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Environmental Contamination and Toxicology

Inclusion Effects of Highly Water-Soluble Cyclodextrins on the Solubility, Photodegradation, and Acute Toxicity of Methyl Parathion

Q. Zeng,[1,2] B. Liao,[2] Y. Luo,[1] C. Liu,[2] H. Tang[1]

[1] State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Post Office Box 2871, Beijing 100085, People's Republic of China
[2] College of Resources and Environment, Hunan Agricultural University, Changsha 410128, People's Republic of China

Cyclodextrins, cyclic oligosaccharides formed from the enzymatic degradation of starch by bacteria, have a doughnut-shaped structure with a relatively hydrophilic exterior and a relatively hydrophobic interior hole. Low-polarity organic substances with a size and shape complementary to the hole form inclusion complexes with cyclodextrins (Wang et al. 1993). Due to their unique ability to form inclusion with various organic solutes, cyclodextrins are widely used in chemistry, pharmaceutical science, and several other fields. Also some works show that cyclodextrins have the potential use as an in-situ flushing agent in environmental remediation (Boving et al. 1999; Martinez et al. 2000). It can increase the apparent aqueous solubilities of low-polarity organic compounds and enhance their desorption and transport in soil (Brusseau et al. 1994). In order to suppress toxicity in activated sludge systems, β -cyclodextrin was used to form inclusion complexes with pesticides and related compounds, leading to enhanced biological detoxification of industrial wastewater (Olah et al. 1988).

The use of organophosphate insecticides to control a wide variety of insect pests has increased extensively in recent years due to the restriction on chlorinated hydrocarbons as pesticides. The toxicity of organophosphates arises from their inhibition of the enzyme acetylcholinesterase which is essential for the transmission of nerve impulse. The environmental fate of the organophosphorus insecticides may be different according to both the agricultural practice and the physic-chemical characteristics of the various compounds. The degradation process of organophosphorus insecticides is generally faster in respect to other organochlorines or carbamate agrochemicals (Sattar, 1990). Usually, they are known as nonpersistent agrochemicals and have lower residual effects on terrestrial and aquatic ecosystems. Investigation of the photolysis of pesticides is important in environmental chemistry because it presents basic data on their fate and persistence in natural environments exposed to sunlight acting.

The wide application of methyl parathion has provided many occasions for this

Correspondence to: Q. Zeng

insecticide to enter aquatic environment and cause damage to the aquatic biota. Compared to other organophosphorus insecticides, it is considered more persistent and shows a greater affinity to soil and sediments (Kamiya et al. 1995). An important consequence of the widespread use of insecticides is that toxic chemicals may impair the ability of non-target organisms to survive and reproduce in affected habitats. The toxicity of organic pesticides in the environment is largely dependant on their physical and chemical properties. Recently, aqueous solubility enhancement of hydrophobic organic pollutants by cyclodextrins was studied (Boving et al. 1999; Martinez et al. 2000). The tadpoles are widely distributed in China. They are common in ponds and rice paddies. However, little published information is available on the sensitivity of tadpoles to methyl parathion in the presence of cyclodextrins or their highly water-soluble derivates. In addition, the structure-activity correlations of the inclusion effect of cyclodextrins on the photolysis of organophosphorus pesticides need to be evaluated. The present study was initiated to determine the effect of the highly water-soluble cyclodextrins on photodegradation, acute toxicity of methyl parathion to tadpoles.

MATERIALS AND METHODS

β-cyclodextrin (99%, pure) was purchased from Shanghai Chemical Co. Inc. HPCD and MCD were prepared referring to the methods reported in the reference (Che et al. 1997) and the products of HPCD and MCD were identified by IR spectrum and XRD. Methyl parathion (99%, pure) was obtained from Hunan Pesticides Company, China. Methanol, used for standard preparation and sample dilution, was of spectrum grade. Other reagents were of analytical grade.

The tests for enhancing apparent aqueous solubility of methyl-parathion with β-CD, HPCD and HECD was carried out by using the generator column approach (Wang et al. 1993). The generator column used in this work was packed with prewashed quartz sands coated with excess pesticides (0.1-0.5g). The column was plugged with glass wool at both ends and contained a large pore-diameter fritted disc sealed at outlet. 25ml of distilled water, or 25ml of solutions containing different concentrations of HPCD or MCD was 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.

