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Toxic effects of acetochlor and methamidophos on earthworm *Eisenia fetida* in phaiozem, northeast China

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Abstract: Acetochlor and methamidophos are two important agrochemicals which are widely applied to agricultural production in northeast China. The investigation on the earthworm *Eisenia fetida* as an important type of soil animals exposed to single and binary-combined contamination of acetochlor and methamidophos was thus carried out. The single toxic effect test showed that the two agrochemicals had their toxicity to the earthworms living in phaiozem. Acetochlor had a stronger acute toxic effect on the earthworms than methamidophos. The mortality of the earthworms exposed to individual acetochlor and methamidophos changed with an increase in the exposure time and the exposed concentrations. The LD_{50} value of acetochlor and methamidophos toxic to the earthworms was 115.6—275.3 and 29.5—228.6 mg/kg, respectively. The weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects of acetochlor and methamidophos in phaiozem. When considering both the mortality and the body-weight change, the combined pollution of acetochlor and methamidophos in phaiozem resulted in their synergic toxic effects on the earthworms.
Keywords: ecological safety; earthworm *Eisenia fetida*; acetochlor; methamidophos; combined pollution; soil ecotoxicology

Introduction

With the increase in types and amount of pollutants releasing into soil ecosystems, research on the joint effects of more than one pollutant in soil-plant, soil-animal and soil-microorganism systems is increasingly concerned (Zhou and Gao, 1994; Zhou *et al.*, 1997; Cheng and Zhou, 2002; Hu *et al.*, 2004; Scott-Fordsmand *et al.*, 2004; Ye *et al.*, 2004; Song *et al.*, 2005). In agricultural ecosystems, the combined pollution is becoming evident because more and more agrochemicals applied to increase soil fertility for agricultural production and soil health for the quality of agricultural products are changing into toxic substances (Zhou *et al.*, 1997; Scott-Fordsmand *et al.*, 2004; Ye *et al.*, 2004; Gao *et al.*, 2005; Yu *et al.*, 2005). In view of the importance of ecological safety, more and more attention has been paid to soil ecotoxicology of earthworms in recent years (Abdul *et al.*, 1996; Furst, 2002; Johnson *et al.*, 2002; Miyazaki *et al.*, 2002; Scott-Fordsmand *et al.*, 2004; Zhou *et al.*, 2004; Ma, 2005).

Phaiozem is a typical soil with high fertility for agricultural production, which is mainly distributed in northeast China and centered in Heilongjiang Province and Jilin Province of China (Shen, 1998). According to Meng and Zhang (1998), the total area of phaiozem is approximately 60000 km^2, although the estimate is presently controversial. From the year 1970 to 2000, the food production from phaiozem increased 7 times. In particular, chemical fertilizers and pesticides used to maintain the agricultural productivity of phaiozem have been contributing to the main increase in food production in the last decades in this area (Shen, 1998; Xiao *et al.*, 2004). Nowadays the phaiozem area in northeast China has become an important commodity grain base in the whole nation (Shen, 1998; Guo and Zhou, 2004). However, excessive use of agricultural chemicals has adversely affected the quality of groundwater, and significantly changed the pedological properties of phaiozem (Liang *et al.*, 2001; Guo and Zhou, 2004; Yu *et al.*, 2005). Acetochlor and methamidophos are two of the most important agrochemicals with good sales and high dosage in one of the most important agricultural area of China (Liang *et al.*, 2001; Zhou and Huang, 2001; Zhou *et al.*, 2004; Zhou, 2005). It has been proposed by our recent investigation and other researchers that soil animals such as earthworms and eelworms that are significant contributors to biomass and fertility of many temperate soils (Liang *et al.*, 2001; Garca and Fragoso, 2002; Zhang *et al.*, 2004) are becoming extinct in the area, and the security of soil ecosystems in the area has been threatened. However, relationships between death and extinction of earthworms in the area and the application of acetochlor and methamidophos are still vague. Although the mutagenicity of acetochlor to male rat germ cells (Ashby *et al.*, 1997) and sub-chronic administration of low doses of methamidophos in male and female rats (Temerowski and van der Staay, 2005) were reported, there is no literature involving in toxic action of earthworms by acetochlor and methamidophos. Thus, joint toxic effects of acetochlor

