.05) on the algal growth relative to control values were 9.3, 0.07 and 3.9 ppm in unialgal cultures of *Chlorella*, *Scenedesmus* and *Pseudanabaena*, respectively.

The concentrations of chlorsulfuron that caused significant effects ($P<0.05$) on the algal growth ranged from 20.7-185 ppm for *C. saccharophila,* from 0.10-0.66 ppm for *S. acutus* and from 6.6-46.6 ppm for *P. galeata* (Table 1). The pH values of the culture media from 0 to 72 hours ranged from 7.5-9.0.

Table 1. Exponential regression equations of *C. saccharophila, S. acutus* and *P. galeata* growth, at different concentrations of chlorsulfuron, for 96 hours.

Species	Concentrations (ppm)*	Regression equations**	R^2 (%)***
Chlorella saccharophila	0.0 ± 0.0 [a]	Ln Y=9.50 + 0.075X	0.95
	20.7 ± 1.2	Ln Y=9.39 + 0.073X	0.95
	48.4 ± 1.8	Ln Y=9.31 + 0.072X	0.96
	106.6 ± 1.5	Ln Y=9.18 + 0.064X	0.98
	184.7 ± 4.5	Ln Y=9.25 + 0.031X	0.95
Scenedesmus acutus	0.00 ± 0.00 [a]	Ln Y=9.10 + 0.060X	0.97
	0.10 ± 0.01	Ln Y=9.00 + 0.058X	0.97
	0.15 ± 0.01	Ln Y= 8.99 + 0.055X	0.97
	0.33 ± 0.01	Ln Y= 8.97 + 0.047X	0.98
	0.66 ± 0.02	Ln Y= 8.98 + 0.043X	0.97
Pseudanabaena galeata	0.0 ± 0.0 [a]	Ln Y= 9.27 + 0.077X	0.99
	6.6 ± 0.1	Ln Y=9.20 + 0.076X	0.99
	12.7 ± 0.9	Ln Y=9.08 + 0.074X	0.99
	24.4 ± 0.7	Ln Y=8.81 + 0.070X	0.98
	46.6 ± 1.7	Ln Y= 9.22 + 0.034X	0.99

*: Concentrations of chlorsulfuron that caused significant effects ($P < 0.05$) on the algal growth with respect to control [a]. Mean of six values ± S.D.
**: Slopes shown the average specific growth rate Y=N° organisms/ml, X = Time (Hours).
***: Correlation coefficient.

Table 1 shows the regression equations of growth of three species for each herbicide treatments. The slope of each equation represents the average specific growth rate which decreased progressively with increasing concentration of chlorsulfuron. At 185, 0.66 and 46.6 ppm of chlorsulfuron the growth of *ChloreIla, Scenedesmus* and *Pseudanabaena* was significantly decreased, respectively.

The difference in sensitivity between the two chlorophyceas was greater than between the chlorophycea *Scenedesmus* and the cyanobacteria *Pseudanabaena*. *Scenedesmus* was strongly inhibited by chlorsulfuron after 96 hours at concentrations of 0.66 ppm while *Chlorella* and *Pseudanabaena* showed no inhibition at 9.3 ppm and

3.9 ppm, respectively. A chlorsulfuron concentration of 46.6 ppm strongly inhibited *Pseudanabaena galeata* growth, whereas a greatest concentration, 185 ppm, was necessary for producing similar inhibition effects on *Chlorella* growth.

As reported by Dyer *et al.* (1982), the growth of the alga *Chlorella sorokiniana* was inhibited by chlorsulfuron at 5 to 50 ppm but was not affected by levels below 1 ppm. In our study, concentrations of 20.7 and 48.4 ppm inhibited *Chlorella saccharophila* growth and this green alga proved to be resistant to chlorsulfuron concentrations below 9 ppm.

The 96 hr E_bC_{50} values for three species of phytoplankton for chlorsulfuron are listed in Table 2. The two green algae tested responded very differently to chlorsulfuron; *C. saccharophila* was less sensitive to the herbicide while *S. acutus* was more sensitive. Sensitivity of the cyanobacteria *Pseudanabaena* was intermediate between *Sceneadesmus* and *Chlorella.*

Table 2. Probit regression equations and E_bC_{50} (0-96 hours) values of chlorsulfuron for the three species.

	Probit regression equations*	E_bC_{50} (95% confidence limits) $mg \bullet L^{-1}$
C. saccharophila	Y = -0.03 + 1.261 X	54.0 (41,5 - 66.9)
S. acutus	Y = 6.29 + 0.866 X	0.22 (0.19 - 0.26)
P. galeata	Y = 0.87 + 1.479 X	16.3 (13.7 - 19.0)

*: Y = Probit of % inhibition; X = Ln (mg of chlorsulfuron/liter).

Chlorsulfuron 96 hr E_bC_{50} value was 54.0 (41.5-66.9) ppm for *Chlorella saccharophiia* growth at pH 8.2-9.0, in the present study. This E_bC_{50} value are much higher than those reported by Fahl *et al.* (1995) for metsulfuron-methyl, chlorsulfuron, triasulfuron and tribenuron-methyl on *Chlorella fusca*, at pH 6.5. They found that these sulfonylurea herbicides affected growth and reproduction of *Chlorella fusca* at concentrations between 0.08 and 1.2 ppm. These authors reported a pH-dependent algal toxicity of sulfonylurea herbicides and showed that the algal toxicity of chlorsulfuron was enhanced by a factor of 25 when the pH was lowered from 6.5 to 5.0.

