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## THE EFFECTS OF THREE SULFONYLUREA HERBICIDES AND THEIR DEGRADATION PRODUCTS ON THE GREEN ALGAE *CHLORELLA PYRENOIDOSA*\*

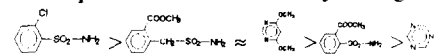
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### ABSTRACT

The effects of three sulfonylurea herbicides, metsulfuron-methyl, chlorsulfuron and bensulfuron, and their main degradation products on the green algae (*Chlorella pyrenoidosa*) were studied through 96-hour algae growth inhibition tests and organic nitrogen bioavailability tests. Experimental results indicated that, the acute toxicity of these sulfonylurea herbicides was much higher (about 2 or 3 orders) than their corresponding degradation products, and nitrogen of the degradation products could be used by the algae as

the only nitrogen source to stimulate their growth in the order 

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### INTRODUCTION

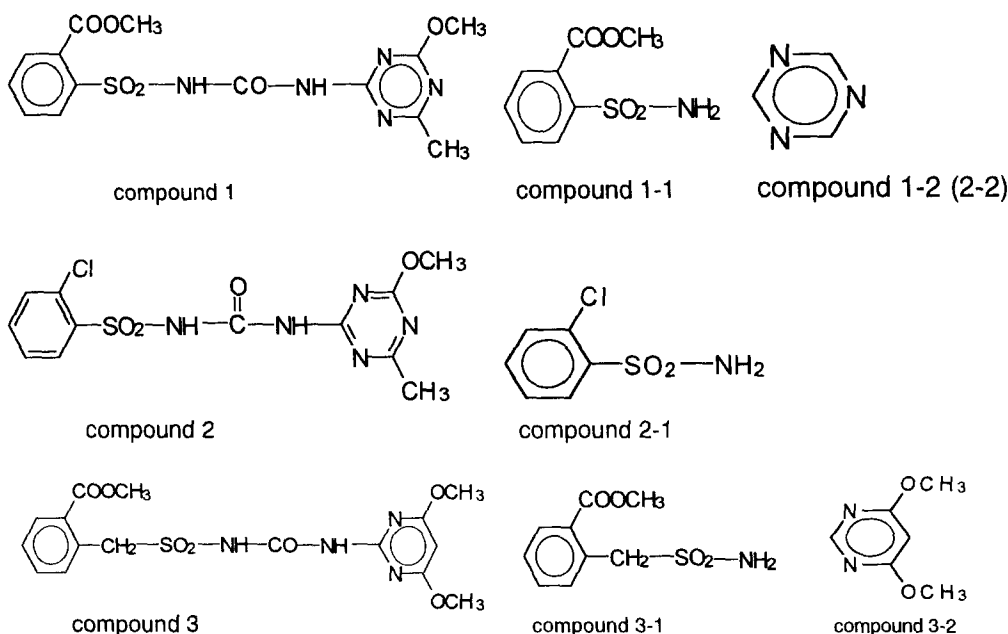
Metsulfuron-methyl, chlorsulfuron, bensulfuron are sulfonylurea herbicides. They display high herbicidal activity and very low mammalian toxicity and they are widely used in agriculture[1,2]. They exist, at neutral or basic pH values, almost exclusively in the anionic form which are much less susceptible to their main degradation pathway, hydrolysis[2,3]. Much research concerning the toxicity of sulfonylurea herbicides on weeds and crops has been reported [1,4,5], there are few reports about their effects on aquatic organisms. In this study, the 96-hour algae growth inhibition toxicity of metsulfuron-methyl, chlorsulfuron, bensulfuron and their main degradation products to the green algae (*Chlorella pyrenoidosa*), as well as bioavailability of organic nitrogen of the degradation products by the tested green algae was studied.

### MATERIALS AND METHODS

**Chemicals** Three sulfonylurea herbicides (purity>95%) and their corresponding degradation products, metsulfuron-methyl (compd. 1), chlorsulfuron (compd. 2) and bensulfuron (compd. 3), and their degradation products, compd. 1-1, 1-2, 2-1, 2-2, and 3-1, 3-2 were used. The chemical structures were shown as below.

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**Algae** *Chlorella pyrenoidosa* used in this work was supplied by the Institute of Aquatic Organisms, Academia Sinica, Wuhan PRC. Stock culture was maintained on solid (agar) medium which was identical in composition to Bold Basal Medium (BBM) [6] at 4 °C.

