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Effect of herbicide acetochlor on cytochrome P450 monooxygenases and GST of earthworms *Eisenia fetida*

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Abstract: To assess the sublethal toxicity of the herbicide acetochlor to earthworms and to find out biomarkers possible inducted under acetochlor exposure, *Eisenia fetida* was exposed to artificial soils supplemented with different concentrations of acetochlor(5, 10, 20, 40 and 80 mg/kg soil). Effects of the acetochlor on cytochrome P450 monooxygenases *p*-nitroanisole O-demethylase(ODM), aldrin epoxidase(AE) and glutathione-S-transferases (GSTs) activities were determined. The results revealed cytochrome P450 monooxygenases were elevated with increasing concentrations of acetochlor, and the AE activity increased significantly compared with control at the concentration of 80 mg/kg ($P<0.05$). However, ODM activity from *E. fetida* was not induced significantly by acetochlor at all treatments($P>0.05$). Sodium dodecyl sulfate-polyacryamide gel electrophoresis(SDS-PAGE) showed that one protein band was visualized and no evident differences were found in protein profiles between treatments and control. The GST activity increased significantly with longer duration($P<0.05$) and increasing concentrations of acetochlor exposure($P<0.05$). This study showed that the monooxygenases and GSTs activities in *E. fetida* could be induced by acetochlor, and thus, the AE and GST could be used in sublethal assays for soil contamination surveys and GST could be used as biomarkers of acetochlor exposure in *E. fetida*.

Keywords: acetochlor; *Eisenia fetida*; *p*-nitroanisole O-demethylase; aldrin epoxidase; GST

Introduction

Acetochlor is one of the most widely used herbicide all over the world with a consumption of more than 10^4 t/a in China (Ye, 2003). It showed significant carcinogenicity to the rat nasal epithelium (Green *et al.*, 2000), and has been classified as a B-2 carcinogen by the USEPA(1984). The 14 d(half lethal dose (LD_{50}) of acetochlor to *Eisenia fetida* is 155.8 mg/kg in black soil, and its toxicity is stronger than Cu (Liang and Zhou, 2003). Acetochlor and Cu^{2+} had a synergic inhibitory effect on the amount of culture-dependent viable bacteria and the activity of soil dehydrogenase (Zhang *et al.*, 2004). However, there were few reports about the impact of physiology and biochemical of acetochlor to non-target soil organisms such as earthworms.

Enzymatic activities are regarded as fast and prognostic indices of individual reaction to the environmental stress, and should allow prediction of the consequences of pollution at population and ecosystem level (Cortet *et al.*, 1999; Scott-Fordsmand and Weeks, 2000). Metabolism of hydrophobic xenobiotics into more polar compound includes two phase. The phase I is the addition of the oxygen to lipophilic compound metabolized by monooxygenases. The microsomal cytochrome P450 dependent monooxygenases constitute an extremely important metabolic system, and have numerous functions and roles, including growth, development, feeding, resistance to pesticides and tolerance to plant toxins. It can lead to the formation of production they are detoxic or more toxic than the parent compound (de Bethizy and Hayes, 1994). In *Lumbricus terrestris*, cytochrome P450 and b5, aldrin epoxidase and ethoxycoumarin-O-dealkylase (ECOD) have been detected (Khan *et al.*, 1972; Milligan *et al.*, 1986; Berghout *et al.*, 1991). The cytochrome P450 monooxygenases system has been characterized and partially purified in the annelid *E. fetida andrei* (Achazi *et al.*, 1998).

The phase II is that the oxidated production of phase I conjugated with molecule such as glutathione. Glutathione-S-transferases (GSTs) are involved in phase II metabolism. They form a superfamily of mostly cytosolic enzymes found in almost all organisms. GSTs catalyze the conjugation of the glutathione (GSH) to endogenous of exogenous electrophilic substrates, and the productions are usually more polar, less toxic and easy excretion (Pascal and Scalla, 1999). These enzymes have been studied in earthworms and especially in *E. fetida andrei* (Stenersen and Oien, 1981; Saint-Denis *et al.*, 1998). Six groups of isoenzymes with particular substrate specificities have been isolated in *E. andrei* by Borgeraas *et al.*(1996).

