Uptake and Phytotransformation of Organophosphorus Pesticides by Axenically Cultivated Aquatic Plants

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The uptake and phytotransformation of organophosphorus (OP) pesticides (malathion, demeton-S-methyl, and crufomate) was investigated in vitro using the axenically aquatic cultivated plants parrot feather (*Myriophyllum aquaticum*), duckweed (*Spirodela oligorrhiza* L.), and elodea (*Elodea canadensis*). The decay profile of these OP pesticides from the aqueous medium adhered to first-order kinetics. However, extent of decay and rate constants depended on both the physicochemical properties of the OP compounds and the nature of the plant species. Malathion and demeton-S-methyl exhibited similar transformation patterns in all three plants: 29−48 and 83−95% phytotransformation, respectively, when calculated by mass recovery balance during an 8-day incubation. No significant disappearance and phytotransformation of crufomate occurred in elodea over 14 days, whereas 17−24% degraded in the other plants over the same incubation period. Using enzyme extracts derived from duckweed, 15−25% of the three pesticides were transformed within 24 h of incubation, which provided evidence for the degradation of the OP compounds by an organophosphorus hydrolase (EC 3.1.8.1) or multiple enzyme systems. The results of this study showed that selected aquatic plants have the potential to accumulate and to metabolize OP compounds; it also provided knowledge for potential use in phytoremediation processes.

Keywords: *Phytotransformation; phytoremediation; organophosphorus pesticides; enzymatic degradation; organophosphorus hydrolase*

INTRODUCTION

A critical aspect of environmental fate assessment is the extent to which contaminants are accumulated and subsequently degraded by plant materials. Such information is required to predict phytoremediation potential as well as phytotoxicity and other potential impacts of chemical release into the environment. Phytotransformation processes that rely on plant enzymes to degrade or transform contaminants offer a cost-effective, innovative technique for treatment of selected hazardous pollutants at a variety of contaminated sites (Schnoor et al., 1995; Medina and McCutcheon, 1996).

Aquatic plants have great potential to function as in-situ, on-site biosinks and biofilters of aquatic pollutants because of their abundance and limited mobility. Elodea has been successfully used to accumulate selected heavy metals through its root system as well as by uptake through the plant body (Kähkönen and Manninen Pentti, 1998). Also, duckweed was proven to be a good accumulator of Cd, Se, and Cu and a moderate accumulator of Cr from water with concentrations of the metals ranging from 0.1 to 10 mg/L (Zayed et al., 1998). The results of Hughes et al. (1997) showed the ability of parrot feather to uptake and transform the munition TNT. In addition, selected plant tissue/cell cultures have been used in a number of pesticide metabolism studies to evaluate their suitability as model systems for bioremediation by whole plants (Hughes et al., 1997; Burken and Schnoor, 1997).

Organophosphorus (OP) pesticides have contaminated environmental compartments in many countries (Bollag and Liu, 1990; Lai et al., 1995). The land area affected by the expanding application of these pesticides has exceeded 20 million acres in the United States, significantly increasing the risk of contaminating food, groundwater, and other water resources (Salt et al., 1995). It has been reported that OP compounds are transformed by a variety of different processes in the environment. In addition to photochemical (Kamiya and Kameyama, 1998) and hydrolytic (Lacorte et al., 1995) reactions that occur effectively on plant/soil surfaces or in aquatic systems, biological processes, often showing more chemical specificity, are thought to be responsible for attenuating the toxicity and effecting the fate of OP pesticides. Most bioremediation studies of OP compounds have been limited to microorganisms or plant-associated microflora (Zayed et al., 1998; Salt et al., 1995; Burken and Schnoor, 1996; Levanon, 1993), so the ability of plants to transform OP pesticides without the participation of associated microbes remains arguable (Schnoor et al., 1995; van der Krol et al., 1995). Thus, to delineate plant-mediated pathways, data must be obtained using axenic plants, plant tissues, or cell cultures in the absence of microorganisms.

In this study, three axenically cultivated aquatic plants: parrot feather (*Myriophyllum aquaticum*), duckweed (*Spirodela oligorrhiza* L.), and elodea (*Elodea canadensis*), were investigated in vitro for their ability

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10.1021/jf9904968 CCC: $19.00
Published on Web 11/17/2000

to transform three OP pesticides: malathion, demeton-S-methyl, and crufomate (also called ruelene). These aquatic plants are widespread, free-floating aquatic macrophytes, a source of food for waterfowl, and a shelter for small aquatic invertebrates. They grow quickly and reproduce more rapidly than other vascular plants (Zhang and Jin, 1997).

