geous, Quebec. They were filled with lake water, algae, plants and sediment in various combinations. Each system was sprayed by hand with aqueous 10% fenitrothion EC formulation (1% Atlox 3409 and 1% Aerotex 3470) to simulate an aerial spray treatment equivalent to 4 oz/acre. The lake water temperature varied from 19–23 °C and the pH from 7.0–7.5 during the 14-day study. The lake water was analyzed periodically and a half-life of 1.5–2 days determined for fenitrothion in the systems. Despite absorption by the PVC, the observed half-life is consistent with the values, 0.73–5 days reported for shallow ponds (1 m deep) and small lakes (Symons, 1977). Thus, the half-life of fenitrothion in the field experiments is much less than the values determined in the laboratory study at the same pH and temperature but in the absence of light. Although surface evaporation (Marshall and Roberts, 1977), absorption by aquatic plants, algae and sediment will contribute to the disappearance of fenitrothion, the hydrolysis study and the formation of oxidation products in the field study strongly suggest that photolytic or microbial processes are the primary means of its removal from natural water systems.

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Enzymatic Detoxification of Waste Organophosphate Pesticides

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A bacterial enzyme preparation which could hydrolyze eight organophosphate pesticides was examined for its ability to detoxify pesticides derived from pesticide containers, spray tanks, and spray solutions and from industrial pesticide production facilities. The commercial pesticide formulations, parathion emulsifiable concentrate (EC) 48%, Dursban EC 50%, Diazinon EC 25%, cyanophos EC 50%, and methyl parathion wettable powder 25%, were examined at 0.02–0.04% (spray solution) and at 1% (container residue) concentrations. The enzyme preparation could hydrolyze these pesticides significantly faster than chemical hydrolysis procedures currently recommended, had a half-life in diluted formulated pesticide solutions ranging from 27 to 80 h, and was not strongly inhibited by the detergent and solvent ingredients in the commercial formulations. Residual amounts of formulated ethyl parathion in industrial pesticide containers could be enzymatically hydrolyzed (>95%) in 16 h using 13–16 mg of enzyme preparation/L of treated pesticide-containing waste water.

The use of pesticides throughout the world has been expanding quite rapidly since the first introduction of synthetic chemicals for use in crop protection, and growth rates of 6–8% per year (Doyle, 1975) are expected for the next several years. In 1980, it is estimated that 2.27 billion kg of pesticides will be produced (Munnecke, 1978). Hazardous wastes are generated in this section of the chemical industry at both the consumer and producer levels and in order to insure safe production, handling, and utilization of these agricultural chemicals, environmental guidelines and regulations governing these procedures have been established. Pesticide producers and formulators have a seemingly wide range of pesticide waste disposal technology available for meeting these air and water point source discharge requirements; however, once the pesticides have been distributed to the individual consumer, the methods available for the disposal and/or detoxification of excess pesticides, pesticide containers, and spray

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tanks and spray solutions are severely reduced, and in most situations, no detoxification technology exists.

The goal of this research was to develop enzymatic methods for pesticide detoxification which could be used primarily by the untrained consumer anywhere in the world with little added expenses.

In previous research, a mixed microbial culture was adapted to growth on parathion, an organophosphate insecticide (Munnecke and Hsieh, 1974). A cell-free enzyme preparation was obtained from this culture which could hydrolyze the organophosphate insecticides ethyl parathion, methyl parathion, paraoxon, Diazinon, Dursban, EPN, cyanophos, fenitrothion, and triazophos at rates significantly faster than chemical hydrolysis with sodium hydroxide (Munnecke, 1976). The enzyme activity for pesticide hydrolysis was stable at temperatures up to 45–50 °C, had a temperature and pH optimum for expression of enzymatic activity of 35 °C and pH 8.5–9.0, and was sufficiently stable for consideration for use in waste treatment systems.

