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# Bioavailability of bound residue derived from $^{14}$C-labeled chlorsulfuron in soil and its mechanism of phytotoxicity

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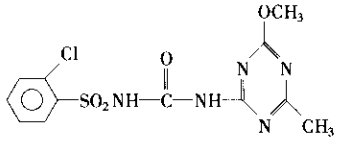
**Abstract**: The bioavailability of bound residue (BR) derived from $^{14}$C-labeled chlorsulfuron in soil and effect of the main components of the BR on growth of rape (*brassica napus*) and rice (*Oryza sativa* L.) were investigated. The results showed that the BR with the concentration of 0.28 and 0.56 nmol/g air-dried soil, which was calculated by special radioactivity of $^{14}$C-labeled chlorsulfuron parent compound, resulted in significant depression effect on growth of rape seedling. It was assured that the main components (2-amino-4-methoxyl-6-methyl-1,3,5-triazine, 2-amino-4-hydroxyl-6-methyl-1,3,5-triazine, and 2-chloro-benzenesul-fonamide) of the BR did not inhibit the growth of rape and rice. LC-MS analysis demonstrated that the parent compound previously bound to the soil matrix could be again released and transformed into methanol-extractable residue during the course of rape growth. It was concluded that the molecular leading to the phytotoxicity to rape and rice in the BR is still the parent compound.

**Keywords**: chlorsulfuron; bound residue; bioavailability; phytotoxicity; mechanism

## Introduction

2-chloro-N-[4-methoxy-6-methyl-1, 3, 5-triazine-2-yl] amino carbonyl benzenesulfonamide, chlorsulfuron, is one of sulfonylurea herbicides with the property of high activity, broad spectrum and low toxicity to animals (Levitt, 1981; Beyer, 1988; Brown, 1990). Its structure is expressed as follows:



Chlorsulfuron is used in both pre- and post-emergent application at labeled rates of 4—18.75 gAI/hm$^2$ (Strek, 1998a). Its target enzyme is acetolactate synthase (ALS) (Ray, 1984), which is the first common enzyme in the biosynthesis of branched amino acids specific to plants and microorganism. The mode of action (Ray, 1984; Blair, 1988), degradation route (Sabadie, 1992; Hemmamda, 1994; Strek, 1998a; 1998b), the fate and environmental behavior of chlorsulfuron (Anderson, 1985; Joshi, 1985; Thirunarayanan, 1985; Fredrickson, 1986; Mersie, 1986; Moyer, 1989; Walker, 1989) and different sensitivity on crops (Brown, 1990; Moyer, 1990) have been extensively investigated. Many studies have focused on the phytotoxicity of chlorsulfuron to substitution crop such as rape, rice and sugar beet (Moyer, 1990; Kotoula, 1993; Nicholls, 1998). Some groups have found that the phytotoxicity of chlorsulfuron appears to be linked to its bound residues (BR) in soil (Chen, 1996; Sun, 2000). It has been reported that the $^{14}$C-BR content of $^{14}$C-chlorsulfuron in seven kinds of soil was significantly negatively related to soil pH and positively related to the clay content, and the maximum value of the BR accounted for about 53.5% of applied amount (Ye, 2000).

Some aqueous hydrolysis products and degradation products of chlorsulfuron in soil and humic acids have been identified (Sabadie, 1992; 1993; Strek, 1998a). Recently studies have demonstrated that the BR composition of $^{14}$C-chlorsulfuron in soil was composed of $^{14}$C-labeled 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine, $^{14}$C-labeled 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine, 2-chloroben-zenesulfonamide and $^{14}$C-labeled chlorsulfuron parent compound (Ye, 2000), which is a basis for explaining mechanism of phytotoxicity induced by the BR.

The objective of this study was to investigate bioavailability of bound residue derived from $^{14}$C-chlorsulfuron in soil and effect of the main components of the BR on growth of rape (*brassica napus*) and rice (*Oryza sativa* L.). On the other hand, the possibility that chlorsulfuron parent compound in the form of BR was again released and transformed to extractable residue during the process of rape growth was also studied.

## 1 Materials and methods

### 1.1 Reagent

$^{14}$C-labeled chlorsulfuron (triazine-4-$^{14}$C; special radioactivity, $1.8 \times 10^{10}$ Bq/mol; radiochemical (purity > 95%; Ye, 2002). 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine (purity > 97%), 2-chloro-benzenesulfonamide (purity > 97%). 0.1 mol/L $Na_2CO_3$-$NaHCO_3$ buffer (pH = 10.0), dimethylbenzene, glycol-ether, ethyl acetate, ethanolamine, 2, 5-diphenyloxazole (PPO), 1, 4-di-[2'-(5'-

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phenyloxazolyl)]-benzene (POPOP), the all solvents mentioned above were analytical grade. Acetonitrile, acetic acid and methanol were HPLC grade.