To characterize such plants and their enzyme-based transformation capabilities more fully, the enzymes involved in pesticide metabolism are currently under study in our laboratory. Organophosphorus hydrolase (OPH; EC 3.1.8.1) comprises a family of enzymes involved in the transformation of OP compounds with P−O, P−S, P−CN, and P−F bonds; these enzymes are present in aquatic plants and have been shown to have variable substrate specificities (Hoskin et al., 1995; Morita et al., 1996). The enzymatic hydrolysis of the three OP pesticides using duckweed enzyme extracts was also studied here. A direct link between hydrolysis by this extract and biodegradation of OP compounds by axenically cultured duckweed would provide strong evidence for the metabolic pathways involved in phytotransformation.

MATERIALS AND METHODS

Reagents. Analytical grade (>99% purity) malathion [[(dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester], demeton-S-methyl [*O*,*O*-methyl *S*-[2-(ethylthio)ethyl]-phosphorothioate], and crufomate [methylphosphramidic acid 2-chloro-4-(1,1-dimethylethyl)phenyl methyl ester] were obtained from Chem Service (West Chester, PA). Deionized water (Nanopure II; Boston MA) was used in all studies without further purification. All other chemicals were of reagent grade or better. OP solutions were prepared by diluting the appropriate volumes from a 1000 mg/L methanol stock solution in water.

Axenic Plant Incubations. Axenic parrot feather was supplied by the Department of Biochemistry, University of Georgia (UGA; Athens, GA). These plants were propagated vegetatively in culture boxes on NH_4^+-free Murashige Skoog (MS) medium (Sigma Plant Culture, St. Louis, MO) supplemented with agar. Axenic plants were maintained at room temperature under light from cool white and broad spectrum bulbs for a 12-h photo period at a light intensity of 100 μeinstein/m^2 (Schnoor et al., 1995).

Duckweed was taken from ponds and wetlands in the Athens, GA, vicinity (33° 55.919′ N and 83° 22.233′ W). After the plants were cleaned thoroughly under gentle running water to remove adhering algae and insect larvae, they were transferred to and cultured in 75-L plastic containers with half-strength Hoagland's culture solution (Sigma Plant Culture) at pH 7.0; they were later transferred to a phosphate-deficient culture solution. All of the containers were maintained at 25 °C and at an irradiance of 400 μmol of photon flux density m^{-2} s^{-1}. After cultivation for 3 weeks, the duckweed was rinsed with flowing tap water for ~10 min and then transferred into sterilized water containing 1% sodium hypochlorite. This solution was stirred with a magnetic bar for 15 min; the plants were then removed, rinsed with sterilized water, and used for the experiment.

Elodea was collected from Lake Herrick (33° 55.929′ N, 83° 22.247′ W), a small oligothropic pond in Athens, GA. The procedures of culture propagation and sterilization were similar to those for duckweed as described above except the concentration of sodium hypochlorite was 5%. These plants were cultivated for 1 week in the laboratory before use in the experiments.

Uptake and Phytotransformation Studies. All uptake and phytotransformation experiments were conducted in a fume hood and performed in triplicate. Stems and feathery leaves totaling 2 g (fresh weight) of axenic plants were placed in 50-mL Erlenmeyer flasks fitted with Styrofoam stoppers; each flask contained 20 mL of sterile hydroponic culture medium (Hoagland nutrient solution adjusted to pH 7.0 with 1.0 N NaOH, Sigma) spiked with 1 ppm of malathion, 10 ppm of demeton-S-methyl, or 10 ppm of crufomate. These mixtures were incubated in a rotating incubator (50 rpm; Innova 4230, Edison, NJ) at 22 °C under fluorescent lights. The aqueous culture medium was sampled (0.5 mL) at time intervals of 0, 4, 8, 16, and 24 h and then daily for 8 days except crufomate (for 14 days); the aqueous phase was extracted twice with 0.5 mL of *n*-hexane. The extracts were analyzed without further cleanup by gas chromatography (GC; see below). Four controls were carried out in parallel: (1) sterilized water solution with pesticides; (2) culture medium solution with pesticides; (3) autoclaved plant with culture medium and pesticides (added after autoclaving); and (4) plant with culture medium but without pesticides. Throughout the experiments aliquots of culture medium were randomly collected and sent to the Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, for a total bacterial colony count, in which three media (basic blood agar, cooked meat broth, and thioglycollate broth) were used. The total bacterial count was always <50 colony-forming units (CFU)/mL.

