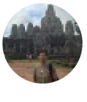
Acute Toxicity of Organophosphorus and Pyrethroid Insecticides to Bombyx mori</I

ARTICLE *in* JOURNAL OF ECONOMIC ENTOMOLOGY · MAY 2008

Impact Factor: 1.61 · DOI: 10.1603/0022-0493(2008)101[360:ATOOAP]2.0.CO;2 · Source: PubMed



Xiao-Yue Hong
Nanjing Agricultural University

Acute Toxicity of Organophosphorus and Pyrethroid Insecticides to *Bombyx mori*

ZHI-YONG ZHANG,[1,2] DONG-LAN WANG,[2] ZHI-JUAN CHI,[1,2] XIAN-JIN LIU,[2,3] AND XIAO-YUE HONG[1,3]

J. Econ. Entomol. 101(2): 360–364 (2008)

ABSTRACT Acute toxicities of two organophosphorus insecticides (dichlorvos and phoxim), four pyrethroid insecticides (permethrin, tetramethrin, bifenthrin, and ethofenprox), and their combined uses to the third instar of the silkworm, *Bombyx mori* (L.), were determined by feeding the insect with the insecticide-treated mulberry, *Morus albus* (L.), leaves. Twenty-four and 48 h after treatment, toxicity levels of all insecticides to the silkworm were in the very high or high range, and the LC_{50} values of permethrin, tetramethrin, bifenthrin, ethofenprox, dichlorvos, and phoxim were 1.60 and 0.75, 3.86 and 2.83, 0.09 and 0.06, 2.87 and 0.80, 6.63 and 4.11, and 1.05 and 0.45 mg liter^{-1}, respectively. The toxicity levels of 50:50 binary mixtures of organophosphorus and pyrethroid insecticides to the silkworm were in the high or middle range. Twenty-four and 48 h after treatment, the LC_{50} values of 50:50 binary mixtures permethrin + phoxim, permethrin + dichlorvos, tetramethrin + phoxim, tetramethrin + dichlorvos, bifenthrin + phoxim, bifenthrin + dichlorvos, ethofenprox + phoxim, and ethofenprox + dichlorvos to the silkworm were 1.49 and 0.85, 1.24 and 0.79, 2.20 and 1.08, 14.62 and 13.16, 0.33 and 0.13, 0.12 and 0.10, 2.81 and 1.37, and 4.82 and 3.00 mg liter^{-1}, respectively. Based on the combinations coefficient values, the toxicities of binary mixtures of organophosphorus and pyrethroid insecticides had additive effect except for the binary mixture of etramethrin + dichlorvos, which showed antagonism effect.

KEY WORDS insecticide, silkworm, toxicity

Sericulture has a history of >5,000 yr in China, and it has been very popular particularly in eastern and southern parts of the country where the climate is suitable for the growth of mulberry, *Morus albus* (L.), trees and silk products are major sources of income for farmers (Wang 2003, Zhang 2005). In recent years, one problem in sericulture has become prominent in China, i.e., the sensitivity of the silkworm, *Bombyx mori* (L.), to many pesticides, and the death of silkworm (Cui et al. 2003, Wu et al. 2006). This is primarily due to the application of pesticides in the control of pests in crop fields that are close to mulberry orchards and also to the direct control of mulberry pests (Han 1997, Cui et al. 2003, Etebari et al. 2007).

Previous studies indicated that organophosphorus and pyrethroid insecticides were commonly used in China (Zhang et al. 2006). Frequent use of pesticides can cause accumulation of pesticide residues in the environment. Thus, pesticides can have secondary toxic effects on the nontarget organisms (Vyjayanthi and Subramanyam 2002). Permethrin, tetramethrin, bifenthrin, and ethofenprox are members of the pyrethroid family. They are used in agriculture for controlling pests of vegetables, cotton, corn, tea trees, and fruit trees, and in houses (Dominguesa et al. 2005, DeLorenzo et al. 2006). But because of the high toxicity of these pyrethroids to aquatic organisms, products are registered as "restrictedly applied pesticides." Dichlorvos and phoxim belong to the organophosphorus family of insecticides. They are effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies, and they also are used to treat a variety of parasitic worm infections in dogs, livestock, and humans (Zhu et al. 2002, Yarsan and Cakir 2006).