Eisenia fetida, because of its low cost, easy handing and the standardization of acute and

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subchronic ecotoxical test(OECD, 1984), was used in our experiments. We exposed earthworms to artificial soils supplemented with increasing concentrations of acetochlor, the aim was (1) to understand the biotransformation of acetochlor to the earthworm *E. fetida*; (2) to assess the sublethal toxicity of the herbicide acetochlor to earthworms and explore the potential biomarkers for acetochlor biomonitoring.

1 Materials and methods

1.1 Earthworms and chemicals

The adult earthworms *Eisenia fetida* with well developed clitellum (about 300—400 mg wet weight) were obtained from a synchronized culture in our laboratory and selected for this test. Animals were acclimated at the test temperature for 7 d to artificial soil substrates prior to test.

The acetochlor [2'-ethyl-6-methyl-N-(ethoxymethyl)-2-chloroacetanilide] was 90% miscible oil reagent and was obtained from WU County Chemistry Factory, Zhejiang Province, China.

1.2 Treatments

The test substrate was artificial soil prepared according to OECD guideline 207 (OECD, 1984). It comprised (by dry weight) of 10% finely ground sphagnum peat, 20% kaolin clay, 70% industrial fine sand, with pH adjusted to 6.5 by addition of calcium carbonate.

Earthworms were exposed to an increasing range concentration of acetochlor (5, 10, 20, 40 and 80 mg/kg dry soil). 500 g substrate was placed in each polyethylene plastic containers (16 cm × 10.5 cm × 5 cm). The low volume concentration recommended for the agricultural application(1500 — 3000 ml/hm^2) was converted for the surface area of the test containers (Ma *et al.*, 2004). This is equivalent to 5 — 10 mg/kg dry soil. Acetochlor was dissolved in 5 ml acetone and then mixed into a small quantity of fine quartz sand. The solvent is then removed by evaporation for at least one hour and the sand then mixed thoroughly with the premoistened artificial soil. The final water content was adjusted to 50% of the maximum water holding capacity. Controls were prepared in a similar way except 5 ml acetone added only. Groups of ten worms were placed in individual test container. For each concentration, three replicates were prepared. The container lids were perforated to allow aeration. Earthworms were placed in a climate chamber with 20 ± 1℃ and 12D/12L photoperiod.

1.3 Preparation of microsomes

Microsomes were prepared based on the method of Lee and Scott(1989) and Qiu *et al.*(2003). *E. fetida* was kept on moist filter at room temperature up to 48 h to remove gut contents after 40 d of acetochlor exposure. Earthworms were transferred into a glass homogenizer, and were homogenized in ice-cold homogenization buffer (0.1 mol/L sodium phosphate buffer, pH 7.5, containing 10% glycerol, 1 mmol/L EDTA, 0.1 mmol/L dithiothreitol (DTT), 1 mmol/L 1-phenyl-2-thiourea (PTU), and 1 mmol/L phenylmethylsulfonyl fluoride(PMSF)). After filtering the crude homogenate through two layers gauze, the crude homogenate was centrifuged at 10000 g for 15 min (Beckman J2-MC). The supernatant were centrifuged at 100000 g for 1 h (Hitachi CP70C) and microsomes were sedimented. The microsomal pellet was resuspended in homogenisation buffer supplemented with 20% glycerol, and aliquots stored at -70℃.

1.4 Monooxygenases assays

Aldrin epoxidation (AE) activity was determined according to the method of Lee *et al.*(1989). The 1 ml reaction mixture consisted of 0.36 mmol/L of NADPH (β-nicotinamide-adenine dinucleotide phosphate), 0.1 ml of microsomal sample, and 0.1 mol/L sodium phosphate buffers, pH 7.4. After a 2-min preincubation period at 37℃, the reaction was initiated by the addition of aldrin dissolved in methanol (final assay concentration 0.2 mmol/L). The reaction was allowed to proceed at 30℃ for 10 min in water bath and then stopped by the addition of 3 ml acetone. The reaction production (dieldrin) was extracted with petroleum ether (60 —90℃, 2 ml) by vortexing the tube 1 min. The amount of dieldrin formed in each assay was quantified by gas chromatography after drying over anhydrous sodium sulfate.