Analysis of Plant Materials. At the end of the incubation (eighth day), the plants were rinsed thoroughly with water, blotted dry, finely chopped with pruning shears, and ground up in liquid nitrogen using a mortar and pestle. Then the plant materials were sonicated twice in an ultrasonic bath (Branson 5120, Danburg, CT) in a 10 mL mixture of acetonitrile and hexane (v/v = 1:1) for 20 min at 30 °C. The extracts containing malathion or demeton-S-methyl were then cleaned up by passage through a silica gel (1 g) column (Silica Gel SPE, Baker, Phillipsburg, NJ), and compounds were eluted with 10 mL of 10% ethyl acetate in hexane. Crufomate-containing extracts were passed through a C-18 (0.5 g) column (LC-18 SPE, Supelco, Bellefonte, PA) and eluted with 6 mL of methanol. These eluting solvents were evaporated with nitrogen at room temperature, and residues were redissolved in 1 mL of hexane for subsequent GC analysis.

Parent compounds were analyzed by an HP 5890 series II GC equipped with a ^{63}Ni electron capture detector and a capillary column (30 m, 0.53 mm i.d.) coated with 95% dimethyl−5% diphenyl polysiloxane at 0.5 μm film thickness (RTS-5, Restek Corp., Bellefonte, PA). The column oven was programmed from an initial temperature of 120 °C for 2 min to 220 °C at a rate of 20 °C/min, held for 1 min, and then ramped at a rate of 4 °C/min to 240 °C with a final hold time of 10 min. The detector and injector were maintained at 300 and 250 °C, respectively; the injector was in the split mode at 1:40. Helium was the carrier gas at 30 cm/s, and nitrogen was the makeup gas at 30 mL/min. Injection volume was 1 μL in *n*-hexane. To determine analytical recovery, pesticides were spiked into the aqueous growth medium and the ground plant materials. Recoveries ranged from 94 to 98% ± 4.9 and from 88 to 105% ± 6.8 (RSD), respectively.

Enzymatic Degradation Studies. Enzyme extraction and enzyme purification were modified according to Morita et al. (1996) and are briefly described as follows. Three grams of duckweed, grown in Hoagland's solution, was dried and powdered in liquid nitrogen and then homogenized with a Waring blender for further disruption of cell wall material in an extraction buffer [50 mM tris/maleate, pH 8.5, containing 0.2% poly(vinylpyrrolidone), 30% *n*-butanol, and 5 μM phenylmethanesulfonyl fluoride]. After filtration through double-layered cheesecloth, the extraction buffer was centrifuged at 15000*g* for 15 min at 4 °C. The suspension of this crude extract was cleaned up and purified in a process involving size exclusion chromatography and ammonium sulfate precipitation. To assay the degradation of OP compounds, 1 mL of plant enzyme extract was diluted with 4 mL of culture medium and mixed with 50 μL of 100 μg/mL malathion (3.0 μM), 1000 μg/mL demeton-S (39 μM), or 1000 μg/mL crufomate (34 μM) in methanol. Samples were incubated in a rotating incubator (50 rpm) at 22 °C with fluorescent lighting. At time intervals of 0, 4, 8, 16, 24, and 48 h, 0.5 mL of the aqueous culture medium

was extracted twice with 0.5 mL of hexane and analyzed by GC as described above. Both water and culture medium containing OP compounds but no enzyme extracts were used as controls. All experiments were replicated at least three times.

Data Analysis. The first-order decay model ($C_t/C_0 = e^{-kt}$) was used to estimate the kinetic parameters of OP pesticide disappearance from the medium, where C_t is the concentration of the pollutant at time t, C_0 is its initial concentration (0.5 ppm), and k is the first-order rate constant. The k was obtained as the absolute value of the slope of the line obtained by least-squares analysis. The half-life, $t_{1/2}$, corresponding to the period of time by which the pesticide concentration is equal to half of the initial concentration, is given by $t_{1/2} = \ln(2)/k$. Mean values and standard deviations were calculated for each test compound on the basis of the values obtained for each kinetic run. These values were compared by analysis of variance (ANOVA). All statistical analyses were performed on an IBM computer using the Excel software program. The significance level was set at 0.05. The bioconcentration factor (BCF) was defined as the ratio between concentrations of OP compounds extracted from the plants (mg/kg) and from the culture media (mg/L) at harvest.