Photolysis of the methyl parathion was performed in a photochemical setup. 50ml of each solution of methyl-parathion dissolved in an aqueous solvent consisting of methanol or in the same solvent in the presence of 6g L^{-1} HPCD, and MCD was separately irradiated with a low pressure mercury lamp (Model L-15W, $\lambda max=254$ nm, Tianjing G&C Co., Ltd). All experiments were carried out at room temperature (25±1°C). The 1 mL aliquots for different irradiation times were withdrawn and diluted with 1:1 methanol/water solution in 10mL volumetric flasks. The role of methanol is to decompose the formation of HPCD or MCD-solute complexes, thereby keeping the UV spectrum of pesticides unchanged. The concentrations of all samples were measured on UV-VIS spectrophotometer (Shanghai Chemical Instrument Co. Inc.). The wavelength used for UV detection was 276 nm for methyl parathion solutions. The effects HPCD and MCD on the UV spectra of the pesticides were negligible within the range of experimental concentrations.

The average total length of tadpoles (*Nana limnocharis*) was 12mm. Before the test, the tadpoles were acclimatized for three days in glass aquaria at room temperature. During the test period, the tadpoles were not fed. Because of the low solubility of methyl-parathion in pure water a stock solution of pesticide (10,000 mg L^{-1}) in methanol was prepared. Test aquaria were wide-mouth glass jars of 12 liters. Each contained 10 liters of test solution and 10 tadpoles for the bioassay. After preliminary tests, the nominal concentrations chosen for static acute toxicity of methyl-parathion were: 0, 0.50, 1.0, 2.0, 3.1, 4.8, 7.8, and 12.0 mg L^{-1}. The physico-chemical properties of water quality were as follows: DO≥4mg L^{-1}, T=20±1℃, pH=6.5-6.8. In order to evaluate the effects of photodegradation on acute toxicity of methyl-parathion, two sets of pesticide solutions with or without HPCD or MCD were prepared for 3h decay in the sunlight before the tested animals were added into glass jars. The number of tadpole that died in each concentration was recorded after 30 minutes and 1, 2, 4, 8, 12, and 24 h thereafter. Dead specimens were removed immediately. The total time of exposed experiment was 10 days. All treatments in experiments were set up with three replications. The data from the exposed test were statistically processed by using SAS Statistical Analysis, including calculation of average values, median lethal concentrations (LC_{50}), and 95% confidence (Barr et al. 1979).

RESULTS AND DISCUSSION

Due to the advantage of their high water solubility, chemically modified cyclodextrins, such as hydroxypropyl-β-cyclodextrin (HPCD) and methyl-β-cyclodextrin (MCD), show great potential use as solubility enhancing agents in comparison of β-cyclodextrin. The effect of HPCD and MCD on aqueous solubility of methyl-parathion was investigated (Figure 1). The relative solubility (apparent solubility/aqueous solubility) of this pesticide was significantly increased in HPCD, and MCD solutions. This increase was linear with respect to

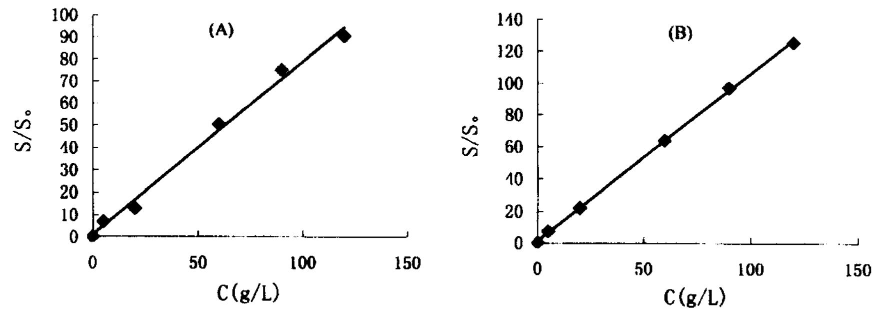

Figure 1. The relation between concentration of HPCD (A) and MCD (B) solubilization of methyl-parathion