The use of an enzyme in a waste treatment system is dependent upon its biochemical characteristics, that is, its ability to perform the required enzymatic function under the industrial conditions expected, and the cost for such a treatment. It is possible to decide whether an enzyme treatment system is practical only when both the biochemical and economical aspects are known. This paper will discuss the biochemical aspects involved with the use of a soluble pesticide hydrolyzing enzyme preparation for the detoxification of waste pesticides derived from the consumer and industrial sections of the pesticide industry. The examination of immobilized enzymes for treating waste waters from production and formulation facilities will be discussed in another paper (Munnecke, 1979), while the technological aspects concerning the production of this pesticide hydrolyzing enzyme will be discussed in a third paper (Munnecke and Fischer, 1979).

MATERIALS AND METHODS

Enzyme. An enzyme preparation which could hydrolyze organophosphate pesticides was obtained from a mixed bacterial culture grown on parathion (PAR) [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate] as the sole carbon and energy source (Munnecke, 1976). This culture was grown in 5- or 12-L fermentors in Braunschweig (Munnecke and Fischer, 1979) or in a 2000-L fermentor by Dr. Baumgarten, Bayer A. G., Wuppertal. The dry cell preparation obtained from Bayer A. G. was suspended (1 g/100 mL) in 100 mM phosphate buffer, pH 7.5, and sonified for 12 s/mL using a MSE model 150 sonifier, 150 Watt at its highest setting, while cells from the 5- and 12-L fermentors were removed from the fermentation broth by centrifugation (10000*g*, 15 min), resuspended in 100 mM phosphate buffer, and then sonified. The crude enzyme extract obtained after sonification was used directly in hydrolysis experiments. The specific activity of these enzyme preparations ranged from 1.0 to 6.0 μmol of PAR hydrolyzed/min·(mg of protein) under the assay conditions described in this section. An enzyme unit is defined as 1 μmol of PAR hydrolyzed/min at 22 °C.

In experiments involving pretreatment of the crude enzyme preparation, the enzyme preparation was produced as follows. *Pseudomonas alcaligenes*, a pure culture isolated from the mixed culture, was grown in a nutrient broth medium, harvested by centrifugation (10000*g*), washed with distilled water, and recentrifuged, and the cell pellet was then suspended in 100 mM phosphate buffer, pH 7.5, and divided into four portions. To portion A, 1 mM cobalt chloride was added, and to portion B, nothing was added. To portion C, 1% toluene was added and portion D received no additive. After 30 min, portion A and B were sonified for 12 s/mL, while C and D were not sonified. All samples A–D were then frozen with liquid nitrogen and dried under vacuum for 48 h. The four dried preparations were assayed by dissolving 10 mg in 10 mL of 100 mM phosphate buffer, pH 7.5, and then adding 0.1-mL aliquots to 5.0 mL of parathion test solution.

Chemicals. Technical PAR [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate] (97.8%) was used as a 1% methanol solution, or as a formulated 48% emulsifiable concentrate (EC). Dursban [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] as a 25% EC, Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] as a 48% EC (BASF, Ludwigshafen), cyanophos [*O,O*-dimethyl *O*-(*p*-cyanophenyl) phosphorothioate] as a 48% EC, methyl parathion [*O,O*-dimethyl *O*-(*p*-nitrophenyl) phosphorothioate] as a 25% wettable powder, and triazophos [1-phenyl-3-(diethoxythiophosphoryloxy)-1,2,4-triazol] as a 25% EC (Hoechst A.G. Frankfurt) were all obtained from Bayer A. G., Wuppertal, unless otherwise indicated. Waste water from the production of PAR was also provided by Bayer A.G., Wuppertal.

Some common ingredients used in formulating industrial detergents—ethoxylated nonyl phenol, sodium soap, sodium tripolyphosphate, sodium perborate, and alkyl benzene sulfonate—were provided by Novo Industries, Copenhagen. All other chemicals and solvents used in these experiments were obtained from Merck, A. G., Darmstadt.

Methods of Analysis. Gas chromatographic pesticide determinations were made using a flame ionization detector (Hewlett Packard 5750 G gas chromatograph) equipped with a 6% SE 30 Chromosorb W packed 1.85 m, 6 mm i.d. glass column. Detector and injector temperatures were 350 and 275 °C, respectively, and column temperatures ranged from 190 to 230 °C. Pesticides were first extracted out of the aqueous solution into hexane (1:1) and then injected.