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Table 2 Concentrations of Cu, Zn, Pb, Cd and P (mg/kg) in shoots and roots of *Vicia faba* with mycorrhizal (GM) and without inoculation treatment (NM) in the soil

	Treatment	Cu	Zn	Pb	Cd	P
Shoot	NM	10.9±3.4^a	86±2.7^a	3.3±1.1^a	2.4±0.8^a	1991±176.6^b
	GM	15±2.0^a	116±20.2^a	0.27±0.1^b	1.9±0.7^a	2222±23.7^a
Root	NM	724±23.1^b	566±45.3^b	273.1±2^a	55.5±9.4^a	1779±44^a
	GM	997±106.4^a	831±16.2^a	370.5±19.6^a	73.5±1.8^a	1414±53.4^b

Notes: Results are expressed as the means±*SE* (n=3); different letters following the figures in the Table indicate significant difference in the means of inoculation and non-inoculated treatments by LSD at 5% level

Table 3 The ratio of Cu, Zn, Pb, Cd and P content of root to that of shoot under different treatments in the contaminated soil (*R/S*)

Treatments	Cu	Zn	Pb	Cd	P
NM	35.2±4.2^a	3.2±0.3^a	35.8±2.5b^b	11.3±1.9^b	0.4±0.07^a
GM	49.6±9.9^a	5.3±1.0^a	987.6±216.3^a	28.4±5.2^a	0.4±0.04^a

Notes: It is the same as Table 2

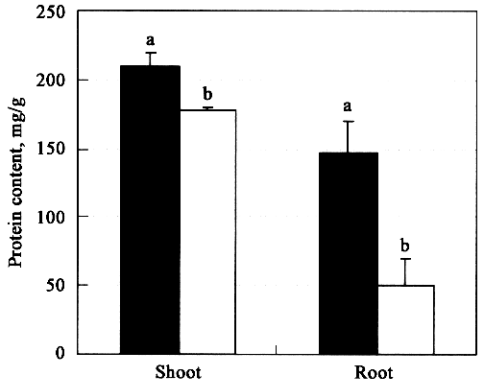


Fig.2 Shoot and root protein concentration of *Vicia faba* grown in soil with combined contamination
The same as Fig.1

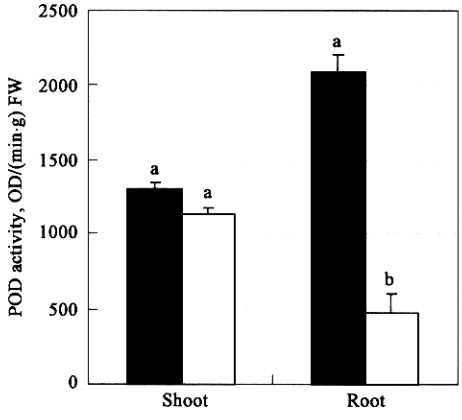
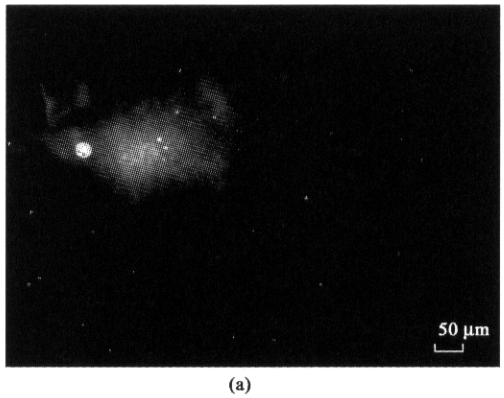


Fig.3 Shoot and root POD activity of *Viaia faba*
The same as Fig.1

Typical comet images of DNA damage in the leaves of *Vicia faba* are shown in Fig.4. In the treatments with GM the comet images had smaller comet tails and fainter fluorescent density, which indicates that there was less DNA migration from the nuclei. The tail length, tail DNA, TM value and OTM value were used to quantify DNA damage (Fig.5a, b, c). All these parameters were significantly lower in GM plants compared to NM plants, showing that the colonization of GM decreased the DNA damages induced by the HMs.