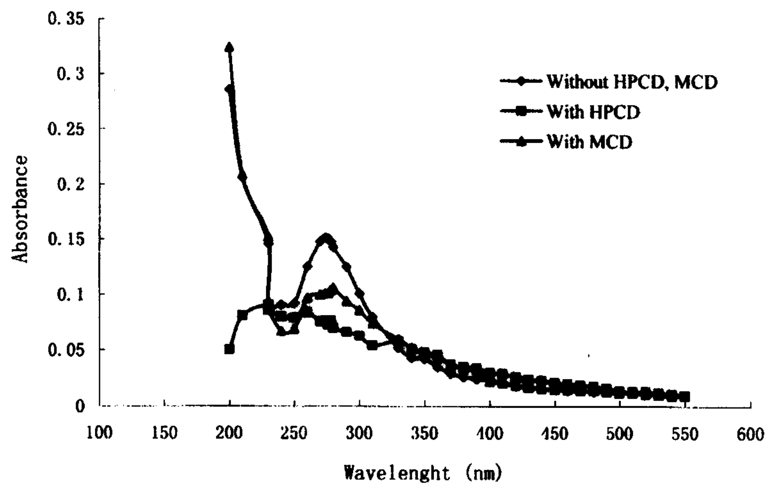

Figure 2. Spectra of photodegradation of methyl-parathion solution

the concentrations of cyclodextrins, being about 53 and 64 times higher in the presence of 60 g L^{-1} HPCD and MCD than in water alone, respectively. Straight lines in the diagrams, with the slopes less than 1, indicate formation of a 1:1 inclusion complex (Wang et al 1993). Because of being capable of forming inclusion complexes with various organic compounds, cyclodextrins can be regarded as a potent influence on their residual persistence in natural environments exposed to action of the sunlight (Kamiya et al 1995). Figure 2 shows a sequence of spectra measured during separately illumination of the solutions containing initially 6.0 mg L^{-1} methyl-parathion with or without 6g L^{-1} HPCD and MCD for different times. It was found that photodegradation rate of the pesticide was considerably influenced by species of the hosts of cyclodextrins.

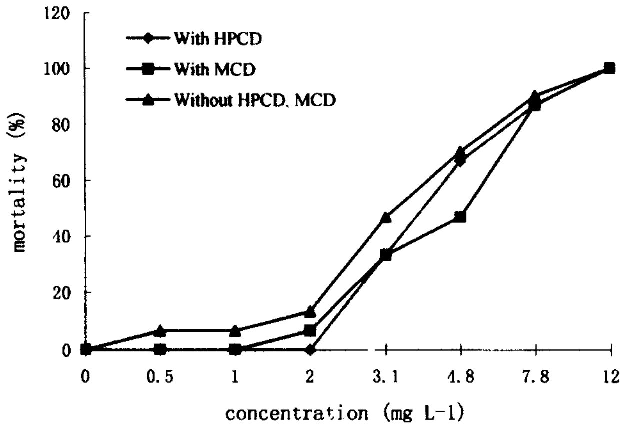

Figure 3. Culmulative mortality of tadpoles exposed to different concentration of methyl-parathion. Each point represents the mean of three replications of 10 animals.

The fact that HPCD and MCD can lead to more rapid rates of pesticide decay clearly demonstrates that they act as the photocatalysts. The above results indicate that cyclodextrins actually exert inclusion effects on the photolysis rates of pesticide. Whether these are inhibitive or promotive to the photodegradation rates of the included pesticide may be dependent on the difference in the sizes of the cavities of cyclodextrins (Kamiya et al 1995). Deeply inclusion of the pesticides will prevent them from interaction with the secondary hydroxyl groups and water and decrease the photolysis rate. The significantly promotion effect of cyclodextrins on the photolysis of methyl-parathion may be caused by a moderate inclusion depth which allows the reaction site of pesticide to be reached easily. Mortality of tadpoles at different concentrations of methyl parathion in acute toxicity tests is presented in Figure 3. Variability in mortality rate among replicate samples was low. Mortality increased with pesticide concentrations. A sharp increase in mortality occurred between 3.1 and 12.00 mg L^{-1}. The probit method for calculating the LC_{50} values was applicable because the relationship between probit and dose could be adequately described by regression lines. The 48h LC_{50} values for tadpoles exposed to methyl parathion with or without cyclodextrins were 3.9, 4.3, 3.3 mg L^{-1}, respectively. The experimental results showed that the acute toxicity has not significant change in the present of highly water-soluble cyclodextrins ($P>0.05$). However, at sublethal concentrations, significant effect of methyl-parathion on the physiological processes of tadpoles, including behavioral changes of tadpoles such as surfacing and rapid movements, could be still observed at concentrations as low as 1.0 mg L^{-1}. It suggested that tadpoles would be significantly affected at concentrations considerably lower than those