Spectrophotometric measurements of PAR and methyl PAR degradation were possible by measuring the concentration of their yellow metabolite, *p*-nitrophenol after it was determined that equal molar quantities of *p*-nitrophenol resulted from parathion hydrolysis. The pH of aqueous samples was adjusted to pH 8.5 and *p*-nitrophenol concentration measured at 410 nm using a dual-beam Shimadzu UV-200 spectrophotometer.

Acid formation during the hydrolysis of the pesticides was also used to determine hydrolysis kinetics. Pesticides were added to a 5 mM phosphate buffer, pH 8.5, and the enzyme was then added and the solution continually mixed with a magnetic mixer. The pH was maintained constant during the hydrolysis by titration with 0.034 N NaOH. Two moles of acid were derived from 1 mol of hydrolyzed pesticide under the assay conditions. Alkoxy groups were not hydrolyzed in these experiments. Protein determinations were conducted using a modified Lowry method (Miller, 1959).

Enzyme Assay Methods. The standard enzyme assay solution contained 10 mg/L of PAR, added as a 1% methanol solution to 10 mM Tris/HCl buffer, pH 8.5. Enzyme was then added (0.01–0.25 mL) to 5 mL of test solution and vortexed, and the rate or enzymatic production of *p*-nitrophenol was measured spectrophotometrically. To determine the inhibitory effect of chemicals on enzyme activity, various chemicals were added to the assay solution, and the enzyme activity was then assayed.

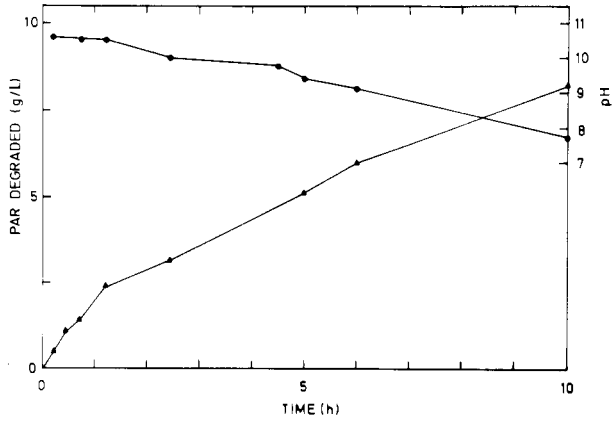

Figure 1. Enzymatic hydrolysis of parathion in a commercial formulation. See text for details. Initial PAR concentration (10 g/L), (●) pH data, (▲) grams/L of PAR degraded.

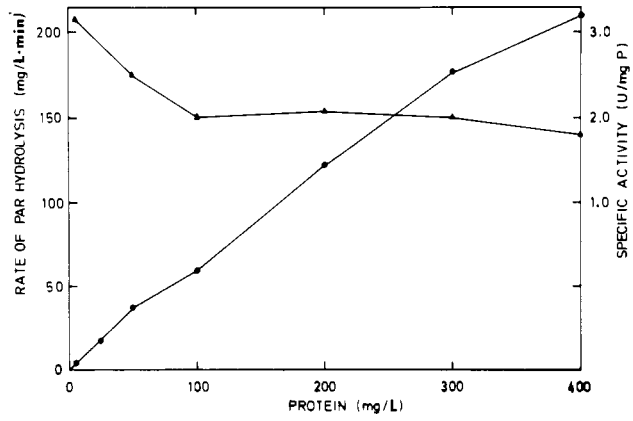

Figure 2. Effect of protein concentration on both the rate of parathion hydrolysis and the specific activity of the enzyme preparation. Rate of parathion hydrolysis is noted by ●, specific activity by ▲.

In stability studies, a 10-μL aliquot of the enzyme in the test solution stored at 22 °C was periodically removed over a 5-day period and its activity measured in the standard assay solution.