**Ninety-six-hour Algae Growth Inhibition Test** Ninety-six-hour preculture of the algae in BBM was prepared from the stock culture and was repeated three times. *Chlorella pyrenoidosa* was cultured under germ-free conditions at  $20 \pm 1$  °C with illumination under fluorescent light (4500 lux, 24h/day) (*Method A*) and regular shaking. Log EC<sub>50</sub> (effective concentration of a tested chemical causing 50% algae growth inhibition), was measured according to reference [6]. Briefly speaking, portions (5mL each) of cell suspension ( $6 \times 10^5$  cells/mL) at logarithmic phase in growth curve were pipetted into five 100 mL culture vessels in which different amounts of the tested compound were dissolved in 25 mL germ-free BBM, and the cultivation was continued in the same conditions as mentioned above. After 96-hour exposure, the optical density of the cultures was measured at 680 nm on a spectrophotometer. Experiments were done in triplicate.

**Bioavailability of the Organic Nitrogen** BBM was modified by dropping the level of NaNO<sub>3</sub> below the normal and adding additional NaCl to keep the sodium supply constant. The algae acclimated to the low inorganic nitrogen medium were obtained through 96-hour-preculture in modified and germ-free BBM. For the experiment, degradation products at the concentration of EC<sub>10</sub> (effective concentration causing 10% algae growth inhibition, from the 96-hour algae growth inhibition tests) replaced NaNO<sub>3</sub> as the only N source. Triplicate 100mL culture vessels, each containing 25mL germ-free solution mentioned above and 5mL acclimated algal suspension, were cultivated for about 10 days until the growth curve by optical density reached the stationary phase. After the cultivation, the cells were harvested by centrifuging (4000rpm, 20 min.) and resuspended three times in distilled water whereafter the algae were dried for 3 hours at 104 °C. The algae samples were analyzed for cell nitrogen content (Kjeldahl Method). The tested compound remained in the culture medium was determined by RP-HPLC (the analysis was carried out by employing methanol-water (2:1 v/v) at a wavelength of 230 nm using HP1050 series HPLC equipped with a 100 × 4.6mm i.d. HP-ODS column).

## RESULTS AND DISCUSSION

### Ninty-six-hour Algae Growth Inhibition Toxicity of the Sulfonylureas and their Corresponding Degradation Products

The inhibition rates (p%), derived from the optical density of 96-hour-exposed algae suspension, were linearly regressed with logarithmic concentrations of the tested compound, and Log EC<sub>50</sub> was obtained. The results were listed in Table 1.

Table 1. Dose Response Relationship of three Sulfonylurea Herbicides and their corresponding Degradation Products to *Chlorella pyrenoidosa*

Compounds	Regression equation	Prob. level (F-test)	r	SE	Log EC <sub>50</sub> (mg/L)
1	P% = 0.60 + 0.48 Log C	0.020	0.929	0.16	-0.21
1-1	P% = - 0.71+ 0.42 Log C	0.004	0.813	0.03	2.89
1-2	P% = - 0.29 + 0.40 Log C	0.002	0.985	0.06	1.97
2	P% = 0.65 + 0.38 Log C	0.040	0.895	0.16	-0.39
2-1	P% = - 2.26 + 1.34 Log C	0.005	0.975	0.10	2.06
2-2	P% = - 0.29 + 0.40 Log C	0.002	0.985	0.06	1.97
3	P% = 0.70 + 0.26 Log C	0.004	0.976	0.03	-0.77
3-1	P% = - 1.27 + 0.95 Log C	0.005	0.941	0.10	1.86
3-2	P% = - 1.24 + 0.78 Log C	0.017	0.940	0.09	2.24

It can be seen from Prob. level (less than 0.05), r (regression coefficient, larger than 0.80 ) and SE (Standard Error, small) in Table 1 that linear relationship between prohibition rate and logarithmic concentration were significant.

From Table 1, we can see that the toxicity of the sulfonylureas to *Chlorella pyrenoidosa* was greater by 2 to 3 orders than their corresponding degradation products. For example, Log EC<sub>50</sub> of metsulfuron methyl (compd. 1) was -0.21 while those of its corresponding degradation products of compd. 1-1 and 1-2 were 2.89 and 1.97 respectively, that is, Log EC<sub>50</sub> of compd. 1-1 and 1-2 were larger by 3.10 and 2.18 than the parent compound. So, the degradation of these sulfonylureas would lead to a rapid decrease in acute toxicity to the green algae.