### 1.2 Equipment

HPLC system (Waters 510, 680 and 996 etc., Waters Co., USA), water purification system (Milli-Q, Millipore Co., Milford, MA), solvent filtration module (Waters Co., USA), centrifugator (Universal 32, Hettich Co., Germany), decompression rotary evaporator (R-201, Institute of Shenke Machine, Shanghai, China), 4.6 × 250 mm $C_{18}$ column (Supelco discovery $C_{18}$, Sigma-Aldrich Co., USA), $C_{18}$ solid phase extraction column (SPE, 10 × 150 mm, 7 μm), biological oxidizer (OX-600, Harvey Instrument Co., USA), liquid scintillation counter LSC, Wallac 1414, Wallac Co., Finland), LC-MS (LC, Waters 2690 separation module; MS, Micromass Quattro LC, Waters Co., USA), and so on.

### 1.3 Preparation of $^{14}$C-BR soil

The tested soil, Fluvio marine yellow loamy (pH($H_2O$) 6.50; OM 30.5 g/kg; CEC 10.83 cmol/kg; clay 8.0%; silt 71.3%; sand 20.8%), was taken from the surface layer (0—15 cm) in the test field of Huajiachi Campus, Zhejiang University, and passed through a 1 mm sieve. 240 g of each soil samples in four replication was put into 500 ml flask with a rubber plug, respectively. $1.25 \times 10^5$ Bq of $^{14}$C-labeled chlorsulfuron dissolved in methanol was added into each soil sample, and stirred in a vent hood. After all the methanol were removed, distilled water was added into the soils until the water content was adjusted to 60% of soil water holding capacity (WHC), continue to stir the soil until it was uniform. In order to prevent the escape of $^{14}$C-$CO_2$, a 20 ml-vial filling with 10 ml 0.5 mol/L NaOH was hung under the rubber plug. The flasks were maintained at 25 (± 1)℃ in the dark. During the course of incubation, the water content was kept constant by adding distilled water every day. At the 90 d of incubation, all spiked soil was sampled and divided into 12 parts, and then each part was extracted by methanol in a vibrator for five times continuously until all the extractable residues of $^{14}$C-labeled chlorsulfuron in soil was thoroughly removed. In each step mentioned above, the spiked soil sample was extracted for 2 h and subjected to centrifugate (4000 r/min) for 15 min. The efficiency of the continuous methanol-extraction for extractable residues of $^{14}$C-labeled chlorsulfuron in soil is higher than that of Soxhlet extraction method (24 h). The residual soil after the continuous methanol extraction was termed as $^{14}$C-BR sample in this paper.

The methanol remained in the $^{14}$C-BR soil samples were thoroughly removed in a vent hood. Subsequently, 1 g of the $^{14}$C-BR soil was combusted using a biological oxidizer, the $^{14}$C-$CO_2$ released from the $^{14}$C-BR soil was absorbed with liquid scintillation cocktail (10 gPPO + 0.4 gPOPOP + 175 ml ethanolamine + 325 ml glycol-aether + 500 ml dimethylbenzene), and then the total radioactivity of the $^{14}$C-BR soil was determined by LSC. The processes mentioned above were replicated for six times.

### 1.4 Experiment for examining bioavailability of $^{14}$C-BR derived from $^{14}$C-chlorsulfuron in soil

The soil used for bioavailability experiment was a mixture of $^{14}$C-BR soils and fresh soils (Fluvio marine yellow loamy). The content of $^{14}$C-BR in the tested soil was respectively adjusted to 0.28 nmol/g and 0.56 nmol/g air-dried soil (marked as $BR_1$ and $BR_2$), which was calculated by special radioactivity of $^{14}$C-labeled chlorsulfuron parent compound. 1.5 kg of the each treated soil was put into a pot, a proper amount of water was added and 20 oilseeds of rape (*brassica napus* L.) cv. 605 were sown. All treatments and control were replicated for four times. The growth of rape seedlings was examined after 45 d of sowing.

### 1.5 Experiment for investigating effect of the main components of the BR on growth of rape and rice

The experimental setting for examining effect of the main components of BR derived from chlorsulfuron on rape growth is listed in Table 1. 20 oilseeds of rape (*brassica napus* L.) cv. 605 were sown in each pot with 1.5 kg air-dried soils. All treatments and control were replicated for four times. The height of plant, the dry weight of shoot and root was examined at three-leaf stage. 12.5 kg air-dried soils of each pot were used at the whole growth stage. Each pot transplanted four rape seedlings. Yield and agronomic characters of rape were investigated at harvest time.