The rate of enzymatic hydrolysis of formulated pesticides was measured in a 100 mM carbonate buffer, pH 10.5. The pesticides were added at concentrations from 100 to 10 000 mg of active pesticide/L, and then enzyme preparation was added (final protein concentration, 25 mg/L), mixed, and allowed to sit at room temperature (19–22 °C). The pH was periodically checked and adjusted with 1 N NaOH if below 9.5. Kinetic measurements were conducted by the procedures mentioned above.

For pesticide container detoxification studies, the amount of PAR remaining in a given size container was determined by placing a known volume of formulated PAR into the container, rolling and shaking the container, and then measuring volumetrically how much could be poured out of the container, and this was subtracted from the initial known volume to determine the residual amount. Appropriate amounts of water, sodium carbonate–bicarbonate buffer, and enzyme, in that order (see Table IV), were added, and the container was shaken and allowed to sit for 16 h. The rate and extent of degradation was followed by determining the concentration of *p*-nitrophenol.

RESULTS

Hydrolysis. The kinetics of enzymatic hydrolysis of a 1% solution of formulated parathion (PAR) are shown in Figure 1. As the hydrolysis by 25 mg of protein proceeded over the 10-h experiment, the rate of hydrolysis decreased. Since the optimal pH for enzymatic hydrolysis is above pH 9, when the pH sinks as a result of 2 mol of acid released per mole of PAR hydrolyzed, the rate of hydrolysis also decreases. To maintain appropriate conditions for enzymatic hydrolysis of pesticide residues in containers, buffering salts were thus required. Of the two buffer systems which could be considered for industrial uses (phosphate and carbonate), carbonate buffer, due to its high buffering capacity at high pH values, proved the best. The pH of the waste pesticide solution after complete pesticide hydrolysis was controlled by the amount of carbonate used per gram of PAR hydrolyzed. The enzyme preparation had its highest expressed activity when the pesticide solution remained throughout the hydrolysis reaction above pH 9.5 (Munnecke, 1976), but since such a high final effluent pH would be environmentally undesirable, it was necessary to compromise and select a carbonate/pesticide ratio that would allow for high expressed enzyme activity during the entire hydrolysis reaction and yet produce a neutral, final pH. A carbonate/PAR (g/g) ratio of 0.35 was determined best for this purpose and allowed for more than 80% enzyme efficiency while producing a final effluent between pH 7.0 and 8.0.

Table I. Effect of Initial Formulated Pesticide Concentration on the Specific Activity of the Crude Enzyme Preparation

	specific activity[a] (units mg) of crude enzyme at various pesticide concentrations			
	active ingredient, mg/L			
pesticide	100	1000	5000	10 000
parathion, 48% EC	2.5	2.3	2.2	1.9
methyl parathion, 25% WP	1.8	2.3	2.8	2.6
cyanophos, 48% EC	2.2	3.0	3.5	3.0
Diazinon, 25% EC	0.2	0.4	0.6	0.4
Dursban, 48% EC	0.2	0.1	0.0	0.0

[a] Expressed in micromoles of pesticide hydrolyzed/min·mg of protein, 25 mg of protein/L added.

Most of the experiments reported here were conducted with less than 25 mg of protein/L of pesticide-containing waste water. However, higher concentrations of enzyme preparation increased the rate of hydrolysis (Figure 2). Thus, raising the protein concentration from 25 to 400 mg/L, a 16-fold increase, improved the rate of hydrolysis 11-fold. The most efficient use of the protein occurred, however, at lower protein concentrations. The expressed specific activity of the protein at 10 mg/L was 3.16 units/mg, while at 400 mg of protein/L, only 1.85 units/mg of protein was expressed (Figure 2).