RESULTS AND DISCUSSION

Uptake and Phytotransformation by Parrot Feather. The results demonstrated that ~8.6% of malathion disappeared from the viable parrot feather culture medium with 16 h of incubation. After that, the concentration in the culture medium decreased gradually; at the end of incubation (8 days) a total of 83% had disappeared (Figure 1). An initial decrease in malathion concentration was also observed with autoclaved plants; this was attributed to sorption on the dead plant surface. Thereafter, the concentration remained constant. Similar trends for demeton-S-methyl and crufomate were observed but to a lesser extent (2.1 and 1.8% at 16 h; 78 and 58% at 8 days, respectively, Figure 1). After 8 days, the crufomate concentration continued to decline slowly, and ~77% had disappeared at the end of the experiment (14 days).

The concentration of malathion in the parrot feather culture medium followed a logarithmic decay, with a first-order decay constant of 0.012 h^{-1}. This 58-h half-life was achieved with a mass ratio of plant to medium of ~0.1 (20 mL of medium to 2 g of parrot feather). The half-lives ($t_{1/2}$) and disappearance rate constants (k) for all three OP compounds are given in Table 1. Mass recovery at the end of the studies showed that about 29, 82, and 17% of malathion, demeton-S-methyl, and crufomate, respectively, were degraded and/or bound in a nonextractable manner with plant material (Figure 2). Very little demeton-S-methyl was extractable from the plant, as indicated by its smaller BCF value with parrot feather (Table 2). This indicates that most of the demeton-S-methyl disappearance was due to degradation rather than reversible sorption.

Before undergoing transformation, OP pesticides must make contact and be taken up by the plants, which may involve an active process specific to the compound and/or a passive process. It is not clear whether transformation occurs on the surface or during transport into the plant tissue (Schnoor et al., 1995). At any rate, metabolism of the OP compounds is mediated by enzymes after they reach the plant cells. This transformation is dependent on three factors: (1) physicochemical properties of the compounds, (2) plant species, and (3) environmental factors. In the case of parrot feather, the physical and chemical characteristics of the OP compounds appear to be important in determining their susceptibility to biotransformation processes. Unlike malathion, which contains P═S and P−S bonds, demeton-S-methyl contains a P═O bond and a thiono group, whereas crufomate contains a P═O bond, a P−N bond, and a chlorophenyl moiety. These differences in molecular structure likely determine metabolic pathways, which are mediated by different or multiple enzymes (Tarrant et al., 1992). The disappearance of malathion (half-life of 58 h) was in good agreement with the results of other investigations using soybean and wheat suspension cultures that showed malathion to be metabolized at 70−90% and with a nonextractable residue of 6−11% within 48 h (Schmidt et al., 1988). These results suggest that axenic parrot feather contains enzymes that can metabolize OP compounds. Although no attempt was made to carry out a mass balance using radiolabled OP compounds, the high efficiencies of OP compound recovery from the autoclaved plants confirm that neither abiotic reactions (e.g., reaction with the medium or volatilization) nor losses in sampling can explain the disappearance of OP compounds observed with viable plants; plant-mediated degradation and irreversible binding are likely the main mechanisms of disappearance.