In the experiment, we observed that algal growth can be stimulated at low concentration of compound 1-1, 1-2, 2-1, 3-1 and 3-2 (the degradation products of the tested sulfonylureas). We also found from the regression equations in Table 1 that the interceptions (Prob. level by t-test were less than 0.05) of the parent compounds were positive ( $0.60 \pm 0.08$ ,  $0.65 \pm 0.08$  and  $0.70 \pm 0.02$  for compound 1, 2 and 3); while those of the degradation products were negative ( $-0.71 \pm 0.12$ ,  $-0.29 \pm 0.07$ ,  $-2.26 \pm 0.36$ ,  $-1.27 \pm 0.33$  and  $-1.24 \pm 0.38$  for compound 1-1, 1-2, 2-1, 3-1 and 3-2 respectively) and in the order of  $2-1 < 3-1 \approx 3-2 < 1-1 < 1-2$ . The negative interceptions implied that, at low concentrations of the degradation products (1mg/L, for example), the inhibition rates were negative, that is, no algae growth inhibition but stimulation occurred. The stimulating effects were in the order of compd.  $2-1 > 3-1 \approx 3-2 > 1-1 > 1-2$ . Therefore the degradation products of the tested sulfonylureas, at low concentrations, would not inhibit but stimulate the algae growth.

### Bioavailability of Organic Nitrogen of the Degradation Products

To study the stimulating effects, we focused on the absorption rates of the degradation products and their organic nitrogen bioavailability by *Chlorella pyrenoidosa*. Since the tested compounds are soluble in water, their volatility during the cultivation was ignored. Absorption rate of the tested compound was derived from the difference between its initial concentration (expressed with mg N/L culture) and the concentration after 10-day-cultivation. Organic nitrogen bioavailability was expressed with cell nitrogen content (mg N/g cell dry matter) and algae growth stimulating rate which was derived through dividing the difference of algal optical density between the treated culture (cultured in organic N source) and the control (no nitrogen in medium) by that of the control. The results were shown in Table 2.

Table 2. Absorption and Bioavailability of Organic Nitrogen from the Degradation Products of Sulfonylurea Herbicides by *Chlorella pyrenoidosa*

Nitrogen sources	Initial concentration (mg N/ L)	Concentration after cultivation (mg N / L)	Absorption rate (%)	Cell nitrogen content (mg N/g cell dry matter)	Algae growth stimulating rate (%)
compd. 1-1	4.24	0.33	92.22	20.69	6.90
compd. 1-2	5.26	0.44	91.62	21.08	3.15
compd. 2-1	1.73	0.19	89.02	21.74	28.6
compd. 3-1	2.03	0.34	83.33	21.40	12.1
compd. 3-2	10.89	0.49	95.50	20.60	13.8

We can see from Table 2 that the absorption rates of the tested chemicals reached more than 80% after the 10-days-cultivation, and there was no significant difference in absorption rates between different nitrogen sources. It showed that the algae could absorb the tested compounds.

There was no significant difference between cells cultured in different nitrogen sources as to cell nitrogen content: the largest value was 21.74 mg N/g cell dry matter for compound 2-1, and the smallest was 20.60 mg N/g cell dry matter for compound 3-2; that is, cell nitrogen contents were at similar levels though they were cultured in different organic nitrogen sources. It seemed that the algae did not utilize the absorbed compounds completely. Reports have shown that, the algae growth was N-limited when N:P ratio dropped below a certain constant (e.g., N:P ratio of 30 for *Scenedesmus* [7]) in a chemostat culture. In this experiment, the P concentration was constant at normal level of BBM and N concentration was varying and lower than the normal level of BBM. That algal cells did not utilize the absorbed organic N completely was not surprising because the only nitrogen source available were the degradation products.

Although no significant difference was evident in the absorption rate and cell nitrogen content, there were differences in the growth stimulation ( in optical density ) rate between compounds, and, as shown in Table 2, the stimulation rates of the different compounds were in the order of compd. 2-1>3-2  $\approx$  3-1>1-1>1-2, which was the same as showed by interceptions of the regression equation from acute toxicity test.

## SUMMARY

Although the sulfonylurea herbicides, metsulfuron-methyl, chlorsulfuron and bensulfuron, were toxic to the green algae, *Chlorella pyrenoidosa*, their degradation would likely lead to a rapid decrease in toxicity to the green algae. Low concentrations of the degradation products would significantly stimulate the algal growth, and they can be used as the only nitrogen sources.

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