Previous studies indicated that this enzyme preparation hydrolyzed PAR and other organophosphate pesticides when they were in solution as technical grade chemicals (Munnecke, 1976). The enzymatic hydrolysis of five of these pesticides in commercial formations at four concentrations was examined, and the results are shown in Table I. At 1000 mg (active pesticide)/L substrate concentrations, the relative activity of the enzyme preparation for pesticide hydrolysis varied from those previously measured for the technical chemicals at low, water-soluble concentrations (relative activity PAR = 1.0, triazophos, 1.45, cyanophos 0.07, methyl parathion 0.20, Dursban 0.20, Diazinon, 0.41) Munnecke, 1976). Thus, cyanophos and

Table II. Enzymatic and Chemical Degradation of Formulated and Technical Parathion[a]

test solution	rate of hydrolysis, mg/L·min			
	shaking flask		standing flasks	
	enzymatic	chemical	enzymatic	chemical
parathion, technical (5 g/L)	0.39	0.005	0.07	0.01
parathion, technical (5 g/L) + 0.1% Triton X-100	1.60	0.019	0.06	0.001
parathion, 48% EC (5 g of active PAR/L)	24.3	0.03	18.0	0.015

[a] Test conditions: chemical hydrolysis by 0.1 N NaOH, enzymatic with 50 mg of enzyme preparation/L, 200 mM carbonate buffer, pH 10.5, shaking flasks (110 rpm) or standing flasks, incubation temperature, 22 °C.

methyl PAR were hydrolyzed faster than parathion at 5000 mg/L substrate concentrations, while only triazophos was hydrolyzed faster at low substrates concentrations (13 mg/mg/L) L) in earlier reported work (Munnecke, 1976). Linear degradation curves for Dursban, parathion, and Diazinon were observed at higher concentrations (100–10 000 mg/L), whereas nonlinear degradation kinetics for methyl PAR and cyanophos were measured at these concentrations.

When two pesticides were in solution simultaneously at equal molar concentrations, there occurred three types of enzymatic hydrolysis kinetics. With the combination triazophos EC–PAR EC, triazophos was preferentially hydrolyzed. With the combination PAR EC–methyl PAR, PAR was first degraded and methyl PAR degradation was delayed until PAR was completely hydrolyzed, while with the combination PAR EC–cyanophos EC, both pesticides were simultaneously degraded.

The enzymatic and chemical degradation of large quantities of technical PAR were also examined and compared to the rates of degradation of formulated, and Triton X-100 solubilized PAR (Table II). In all cases, enzymatic hydrolysis by 50 mg/L of protein was faster than chemical hydrolysis by 0.1 N NaOH. A comparison of the rates of enzymatic hydrolysis of technical and formulated parathion indicates that the kinetics of PAR solubilization drastically limits the rate of enzymatic hydrolysis, whereas the low solubility of PAR (76 μM) did not as significantly affect the rate of chemical hydrolysis. Shaking the samples increased the rate of enzymatic hydrolysis of technical PAR more than fivefold, but only slightly (less than 25%) in the case of formulated PAR. The addition of Triton X-100 (0.1%) to technical PAR increased the hydrolysis rate only if the samples were shaken.

Stability. The stability of parathion hydrolase activity in various solutions was examined to determine if this enzymatic activity would be sufficiently stable for commercial use. The enzyme preparation in formulated pesticide solutions (0.2–0.4% active ingredient), pH 8.5–10.5, at 21 °C had half-lives ranging from 27 to 50 h, while at higher concentrations (1%) at pH 10.5, the half-lives ranged from 43 to >50 h (Table III). With Dursban, the enzyme preparation quickly lost approximately 50% of its activity but the remaining activity decreased more slowly. Industrial waste water from the production of PAR did not inactivate the enzyme preparation if the pH of the waste water was first adjusted to within a range of 8.5–10.5. Certain detergent components at 0.1% shorten the half-life of the enzyme preparation to 23–35 h.

Inhibition. Various chemicals which might be in pesticide waste waters were examined to determine if they would significantly inhibit enzymatic pesticide hydrolysis. In an earlier study, organic solvents were examined (Munnecke, 1976), while in this study, various salts, detergent ingredients, and pesticide formulations were examined. The only truly strong inhibitors were the detergent ingredients, sodium perborate (26% I at 9.1%) and alkyl benzenesulfonate (100% I at 0.1%) (Table IV). The ingredients of formulated parathion, as well as chemicals in waste water from PAR production inhibited the enzymatic activity by 24 and 15%, respectively. Common salts,