(a)

(b)

Fig.4 Comet images formed by DNA migration in electrophoresis field in the cells of *Vicia faba* leaves
a. NM plants; b. GM plants

3 Discussion

Fuyang District environmental problems associated with fast development of township and households during mine exploitation, appearing in China. Large area of arable land is occupied and contaminated with solid wastes and sludge from mine refining containing many kinds of HMs. The farmland has also been contaminated by irrigation with the wastewater from the factory. This mode of irrigation causes deterioration in soil quality.

It has been suggested that mycorrhizas are beneficial to plant nutrient uptake especially to P uptake (Smith and Read, 1997; Jayachandran and

(Ashby *et al.*, 1997), and toxic to ecosystems (Archer and Shogren, 2001; Nemeth-Konda *et al.*, 2002). The experiment showed that the toxic response of the earthworms to acetochlor was very acute and strong. There was an obvious poisonous effect of acetochlor on the earthworm *Eisenia fetida* even when acetochlor in soil was at the low tested concentration ($\leqslant$ 164.0 mg/kg). After they were exposed to the high concentrations (including 337.0, 428.0 and 545.0 mg/kg) of acetochlor only in several seconds, the tested earthworms twisted and leaped violently. With an increase in the exposure time, the tache of the earthworms became bagged, and the earthworm bodies became soft and rotten partially, in particular, some earthworms lost the capability of getting away from acetochlor pollution in soil. Obviously, the skin infiltration of earthworms could be the main toxic mode of acetochlor acting on earthworms (Gustin *et al.*, 2005).

The mortality of the earthworms exposed to acetochlor increased with the exposure time. There were different shapes of the curves to depict the toxic effects which varied with the concentration of acetochlor. When the concentration of acetochlor was equal to the mean (337.0 mg/kg) of MLC and LC_{100}, the caved curves were in the "S"-like shape. When it was lower than the concentration, the shape of the curves was concave. This indicated that the death of the earthworms under the concentrations appeared slowly at the beginning, then quickly. While it was higher than the concentration, the shape of the curves was convex, which means that the death of earthworms under the concentrations took place quickly first, then slowly. The mortality of the earthworms was also related to the concentration of acetochlor in phaiozem. The changing trend can be depicted using following regression equation:

$$Y(t)=a\ln^2 X_1+b\ln X_1+c \quad (1)$$

Where X_1 is the concentration (mg/kg) of acetochlor in soil, $Y(t)$ is the mortality of earthworms during various exposure time, t is the exposure time. $Y(t)$ changed with the exposure time. According to the Equation (1), LD_{50} of acetochlor to the earthworms was 115.6—275.3 mg/kg. The value of LD_{50} decreased with an increase in the exposure time. In other words, the ecological toxicity of acetochlor to the earthworms strengthened with an increase in the exposure time.

According to Table 3, the weight change of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects of acetochlor. Generally speaking, the body weight of the tested earthworms can be lost observably, when there is no dead earthworm under the low concentration of acetochlor. Of course, the weight change of the earthworms can be determined when the exposed time is too short to induce the death of earthworms (Abdul *et al.*, 1996; Scott-Fordsmand *et al.*, 2004). The loss in body weight changing with time was obviously related to the amount of acetochlor added to the tested soil.