The 96 hr E_bC_{50} for chlorsulfuron in the present study was 0.22 (0.19-0.26) ppm for *Scenedesmus acutus.* This value is only slightly higher than the EC_{50} value of 0.11 ppm reported by Grossmann *et al.* (1992) for the inhibition of reproduction in the same species exposed to chlorsulfuron. An EC_{50} value of 0.31 ppm (with 95% confidence limits of 0.29-0.33 ppm), for effects on the growth rates of the green algae *Selenastrum capricornutum* has been published by Källqvist and Romstad (1994), and are in agreement with the level of toxicity found in this study for the green alga *Scenedesmus acutus. The* E_bC_{50} value obtained for *S. acutus* in our assays are also in agreement with observations reported by Hartnett *et al.* (1987), who observed growth inhibition of *Chlamydomonas reinhardii* on agar plates by sulfometuron-methyl at 0.35 ppm.

The 72 hr EC_{50} value of 10 ppm reported for the cyanobacteria *Microcystis aeruginosa* exposed to chlorsulfuron by Källqvist and Romstad (1994) was in the same range as for the *Pseudanabaena galeata* with a E_bC_{50} of 16.3 (13.7-19.0) ppm. However, Källqvist and Romstad (1994) found a E_bC_{50} (72 hr) lower, of 0.15 ppm for the cyanobacteria *Synechococcus leopoliensis*.

Sulfonylureas were the least toxic compounds on phytoplankton in a series of field and laboratory studies with several pesticides. The effects of 4 pesticides (chlorsulfuron, propiconazole, dimethoate and glyphosate) on lake phytoplankton communities were studied in a mesocosm experiment by Kallqvist *et al*. (1994). At 0.1 ppm all pesticides affected the biomass development, measured as chlorophyll *a*, with the exception of chlorsulfuron. In our studies, chlorsulfuron was found to be less toxic for the three species of phytoplankton than thiobencarb (Sabater and Carrasco, 1996).

The sulfonylurea herbicides chlorsulfuron, ethametsulfuron-methyl, metsulfuron-methyl and triasulfuron caused little or no inhibition of *Cyclotella meneghiana* and *Nitzschia sp.* (diatom), *Scenedesmus quadricauda* and *Selenastrum capricornutum* (green algae) and *Microcystis aeruginosa, Pseudanabaena sp., Oscillatoria sp., Aphanizomenon flos-aquae* and *Anabaena inaequalis* (cyanobacteria) at the EEC (Expected Environmental Concentration) level exposure range of 0.003 to 0.020 ppm (Peterson *et al.* 1994).

Thompson *et al.* (1993) showed that chronic exposure to the sulfonylurea metsulfuron-methyl by the phytoplankton community of a forest lake at 1.0 ppm (approx. 40 x the worst case EEC) induced slight transient effects only in the Cyanophyta group, while chronic exposure to hexazinone resulted in concentration-dependent reductions in biomass of all dominant phytoplankton groups (Cyanophyta, Chlorophyta, Chrysophyte, Cryptophyta and Bacilliarophyceae).

Toxicity data for other aquatic organisms, such as waterfleas, fish or the fluorescent *Photobacterium phosphoreum* for the chlorsulfuron have been published. A microtox toxicity analysis using *Photobacterium phosphoreum* as an indicator species measured an EC_{50} of 7.8 ppm (Dyer *et al.* 1982). In a series of laboratory tests, Hessen *et al.* (1994) found that chlorsulfuron was only slightly toxic to the cladocerans *Daphnia magna* and *Daphnia pulex* (LC_{50} > 100 mg/1), whereas other pesticides such as propiconazole, dimethoate and glyphosate had lower LC_{50}, of 0.02 and 10 ppm. Chlorsulfuron 4-day LC_{50} values obtained by Grande *et al.* (1994) for *Salmo trutta* were 40 ppm and chlorsulfuron LC_{50} (96-h) for bluegill and rainbow trout was > 250 mg/1 (Worthing and Hance 1991). The green algae are therefore up to three orders of magnitude more sensitive to sulfonylureas than fish and waterfleas (Fahl *et al.* 1995).

Environmental concentrations of chlorsulfuron of 0.6 µg/1 have been reported by Nilsen (1989) in freshwaters and by Bechmann et *al*. (1990) in a treated field. These concentrations are far below the effective concentration for the inhibition of algal growth as determined in this study and observed in the literature.

The study of the toxicity of chlorsulfuron on three species of phytoplankton have shown that the variation in sensitivity may be considerable: EbC_{50} values for the three species ranged from 0.22 to 54.0 ppm. *Scenedesmus* was the most sensitive alga and *Chlorella* the most resistant. The results of the algal toxicity tests showed that thiobencarb (Sabater and Carrasco 1996) was more toxic than chlorsulfuron and the green alga *Chlorella saccharophila* was more resistant to this sulfonylurea than other freshwater organisms.

Acknowledgments. This work was supported by a grant from Comisión Interministerial de Ciencia y Tecnología n° AMB 92-1277.

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