Table III. Stability of Parathion Hydrolase Activity in Various Solutions

test solution	pH	half-life, h
industrial pesticide production waste water	8.5	>50
parathion, 48% EC		
0.2%	8.5	27
0.2%	10.5	>50
1.0%	10.5	>50
Dursban, 50% EC		
0.2%	10.5	>50
1.0%	10.5	44[a]
Diazinon, 25%		
0.4%	10.5	44
1.0%	10.5	>50
cyanophos, 50% EC		
0.2%	10.5	40
1.0%	10.5	>50
methyl parathion, 25% WP		
0.4%	10.5	>50
2.0%	10.5	43
detergent components		
0.1%	9.8	23-35
control		
5 mM phosphate buffer	8.5	80

[a] Within 1.5 h the enzyme had lost 50% of its activity. Half-life reported is for remaining activity.

Table IV. Influence of Various Chemicals on Parathion Hydrolase Activity

test solution[a]	concn, %	inhibition,[b] %
control, 10 mM Tris		0
pesticide related		
parathion, 48% EC	0.04	15
parathion, 48% EC	1.0	24
waste water from parathion production	full strength	15
p-nitrophenol	0.2	0
detergent components		
ethoxylated nonylphenol	0.1	0
sodium soap	0.1	0
sodium tripolyphosphate	0.1	0
sodium perborate	0.1	26
alkyl benzenesulfonate	0.1	100
salts		
magnesium sulfate	5.0	7
calcium chloride	5.0	+7
potassium nitrate	5.0	+13
potassium phosphate	5.0	+37

[a] Tests were conducted in Tris, 10 mM buffer, pH 8.5, with 15 mg of protein/L of enzyme solutions. Waste water was made pH 8.5 and parathion then added, 10 mg/L. [b] Numbers marked with a + indicate percent stimulation, no sign represents percent inhibition.

Table V. Enzymatic Detoxification of Pesticide Containers Containing Formulated Parathion Residues

container	PAR EC residue,[a] g	active PAR, g	water added, L	carbonate added, g	enzyme added, mg	pesticide degraded, %
1 L, metal	5.8	2.8	0.58	1.4	8.4	97
5 L, metal	23.8	11.4	2.40	5.7	35.0	94
10 L, metal	52.8	25.4	5.28	12.7	82.0	94
20 L, metal	67.6	32.4	6.76	16.2	101.0	98
50 L, metal	81.0	38.9	8.10	19.4	120.0	94
200 L, metal	177.0	85.0	17.70	42.5	212.0	94

[a] Average from three runs.

Table VI. Use of an Enzyme-Detergent for Detoxification of Parathion Absorbed to Cloth

		wash cycle, 45 °C					
		wash phase (15 min)		rinse 1 (15 min)		rinse 2 (15 min)	
solution	PAR hydrolysis (30 min), %[b]	cloth,[a] %	degradation,[b] %	cloth,[a] %	degradation,[b] %	cloth,[a] %	degradation,[b] %
detergent, 0.25%, pH 9.8	0.0	22.0	0.0	12.0	0.0	2.7	0.0
detergent + enzyme, 50 mg/L	100.0	5.0	74.0	2.0	88.0	0.1	99.9

[a] Percent of parathion remaining in the cloth. [b] Degradation of parathion.

at concentrations of 5%, did not significantly inhibit and generally stimulated the hydrolysis. Since practical grade salts were used in this study, the increase in enzyme activity may have been produced by contaminating divalent salts such as cobalt and magnesium which are known to enhance the activity of this enzyme preparation (Fischer and Munnecke, 1979).