Table 3 Mortality and the body-weight change of the earthworms exposed to various concentrations of acetochlor and methamidophos

Pollutant	Concentration, mg/kg	6-d exposure		12-d exposure		18-d exposure	
		Mortality, %	Weight change, mg	Mortality, %	Weight change, mg	Mortality, %	Weight change, mg
Acetochlor	0	0	0.022±0.0018	0	0.046± 0.0022	0	0.106 ± 0.0013
	164.0	0	-0.020±0.0005	4.69	-0.040 ± 0.0015	11.37	-0.053 ± 0.0034
	209.0	0	-0.055 ± 0.0035	32.81	-0.062 ± 0.0033	65.62	-0.112 ± 0.0072
	265.0	12.50	-0.066 ± 0.0043	51.56	-0.073 ± 0.0061	100	/
	337.0	75.00	-0.087 ± 0.0037	75.88	-0.099 ± 0.0054	100	/
	428.0	100	/	100	/	100	/
	545.0	100	/	100	/	100	/
Methamidophos	0	0	0.010 ±0.0007	0	0.045 ± 0.0011	0	0.060 ± 0.0017
	118.4	6.25	-0.061± 0.0033	46.87	-0.046± 0.0009	65.75	-0.081± 0.0025
	151.2	18.75	-0.088± 0.0017	65.63	-0.069 ±0.0016	89.67	-0.109± 0.0055
	192.0	43.75	-0.094 ±0.0054	88.32	-0.094 ±0.0041	100	/
	244.0	56.25	-0.119 ±0.0078	100	/	100	/
	310.4	68.70	-0.065 ±0.0023	100	/	100	/
	394.4	87.50	-0.037 ±0.0021	100	/	100	/

2.2 Toxic effects of methamidophos

Methamidophos is regarded as an insecticide with strong toxicity to living organisms and ecosystems (Temerowski and van der Staay, 2005; Yu *et al.*, 2005). However, the toxicity of methamidophos to the earthworms was weaker than that of acetochlor. After having been exposed to methamidophos, there was no visible poisonous symptom of earthworms at the beginning. With an increase in the exposure time, the earthworm bodies became red and swollen, stiffened, and crooked, the tache of the earthworms became tumefacient, and the back of the earthworms seeped yellow body fluids after it was filliped. This might be attributed to the fact that methamidophos can

dominatingly invade into earthworm bodies by mouth (Abdul *et al.*, 1996; Zhou *et al.*, 2004). Different from acetochlor, the skin contact of earthworms is not main toxic pattern of methamidophos acting on earthworms (Kikuchi *et al.*, 2000; Zhou *et al.*, 2004).

The mortality of earthworms exposed to methamidophos under experimental conditions had also an increasing trend with time. The shape of each caved curve was similar, just like "S", which means that the death of earthworms took place slowly at the first 2—6 d of an exposure to methamidophos, then quickly, and slowly afterwards.

There were significant positive correlations between the mortality of earthworms and the logarithmic value of methamidophos concentrations in phaiozem. The corresponding regression equations can be expressed as follows:

$$Y = a\ln X_2 + b \qquad (2)$$

Where X_2 is the concentration of methamidophos in soil. According to the Equation (2), LD_{50} of the earthworms exposed to methamidophos was 29.5—228.6 mg/kg. Similar to acetochlor, the value of LD_{50} also decreased with an increase in the exposure time. This indicates that the toxicity of methamidophos to earthworms is the function of the exposure time (Zhou *et al.*, 2004).

According to Table 3, it was also shown that the body-weight change of earthworms was a more sensitive indicator compared to the mortality in diagnosing toxic effects of methamidophos, in particular, when the concentration of methamidophos in soil was low and the exposure time was short. However, there were no regular dose-effect relationships between the body-weight change of earthworms and the concentration of methamidophos in soil.