The dieldrin quantification were analyzed with HP5890 series gas chromatography equipped with electron capture detector under the following conditions: column HP-1 (polydimethylsiloxane 25 m × 0.32 mm I.D.); injector, detector and column temperatures were 250, 300 and 230℃, respectively; injections 1 μl. The retention time of dieldrin using this method was 5.5 min. Quantification of dieldrin (purity 99.5%, Sigma Company) was injected to obtain a standard curve (external standard method)

p-Nitroanisole O-demethylase (ODM) activity was assayed according to the method described by Hansen and Hodgson(1971) and Qiu *et al.*(2003). The 2 ml standard reaction mixture contained 0.36 mmol/L of NADPH, 0.5 ml of microsomal sample, and 0.1 mol/L sodium phosphate buffers, pH 7.8. The reaction was initiated by the addition of *p*-nitroanisole dissolved in ethanol (final assay concentration 3 mmol/L). Reaction mixture was incubated 30 min at

25℃ with water bath and then terminated by the addition of 0.5 ml 1.0 mol/L HCl. The production *p*-nitroanisole was extracted with 2.5 ml $CHCl_3$, and then centrifuged (3000 × g, 15 min). The $CHCl_3$ fraction was back-extracted with 0.5 mol/L NaOH. The OD_{400} (optical density at 400 nm) value of NaOH solution was recorded, and the quantity of the production was determined by using an experimentally standard curve.

1.5 GST activity assays

Triplicates of three living earthworms pooled together were used for GST activity assays. Before being homogenized, earthworms were placed on a damp filter to remove defecation. The earthworms were homogenized in Tris buffer (100 mmol/L, pH 7.5) used glass homogenizer. The homogenate was centrifuged at 10000 r/min for 30 min to yield the postmitochondrial (supernatant: S9) fraction. Aliquots were frozen and stored at -70℃.

Glutathione-S-transferases(GSTs) activities were measured according to the method described by Habig and Hodgson (1974) and Saint-Denis *et al.* (1998), 1-chloro-2, 4-dinitro-benzene (CDNB) was used as substrate. The assay mixture, total volume 1 ml, consisted of 100 mmol/L Tris buffer, pH 6.8, 2 mmol/L GSH, 1 mmol/L CDNB, 50 μl sample proteins. The reaction was conducted at 37℃ and initiated by addition of GSH. OD_{340} value was measured.

1.6 SDS-PAGE

SDS-PAGE of solubilized microsomes from *E. fetida* was carried out by a modified method described by Laemmli (1970). The stacking gel contained 4% acrylamide and separating gel contained 12% acrylamide. Protein samples (15 μg/well) were boiled for 5 min at 95℃, and applied to each well in the gel. Gel was run at 12 mA in Bio-Rad Protein II electroperature unit. Molecular weight protein standards were run in parallel with the microsomal proteins. Bands were stained with 0.125% Coomassie brilliant R-250. Electrophoresis profiles were analyzed using the Bio-Rad video system.

1.7 Protein determination

Total protein contents determinations were performed with the method of Lowry *et al.*(1951).

1.8 Statistical analysis

The data in this study were analyzed by the use of the SPSS program(Standard Version 10.0, SPSS Inc.). LSD test was used to determine the differences between treatments and control. A two-way ANOVA was employed to determine the differences between duration and concentrations. The probability level used for the statistical significance was $P<0.05$. All the values were presented as mean ± *SD*.

2 Results

2.1 Monooxygenases activity

The control earthworms had the lowest activity of aldrin epoxidase (AE) of 3.46 ± 1.15 pmol/(mg protein·min). At the lowest concentration, the AE activity reached 130.3% of the control, but no significant differences were found between them ($P>0.05$). Induction increased when earthworms were treated with increasing concentrations of acetochlor (Table 1). At the highest concentration of 80 mg/kg, the AE activity increased up to 2.57 fold of the control, and there were significant differences between them ($P<0.05$). This showed that acetochlor had induction effect on AE activity of *E. fetida*.