Applications. Containers. After the ability of the enzyme preparation to degrade formulated and technical pesticides in laboratory glassware had been examined, it was then necessary to determine if residual pesticide in the actual pesticide containers could also be completely degraded by the addition of the enzyme preparation. The amount of pesticide residue remaining in an "empty" container was dependent upon the type and size of the container and the pesticide formulation it contained (Table V). The amount of the parathion emulsifiable concentrate (PAR EC) residue, for instance, ranged from 4.5 g in a 1-L metal container to 177 g (PAR EC) for a 200-L container. Sufficient water was added to these containers to bring the formulated pesticide concentration below 1%, and carbonate buffer and parathion hydrolase activity were added (see Table V for amounts) and the containers briefly shaken. After 16 h, the amount of pesticide remaining was determined by measuring *p*-nitrophenol concentration and calculating the amount of pesticide remaining. The percent degradation is reported in Table V. Thus, without the need for continuous agitation, PAR EC could be 94–98% degraded in the pesticide container during this reaction time. Higher temperatures, of 37–50 °C, increased the rate of pesticide degradation by approximately 20–40%, while doubling the incubation time approximately halved the required amount of enzyme.

Enzyme Preparation. Fresh cell preparations which were not sonified expressed only 2% of the activity of the same cells which were opened by sonification. Therefore, it was necessary to determine how to pretreat the cells before lyophilization in order to achieve a higher enzyme efficiency during the proposed commercial applications of this enzyme preparation. Results indicated that pretreatment involving cell sonification in a 100 mM phosphate buffer containing 1 mM cobalt chloride before lyophilization produced a dried enzyme preparation which contained 90–105% of the initial activity of the nonfrozen, nonlyophilized by sonified, cobalt-treated cells, and represented a two–four-fold increase in expressed activity over nonpretreated, lyophilized cells. The lyophilization process increased the enzyme activity of the nontreated cells by 25-fold and these dried cells had the same activity as cells pretreated by sonification without cobalt present. The increase due to cobalt was more pronounced the longer the cells were incubated after sonification and before lyophilization. After 3 days, the cobalt treated cells had twice the activity of sonified cells without cobalt, and after 7 days, four times more activity. Toluene treated cells did not show a significant increase in enzyme activity in comparison to nontreated, lyophilized cells.

In addition to higher expressed activity, sonified cobalt pretreated cell preparations easily went into solution whereas the untreated cell preparation was more difficult to solubilize. The nontreated cell preparation was shown to have a storage half-life of more than 11 months (Munnecke and Fisher, 1979) and the storage stability of pretreated enzyme preparations is also good but had not been accurately determined.

Enzyme Detergent. The use of this crude enzyme preparation to degrade PAR which is not in solution but absorbed onto cloth was examined. PAR (500 mg) was allowed to absorb for 4 h onto a 5 × 5 cm portion of cotton cloth before this was placed into either a commercial detergent solution (0.1%, pH 9.8) or the same detergent solution, with 50 mg of enzyme preparation/L. Preliminary experiments at 30 °C showed that after 30 min, all the PAR was degraded in the enzyme-detergent solution, whereas no significant (less than 1%) PAR degradation occurred in the detergent solution (Table VI). In a second experiment, a wash cycle was simulated and results showed that with an enzyme detergent wash 99.9% of the pesticide was degraded (0.1% remained in the cloth), whereas in the detergent wash, no degradation occurred, 2.7% of the PAR remained in the cloth, and the rest was found in the rinse waters. In a normal wash with 4–5 kg of clothing, it would be expected that a portion of this solubilized PAR would be reabsorbed by the noncontaminated clothing.

DISCUSSION

Enzyme kinetics for PAR hydrolysis did not show expected, classical linear kinetics (Figures 1 and 2). In Figure 1, this can partially be attributed initially to inactivation and inhibition of the enzyme by the formulation chemicals (Tables III and IV) and secondly to the effect the changing pH had on enzyme activity during the second half of the experiment. In the experiment relating enzyme hydrolysis kinetics to enzyme concentration (Figure 2), the premature

departure from direct linear increases in enzyme kinetics with increasing protein concentration is probably due to the fact that in the 1000 mg/L PAR C solution used in these experiments, the concentration of water solubilized PAR is not more than 20 mg/mL and thus substrate availability and solubilization kinetics may be limiting the rate of hydrolysis.

Differences in enzyme specific activity reported in previous research (Munnecke, 1976) and in this current report may result from varying degree of inhibition of enzyme activity by formulation chemicals. Since pesticide formulations without the pesticide were not available, it was not possible to evaluate how strongly the various formulation chemicals inhibited enzymatic activity.