2.3 Joint effects of acetochlor and methamidophos

Joint effects of acetochlor and methamidophos on the mortality of earthworms were obviously affected by changing concentration combinations of acetochlor and methamidophos added to the soil. According to the changes in the mortality of earthworms by the two pesticides, the joint toxicity of acetochlor and methamidophos was higher than the addition of individual acetochlor or methamidophos toxicity to the earthworms. The interaction between acetochlor and methamidophos was always synergistic compared with the toxicity of individual acetochlor and methamidophos in acting on the earthworms (Table 4). In other words, the combined pollution of acetochlor and methamidophos in phaiozem resulted in their synergic toxic effects on earthworms as an important component of agricultural ecosystems.

Due to the synergism, all the tested earthworms were dead only after a 10-d exposure when the low concentration (164.0 mg/kg) of acetochlor combined with various concentrations of methamidophos, and all the tested earthworms were died only after a 6-d exposure when the high concentration (265.0 mg/kg) of acetochlor combined with various concentrations of methamidophos.

Table 4 Joint effects of methamidophos (Met) and acetochlor (Ace) on the mortality of earthworms after a 6–d exposure

Ace, mg/kg	Mortality, %	Met, mg/kg	Mortality, %	Ace+Met, mg/kg	Mortality, %	Mode of joint action
164	0	118.4	6.25	164+118.4	25.00	Synergism
164	0	151.2	18.75	164+151.2	31.25	Synergism
164	0	192.0	43.75	164+192.0	62.50	Synergism
164	0	244.0	56.25	164+224.0	81.25	Synergism
164	0	310.4	68.75	164+310.4	100	Synergism
265	12.5	118.4	6.25	265+118.4	25.00	Synergism
265	12.5	151.2	18.75	265+151.2	31.25	Synergism
265	12.5	192.0	43.75	265+192.0	62.50	Synergism
265	12.5	244.0	56.25	265+244.0	81.25	Synergism
265	12.5	310.4	68.75	265+310.4	100	Synergism

Table 5 Weight changes of the earthworms by combined pollution of acetochlor (Ace) and methamidophos (Met)

Exposure time, d	Ace+Met, mg/kg	Weight change (mg) by combined pollution	Addition of weight changes by single Ace and Met	Mode of joint action
6	164 + 118.4	-0.088 ±0.0013	-0.081 ± 0.0011	Synergism
6	164 + 151.2	-0.112± 0.0053	-0.108± 0.0033	Synergism
6	164 + 192.0	-0.126± 0.0033	-0.114 ±0.0028	Synergism
6	164 + 224.0	-0.144± 0.0078	-0.139 ± 0.0031	Synergism
6	265 + 118.4	-0.138± 0.0023	-0.127 ± 0.0046	Synergism
6	265 + 151.2	-0.159± 0.0035	-0.154± 0.0038	Synergism
6	265 + 192.0	-0.171± 0.0047	-0.160± 0.0066	Synergism
6	265 + 244.0	-0.195± 0.0035	-0.185 ±0.0051	Synergism
10	164 + 118.4	-0.098± 0.0012	-0.089 ±0.0019	Synergism
10	164 + 151.2	-0.116± 0.0019	-0.106 ±0.0021	Synergism
10	164 + 192.0	-0.125± 0.0043	-0.123 ±0.0021	Synergism
10	164 + 224.0	-0.157± 0.0027	-0.157 ±0.0028	Synergism
10	265 + 118.4	-0.134 ±0.0051	-0.099 ±0.0008	Synergism
10	265 + 151.2	-0.178± 0.0044	-0.173± 0.0036	Synergism
10	265 + 192.0	-0.186± 0.0036	-0.168 ±0.0025	Synergism
10	265 + 244.0	-0.201± 0.0081	-0.193± 0.0037	Synergism

Obviously, combined pollution of acetochlor and methamidophos had an adverse influence on the growth and development of earthworms. The body weight of earthworms changed with changing concentration combinations of acetochlor and methamidophos added to the soil. The weight loss of earthworms by combined pollution of acetochlor and methamidophos was bigger than the summation of the weight loss of earthworms by individual acetochlor and methamidophos (Table 5). In other words, the interaction between acetochlor and methamidophos

had synergic toxic effects on the growth and development of the earthworms. Thus, we suggest that we should avoid the simultaneous application of acetochlor and methamidophos in agricultural production in order to sustain soil health (Zhou, 2005; Yu *et al.*, 2005).

