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Effect of β-Cyclodextrin Compounds on the Solubilization of Three Selected Pesticides and Their Toxicity with Methyl Parathion to *Rana tigrina* Tadpoles

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The application of pesticides has become inevitable to protect crop plants from pests and diseases. At the same time, pesticide pollution in the environment has caused increasing concern among the public. Some hydrophobic organic pesticides have proven to be difficult to remove from soil and have limitations for extensive application, due in part to their low water-solubilities. Agents such as organic cosolvents and surfactants have been considered for improving solubility of hydrophobic pesticides (Kile and Chiou 1989). However, both cosolvents and surfactants have disadvantages for such applications. For example, the solubilization effect of cosolvents is usually not significant until their volume-fraction concentrations are above 10%. Surfactants may form high-viscosity emulsions that are difficult to remove. As an alternative, cyclodextrins may have potential for use as solubility-enhancement agents for hydrophobic organic pesticides.

Cyclodextrins are cyclic oligosaccharides formed from the enzymatic degradation of starch by bacteria. The unique property of these compounds is that they have a hydrophilic shell and relatively apolar cavity. They can solubilize low-polarity organic compounds through the formation of water-soluble inclusion complexes (Bender and Komiyama 1978). Among the three cyclodextrin homologues (α, β and γ), β-cyclodextrin (β-CD) is produced at commercial scales and is the least expensive. Unfortunately, the low water-solubilities of β-CD and its inclusion complexes limit its application as a solubility-enhancement agent. Thus, β-CD is often chemically modified to enhance its water solubility. Its derivatives, such as hydroxypropyl-β-cyclodextrin (HPCD) and hydroxyethyl-β-cyclodextrin (HECD), were found to be very water-soluble, indicating much stronger solubilization power in comparison to β-CD. This led to their potential use in the remediation of contaminated soil and groundwater (Boving et al. 1999; Brusseau et al. 1994; Wang and Brusseau 1993). However, most of the information available on the application of cyclodextrins and their derivates in environmental remediation has been confined to enhancing the solubility and

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desorption of contaminants. Little attention has been paid to their toxicity with pesticides. The objective of this paper is to evaluate the ability of β-CD, HPCD and HECD to increase the aqueous solubility of three selected hydrophobic organic pesticides (methyl parathion, carbofuran, pentachlorophenol), and to evaluate their toxicity with methyl parathion to *Rana tigrina* tadpoles, so as to find a new method for improving the application of hydrophobic organic pesticides and enhancing their removal from the environment.

MATERIALS AND METHODS

β-cyclodextrin (99%, pure) was purchased from Shanghai Chemical Inc., China HPCD and HECD were prepared as reported by Che et al. (1997) and the products of HPCD and HECD were identified by their infrared spectra and X-ray diffraction. Methyl parathion (99%, pure) was obtained from Hunan Pesticides Company, China. Carbofuran (99%, pure) and pentachlorophenol (99%, pure) were purchased from Beijing Institute of Chemical Industry, China. Methanol, used for standard preparation and sample dilution, was of spectrum grade. Other reagents were of analytical grade.

The generator column approach (Wang and Brusseau 1993) was used to determine the solubilization of the pesticides by β-CD, HPCD and HECD. The generator column used was a 40-cm long glass chromatography column packed with prewashed quartz sands coated with excess pesticides (0.1-0.5 g). The column was plugged with glass wool at both ends and contained a large pore-diameter fritted disc sealed at the outlet. 25 mL of distilled water, or 25 mL of solutions containing different concentrations of β-CD, HPCD or HECD were passed through this column, and a fraction of the effluent was immediately analyzed for solute concentration. The remaining effluent was then repeatedly passed through the column so as to obtain a relatively constant effluent concentration. All experiments were carried out at room temperature (25°C). The concentration of all samples was measured by UV-VIS spectrophotometry (Shanghai Chemical Instrument Inc., China). The wavelengths used for UV detection were 276 nm for methyl parathion, 280 nm for carbofuran and 220 nm for pentachlorophenol. During solubility measurement, 0.5-1 mL of effluent was withdrawn and diluted with a 1:1 methanol/water solution in 10 mL volumetric flasks. The role of methanol is to inhibit the formation of β-CD, HPCD or HECD-solute complexes, thereby keeping the UV spectrum of pesticides unchanged. The effects of β-CD, HPCD and HECD on the UV spectra of the pesticides were negligible within the range of experimental concentrations.