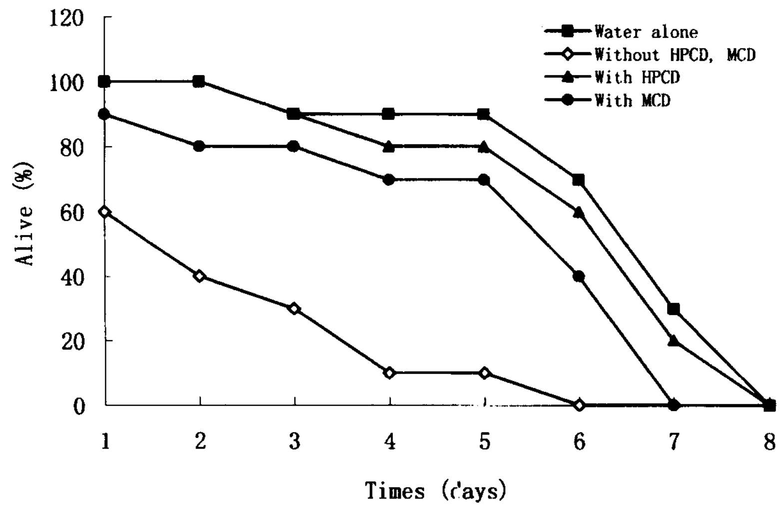

Figure 4. Effect of methyl-parathion photodegradation in the presence of HPCD and MCD on the survival patterns of tadpoles. Each point represents the mean ofthree replicates of 10 animals. Significant ($P < 0.01$) difference in survival relative to treatment without HPCD and MCD is indicated by closed symbols.

that would cause acute toxicity. Acute toxicity of methyl-parathion to other aquatic organisms has been documented. For example, Guzzella et al. (1997) reported the sensitivity of the marine organism and demonstrated that the toxicity of micropollutants depended on the medium compositions and the species of organism.

The remarkable difference in the toxicity to tadpoles was induced by pesticide photilysis in the presence of cyclodextrins. Survival curves of tadpoles in acute toxicity tests are presented in Fig. 4. The concentration of methyl-parathion for each treatment was 7.8 mg L^{-1}. After 3h photodecay in the sunlight, significant ($P < 0.01$) effect of methyl parathion on survival was observed in all treatments. Mortality rate of methyl-parathion to tadpoles in the presence of HPCD and MCD was considerabIy lower than that of control, which may be ascribed to their promotion photogradation of methyl-parathion. No significant difference in survival pattern was found between cyclodextrin treatment and water treatment alone. Mortality began after 24 hours and no individual survived longer than 96 hours for methyl-parathion in the treatment without cyclodextrins.

In general, the photodegradation of organic pesticides occurs commonly in natural water, therefore, the interaction of toxicity induced by the sunlight is an important factor which must be taken into account when assessing the hazards of environmental pollutants to aquatic life and for setting valid water quality standards for diverse uses (Buckland et al 1987; Gal et al 1992). Additional studies on the effects of toxicants, singly and in mixtures, on biochemical and physiological processes are need to gain more knowledge of interactions between environmental factors and the toxicants.

REFFERENCES

Barr AJ, Goodnight JH, Sall JP, Helwig JAT (1979) User's guide to SAS Statistical Analysis, Institute Press, North Carolina

Boving TB, Wang XJ, Brusseau ML (1999) Cyclodextrin-enhanced solubilization and removal of residual-phase chlorinated solvents from porous media . Environ Sci Technol 33:764-770

Brusseau ML, Wang X, Huang QG (1994) Enhanced transport of low-polarity organic compounds through soil by cyclodextrin. Environ Sci Technol 28: 925-956

Buckland SJ, Davidson RS (1987) The photodegradation of parathion and chlomephos. Pest Sci 19: 61-66

Che BQ, Ling Y, Dai RJ (1997) Synthese and property of some highly hydro-soluble cyclodextrin chiral selectors. Chinese Chem Reag 19:129-130, 133

Gal E, Aires P, Chamarro E, Esolugas S (1992) Photochemical degradation of parathion in aqueous solutions. Water Res 26: 911-915

Guzzella J, Gronda A and Colombo L (1997) Acute toxicity of organophosphorus insecticides to marine invertebrates. Bull Environ Contam Toxicol 59:313-320

Kamiya M, Nakamura K (1995) Cyclodextrin inclusion effects on photodegradation rates of organosphorus. Environ Int 21: 299-304

Khangarot BS, Ray PK (1990) Acute toxicity and toxic interaction of chromium and nikel to common guppy poecilia reticulata (Peters). Bull Environ Contam Toxicol 44:832-839

Martinez JIP, Gines JM, Morillo E (2000) Improvement of the desorption of the pesticide 2,4-D via complexation with HP-β-cyclodextrin. Pest Manage Sci 56:425-430

Olah J, Cserhati T, Szejtli I (1988) β -cyclodextrin enhanced biological detoxification of industrial wastewaters. Wat Res 22:1345-1351

Wang XJ, Brusseau ML (1993) Solubilization of some low-polarity organic compounds by hydroxypropyl- β -cyclodextrin. Environ Sci Technol 27: 2821-2825