3 Conclusions

Acetochlor had a strong toxic effect on the earthworms *Eisenia fetida* because it mainly exerted its toxic action by way of the skin infiltration. On the contrary, methamidophos entered into the earthworm bodies and exerted its toxic action by mouth dominatingly. The mortality of earthworms by individual acetochlor or methamidophos changed with an increase in the exposure time and the concentration of individual acetochlor or methamidophos. The value of LD_{50} of acetochlor and methamidophos decreased with an increase in the exposure time. The body-weight change of earthworms was a more sensitive toxic indicator of acetochlor or methamidophos compared to the mortality of earthworms. However, there was no regular dose-effect relationship based on the body-weight change of the earthworms. The combined pollution of acetochlor and methamidophos resulted in obviously synergistic effects on the mortality and synergistic stress on the body-weight change of the earthworms, thus inducing the unsafety of soil ecosystems with an increase in earthworm ecotoxicity.

References:

Abdul M M, Abdul R, Bouche M B, 1996. Earthworm toxicology: from acute to chronic test[J]. Soil Biol Biochem, 29(3/4): 699—703.

Archer D W, Shogren J F, 2001. Risk-indexed herbicide taxes to reduce ground and surface water pollution: an integrated ecological economics evaluation[J]. Ecol Econ, 38: 227—250.

Ashby J, Tinwell H, Lefevre P A *et al.*, 1997. Evaluation of the mutagenicity of acetochlor to male rat germ cells [J]. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 393(3): 263—281.

Cheng Y, Zhou Q X, 2002. Ecological toxicity of reactive X-3B red dye and cadmium acting on wheat (*Triticum aestivum*)[J]. Journal of Environmental Sciences, 14(1): 136—140.

Domínguez J, Velando A, Ferreiro A, 2005. Are *Eisenia fetida*(Savigny, 1826) and *Eisenia andrei* (Oligochaeta, Lumbricidae) different biological species?[J]. Pedobiologia, 49(1): 81—87.

Furst A, 2002. My saga with earthworms [J]. Food and Chemical Toxicology, 40: 789—791.

Gao H J, Jiang X, Wang F *et al.*, 2005. Residual levels and new inputs of chlorinated POPs in agricultural soils from Taihu Lake region [J]. Pedosphere, 15(3): 301—309.

Garca J A, Fragoso C, 2002. Growth, reproduction and activity of earthworms in degraded and amended tropical open mined soils: laboratory assays[J]. Appl Soil Ecol, 20: 43—56.

Guo G L, Zhou Q X, 2004. Contaminative trends of heavy metals in phaiozem of northeast China [J]. Journal of the Graduate School of the Chinese Academy of Sciences, 21(3): 386—392.

Gustin C A, Moran S J, Fuhrman J D *et al.*, 2005. Applicator exposure to acetochlor based on biomonitoring [J]. Regulatory Toxicology and Pharmacology, 43(2): 141—149.

Hong X, 1987. Analytical methods for environmental pollution [M]. Beijing: Science Press. 487.

Hu X F, Wu H X, Hu X *et al.*, 2004. Impact of urbanization on Shanghai's soil environmental quality [J]. Pedosphere, 14 (2): 151—158.

Johnson D L, Jones K C, Langdon C J *et al.*, 2002. Temporal changes in earthworm availability and extractability of polycyclic aromatic hydrocarbons in soil[J]. Soil Biol Biochem, 34: 1363—1370.

Kikuchi M, Sasaki Y, Wakabayashi M, 2000. Screening of organophosphate insecticide pollution in water by using daphnia magna[J]. Ecotoxicol Environ Saf, 47: 239—245.