The experimental setting for testing effect of the main components of the BR derived from chlorsulfuron in soil on growth of rice seedling is listed in Table 2. 15 rice seeds of Xiushui-11 (*Oryza sativa* L.) were germinated and sown in each pot with 10 kg soils. The height of plant, the dry weight of shoot and root were examined after 35 d of growing.

### 1.6 The extraction of the released residue from $^{14}$C-BR soil after planting rape and identification method of LC-MS

After the oilseeds were sown for 45 d, the $BR_1$ soils corresponding to 20 g air-dried weight were extracted by methanol in a vibrator for five times continuously. In each

**Table 1 The experimental setting for examining effect of the main components in the BR derived from chlorsulfuron on growth of rape**

| Treatment | Conc. (nmol/g soil) and the serial number | | | | | |
|---|---|---|---|---|---|---|
| T | 0(CK) | 0.28($T_1$) | 0.56($T_2$) | 1.68($T_3$) | 2.80($T_4$) | 3.92($T_5$) |
| C | 0(CK) | 0.28($C_1$) | 0.56($C_2$) | 1.68($C_3$) | 2.80($C_4$) | 3.92($C_5$) |
| T + C | 0(CK) | 0.28($TC_1$) | 0.56($TC_2$) | 1.68($TC_3$) | 2.80($TC_4$) | 3.92($TC_5$) |

Notes: T. 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine; C. 2-chlorobenzenesulfonamide; T + C. 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine and 2-chlorobenzenesulfonamide were applied in combination

**Table 2 The experimental setting for testing effect of the main components in the BR derived from chlorsulfuron in soil on growth of rice seedling**

| Treatment | Conc.(nmol/g soil) and the serial number | | | | | |
|---|---|---|---|---|---|---|
| T | 0(CK) | 0.56($T_1$) | 1.68($T_2$) | 2.80($T_3$) | 3.92($T_4$) | 5.04($T_5$) |
| C | 0(CK) | 0.56($C_1$) | 1.68($C_2$) | 2.80($C_3$) | 3.92($C_4$) | 5.04($C_5$) |
| T+C | 0(CK) | 0.56($TC_1$) | 1.68($TC_2$) | 2.80($TC_3$) | 3.92($TC_4$) | 5.04($TC_5$) |

Notes: T. 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine; C. 2-chlorobenzenesulfonamide; T+C. 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine and 2-chlorobenzenesulfonamide were applied in combination

step, the soil sample was extracted for 2 h and subjected to centrifugate (4000 r/min) for 15 min. All the extracting solutions were combined and concentrated under decompressed condition at 60℃. Subsequently, 20 ml buffer solution of 0.1 mol/L $Na_2HCO_3$-$Na_2CO_3$ was added and the methanol was removed from the mixed solution by distillation. The remained solution was filtered through a 0.45 μm ultra-filtrate membrane and adjusted to pH 3.3 with 0.5 mol/L HCl, and then the filtrate was passed through a $C_{18}$ pretreated column(SPE) at flow rate of 3 ml/min. After all filtrate solution was passed through the SPE column, the water-soluble impurity was removed by 0.1% acetic acid solution. The $^{14}$C-component hold on the SPE column was eluted by ethyl acetate at flow rate of 2 ml/min. The elution was collected in a condense tube and the solvent was thoroughly vaporized. The residue was dissolved in methanol and subjected to LC-MS analysis. The analysis condition for LC-MS was as follows: A 4.6 × 250 mm Discovery $C_{18}$ column was used as the separation column, the flow rate of mobile phase was 1 ml/min in a gradient elution mode (min/% A: 0—2/90, 2—12/65, 12—20/65, 20—33/20, 28—33/20, 30—35/90). Solution A was a mixture of water and 0.1% glacial acetic acid (1 + 1 by volume), solution B was a mixture of acetonitrile and 0.1% glacial acetic acid(1 + 1 by volume). A photodiode array detector was used. The mass spectrometer was Micromass Quattro LC. Electrospray positive ion($ES^+$) and negative ion($ES^-$) full scan(100—600 amu) was performed respectively, meanwhile, the selected ion with 358 of m/z for $ES^+$ scan was also detected. The injection volume of the sample was 40 μl.

## 2 Results

### 2.1 Phytotoxicity produced by bound residue derived from $^{14}$C-chlorsulfuron in soil

The growth of rape seedling was investigated in the soil containing BR of $^{14}$C-chlorsulfuron (Fig. 1). Significant depression effect on growth of rape seedling was observed in the spiked soils($BR_1$ and $BR_2$) after sown for 45 d. The rape seedling treated with 0.28 nmol/g air-dried soil($BR_1$) grew more slowly and weakly than the control. Meantime, the rape seedling treated with 0.56 nmol/g air-dried soil($BR_2$) all died. These results suggested that BR of $^{14}$C-chlorsulfuron had significant phytotoxicity on growth of rape seedlings. It has been reported that BR derived from $^{14}$C-chlorsulfuron in soil also had significant phytotoxicity on growth of rice seedlings (Chen, 1996; Sun, 2000). Therefore, it is essential to investigate the mechanism of phytotoxicity induced by BR derived from $^{14}$C-chlorsulfuron in soil.