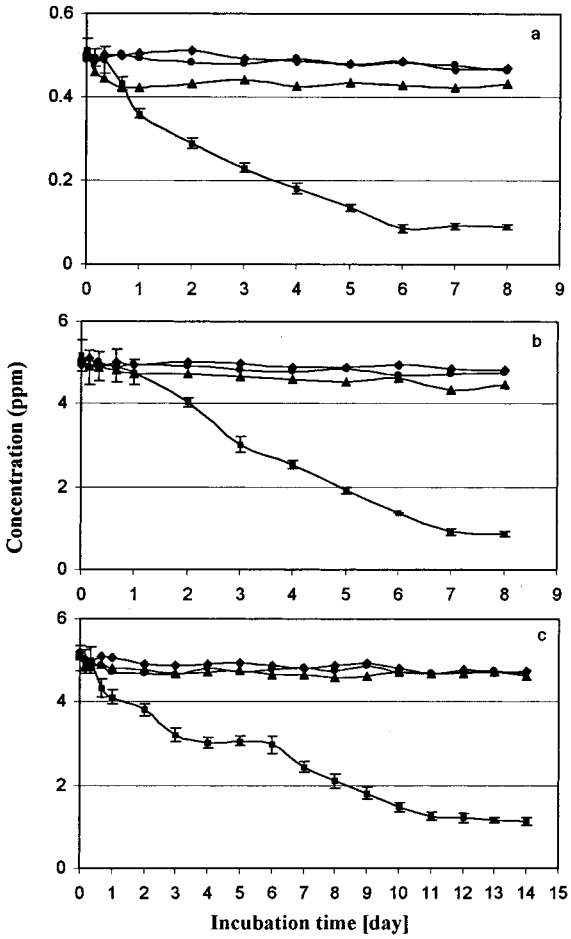

Figure 1. Uptake of malathion (a), demeton-S-methyl (b), and crufomate (c) exposed to axenically cultivated parrot feather (*M. aquaticum*) under controlled conditions: control (◆); culture medium (●); autoclaved parrot feather (▲); viable parrot feather (■). Concentrations of the residual pesticide in the liquid medium were determined by GC-ECD analysis. Error bars represent ±1 standard deviation. See text under Materials and Methods for details.

Table 1. Disappearance Rate Constants (k) and Half-Lives ($t_{1/2}$) of OP Pesticides Incubated with Axenic Aquatic Plant or Enzyme Extract of Duckweed

	malathion				demeton-S-methyl				crufomate			
plant	C_0 (ppm)	k (h^{-1})	r^2	$t_{1/2}$ (h)	C_0 (ppm)	k (h^{-1})	r^2	$t_{1/2}$ (h)	C_0 (ppm)	k (h^{-1})	r^2	$t_{1/2}$ (h)
parrot feather	0.5	0.012	0.91	58	5.0	0.011	0.93	69	5.0	0.005	0.86	139
duckweed	0.5	0.014	0.92	49	5.0	0.012	0.89	57	5.0	0.007	0.90	99
elodea	0.5	0.006	0.88	116	5.0	0.056	0.87	12	5.0	0.003	0.91	2302
enzyme extract of duckweed	3.0[a]	0.019	0.94	36	39[a]	0.013	0.92	53	34[a]	0.009	0.90	78

[a] In μM.

Table 2. Some Physicochemical Properties of OP Pesticides and Their BCF with Axenically Cultured Plants

					BCF[c] (L/kg)		
compound	water solubility[a] (ppm)	vapor pressure[a] (mmHg)	K_{ow}[b] (mL/mL)	K_{oc}[b] (mL/g)	parrot feather	duckweed	elodea
malathion	145	4×10^{-5}	280	134[d]	3.0	23	1.2
demeton-S-methyl	3300	4.8×10^{-4}	21	66	0.023	13	0.37
crufomate	<1	4.9×10^{-5} [d]	1585[d]	759[d]	2.7	2.6	0.046

[a] The data were taken from *Wauchope's Pesticide Properties: A Selection of Fields from the USDA–ARS Interim Pesticides Database*, version 1.0; U.S. Department of Agriculture, Washington, DC, 1988. [b] From *The Pesticide Manual*, 10 ed.; British Crop Protection Council, Lavneham, Suffolk, and Royal Society of Chemistry, London, U.K., 1994. [c] The bioconcentration factor (BCF) was calculated as described under Materials and Methods [d] The data were calculated by SPARC.

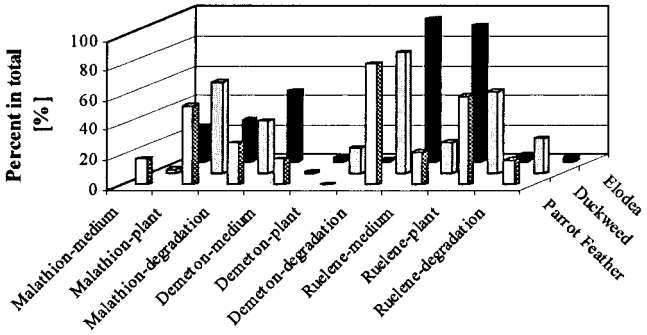

Figure 2. Distribution and mass balance of OP compounds from the axenic plant incubations. OP compounds in both medium and plant were extracted and determined by GC-ECD analysis at the end (8 days) of incubation except crufomate (ruelene) (14 days). The amounts are reported as percentages of the initial OP concentration in the medium. "Plant" refers to the amount extracted from the plant, whereas "degradation", calculated as the difference between the initial spiked concentration and the sum of recovered concentrations from the medium and the plant, refers to the amount of OP that is not extractable from the plant and is an indication of that metabolized and/or irreversibly bound.