Li Y K, 1983. The routine analytical methods in soil science and agricultural chemistry[M]. Beijing: Science Press.

Liang W J, Zhang W M, Li W G, 2001. Effect of chemical fertilizer on nematode community composition and diversity in the black soil region[J]. Biodiver Sci, 9: 237—240.

Lu R K, 2000. Analytical methods in soil science and agricultural chemistry [M]. Beijing: China Agricultural Science and Technology Press.

Ma W C, 2005. Critical body residues (CBRs) for ecotoxicological soil quality assessment: copper in earthworms [J]. Soil Biology and Biochemistry, 37(3): 561—568.

Meng K, Zhang X Y, 1998. Degradation and ecological restoration of phaiozem in the Songnen Plain of China [J]. Chinese Journal of Soil Science, 29(3): 100—102.

Miyazaki A, Amano T, Saito H *et al.*, 2002. Acute toxicity of chlorophenols to earthworms using a simple paper contact method and comparison with toxicities to fresh water organisms [J]. Chemosphere, 47: 65—69.

Nemeth-Konda L, Füleky G, Morovjan G *et al.*, 2002. Sorption behaviour of acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian agricultural soil [J]. Chemosphere, 48(5): 545—552.

Scott-Fordsmand J J, Van Gestel C A M, Weeks J M, 2004. Third international workshop on earthworm ecotoxicology: ecological, physiological, and physicochemical factors in earthworm ecotoxicology[J]. Ecotoxicol Environ Saf, 57(1): 1—3.

Shen S M, 1998. Chinese soil fertility[M]. Beijing: Science Press.

Song Y F, Gong P, Zhou Q X *et al.*, 2005. Phytotoxicity assessment of phenanthrene, pyrene and their mixtures by a soil-based seedling emergence test [J]. Journal of Environmental Sciences, 17 (4): 580—583.

Temerowski M, van der Staay V J, 2005. Absence of long-term behavioral effects after sub-chronic administration of low doses of methamidophos in male and female rats [J]. Neurotoxicology and Teratology, 27(2): 279—297.

van Gestel C A M, Ma W-C, Smit C E, 1991. Development of QSARs in terrestrial ecotoxicology: earthworm toxicity and soil sorption of chlorophenols, chlorobenzenes and dichloroaniline [J]. The Science of the Total Environment, 109/110: 589—604.

Xiao H, Zhou Q X, Liang J D, 2004. Single and joint effects of acetochlor and urea on earthworm *Esisenia foelide* populations in phaiozem [J]. Environmental Geochemistry and Health, 26 (2): 277—283.

Ye C M, Wang C X, Jin L Z, 2004. Environmental chemistry in the 21th century[M]. Beijing: Science Press.

Yu Y, Zhou Q X, He Z L, 2005. Effects of methamidophos and glyphosate on copper sorption-desorption behavior in soils [J]. Science in China(Ser. C), 48(supp. I): 67—75.

Zhang W, Song Y F, Sun T H *et al.*, 2004. Soil nematode as a bioindicator of environmental pollution [J]. Chinese Journal of Applied Ecology, 15(10): 1973—1978.

Zhou Q X, Gao Z M, 1994. Compound contamination and secondary ecological effects of Cd and As in soil-alfalfa ecosystems [J]. Journal of Environmental Sciences, 6(3): 330—336.

Zhou Q X, Huang G H, 2001. Environmental biogeochemistry and global environmental changes[M]. Beijing: Science Press.

Zhou Q X, Kong F X, Zhu L, 2004. Ecotoxicology[M]. Beijing: Science Press.

Zhou Q X, Zhu Y M, Chen Y Y, 1997. Food-security indexes related to combined pollution of chromium and phenol in soil-rice systems [J]. Pedosphere, 7(1): 15—24.

Zhou Q X, 2005. Health soil science: soil health quality and safety of agricultural products[M]. Beijing: Science Press.

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