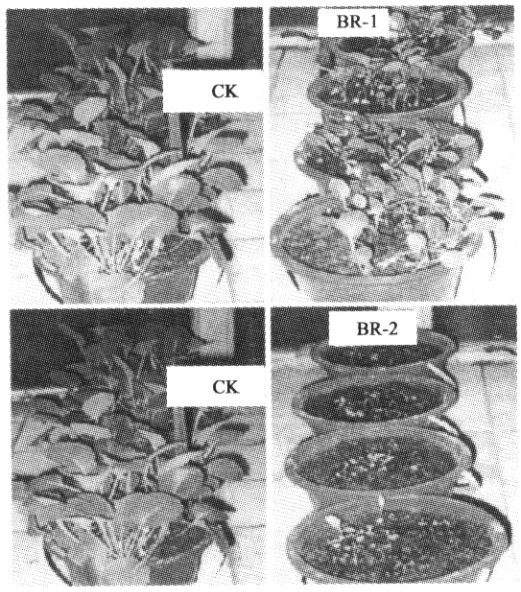


Fig.1 Effect of $^{14}$C-BR derived from $^{14}$C-chlorsulfuron in soil on growth of rappe seedling(after 45 d of sowing)

### 2.2 The effect of main components of BR derived from chlorsulfuron in soil on rape growth

Plant height, dry weight of shoot and root of rape seedling treated with 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine or 2-chloroben-zenesulfonamide or combination with two compounds are shown in Table 3, Table 4 and Table 5. No statistical significant differences were observed among control and different treatments with different concentration ($P > 0.1$). Yield, thousand-grain weight, total numbers of legumen, numbers of branches, plant height and dry weight during the whole growth stage of rape treated with 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine or 2-chlorobenz-enesulfonamide or combination with two components are shown in Table 6, Table 7 and Table 8. No statistical significant differences were also found among control and different treatments with different concentration ($P > 0.1$). These results suggested that 2-amino-4-methoxyl-6-methyl-1, 3,5-triazine or 2-chlorobenzenesulfonamide had no phytotoxic effect on the growth of this kind of rape.

Since 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine was the degradation product of 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine (Sabidie, 1992; 1993; Strek, 1998a). 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine had no phytotoxic effect on rape growth during the whole development stage, that means 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine also

has no phytotoxic effect on rape growth.

**Table 3 Effect of 2-amino-4-methoxyl-6-methyl-1,3,5-triazine on growth of rape seedling**

| Conc., nmol/g soil | Plant height, cm | Dry weight of shoot, mg | Dry weight of roots, mg | Total weight, mg/plant |
|---|---|---|---|---|
| 0 | 18.2 ± 1.4 | 222.6 ± 58.4 | 22.6 ± 8.7 | 245.2 ± 64.7 |
| 0.28 | 18.5 ± 1.2 | 245.8 ± 50.4 | 25.3 ± 9.4 | 271.0 ± 56.6 |
| 0.56 | 18.6 ± 1.5 | 208.8 ± 71.7 | 19.6 ± 6.8 | 228.4 ± 76.6 |
| 1.68 | 18.4 ± 1.3 | 206.5 ± 47.6 | 19.9 ± 6.4 | 226.4 ± 48.8 |
| 2.80 | 18.3 ± 1.6 | 213.4 ± 60.3 | 24.0 ± 10.8 | 237.4 ± 66.6 |
| 3.92 | 18.3 ± 1.4 | 235.1 ± 69.3 | 21.3 ± 7.7 | 256.4 ± 75.0 |

**Table 4 Effects of 2-chlorobenzenesulfonamide on growth of rape seedling**

| Conc., nmol/g soil | Plant height, cm | Dry weight of shoot, mg | Dry weight of roots, mg | Total weight, mg/plant |
|---|---|---|---|---|
| 0 | 18.2 ± 1.4 | 222.6 ± 58.4 | 22.6 ± 8.7 | 245.2 ± 64.7 |
| 0.28 | 18.4 ± 1.2 | 241.1 ± 54.9 | 23.1 ± 7.1 | 264.0 ± 59.0 |
| 0.56 | 18.3 ± 1.5 | 233.0 ± 45.9 | 23.8 ± 7.9 | 256.8 ± 50.0 |
| 1.68 | 18.0 ± 1.3 | 214.8 ± 53.5 | 23.0 ± 9.3 | 238.0 ± 58.0 |
| 2.80 | 18.4 ± 1.4 | 239.4 ± 48.6 | 23.6 ± 5.3 | 263.0 ± 51.3 |
| 3.92 | 18.3 ± 1.4 | 243.6 ± 71.7 | 23.9 ± 8.3 | 268.0 ± 78.0 |