Several studies have been carried out on the toxicity of the aforementioned pesticides to some nontarget organisms, including fish (Bermádez-Saldanîsa Josè et al. 2006, Capkin et al. 2006, Venkateswara 2006, Yan et al. 2006), earthworms (Reinecke and Reinecke 2007), ants and spider (Adu-Acheampong and Ackonor 2005), springtails (Campiche et al. 2006), soil protozoa (Andersen and Winding 2004), plants (Kate and Ling 2004), and plankton (Cedergreen and Streibig 2005). However, little information is available on nontarget effects of pesticide on the silkworm. The main purposes of this study were to 1) evaluate acute toxicity of two organophosphorus (dichlorvos and phoxim) and four pyrethroids (permethrin, tetramethrin, bifenthrin, and ethofenprox) insecticides and the 50:50 binary mixtures of organophosphorus and pyrethroid insecticides to silkworm; 2) comparatively

[1] Department of Entomology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China.
[2] Institute of Food Safety Research and Inspection, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu 210014, China.
[3] Corresponding authors, e-mail: jaasliu@jaas.ac.cn and xyhong@njau.edu.cn respectively.

study the joint toxicity of organophosphorus and pyrethroid insecticides to silkworm; and 3) provide guidelines for pesticide use in mulberry orchards especially in those next to field crops.

Materials and Methods

Sources and Rearing of Silkworm. The eggs of the bivoltine silkworm (hybrid: Feng × 54A) were purchased from the Institute of Silkworm, Chinese Academy of Agricultural Sciences (Zhengjiang, Jiangsu Province, China). Silkworms were reared under laboratory conditions at 25 ± 2°C, 70–85% RH, and a photoperiod of 12:12 (L:D) h (Miao and Bharathi 2003). All silkworms were fed twice per day with fresh *M. alb*a leaves harvested from an irrigated mulberry orchard at Jiangsu Academy of Agricultural Sciences (Nanjing, Jiangsu Province, China), which had not been treated with any pesticides.

Insecticides. Permethrin (technical grade, 90%), tetramethrin (technical grade, 92%), bifenthrin (technical grade, 90%) and phoxim (technical grade, 90%) were obtained from Jiangsu Yangnong Chemical Company Ltd. (Yangzhou, Jiangsu Province, China); ethofenprox (technical grade, 95%) and dichlorvos (technical grade, 95%) were obtained from Dafeng Fengshan Pesticide Company Ltd. (Dafeng, Jiangsu Province, China) and Jiangsu Golden Phoenix Agrochemical Company Ltd. (Changzhou, Jiangsu, China), respectively. The technical grade insecticides were prepared in acetone separately, and the concentrations of stock solutions for each insecticide were 2,000 μg ml^{-1}. The 50:50 binary mixtures stocks of permethrin + dichlorvos, permethrin + phoxim, tetramethrin + dichlorvos, tetramethrin + phoxim, bifenthrin + dichlorvos, bifenthrin + phoxim, ethofenprox + dichlorvos, and ethofenprox + phoxim were prepared by the insecticide stock solutions, and the concentrations of 50:50 binary mixtures were 2,000 μg ml^{-1}. All stock solutions were stored at 4°C until use. Each stock solution was diluted by deionized water for toxicity determining and four drippings Triton X-100 (Shanghai Chemical Reagent Ltd., Shanghai, China) were added for emulsification.

Experimental Design. Based on the methods recommended by the State Environmental Protection Administration of China (SEPAC 1990, Chen et al. 1991, Ma et al. 2005a), we designed an improved method of treating mulberry leaves for use in bioassays. Briefly, fresh mulberry leaves were washed by tap water and then air-dried. After veins were cut, 2.5 g ($\approx$20 cm^2) leaves were immersed in certain concentration of insecticide dilutions for 3 s. Then, the air-dried treated mulberry leaves were chopped to strips $\approx$2 mm in length and placed in the petri dish (9 cm in diameter). Twenty third instars were placed in each of petri dish. Silkworm larvae were allowed to feed on the treated mulberry leaves for 24 and 48 h. Five concentrations were set up for each insecticide and 50:50 binary insecticides mixtures. Three replicates (one dish per replicate) for each concentration were conducted. The cultural conditions were the same as the above rearing conditions. Simultaneously, the carrier controls for each insecticide and mixtures of insecticides had an acetone and Triton X-100 equivalent to the maximum volume of stock insecticide, and the mortality of controls was below 5%.