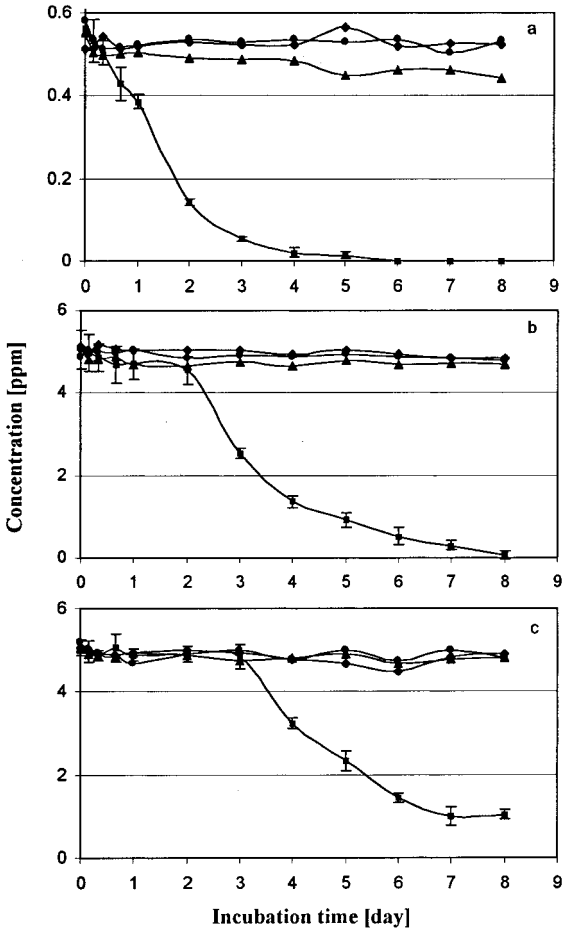

Figure 3. Uptake of malathion (a), demeton-S-methyl (b), and crufomate (c) in the media by axenic duckweed (*S. oligorrhiza* L.) under controlled conditions: control (◆); culture medium (●); autoclaved duckweed (▲); viable duckweed (■). Concentrations are of the residual pesticide in the liquid culture medium as determined by GC-ECD analysis. Error bars represent ±1 standard deviation. See text under Materials and Methods for details.

Uptake and Phytotransformation by Duckweed. When compared to parrot feather, duckweed (Figure 3) showed several significant differences in disappearance kinetics of the three OP compounds: (1) Malathion and demeton-S-methyl disappeared from the medium more rapidly and reached equilibrium after 5 and 8 days, respectively. After reaching this state, the medium concentration of malathion and demeton-S-methyl was lower in duckweed (2.7 and 1.2% of initial concentration, respectively) than in parrot feather (18 and 17%, respectively). (2) The initial time of depletion from the medium was delayed for demeton-S-methyl and crufomate, by 2 and 3 days, respectively, after the beginning of incubation. The delay is not caused by microbial activity, on the basis of the low bacterial colony counts (see Materials and Methods). It is likely attributed to different physicochemical properties of the OP compounds (Table 2) and to differences in plant species, which contain substrate-specific protein carriers for transferring pesticide molecules as well as catalytic enzymes that require induction times (Mohn and Tiedje, 1992). (3) Equilibrium for crufomate was reached more rapidly in duckweed (7 days) than in parrot feather (11 days). (4) Comparison of the kinetic parameters shown in Table 1 reveals that duckweed demonstrated a potential similar to parrot feather for uptake of the OP compounds. However, it generally accumulated (BCF,

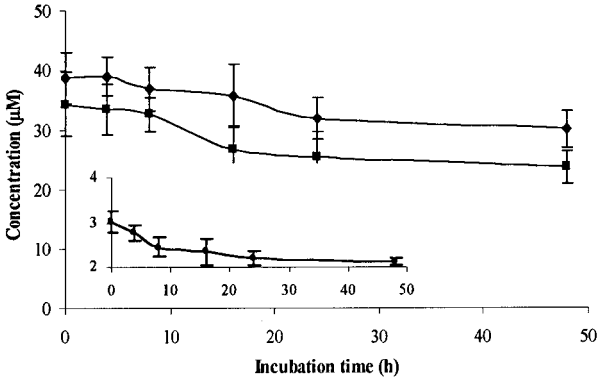

Figure 4. Degradation of OP compounds with the enzymatic extract of duckweed (*S. oligorrhiza* L.): malathion (●); demeton-S-methyl (◆); crufomate (■). Error bars represent ±1 standard deviation. See text under Materials and Methods for details.