Table 1 O-demethylase and aldrin epoxidase activities of earthworms after exposure to different concentrations of acetochlor

Concentrations, mg/kg	Aldrin epoxidase, pmol/(mg protein·min)	*p*-Nitroanisole O-demethylase, pmol/(mg protein·min)
Control	3.46±1.15	1.95±1.13
5.0	4.51±1.43	2.47±1.31
10	7.34±2.08	2.17±0.74
20	7.02±3.70	3.51±2.83
40	6.98±3.24	2.48±0.54
80	8.88±2.53*	3.95±0.69

Notes: *Significant difference compared with control($P<0.05$)

The control earthworms had the lowest activity of *p*-nitroanisole O-demethylase (ODM) activity (Table 1). ODM activity increased when earthworms were treated with acetochlor (Table 1). At 80 mg/kg, the ODM activity increased up to 2.02 fold compared with control. However, no significant difference was found between them($P>0.05$).

2.2 Gel electrophoresis

Cytochrome P450 isozymes have been shown to have molecular masses ranging from 45000 to 60000 Da (Qiu *et al.*, 2003). By SDS-PAGE profiles of the microsomes protein of *E. fetida*, one protein band was visualized in the range of cytochrome P450 molecular masses, and the molecular mass was 58 kDa. However, no evident differences in protein profiles were found between treatments and control. The acetochlor did not induce change of member of cytochrome P450 microsomal protein(Fig.1).

2.3 GST activity

GST activity of earthworm depended on the duration and concentration of acetochlor exposure. It increased when earthworms treated with different

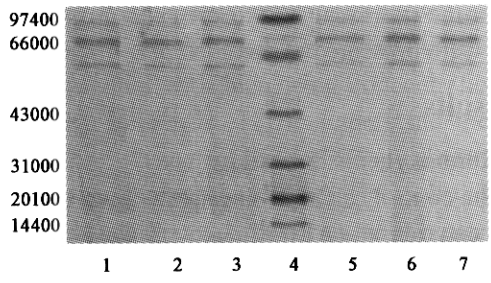

Fig.1 Sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE) patterns of earthworms exposed to different concentrations of acetochlor

Lane 1 represents control, lane 2—3 represent the treatments of 5 and 10 mg/kg concentrations, lane 5—7 represent the treatments of 20, 40, 80 mg/kg concentrations, respectively. Lane 4 represents markers and the molecular is 97400, 66200, 43000, 31000, 20100 and 14400

concentrations of acetochlor after 30 and 60 d(Table 2). After 30 d of exposure, the GST activity increased significantly at the concentration of 40 and 80 mg/kg ($P<0.05$), it reached 105.48% and 106.94% of the control, respectively. After 60 d of exposure, the GST activity increased significantly at the concentration of 5, 20, 40 and 80 mg/kg ($P<0.05$). ANOVA revealed that there were significant influences of durations ($P<0.01$) and concentrations ($P<0.01$) on GST activity. This was showed that GST was induced when earthworms were exposed to higher concentrations and longer duration of acetochlor.

Table 2 GST activities of earthworms after exposure to different concentrations of acetochlor

Concentrations, mg/kg	GST activities, nmol/(mg protein·min)	
	30 d	60 d
Control	128.35±1.46	137.74±1.52
5.0	131.63±6.10	142.04±9.48*
10	127.81±1.15	139.84±2.87
20	126.72±1.40	145.74±6.12*
40	135.39±2.30*	155.66±4.50*
80	137.26±1.07*	150.44±7.94*

Notes: *Significant difference compared with control($P<0.05$)

3 Discussion

Results from our experiments indicated that aldrin epoxidase from *E. fetida* was induced by acetochlor. The AE activity of control earthworm was 3.46 ± 1.15 pmol/(mg protein·min). These observations correlated to the earlier investigations demonstrating aldrin activities in leeches(Khan *et al.*, 1972). Its activity was within the same range of bollworm (*Helicoverpa armigera*) of whole homogenate (6.66 pmol/(mg protein·min); Qiu *et al.*, 2003).