Data Analysis. The mortalities of silkworm at 24 or 48 h after treatment were adjusted by Abbott's formula (Abbott 1925). The death standard for silkworm is immobility. The 50 or 95% of lethal concentration (LC_{50} or LC_{95}) values were determined using probit analysis. The combinations coefficient of binary mixtures of insecticides was analyzed according to the following formula proposed by Smyth et al. (1969):

$$Q = \text{predicted } LD_{50} \text{ of mixture} / \text{observed } LD_{50} \text{ of mixture}$$

$$1/\text{predicted } LD_{50} = P_a/LD_{50} \text{ of component A} + P_b/LD_{50} \text{ of component B}$$

where Q is the combinations coefficient. If the Q value is >2.7, the mixture indicates synergism; if the value is <0.4, the mixture shows antagonism; if the value is >0.4 and <2.7, the mixture has an additive effect (Meng 2000). P_a and P_b are the proportions of components A and B in the mixture, being 0.5 in this study.

The LC_{50} values were analyzed by Data Processing System 7.05 (Zhejiang University) by using probit analysis, but the Q values were calculated by Excel 2002 (Microsoft, Redmond, WA).

Results

Toxicity of Single Insecticides to Silkworm. The acute toxicity results of individual insecticide to silkworm are presented in Table 1. At 24 h after treatment, the LC_{50} values of all tested insecticides were from 0.09 to 6.63 mg liter^{-1}, and the LC_{95} values were from 0.29 to 17.41 mg liter^{-1}, respectively. At 48 h after treatment, the LC_{50} values of all tested insecticides were from 0.06 to 4.11 mg liter^{-1}, respectively, and the LC_{95} values of the insecticides were from 0.09 to 18.01 mg liter^{-1}, respectively. Based on LC_{50} values of these insecticides at 24 or 48 h, the order of toxicity levels of six insecticides tested against silkworm was as follows: bifenthrin > phoxim > permethrin > ethofenprox > tetramethrin > dichlorvos. The LC_{50} or LC_{95} values at 48 h were lower than that at 24 h. With reference to the pesticide acute toxicity to *B. mori* provided by Ma et al. (2005a), the toxicities of all tested insecticides to silkworm ranked high toxicity grade but except for bifenthrin, which was in the extremely high toxicity grade.

Toxicities of 50:50 Binary Mixtures of Insecticides to Silkworm. Data concerning the toxicities of binary mixtures insecticides to silkworm are presented in Table 2. Results indicated that at 24 h after treatment, the LC_{50} values of all tested insecticides mixtures to silkworm were from 0.12 to 14.62 mg liter^{-1}, respectively. And the LC_{95} values of the aforementioned insecticide mixtures were from 0.19 to 31.27 mg liter^{-1}, respectively. At 48 h after treatment, the LC_{50} values

Table 1. Toxicity of single insecticide against silkworm 24 and 48 h after treatment

Insecticide	Time (h)	LC_{50} (95% CI) mg liter^{-1}	LC_{95} (95% CI) mg liter^{-1}	Slope ± SE	χ^2	P	R^2	Toxicity grade[a]
Permethrin	24	1.60 (1.48–1.74)	2.44 (2.13–3.21)	8.97 ± 1.65	1.56	0.67	0.958	High toxic
	48	0.75 (0.57–0.88)	2.12 (1.56–4.49)	3.63 ± 0.84	0.55	0.91	0.971	High toxic
Tetramethrin	24	3.86 (3.54–4.17)	6.08 (5.26–8.60)	8.32 ± 1.83	1.07	0.78	0.953	High toxic
	48	2.83 (2.45–3.21)	5.94 (4.61–12.16)	5.12 ± 1.30	0.43	0.93	0.975	High toxic
Bifenthrin	24	0.09 (0.06–0.11)	0.29 (0.21–0.53)	3.19 ± 0.62	0.94	0.82	0.970	Extreme toxic
	48	0.06 (0.05–0.06)	0.09 (0.08–0.13)	8.19 ± 1.53	1.30	0.73	0.970	Extreme toxic
Ethofenprox	24	2.87 (2.10–4.09)	17.41 (8.81–142.92)	2.10 ± 0.56	0.50	0.92	0.967	High toxic
	48	0.80 (0.55–1.11)	4.95 (2.97–13.04)	2.08 ± 0.37	2.15	0.94	0.958	High toxic
Dichlorvos	24	6.63 (5.60–7.83)	16.75 (12.57–30.61)	4.08 ± 0.80	2.20	0.53	0.937	High toxic
	48	4.11 (3.09–5.32)	18.01 (11.56–45.58)	2.56 ± 0.50	2.67	0.45	0.928	High toxic
Phoxim	24	1.05 (0.80–1.42)	4.89 (2.87–17.64)	2.46 ± 0.55	0.15	0.99	0.996	High toxic
	48	0.45 (0.28–0.60)	2.37 (1.56–5.48)	2.27 ± 0.45	0.14	0.99	0.995	High toxic