Table 2) more of the OP compounds than did parrot feather, except for crufomate (Table 2). Mass recovery data showed also that slightly higher percentages of the OP compounds were degraded by duckweed (Figure 2). These results indicate that, in addition to uptake potential, other factors determine the transformation of the OP compounds. These factors could likely involve different enzyme systems (hydrolases, monooxygenases, etc., see below), isoenzymes (e.g., hydrolases with different substrate specificities), and enzyme cofactors.

Phytotransformation with Duckweed Enzyme Extracts. The duckweed enzyme extract transformed ~25% of malathion after 24 h of incubation; no further transformation occurred before the end of incubation (48 h; Figure 4). Maximum degradation occurred by 8 h. The demeton-S-methyl concentration exhibited no significant change until 8 h, but it was reduced ~15% at 24 h. Similarly, the crufomate concentration decreased ~20% after 16 h of incubation; no further change was observed after that. The mechanism(s) for the delay in the initial degradation of demeton-S-methyl and crufomate relative to that for malathion remains to be elucidated, although uptake processes and enzyme induction can be ruled out as possible factors.

Compared to the whole plant incubation of duckweed, enzyme extracts of duckweed provide mostly higher turnover rates for degradation of OP compounds due to the absence of uptake limitations and translocation processes (Table 1), but the extracts also exhibit less metabolic capacity (van der Krol et al., 1995). The intrinsic enzymatic activities are the same in both cases and are more effective than chemical degradation (Lai et al., 1995). The enzymatic transformation of OP compounds is probably attributed to OPH, which has been well characterized in duckweed (Lai et al., 1995) and has a broad spectrum of reactivity that is capable of hydrolyzing a variety of OP compounds with P−O as well as P−S bonds (Hoskin et al., 1995; Sakai et al., 1993). The most common reaction involves base-catalyzed cleavage at a phosphate ester linkage. Thus, a key variable in the susceptibility of OP compounds to enzymatic hydrolysis is the electron deficiency of the phosphorus atom to which a leaving group is attached (Schwarzenbach and Gschwend, 1990). In addition to OPH, other enzymes may also mediate degradation of OP compounds. These involve mixed function oxidase (P═S bond oxidation; Bond et al., 1997), flavin-containing monooxygenase (P═S bond oxidation; Levi et al., 1992), glutathione *S*-transferase (EC 2.5.1.18, C−S bond cleavage; Edwords and Onen, 1998), and carboxylesterase (EC 3.1.1.1, C−O bond cleavage and O-dealkylation; Lan et al., 1983). Whether the observed degradation of the OP compounds by the enzyme extracts of duckweed was the result of a single- or multiple-enzyme system cannot be determined until more of the physical and chemical properties of these extracts are elucidated.