Cytochrome P450 was of central importance in detoxification and biotransformation usually was used as biomarkers in organisms to indicate the exposure to pollutants (Lee, 1998; D'Alo *et al.*, 2004). In aquatic ecosystem, P450 1A(CYP1A) induction is regarded as one of the most sensitive and specific biomarkers for series of environmental pollutants such as PCBs, PAHs, and related compounds (Bucheli *et al.*, 1995). However, only a few studies about the effects of xenobiotics on AE activity NADPH-supported were reported. Its activity of embryonic turkey liver increased significantly following a 24-h exposure to phenobarbital on day 21 *in vivo*(Perrone *et al.*, 2004). The exposure of midges to 10 mg/L of atrazine in solution for 80 and 160 h resulted in increases of aldrin epoxidase activity of 4- and 9-fold, respectively (Miota *et al.*, 2000). The AE activity of cotton bollworm was induced significantly after treatment with pentamethylbenzene and naphathalene(Qiu *et al.*, 2003). Acetochlor was metabolized in rat by facilitated hydroxylation of the phenyl ring by a cytochrome P450 isoenzyme, and a metabolite of quinoneimine was yielded which showed significant carcinogenicity to the rat nasal epithelium (Green *et al.*, 2000). Acetochlor increased chromatid exchange frequency (SCE) to human lymphocytes (Hill *et al.*, 1997). It also decreased the microbial community diversity of soil (Luo *et al.*, 2004). In our study, exposure of *E. fetida* to acetochlor at 80 mg/kg resulted in significantly increased P450-dependent aldrin epoxidase activity relative to unexposed earthworms. It showed AE was induced by acetochlor and acetochlor had sublethal toxicity to *E. fetida* at the concentrations of 80 mg/kg. However, it should be further studied whether the AE activity can be used as a biomarker for environmental pollutant.

ODM activity can be induced by heavy metal; such as Cd increased significantly the hepatic microsomal *p*-nitroanisole O-demethylase activity of male guinea pigs (Iscan *et al.*, 1993). However, in our study, *p*-nitroanisole O-demethylase (ODM) activity from *E. fetida* was not induced significantly by acetochlor.

By SDS-PAGE of the purified microsomal proteins, one protein band was visualized in the range of cytochrome P450 molecular masses. However, from the electrophoresis photograph, the member of cytochrome was not changed significantly after different concentrations of acetochlor exposure.

GST was used as biomarkers in earthworms both of heavy metal pollution (Lukkari *et al.*, 2004) and of pesticide pollution (Booth *et al.*, 2001) in soil. Saint-Denis *et al.* (2001) observed that in the

earthworm *E. fetida andrei* GST activity decreased already after 2 d of lead acetate exposure. Acetochlor was rapid detoxification in the rats *in vivo* via reaction with GSH (Ashby *et al.*, 1996). In this study, the GST activity in earthworms increased significantly in all treatments. It was sensitive to acetochlor pollution, and then, it can serve as a biomarker for safety evaluation of acetochlor on *E. fetida.*

4 Collusions

The cytochrome P450 monooxygenases activity of *E. fetida* increased after exposure to acetochlor. At the field dose (5—10 mg/kg), the GST activity increased significantly after 60 d exposure. Our study showed that the cytochrome P450 and GST were worked together at detoxicity of acetochlor in *E. fetida* and acetochlor served as a substrate for the cytochrome P450 and GST. Acetochlor had sublethal toxicity to *E. fetida.* If the acetochlor should be any longer used in soil, the population of earthworms and the fauna in soil should be influenced. The GST activity in *E. fetida* can be induced by acetochlor, and thus, the GST could be used as a biomarker for acetochlor pollutants in *E. fetida.* But the AE activity was induced significantly only at higher concentration of acetochlor, and its use as a biomarker to soil pollution should be further study.

References:

Achazi R K, Flenner C, Livingstone D R *et al.*, 1998. Cytochrome P450 and dependent activities in unexposed and PAH-exposed terrestrial annelids [J]. Comp Biochem Physiol, 121C: 339—350.

Ashby J A, Kier L, Wilson A G E *et al.*, 1996. Evaluation of the potential carcinogenicity and genetic toxicity to humans of the herbicide acetochlor[J]. Hum Exp Toxicol, 15: 702—735.

Berghout A G, Wenzel E, Buld J *et al.*, 1991. Isolation, partial purification and characterization of the cytochrome P450 dependent monooxygenase system from the midgut of the earthworm *Lumbricus terrestris* [J]. Comp Biochem Physiol C, 100 (3): 389—396.