[a] Extreme toxic, LC_{50} (at 24 or 48 h): <0.1000 mg liter^{-1}; high toxic, LC_{50} (at 24 or 48 h): 0.1000≈10.00 mg liter^{-1}; moderate toxic, LC_{50} (at 24 or 48 h): 10.0≈1,000 mg liter^{-1}; and low toxic, LC_{50} (at 24 or 48 h): >1,000 mg liter^{-1} (Ma et al. 2005a).

of the insecticides mixtures were from 0.10 to 13.16 mg liter^{-1} respectively, and the LC_{95} values of the insecticides mixtures were from 0.18 to 25.90 mg liter^{-1}, respectively. According to the LC_{50} values at either 24 or 48 h after treatment, the order of toxicity levels for eight binary mixtures of insecticides to silkworm was as follows: bifenthrin + dichlorvos > bifenthrin + phoxim > permethrin + dichlorvos > permethrin+ phoxim > tetramethrin + phoxim > ethofenprox + phoxim > ethofenprox + dichlorvos > tetramethrin + dichlorvos. Based on the data reference in Table 1, the acute toxicity of the binary mixture of tetramethrin + dichlorvos to silkworm was at the moderate toxicity grade, but the others were at the high toxicity grade.

Combination Coefficients of Binary Mixtures of Insecticides. The combination coefficients (Q) of binary mixtures of organophosphorus and pyrethroid insecticides calculated by the method of Smyth et al. (1969) are listed in Table 2. The combination coefficient values of all tested mixtures of insecticides were from 0.25 to 2.08. According to the Q values, the toxicities of binary mixtures of organophosphorus and pyrethroid insecticides were additive effect, except for the binary mixture of tetramethrin + dichlorvos, with a Q value was <0.4, showing antagonism.

Intoxication Symptoms of Silkworm. After treatment with organophosphorus insecticides, we observed that the intoxication symptoms of silkworm included severe blacking and dwindling of body, shaking of body, vomit, and less feeding. The toxicosis symptoms of silkworm caused by pyrethroid insecticides were the body in the shape of an "S" or "C," shortening of body, and less feeding. The intoxication symptoms of silkworm caused by binary mixtures of organophosphorus and pyrethroid insecticides were shortening of body with raising head and body, stiffness of body in the shape of an "S" or "C," vomiting, and reduced feeding.

Discussion

Zhu and Cui (2000) reported that the LC_{50} value of formulations of phoxim to silkworm was 0.7037 μg ml^{-1} 24 h after treatment, and intoxication symptoms of silkworm included shaking of the body, vomiting, and reduced feeding. Ma et al. (2005a) reported that

Table 2. Toxicity of 50:50 binary mixture of insecticides against silkworm 24 and 48 h after treatment