**Table 5 Effects of 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine and 2-chlorobenzenesulfonamide applied in combination on growth of rape seedling**

| Conc., nmol/g soil | Plant height, cm | Dry weight of shoot, mg | Dry weight of roots, mg | Total weight, mg/plant |
|---|---|---|---|---|
| 0 | 18.2 ± 1.4 | 222.6 ± 58.4 | 22.6 ± 8.7 | 245.2 ± 64.7 |
| 0.28 | 18.3 ± 1.3 | 240.3 ± 42.4 | 25.4 ± 5.4 | 265.6 ± 44.2 |
| 0.56 | 18.1 ± 1.3 | 250.3 ± 75.9 | 21.1 ± 6.5 | 271.4 ± 81.4 |
| 1.68 | 18.4 ± 1.4 | 242.7 ± 56.2 | 22.7 ± 9.5 | 265.4 ± 62.2 |
| 2.80 | 17.8 ± 1.1 | 242.2 ± 51.1 | 21.1 ± 6.8 | 263.3 ± 55.9 |
| 3.92 | 17.9 ± 1.4 | 241.4 ± 36.8 | 24.8 ± 6.5 | 266.2 ± 39.7 |

## 2.3 The effect of main component of BR derived from chlorsulfuron in soil on rice growth

Plant height, dry weight of shoot and root of rice seedling treated with 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine or 2-chlorobenzenesulfonamide or combination with two components are shown in Table 9, Table 10 and Table 11, respectively. No statistical significant differences were observed among control and different treatments with different concentration($P > 0.1$). This result indicated that 2-amino-4-methoxyl-6-methyl-1,3,5-triazine or 2-chlorobenzenesulfonamide had also no phytotoxic effect on the growth of rice.

**Table 6 Effects of 2-amino-4-methoxyl-6-methyl-1,3,5-triazine on yield and agronomic characters of rape**

| Conc., nmol/g soil | Plant height, cm | D.W. of shoot, mg | D.W. of roots, mg | Branches /plant | Total number of legumen / plant | Thousand-grain weight, g | Total grain weight, g/plant |
|---|---|---|---|---|---|---|---|
| 0 | 170.4 ± 3.4 | 41.38 ± 0.74 | 11.72 ± 0.61 | 7.8 ± 1.0 | 198.6 ± 9.7 | 3.79 ± 0.18 | 22.54 ± 1.41 |
| 0.28 | 172.2 ± 3.3 | 41.69 ± 0.83 | 11.71 ± 0.57 | 8.5 ± 1.1 | 199.0 ± 11.1 | 3.74 ± 0.23 | 22.30 ± 1.17 |
| 0.56 | 168.8 ± 3.2 | 41.43 ± 0.63 | 11.55 ± 0.66 | 7.7 ± 1.4 | 201.0 ± 9.0 | 3.71 ± 0.12 | 22.38 ± 1.13 |
| 1.68 | 169.2 ± 3.8 | 41.63 ± 0.57 | 11.50 ± 0.31 | 8.0 ± 0.9 | 196.8 ± 8.3 | 3.74 ± 0.17 | 22.06 ± 0.95 |
| 2.80 | 171.0 ± 4.5 | 41.38 ± 0.30 | 11.54 ± 0.36 | 8.0 ± 1.3 | 199.8 ± 6.7 | 3.72 ± 0.16 | 22.28 ± 0.75 |
| 3.92 | 167.7 ± 3.6 | 41.21 ± 0.48 | 11.56 ± 0.17 | 8.0 ± 1.3 | 197.8 ± 7.7 | 3.81 ± 0.12 | 22.63 ± 1.12 |