Rana tigrina tadpoles (approximately 15 days old and mean length 1.18±0.12 cm) were used for acute toxicity tests. Before the tests, the tadpoles were acclimated for three days in the laboratory and fed with pellet feed (40% protein). During the tests, the tadpoles were not fed. The reagents used for acute toxicity tests were methyl parathion (99%, pure) and inclusion complexes of methyl parathion with β-CD, HPCD or HECD which were prepared according to the method reported by Perez-Martinez et al. (2000).

The acute toxicity test methods as described by Sprague (1973) were used for this investigation. The tests were conducted in 12-L glass tanks. The conditions of water quality were as follows: DO≥4 mg/L, T=20-21℃, pH=6.5-6.8. Based on the results of the preliminary tests, for each test reagent, five methyl parathion concentrations (2.0, 3.1, 4.8, 7.8, 12.0 mg/L) and one control concentration were used. In the tests with the inclusion complexes, β-CD, HPCD and HECD controls were used respectively. For each treatment and control, ten healthy *Rana tigrina* tadpoles were added into each tank with 10 L of test solution. There were three replicates per treatment. The total exposure time was 96 hours. During the tests, dead tadpoles were removed each day, and tadpole mortality at 24, 48, 72, 96 hr was noted. The data from the exposure tests were analyzed by SAS Statistical Analysis (Barr et al. 1979). The regression equations between the probability values of tadpole mortality and the logarithm values of treatment concentrations were elaborated to calculate median lethal concentrations (LC_{50}). 95% confidence intervals were also calculated using SAS Statistical Analysis.

RESULTS AND DISCUSSION

The solubilization effects of β-CD, HPCD and HECD on three pesticides are plotted in Figure 1. The results show that the apparent aqueous solubilities of pentachlorophenol and carbofuran are linearly increased with increasing β-CD concentration. Within the range of HPCD and HECD concentrations used, the apparent solubilities of three pesticides in solutions are linearly increased with increasing HPCD and HECD concentrations. The data plot as a straight line with a slope rate less than 1, which may be ascribed to the formation of 1:1 inclusion complexes in solution. The linear relationship can be expressed by

$$S_t=S_0(1+K_sC_0)$$

where S_t is the aqueous-phase concentration of pesticides with β-CD, HPCD or HECD, S_0 is the concentration of pesticides without β-CD, HPCD or HECD, C_0 is the initial concentration of β-CD, HPCD or HECD, and K_s is the stability constant of inclusion complexes for pesticides with β-CD, HPCD or HECD. In this equation, we assume that the concentration of β-CD, HPCD or HECD is