Mixture of insecticides (50:50)	Time (h)	LC_{50} (95% CI) mg liter^{-1}	LC_{95} (95% CI) mg liter^{-1}	Slope ± SE	χ^2	P	R^2	Toxicity grade[a]	Q
Permethrin + phoxim	24	1.49 (1.30–1.76)	3.33(2.49–6.83)	4.71 ± 1.07	0.81	0.85	0.970	High toxic	0.85
	48	0.85 (0.71–0.99)	1.96 (1.57–3.04)	4.55 ± 0.84	0.55	0.91	0.968	High toxic	0.66
Permethrin + dichlorvos	24	1.24 (1.15–1.34)	1.85 (1.63–2.33)	9.54 ± 1.69	0.45	0.93	0.989	High toxic	2.08
	48	0.79 (0.73–0.87)	1.28 (1.09–1.90)	7.96 ± 1.81	0.78	0.85	0.966	High toxic	1.61
Tetramethrin + phoxim	24	2.20 (1.73–2.66)	6.76 (4.97–12.48)	3.38 ± 0.64	0.36	0.95	0.988	High toxic	0.75
	48	1.08 (0.87–1.29)	2.94 (2.25–4.82)	3.79 ± 0.67	1.16	0.76	0.971	High toxic	0.72
Tetramethrin + dichlorvos	24	14.62 (12.49–16.87)	31.27 (25.04–47.87)	4.98 ± 0.90	1.79	0.62	0.962	Moderate toxic	0.33
	48	13.16 (11.61–14.81)	25.90 (21.08–39.85)	5.59 ± 1.09	2.74	0.43	0.920	Moderate toxic	0.25
Bifenthrin + phoxim	24	0.33 (0.28–0.39)	0.80 (0.60–1.47)	4.28 ± 0.84	1.94	0.59	0.953	High toxic	0.50
	48	0.13 (0.11–0.15)	0.31 (0.23–0.57)	4.26 ± 0.83	2.21	0.53	0.949	High toxic	0.81
Bifenthrin + dichlorvos	24	0.12 (0.12–0.13)	0.19 (0.17–0.24)	9.13 ± 1.62	1.76	0.62	0.952	High toxic	1.48
	48	0.10 (0.09–0.11)	0.18 (0.15–0.26)	6.45 ± 1.22	1.44	0.70	0.960	High toxic	1.18
Ethofenprox + phoxim	24	2.81 (2.59–3.03)	4.26 (3.79–5.30)	9.10 ± 1.58	0.48	0.92	0.985	High toxic	0.55
	48	1.37 (1.27–1.47)	2.00 (1.80–2.40)	10.06 ± 1.68	0.87	0.83	0.984	High toxic	0.42
Ethofenprox + dichlorvos	24	4.82 (4.58–5.04)	6.23 (5.79–7.15)	14.76 ± 2.60	1.16	0.76	0.973	High toxic	0.83
	48	3.00 (2.79–3.23)	4.41 (3.94–5.44)	9.84 ± 1.67	4.54	0.21	0.921	High toxic	0.45

[a] Extreme toxic, LC_{50} (at 24 or 48 h): <0.1000 mg liter^{-1}; high toxic, LC_{50} (at 24 or 48 h): 0.1000≈10.00 mg liter^{-1}; moderate toxic, LC_{50} (at 24 or 48 h): 10.0≈1,000 mg liter^{-1}; and low toxic, LC_{50} (at 24 or 48 h): >1,000 mg liter^{-1} (Ma et al. 2005a).

the LC_{50} value of formulations of permethrin to silkworm was 0.3291 $\mu g\ ml^{-1}$ 24 h after treatment, and intoxication symptoms included body shorting. Ma et al. (2006) also showed that the LC_{50} values of formulations of phoxim and dichlorvos were 0.6494 and 1.0713 $\mu g\ ml^{-1}$ respectively, 72 h after treatment. Zhu and Cui (2000) reported that the joint effect of the binary mixture of phoxim and fenpropathrin to silkworm was additive, after exposure 24 h. The intoxication symptoms of silkworm caused by these insecticides are similar to our results, although comparison of LC_{50} values reported earlier with those obtained in this study might not be meaningful, because various factors influenced bioassay results, such as silkworm source (e.g., species, size, and weight), environmental factors (e.g., temperature and humidity), and insecticide formulation.

Organophosphates have previously been shown to inhibit brain critical nervous system enzyme cholinesterase, which functions to rapidly destroy the ubiquitous neurotransmitter acetylcholine (Segall et al. 2003). Pyrethroids owe their insecticidal potency to a rapid pharmacofunctional disruption of the insect neuromuscular system and the secondary consequences of this disruption, rather than to any direct cytotoxicity (Ray and Fry 2006). The health effects of mixtures of pyrethroids and organophosphates deserve careful consideration (Yáñez et al. 2002). The joint effect of two or more compounds may show additive, antagonistic, or synergistic interactions or they may act on different systems and thus not interact. Furthermore, even a single chemical may have multiple effects and affect more than one organ system (Liu et al. 2006).

According to the Pesticide Management Regulations and the Implementation Method of Pesticide Management Regulations by the Chinese government (Ma et al. 2005b), the application registration must be done in China before the pesticide can be produced and the foreign pesticide is imported. The necessary safety and efficiency data and the pesticide sample must be submitted for the application process. Based on the features and the application of the pesticide, at least the toxicity data of the formulation for fish, birds, bees, and silkworm must be provided.