**Table 7 Effects of 2-chlorobenzenesulfonamide on yield and agronomic characters of rape**

| Conc., nmol/g soil | Plant height, cm | D.W. of shoot, mg | D.W. of roots, mg | Branches /plant | Total number of legumen / plant | Thousand-grain weight, g | Total grain weight, g/plant |
|---|---|---|---|---|---|---|---|
| 0 | 170.4 ± 3.4 | 41.38 ± 0.74 | 11.72 ± 0.61 | 7.8 ± 1.0 | 198.6 ± 9.7 | 3.79 ± 0.18 | 22.54 ± 1.41 |
| 0.28 | 168.7 ± 3.9 | 41.12 ± 1.01 | 11.356 ± 0.82 | 8.2 ± 1.2 | 195.5 ± 11.9 | 3.79 ± 0.15 | 22.27 ± 1.72 |
| 0.56 | 168.2 ± 2.5 | 41.45 ± 0.86 | 11.83 ± 0.71 | 8.7 ± 1.5 | 202.8 ± 13.4 | 3.76 ± 0.15 | 22.85 ± 1.33 |
| 1.68 | 170.3 ± 4.3 | 41.82 ± 0.73 | 11.52 ± 0.64 | 8.2 ± 1.3 | 200 ± 13.4 | 3.48 ± 0.15 | 22.45 ± 0.98 |
| 2.80 | 167.8 ± 3.8 | 41.63 ± 0.63 | 11.49 ± 0.21 | 7.7 ± 0.8 | 198.8 ± 11.5 | 3.68 ± 0.14 | 21.95 ± 0.99 |
| 3.92 | 171.7 ± 4.2 | 41.75 ± 0.85 | 11.64 ± 0.41 | 8.2 ± 1.2 | 201.8 ± 10.8 | 3.67 ± 0.10 | 22.22 ± 0.78 |

**Table 8 Effects of 2-amino-4-methoxyl-6-methyl-1,3,5-triazine and 2-chlorobenzenesulfonamide applied in combination on yield and agricultural characters of rape**

| Conc., nmol/g soil | Plant height, cm | D.W. of shoot, mg | D.W. of roots, mg | Branches /plant | Total number of legumen / plant | Thousand-grain weight, g | Total grain weight, g/plant |
|---|---|---|---|---|---|---|---|
| 0 | 170.4 ± 3.4 | 41.38 ± 0.74 | 11.72 ± 0.61 | 7.8 ± 1.0 | 198.6 ± 9.7 | 3.79 ± 0.17 | 22.54 ± 1.41 |
| 0.28 | 172.7 ± 2.6 | 41.16 ± 0.28 | 11.49 ± 0.18 | 8.0 ± 1.4 | 198.8 ± 10.1 | 3.70 ± 0.17 | 22.04 ± 0.62 |
| 0.56 | 167.7 ± 3.2 | 41.26 ± 0.36 | 11.61 ± 0.12 | 8.5 ± 1.0 | 201.3 ± 11.4 | 3.69 ± 0.14 | 22.23 ± 0.69 |
| 1.68 | 169.7 ± 3.8 | 41.28 ± 0.35 | 11.63 ± 0.28 | 8.2 ± 1.5 | 198.3 ± 9.7 | 3.68 ± 0.14 | 22.05 ± 0.93 |
| 2.80 | 172.0 ± 2.8 | 41.38 ± 0.37 | 11.60 ± 0.55 | 8.3 ± 1.0 | 197.7 ± 7.5 | 3.74 ± 0.15 | 22.20 ± 1.23 |
| 3.92 | 170.3 ± 3.7 | 41.43 ± 0.44 | 11.77 ± 0.42 | 7.7 ± 1.4 | 196.8 ± 4.5 | 3.82 ± 0.19 | 22.53 ± 1.07 |

**Table 9 Effect of 2-amino-4-methoxyl-6-methyl-1,3,5-triazine on rice growth**

| Conc., nmol/g soil | Plant height, cm | Dry weight of shoot, mg | Dry weight of roots, mg | Total weight, mg/plant |
|---|---|---|---|---|
| 0 | 50.4 ± 1.4 | 266.8 ± 10.0 | 72.3 ± 10.7 | 339.1 ± 16.6 |
| 0.56 | 50.7 ± 1.2 | 269.5 ± 12.6 | 76.5 ± 11.7 | 346.0 ± 18.3 |
| 1.68 | 50.2 ± 1.3 | 266.1 ± 6.8 | 73.5 ± 12.1 | 339.5 ± 14.4 |
| 2.80 | 50.7 ± 1.4 | 268.3 ± 13.0 | 73.4 ± 10.6 | 341.6 ± 17.3 |
| 3.92 | 50.5 ± 1.3 | 265.2 ± 9.1 | 74.6 ± 13.1 | 339.8 ± 15.8 |
| 5.04 | 50.8 ± 1.4 | 266.0 ± 10.4 | 75.3 ± 10.8 | 341.2 ± 13.6 |

**Table 10 Effects of 2-chlorobenzenesulfonamide on rice growth**

| Conc., nmol/g soil | Plant height, cm | Dry weight of shoot, mg | Dry weight of roots, mg | Total weight, mg/plant |
|---|---|---|---|---|
| 0 | 50.4 ± 1.4 | 266.8 ± 10.0 | 72.3 ± 10.7 | 339.1 ± 16.6 |
| 0.56 | 50.7 ± 1.2 | 266.7 ± 11.4 | 72.7 ± 12.0 | 339.4 ± 17.6 |
| 1.68 | 50.6 ± 1.5 | 265.2 ± 12.1 | 73.9 ± 12.9 | 339.1 ± 18.2 |
| 2.80 | 49.9 ± 1.1 | 264.9 ± 11.3 | 69.1 ± 9.7 | 334.0 ± 12.5 |
| 3.92 | 50.4 ± 1.6 | 268.7 ± 13.1 | 74.9 ± 12.2 | 343.6 ± 21.1 |
| 5.04 | 50.7 ± 1.3 | 267.4 ± 10.7 | 72.3 ± 9.8 | 339.7 ± 16.6 |