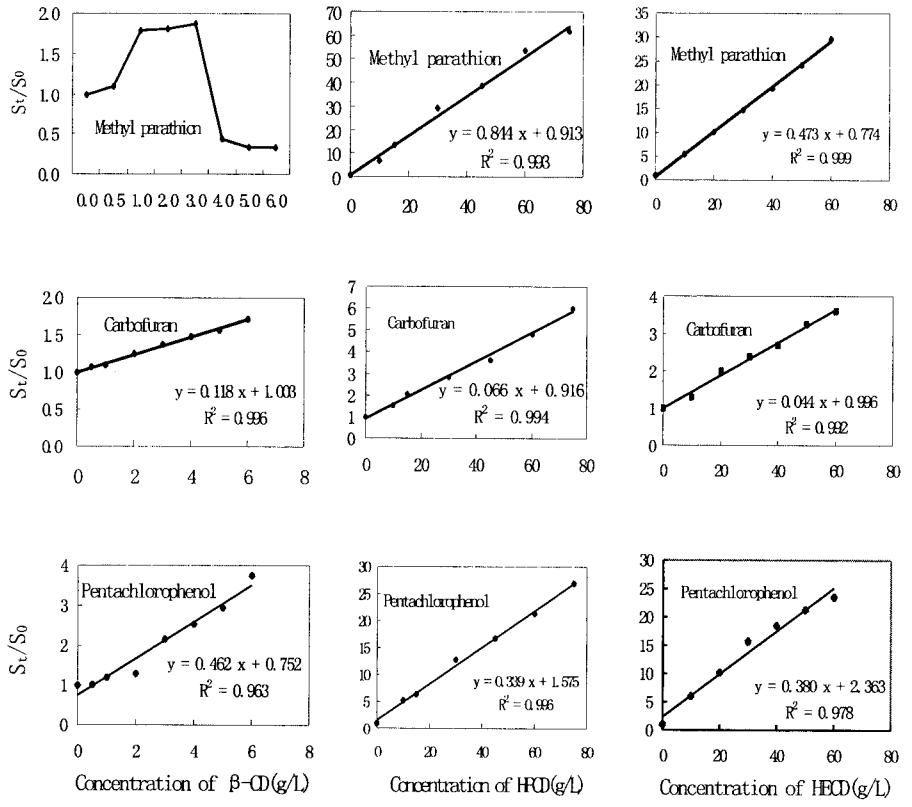

Figure 1. Solubilization curves of three pesticides by β-CD (left), HPCD (middle) and HECD (right) (S_t is the aqueous-phase concentration of pesticides with β-CD, HPCD or HECD; S_0 is the concentration of pesticides without β-CD, HPCD or HECD)

not depleted to an appreciable extent by complexing with pesticides. The relative aqueous-phase concentrations (S_t/S_0) of the pesticides are plotted against the concentration of β-CD, HPCD or HECD.

The solubilization coefficients (K_{s1}, K_{s2} and K_{s3}) of β-CD, HPCD and HECD on the three pesticides, respectively and selected physical and chemical parameters of the pesticides (Lin 1989; Zhu and Cai 1994) are listed in Table 1. Inspection of Table 1 reveals that the order of solubilization effects of β-CD on the three pesticides is pentachlorophenol >carbofuran >methyl parathion; and that solubilization effects of HPCD and HECD on the three pesticides is methyl

parathion >pentachlorophenol >carbofuran.

Table 1. The solubilization coefficients of three pesticides and their selected physical and chemical parameters

Pesticides	Methyl parathion	Carbofuran	Pentachlorophenol
K_{S1} (β-CD)	-0.188	0.118	0.462
Ks2 (HPCD)	0.844	0.066	0.339
Ks3 (HECD)	0.473	0.044	0.380
MV (nm^3)	0.322	0.312	0.257
Solubility (mg/L)	55	700	20
$\log K_{ow}$	3.09	2.29	5.01

Table 1 and Figure 1 show that the solubilization effects of HPCD and HECD on the pesticides are more significant than that of β-CD. This phenomenon is especially obvious for methyl parathion. The aqueous solubility of methyl parathion is increased initially and then decreased as the β-CD concentration increases (Figure 1). The limited water solubility of β-CD itself was considered in an attempt to explain the phenomenon. The water solubility of β-CD is reported to be only 18.5g/L at 25℃ (Szejtli 1982). Thus its inclusion complex with methyl parathion is only slightly water-soluble. When the concentration of β-CD reaches about 3g/L, the aqueous solubility of the complex is maximal, and then with increasing β-CD concentration, the aqueous solubility of the complex is inhibited because of the similar equal-ion effect. The mechanism needs to be studied further. On the contrary, HPCD and HECD are very water-soluble, and the inclusion complexes of methyl parathion with HPCD and HECD are therefore highly water-soluble.

The prerequisite for solutes to fit completely in the cavity of cyclodextrin and form an inclusion complex is that the molecule must be of appropriate shape and size. The size of β-CD cavity is reported to be 0.346 nm^3 (Szejtli 1982). Inspection of Table 1 shows that the molecular volumes (MV) of the three selected pesticides are all smaller than this cavity volume for β-CD. Hence, they can enter into the respective cavities for β-CD, HPCD or HECD and form inclusion complexes. In addition, the octanol/water partition coefficients (K_{ow}) of the compounds have an important impact on the stability of the inclusion complexes (Wang and Brusseau 1993). Because of the apolar cavity of cyclodextrin, only apolar or low-polarity compounds can form stable inclusion complexes. Inspection of Table 1 reveals that the selected pesticides have a large log K_{ow}. Therefore, they can form stable inclusion complexes with β-CD, HPCD or HECD.

The increased solubilization of hydrophobic organic pesticides by β-CD, HPCD and HECD may leave the toxic substances more readily available for degradation by micro-organisms, and therefore reduce their residue in soils. Thus β-CD, HPCD and HECD are suggested for chemical and biological remediation-enhancement techniques (Boving et al. 1999). However, whether β-CD, HPCD and HECD have adverse effects on bioavailability of pesticides needs to be evaluated.