In China, mulberry plants are often damaged by insects such as *Diplosis mori* Yokoyama, *Baris deplanata* Roeloffs, *Phthonandria atrilineata* Butler, *Diaphania pyloalis* Walker, and *Apriona germari* Hope. To control these insect pests, insecticides are applied ineluctably, among which organophosphorus and pyrethroid insecticides or their mixtures account for most of the market. And method for application is often spraying (Han 1997). Our findings clearly demonstrated that the acute toxicity of pyrethroid insecticide bifenthrin is at extreme high grade, it should be prohibited in or near to mulberry garden. Other tested insecticides toxicities to silkworm are at high grade. So, these tested organophosphorus and pyrethroid insecticides are used carefully in mulberry plants. Although the toxicities of some binary mixtures of organophosphorus and pyrethroid insecticides to silkworm have additive effect, even antagonism effect (tetramethrin + dichlorvos), they are still at high or moderate toxicity grade. Therefore, it is critical to select appropriate insecticides when considering control of pests in mulberry orchards. It also is critical to provide long enough preharvest intervals for mulberries harvest and to monitor pesticide residue level in mulberry before harvest, to avoid unnecessary harm to silkworms.

Acknowledgments

We are grateful to Drs. Xiang-Yang Yu and Xin-Ming Zhang (Jiangsu Academy of Agricultural Sciences) for assistance with the research and to Dr. Renjie Hu (California Department of Health Services) for comments and suggestions on an earlier draft. We thank Jiangsu Provincial Department of Science and Technology for financial support for this research (grant BS2007096).

References Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Adu-Acheampong, R., and J. B. Ackonor. 2005. The effect of imidacloprid and mixed pirimiphos-methyl and bifenthrin on non-target arthropods of cocoa. Trop. Sci. 45: 153–154.

Andersen, K. S., and A. Winding. 2004. Non-target effects of bacterial biological control agents on soil protozoa. Biol. Fertil. Soils 40: 230–236.

Bermádez-Saldanîa José, M., and T. D. Cronin Mark. 2006. Quantitative structure—activity relationships for the toxicity of organophosphorus and carbamate pesticides to the rainbow trout *Onchorhyncus mykiss* 62. Pest Manag. Sci. 62: 819–831.

Campiche, S., K. Becker-van Slooten, C. Ridreau, and J. Tarradellas. 2006. Effects of insect growth regulators on the nontarget soil arthropod *Folsomia candida* (Collembola) Ecotoxicol. Environ. Saf. 63: 216–225.

Capkin, E., I. Altinok, and S. Karahan. 2006. Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. Chemosphere 64: 1793–1800.

Chen, R., Z. K. Dai, and D. J. Cai. 1991. The effect of deltamethrin on silkworm and its safety evaluation. China Environ. Sci. 11: 343–347.

Cedergreen, N., and J. C. Streibig. 2005. The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. Pest Manag. Sci. 61: 1152–1160.

Cui, Y., Y. Yang, and J. X. Yuan. 2003. The problems and solving methods of pesticide use in mulberry orchards. China Sericult. 24: 48–49.

DeLorenzo, M. E., L. Serrano, K. W. Chung, J. Hoguet, and P. B. Key. 2006. Effects of the insecticide permethrin on three life stages of the grass shrimp, *Palaemonetes pugio.* Ecotoxicol. Environ. Saf. 64: 122–127.

Dominguesa, V., A. Alvesb, M. Cabralc, and C. Delerue-Matosa. 2005. Sorption behaviour of bifenthrin on cork. J. Chromatogr. A 1069: 127–132.

Etebari, K., A. R. Bizhannia, R. Sorati, and L. Matindoost. 2007. Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. Pestic. Biochem. Physiol. 88: 14–19.

Han, S. Y. 1997. Chemical pesticide control of the main insects in mulberry. Pesticide 36: 6–8.

Kate, W. J., and N. Ling. 2004. Toxicity of the aquaculture pesticide cypermethrin to planktonic marine copepods. Aquacult. Res. 35: 263–270.

Liu, P., X. X. Song, W. H. Yuan, W. H. Wen, X. N. Wu, J. Li, and X. M. Chen. 2006. Effects of cypermethrin and methyl parathion mixtures on hormone levels and immune functions in Wistar rats. Arch. Toxicol. 80: 449–457.