Booth L H, Hodge S, O'Halloran K, 2001. Use of biomarkers in earthworms to detect use and abuse of field applications of a model organophosphate pesticide [J]. Bull Environ Contam Toxicol, 67: 633—640.

Borgeraas J, Nilsen K, Stenersen J, 1996. Methods for purification of glutathione transferases in the earthworm genus *Eisenia* and their characterization[J]. Comp Biochem Physiol, 114C: 129—140.

Bucheli T D, Fent K, 1995. Induction of cytochrome P450 in fish as a biomarker for environmental contamination in aquatic ecosystems[J]. Crit Rev Environ Sci Technol, 25: 200—268.

Cortet J, Gomot-De-Vauflery A, Gomot L *et al.*, 1999. The use of invertebrate soil fauna in monitoring pollutant effects [J]. Eur J Soil Biol, 35(3): 115—134.

D'Alo F, Voso M T, Guidi F *et al.*, 2004. Polymorphisms of CYP1A1 and glutathione S-transferase and susceptibility to adult acute myeloid leukemia[J]. Haematologica, 89(6): 664—670.

de Bethizy J D, Hayes J R, 1994. Metabolism: a determinant of toxicity [M]. In: Principles and methods of toxicology (Hayes A.W. ed.). 3rd ed. New York, NY: Raven Press. 101—148.

Green T, Lee R, Moore R B *et al.*, 2000. Acetochlor-induced rat nasal tumors: Further studies on the mode of action and relevance to humans[J]. Regul Toxicol Pharmacol, 32(1): 127—133.

Habig W H, Pabst M J, Jakoby W B, 1974. Glutathione S-transferases [J]. J Biol Chem, 249(2): 7130—7139.

Hansen L G, Hodgson E, 1971. Biochemical characteristics of insect microsomes: N-and demethylation [J]. Biochem Phamacol, 20: 1569—1572.

Hill A B, Jefferies P R, Quistad G B *et al.*, 1997. Dialkylquinoneimine metabolites of chloroacetanilide herbicides induce sister chromatid exchanges in cultured human lymphocytes [J]. Mutat Res, 12: 159—171.

Iscan M, Coban T, Eke B C *et al.*, 1993. The responses of hepatic monooxygenases of guinea pig to cadmium and nickel [J]. Biol Trace Elem Res, 38(2): 129—137.

Khan M A Q, Kamal A, Wolin R J *et al.*, 1972. *In vivo* and *in vitro* epoxidation of aldrin by aquatic food chain organisms [J]. Bull Environ Contam Toxicol, 8: 219—228.

Laemmli U K, 1970. Cleavage of structural proteins during the assembly of the bacteriophage T4[J]. Nature, 227: 680—685.

Lee R F, 1998. Annelid cytochrome P450[J]. Comp Biochem Physiol C, 121(1—3): 173—179.

Lee S S T, Scott J G, 1989. An improved method for preparation, stabilization and storage of housefly (Diptera: Muscidae) microsomes[J]. J Econ Entomol, 82: 1559—1563.

Liang J D, Zhou Q X, 2003. Single and binary-combined toxicity of methamidophos, acetochlor and copper acting on earthworms *Eisenia fetida* [J]. Bull Environ Contam Toxicol, 71 (6): 1158—1166.

Lowry O H, Rosebrough N J, Farr A L *et al.*, 1951. Protein measurement with a Folin phenol reagent [J]. J Biol Chem, 193: 265—275.

Lukkari T, Taavitsainen M, Soimasuo M *et al.*, 2004. Biomarker responses of the earthworm *Aporrectodea tuberculata* to copper and zinc exposure: differences between populations with and without earlier metal exposure [J]. Environ Pollut, 129: 377—386.

Luo H F, Qi H Y, Zhang H X, 2004. The impact of acetochlor on the bacterial diversity in soil [J]. Acta Microbiologica Sinica, 44(4): 519—522.

Ma Q, Rahman A, Holland P T *et al.*, 2004. Field dissipation of acetochlor in two New Zealand soils at two application rates[J]. J Environ Qual, 33(3): 930—938.