**Table 11 Effects of 2-amino-4-methoxyl-6-methyl-1,3,5-triazine and 2-chlorobenzenesulfonamide applied in combination on rice growth**

| Conc., nmol/g soil | Plant height, cm | Dry weight of shoot, mg | Dry weight of roots, mg | Total weight, mg/plant |
|---|---|---|---|---|
| 0 | 50.4 ± 1.4 | 266.8 ± 10.0 | 72.3 ± 10.7 | 339.1 ± 16.6 |
| 0.56 | 50.8 ± 0.9 | 265.3 ± 10.1 | 76.3 ± 9.2 | 341.6 ± 14.9 |
| 1.68 | 50.1 ± 1.4 | 265.3 ± 8.6 | 68.7 ± 7.3 | 334.0 ± 12.4 |
| 2.80 | 50.3 ± 1.1 | 264.9 ± 10.7 | 70.7 ± 8.2 | 335.7 ± 14.7 |
| 3.92 | 50.6 ± 1.3 | 265.7 ± 9.5 | 71.3 ± 10.4 | 337.0 ± 14.9 |
| 5.04 | 49.9 ± 1.0 | 264.1 ± 9.1 | 68.8 ± 10.1 | 332.9 ± 13.0 |

Take together, except for chlorsulfuron parent compound, other main components in the BR such as 2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine, 2-amino-4-hydroxyl-6-methyl-1, 3, 5-triazine or 2-chlorobenzenesulfonamide had no phytotoxic effect on the growth of crop (rice and rape).

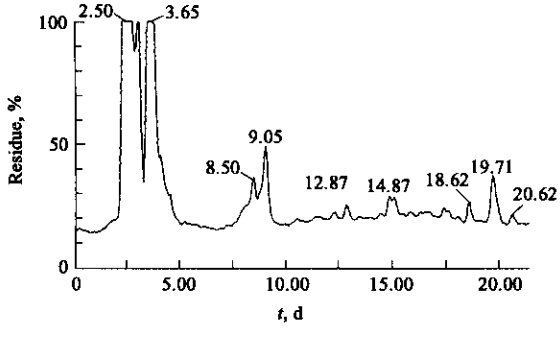


Fig. 2 LC-MS total ion chromatogram (Scan ES$^+$) of methanol-extractable residue in the $BR_1$ soil after 45 d of rape growth

## 2.4 The release of bound parent compound and LC-MS identification

LC-MS analysis results of methanol-extractable residue in the $BR_1$ soil after 45 d of rape growth is illustrated in Fig. 2—5. From selective ion chromatography performed on m/z of 357, a peak with retention time of 20.63 min was observed (Fig.3), which indicated that the compound might have the molecular mass of 357. The mass spectra further verified that the component had molecular weight of 357 and contained the chlorine element in its chemical formula since the chlorine isotope peaks existed in the mass spectra(M: M + 2 = 3:1). Two fragments with the m/z of 141(mw.140 + 1) and m/z of 167(mw.166 + 1) were found in the ES$^+$ scan mode. The deductive structures of the two fragments are shown in Fig. 4. In addition, two fragments with the m/z of 139(mw.140 − 1) and m/z of 190(mw.191 − 1) occurred in mass spectra performed on the ES$^-$ scan mode. The deductive structures of the two fragments are presented in Fig 5. Based on all the above information, it could be concluded that the compound with retention time of 20.63 min was the parent compound ($^{14}$C-labeled chlorsulfuron). Therefore, it could be inferred that the chlorsulfuron parent compound previously bound to the soil matrixes could be again released and transformed into methanol-extractable residue during the course of rape growth.

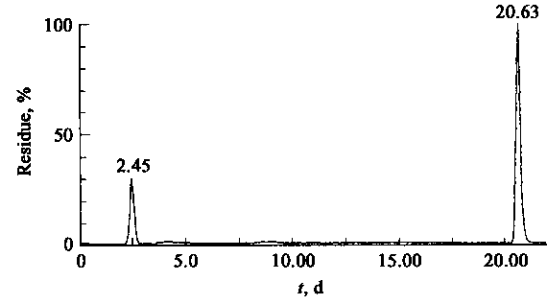


Fig. 3 LC-MS selected ion chromatogram (m/z = 358, Scan ES$^+$) of methanol-extractable residue in the $BR_1$ soil after 45 d of rape growth