Ma, H., H. Jiang, C. J. Tao, L. Liu, and K. Y. Wang. 2005a. Toxicity evaluation of twenty-seven pesticides to *Bombyx mori.* China J. Pestic. Sci. 7: 156–159.

Ma, H., K. Y. Wang, L. Liu, C. J. Tao, and W. G. Qu. 2005b. Advance of research on toxicology and safety evaluation of pesticides to silkworm. Pestic. Sci. Admin. 26: 15–17.

Ma, H., K. Y. Wang, H. Y. Wang, and S. D. Si. 2006. Selective toxicity of chlorfenapyr to silkworm and mulberry pests. Acta Entomol. Sin. 49: 599–603.

Meng, Z. Q. 2000. Environmental toxicology. China Environmental Science Press, Beijing, China.

Miao, Y. G., and D. Bharathi. 2003. Effect of prostaglandin $F_{2\alpha}$on gonadal carbohydrate metabolism of silkworm, *Bombyx mori* L. Prostaglandins Other Lipid Mediat. 70: 259–266.

Ray, D. E., and J. R. Fry. 2006. A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacol. Ther. 1111: 174–193.

Reinecke, S. A., and A. J. Reinecke. 2007. The impact of organophosphate pesticides in orchards on earthworms in the Western Cape, South Africa. Ecotoxicol. Environ. Saf. 66: 244–251.

Segall, Y., G. B. Quistad, S. E. Sparks, D. K. Nomura, and J. E. Casida. 2003. Toxicological and structural features of organophosphorus and organosulfur cannabinoid CB1 receptor ligands. Toxicol. Sci. 76: 131–137.

[SEPAC] State Environmental Protection Administration of China. 1990. Guidelines for chemical pesticide environmental safety evaluation. Pestic. Sci. Admin. 4: 7.

Smyth, H. F., C. S. Weil, J. S. West, and C. P. Carpenter. 1969. An exploration of joint toxic action: twenty seven industrial chemicals intubated in rats in all possible pairs. Toxicol. Appl. Pharmacol. 14: 340–347.

Venkateswara, R. J. 2006. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus.* Pestic. Biochem. Physiol. l 86: 78–84.

Vyjayanthi, N., and M.V.V. Subramanyam. 2002. Effect of fenvalerate-20EC on sericigenous insects I. food utilization in the late-age larva of the silkworm, *Bombyx mori* L. Ecotoxicol. Environ. Saf. 53: 206–211.

Wang, J. X. 2003. Management and pesticide safe use in mulberry pests. Jiangsu Sericult. 2: 6–8.

Wu, J. M., B. G. Chen, and X. H. Kong. 2006. The reasons and of preventing of poisoning of silkworm. Jiangsu Sericult. 2: 16–17.

Yan, D. Y., X. Jiang, G. F. Yu, Z. H. Zhao, Y. R. Bian, and F. Wang. 2006. Quantitative structure–toxicity relationships of organophosphorous pesticides to fish (*Cyprinus carpio*). Chemosphere 63: 744–750.

Yarsan, E., and O. Cakir. 2006. Effects of dichlorvos on lipid peroxidation in mice on subacute and subchronic periods. Pestic. Biochem. Physiol. 86: 106–109.

Yáñez, L., D. Ortiz, J. Calderón, L. Batres, L. Carrizales, J. Mejía, L, Artínez, E. García-Nieto, and F. Díaz-Barriga. 2002. Overview of human health and chemical mixtures: problems facing developing countries. Environ. Health Perspect. 110: 901–909.

Zhang, X. D. 2005. Occurrence and control of mulberry pests in Jiangsu Province. Jiangsu Sericult. 1: 24–25.

Zhang, Z. Y., C. Z. Zhang, X. J. Liu, and X. Y. Hong. 2006. Dynamics of pesticide residues in the autumn Chinese cabbage (*Brassica chinensis* L.) grown in open fields. Pest Manag. Sci. 62: 350–355.

Zhu, L. S., D. F. Fan, J. Wang, J. Zhang, and B. Q. Zhao. 2002. Residue of fenpropathrin, phoxim and their mixture in soils. Plant Nutr. Fertil. Sci. 8: 244–247.

Zhu, L. S., and W. Z. Cui. 2000. Toxicity of fenpropathrin phoxim and its mixture to silkworm. Agroenviron. Prot. 19: 35–37.

Received 19 January 2007; accepted 17 October 2007.