The results of acute toxicity tests with *Rana tigrina* tadpoles exposed to methyl parathion and the inclusion complexes of methyl parathion with β-CD, HPCD and HECD are listed in Table 2. The experimental results show that the LC_{50} values of methyl parathion and the inclusion complexes of methyl parathion with the solvents showed little change. The 96-hr LC_{50} values were all close, which reveals that the presence of β-CD, HPCD and HECD has no significant effect on the toxicity of methyl parathion to *Rana tigrina* tadpoles. Therefore, the addition of β-CD, HPCD and HECD and the formation of inclusion complexes will not decrease the availability of methyl parathion.

The results are not surprising. This is partly because β-CD, HPCD and HECD lack toxicity alone, as shown in Table 2. In addition, in the tests the same concentrations of methyl parathion in each treatment with methyl parathion alone or with the inclusion complexes were used. The mechanism why the formation of inclusion complexes of methyl parathion with cyclodextrin compounds did not affect the toxicity of methyl parathion needs to be studied further. The results may offer useful information for improving the application of hydrophobic organic pesticides with β-cyclodextrin compounds.

In comparison to cosolvents and surfactants, β-CD, HPCD and HECD have some advantages. For example, HPCD and HECD are extremely water-soluble relative to many cosolvents and surfactants; β-CD, HPCD and HECD do not form emulsions as do many surfactants; β-CD, HPCD and HECD are nontoxic and biodegradable, thus posing no hazard to the ecosystem (Brusseau et al. 1994; Wang and Brusseau 1993). The results obtained in this research indicate β-CD, HPCD and HECD may be potentially useful for improving the application of hydrophobic organic pesticides and enhancing their removal from the environment by solubilizing them.

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Table 2. Toxicity of methyl parathion and the inclusion complexes of methyl parathion with β-CD, HPCD and HECD to *Rana tigrina* tadpoles *

Reagent	Treatment time (hr)	Treatment concentration (mg/L) 0	2.0	3.1	4.8	7.8	12.0	Regression equation of LC_{50}	Correlation coefficient	LC_{50} and 95% confidence interval (mg/L)
I	24	0	0	23.3	40	80	96.7	Y=4.458X+1.921	0.989	4.9 (3.6-6.2)
	48	0	0	33.3	66.7	86.7	100	Y=3.839X+2.730	0.995	3.9 (3.0-4.8)
	72	0	0	46.7	70	90	100	Y=3.406X +3.231	0.999	3.3 (2.6-4.0)
	96	0	0	46.7	80	90	100	Y=3.378X+3.356	0.973	3.1 (2.2-4.0)
II	24	0	0	23.3	30	80	90	Y=3.767X+2.258	0.966	5.3 (4.0-6.6)
	48	0	0	33.3	46.7	86.7	100	Y=3.883X+2.528	0.962	4.3 (3.8-4.8)
	72	0	0	33.3	70	90	100	Y=4.260X+2.527	0.995	3.8 (3.5-4.1)
	96	0	0	46.7	80	90	100	Y=3.378X+3.356	0.973	3.1 (2.7-3.5)
III	24	0	0	23.3	40	80	100	Y=3.940X+2.242	0.981	5.0 (3.6-6.4)
	48	0	0	33.3	66.7	86.7	100	Y=3.839X+2.730	0.995	3.9 (3.0-4.8)
	72	0	0	46.7	70	90	100	Y=3.406X+3.231	0.999	3.3 (2.7-3.9)
	96	0	0	46.7	80	90	100	Y=3.378X+3.356	0.973	3.1 (1.8-4.4)
IV	24	0	0	23.3	40	80	100	Y=3.940X+2.242	0.981	5.0 (4.1-5.9)
	48	0	0	33.3	46.7	86.7	100	Y=3.883X+2.528	0.962	4.3 (3.6-5.0)
	72	0	0	46.7	70	90	100	Y=3.406X+3.231	0.999	3.3 (2.6-4.0)
	96	0	0	46.7	70	90	100	Y=3.406X+3.231	0.999	3.3 (2.4-4.2)

* I : Methyl parathion; II : Methyl parathion with β-CD; III: Methyl parathion with HPCD; IV: Methyl parathion with HECD; The treatment concentrations in the table are the concentrations of methyl parathion; the data below the treatment concentrations are tadpole mortality (%); all data are the average values of three treatments; and in the regression equations of LC_{50}, Y denotes the probability values of tadpole mortality and X denotes the logarithm values of treatment concentrations.

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