Milligan D L, Babish J G, Neuhauser E F, 1986. Noninducibility of cytochrome P450 in the earthworm *Dendrobaena veneta* [J]. Comp Biochem Physiol C, 85(1): 85—87.

Miota F, Siegfried B D, Scharf M E *et al.*, 2000. Atrazine induction of cytochrome P450 in *Chironomus tentans* larvae[J]. Chemosphere, 40(3): 285—291.

OECD (Organization for Economic Co-operation and Development), 1984. Test 207: Earthworm, acute toxicity tests [S]. In: OECD Guidelines for Testing of Chemicals (Organization for Economic Co-operation and Development, ed.).

Pascal S, Scalla R, 1999. Purification and characterization of a safener-induced glutathione S-transferase from wheat (*Triticum aestivum*)[J]. Physiol ogia Plantarum, 106: 17—27.

Perrone C E, Ahr H J, Duan J D *et al.*, 2004. Embryonic turkey liver: activities of biotransformation enzymes and activation of DNA-reactive carcinogens[J]. Arch Toxicol, 78(10): 589—598.

Qiu X H, Li W, Tian Y *et al.*, 2003. Cytochrome P450 monooxygenases in the cotton bollworm (Lepidoptera: Noctuidae): tissue difference and induction[J]. J Econ Entomol, 96(4): 1283—1289.

Saint-Denis M, Labrot F, Narbonne J F *et al.*, 1998. Glutathione, glitathione-relatad enzymes, and catalase activities in the earthworm *Eisenia fetida Andrei* [J]. Arch Enviton Contam Toxicol, 35: 602—614.

Saint-Denis M, Narbonne J F, Arnaud C *et al.*, 2001. Biochemical responses of the earthworm *Eisenia fetida Andrei* exposed to contaminated artificial soil: effects of lead acetate [J]. Soil Biol Biochem, 33: 395—404.

Scott-Fordsmand J J, Weeks J M, 2000. Biomarkers in earthworms[J]. Rev Environ Contam Toxicol, 165: 117—159.

Stenersen J, Oien N, 1981. Glutathione-S-transferase in earthworms (*Lumbricidae*): Substrate specificity, tissue and species distribution and molecular weight [J]. Comp Biochem Physiol, 69C: 243 - 252.

USEPA, 1994. Prevention, pesticide and toxic substances, questions and answers, conditional registration of acetochlor [S]. Washington, DC: USEPA, Mar 11, 18

Ye C, 2003. Environmental behavior of the herbicide acetochlor in soil [J]. Bull Environ Contam Toxicol, 71(5): 919—923.

Zhang Q R, Zhou Q X, Zhang H W *et al.*, 2004. Joint effects of acetochlor and Cu^{2+} on indigenous bacterial communities in phaeozem[J]. Acta Scientiae Circumstantiae, 24(2): 326—332.

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Introduction to the State Key Laboratory of Environmental Chemistry and Ecotoxicology (SKLECE)

The State Key Laboratory of Environmental Chemistry and Ecotoxicology (SKLECE) was established in 2004 with the authorization of the Ministry of Science and Technology of China. SKLECE originated from two laboratories formed in 1975, Inorganic Analytical Chemistry Laboratory and Organic Analytical Chemistry Laboratory of the Research Center for Eco-Environmental Sciences. The two laboratories were combined in 1996 into the Open Laboratory of Environmental Chemistry and Ecotoxicology. In 2002, it became the Key Laboratory of Environmental Chemistry and Ecotoxicology, the Chinese Academy of Sciences.

SKLECE has played a leading role in the development of environmental chemistry research in China. Since 1970's the laboratory has led the study on environmental chemistry of persistent toxic chemicals, and developed a series of standardized analytical methods for environmental monitoring. It has also carried out extensive basic research in the areas of environmental analytical chemistry, environmental chemical behavior and ecotoxicology, and made a number of original achievements.

SKLECE has 37 staff members including 1 member of the Chinese Academy of Sciences, 1 member of the Chinese Academy of Engineering, 14 Ph.D supervisors, and 23 senior researchers. Among them there are 5 "Outstanding Young Scientist" and 4 "One Hundred Talents" recipients. There are also 88 graduate students, postdoctoral research assistants and visiting scholars.

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