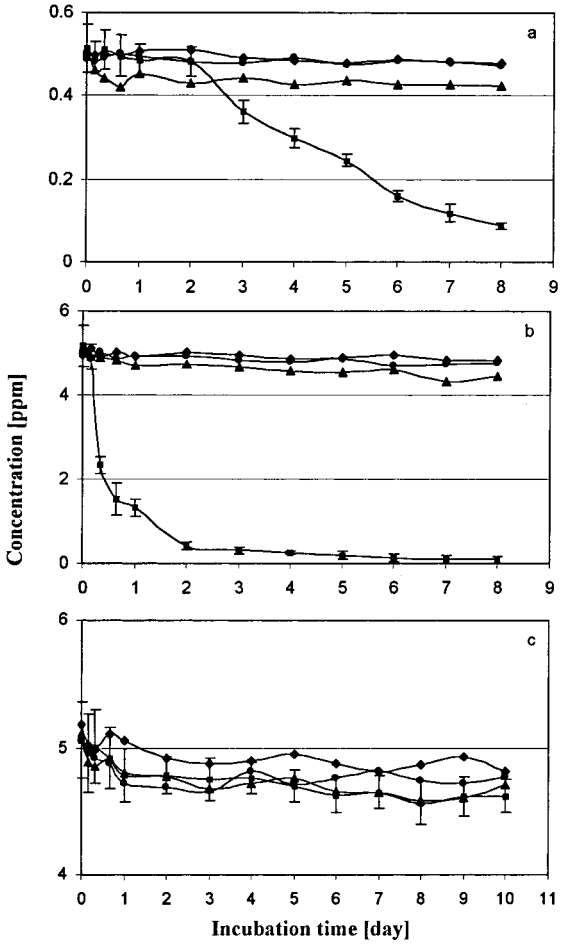

Figure 5. Uptake of malathion (a), demeton-S-methyl (b), and crufomate (c) exposed to axenically cultivated elodea (*E. canadensis*) under controlled conditions: control (◆); culture medium (●); autoclaved elodea (▲); viable elodea (■). Concentrations are of the residual pesticide in the liquid culture medium as determined by GC-ECD analysis. Error bars represent ±1 standard deviation. See text under Materials and Methods for details.

Uptake and Phytotransformation by Elodea. Unlike parrot feather and duckweed, malathion concentrations in elodea did not decrease until 2 days after incubation, but a similar reduction in concentration was observed after that (Figure 5). The reason for this delayed transformation remains unclear. Again, plant uptake may play an important role. Demeton-S-methyl exhibited a different behavior with an initial fast disappearance from the medium, followed by a slower reduction rate; >90% was lost within 48 h. Its short $t_{1/2}$ (Table 1) and low BCF (Table 2) indicate that elodea may be an effective plant for remediation of this OP compound in the environment. This rapid decline and high degree of transformation (>95%; Figure 2) of demeton-S-methyl may be due to its particular metabolic pattern in plants. Generally, demeton-S-methyl first undergoes a sulfoxidation reaction to produce

demeton-S-methyl sulfoxide, which is less stable and continues to degrade to demeton-S-methyl sulfone. This water soluble, nontoxic product can be further stored in cell vacuoles or released into the extracellular space (WHO, 1997; Sandermann, 1992). This type of metabolic process is supported by the fact that a similar decay profile for biodegradation of demeton-S-methyl was observed in all three tested plants.

On the other hand, over the whole experimental period (10 days) very little reduction in crufomate concentration was observed (Figure 5). This lack of disappearance of crufomate was characterized by a very long $t_{1/2}$ (Table 1) and very low BCF (Table 2). The rate of metabolism is unlikely to be the cause of the low BCF; rather, the uptake by the elodea could be the rate-limiting step because >90% of spiked crufomate was recovered from the medium at the end of the incubation (Figure 2). Although crufomate is much more lipophilic than malathion and demeton-S-methyl, it was not able to penetrate the elodea surfaces, indicating that the K_{ow} value is not the only factor that determines plant uptake. Other factors such as various mass-transfer barriers on the elodea surface or the thickness of the wax layer in the elodea subsurface can influence the transport of crufomate across the cell membrane to the cytoplasm (Martinez et al., 1996; Richins et al., 1997). The different patterns in disappearance and transformation of OP compounds presented in this study, therefore, may reflect a common phenomenon of substrate and species specificities.

Conclusions. This study indicated that OP compounds can be taken up by aquatic plants and subsequently transformed as a function of time. The rates of disappearance from the aqueous medium are in the order duckweed > parrot feather > elodea, elodea > duckweed > parrot feather, and duckweed > parrot feather > elodea for malathion, demeton-S-methyl, and crufomate, respectively. Subsequent plant-mediated transformation of OP compounds can be attributed to enzymatic reactions. The intrinsic ability of plant enzymes to transform OP pesticides was confirmed by using tissue enzyme extracts derived from duckweed, which can be manipulated readily in vitro for studies of transformation processes. The physicochemical properties of both the OP compounds and the plant species play a critical role in phytotransformation processes. Increases in our knowledge of plant molecular biology and biochemistry as they relate to phytotransformation will shorten the time required for applying phytoremediation processes to environmental problems.

ACKNOWLEDGMENT

We thank Dr. Tse-Yuan Ou for helping to collect plant samples and for valuable discussions and Dr. Mac Long for SPARC calculations of some physicochemical properties of the OP pesticides. Jimmy Avants provided technical support.

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Received for review May 11, 1999. Revised manuscript received August 15, 2000. Accepted August 16, 2000. This work was funded by a grant from the Strategic Environmental Research and Development Program (SERDP). The National Research Council (NRC) is acknowledged for its financial support.

JF9904968