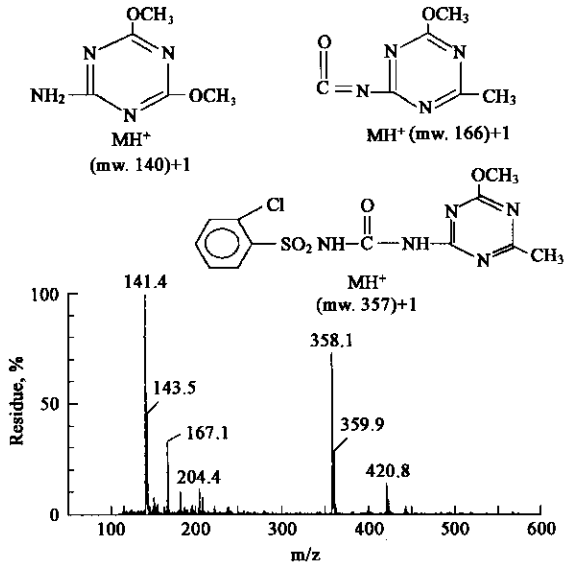


Fig.4 Mass spectrum(ES$^+$) of the compound with the retention time of 20.6 min

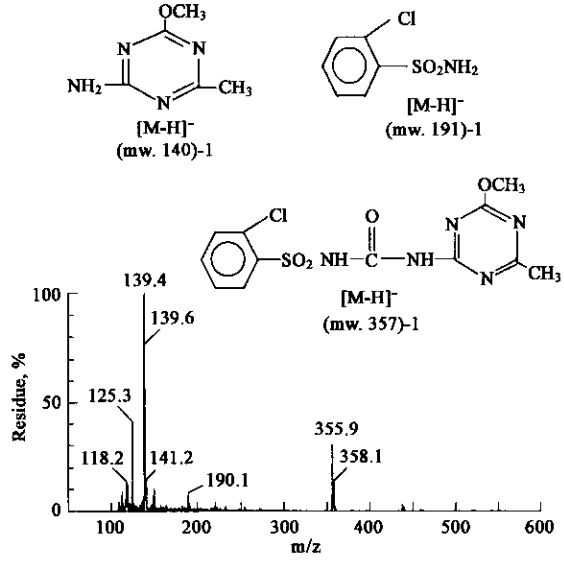


Fig.5 Mass spectrum(ES$^-$) of the compound with the retention time of 20.6 min

## 3 Discussion

The main components of the $^{14}$C-BR derived from $^{14}$C-labeled chlorsulfuron in soil was composed of $^{14}$C-[2-amino-4-methoxyl-6-methyl-1, 3, 5-triazine], $^{14}$C-[2-amino-4-hydroxyl-6-methyl-1,3,5-triazine], $^{14}$C-labeled chlorsulfuron parent compound and 2-chlorobenzene-sulfonamide (Ye, 2000), except for the parent compound, other main components of the BR had no phytotoxic effect on the growth of rape and rice. It could be inferred that the parent compound might play an important role in the phytotoxic effect induced by BR.

Generally, the interaction mode between herbicides and soil was essentially decided by chemical structure of the herbicides and the properties of soils (Bailey, 1970; Khan, 1982; Pignatello, 1996). A given chemical might undergo binding by several mechanism simultaneously (Calderbank, 1989; Senesi, 1992; Gevao, 2000). The heterocyclic ring part of the chlorsulfuron might interact with the acceptor through an electron transfer force, while the benzene ring side might be bound to the acceptor through hydrophobic interaction. The electron draw group on the benzene group and the sulfur atom in the bridge of the molecular might interact with acceptor through electrostatic force, and the oxygen atom in the sulfonyl group might interact with the acceptor through a hydrogen bound. So, chlorsulfuron parent compound might be bound to the soil matrix through a mixed interaction mode.

Khan (Khan, 1982) and Dec (Dec, 1988) proposed that the interactive strength between the bound pesticides and soil matrix might be changed when the soil pH, microorganism community and the activity of soil enzyme changed, since there were diversity interaction mechanism and interaction position. During the course of cultivation, the soil environment could be changed, thus, a part of residues previously bound to the soil matrix might be again released, which causing the retarding phytotoxicity. The results of this paper verified that the chlorsulfuron parent compound previously bound to soil matrix could also be released after planting the rape seedling. This conclusion was accordance with that of many researches in this field (IAEA, 1986; Khan, 1982; Dec, 1988).

Many studies proved that the specific target enzyme of chlorsulfuron is the ALS enzyme (Ray, 1984; Beyer, 1988; Brown, 1990). Therefore, the mechanism of phytotoxicity induced by the bound chlorsulfuron residue in soil could be well explained, the molecular in the BR inducing the phytotoxic effect on the growth of rape and rice was still the parent compound.

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