Although the enzyme appeared to have a higher affinity for Dursban, PAR, Diazinon, and triazophos than it did for methyl PAR and cyanophos, these differences could not be quantified due to possible interferences from formulation chemicals. The hydrolysis rates in part may also be a reflection of the rate of pesticide solubilization.

Organophosphate pesticide detoxification procedures intended for use by the consumer involve the use of strong alkali (1 N or more) for the chemical hydrolysis of the phospho-alkyl ester bond (National Agricultural Chemical Association, 1965; Lawless et al., 1975) with subsequent burial of the treated waste solution. However, this method is slow, large amounts of salt are required, and the final pH of the treated solution is above 13. Previous chemical hydrolysis studies (Munnecke and Hsieh, 1974) indicated that 1 N NaOH hydrolyzed 5 mg of PAR/L·h, a rate 2400 times slower than the rate achieved by 400 mg of protein/L in these studies. Therefore, enzymatic hydrolysis procedures for these pesticides would represent a significant improvement in waste disposal technology. It is envisioned that the enzyme preparation could be distributed directly with the pesticide and could be used at a later date for container detoxification. More enzyme preparation could be obtained later if needed for detoxification of spray solutions and spray equipment.

The enzymatic hydrolysis of PAR or the other examined pesticides is not actually a full detoxification since the metabolites are still somewhat toxic. For instance, 1 g of PAR (LD_{50} oral rat 6 mg/kg) when completely hydrolyzed yields 0.48 g of *p*-nitrophenol (LD_{50} oral rat 300–350 mg/kg). This represents a 100–120-fold reduction in toxicity of the initial pesticide solution. Daughton (1976) and Daughton et al. (1977) reported high in vitro acetylcholinesterase inhibition by dialkyl phosphorus compounds and stated there was a general lack of toxicology on these chemicals. Yet, their in vitro assays may not reflect the in vivo toxicity of these compounds.

As well as being most likely less toxic than the parent pesticides, the metabolites of enzymatic hydrolysis are more water soluble and thus their rate of microbial metabolism is not limited by their solubility or their strong affinity toward insoluble materials. Therefore, the metabolites should be less environmentally persistent due to their greater availability to microbial attack. The microbial metabolism of alkyl phosphates (Cook et al., 1978) and a wide variety of substituted aromatics (National Academy of Science, 1972) by numerous microorganisms have been reported. The true change in toxicity of a pesticide-containing medium after enzymatic hydrolysis of the pesticide can only be accurately measured by conducting pertinent in vivo bioassay experiments which directly relate to the method and type of organism exposed to the treated pesticide waste.

Various research groups have shown that other classes of pesticides can be hydrolyzed by cell-free enzyme preparations (Senior et al., 1976; Sauber et al., 1977; Kearney and Kaufman, 1965; Lanzilotta and Pramer, 1970; Engelhardt et al., 1973; Tiedje and Alexander, 1969; Heritage and MacRae, 1977). Thus, by adapting cultures to growth on different pesticides, it may be possible to obtain enzyme preparations which could remove the specific toxicity of various phenoxy acetate, phenylurea, and phenyl carbamate herbicides. Generally though, no overall detoxification would occur since most of these chemicals are relatively nontoxic and their metabolites have almost equivalent toxicities. But enzymes could be used to remove the specific herbicidal activity from these pesticides and prevent, for instance, cross-contamination of pesticidal spray solutions. Enzymatic detoxification of the toxic dithioate insecticides, as well as some pesticidal phenyl carbamates is possible, however, since there is a large difference in the toxicity of the parent pesticide molecule and the resulting metabolites.

Studies involving the detoxification of large quantities of pesticides in soil have been started. Daughton and Hsieh (1977) reported that active, PAR adapted cultures when added to soil were able to degrade PAR at concentrations up to 10 g/kg of soil. Preliminary experiments by Bayer A.G. (Bayer A.G., 1977; Molhoff, 1978; Schmidt, 1978) with a dried enzyme preparation have indicated that PAR in three different soils (10 k/kg) could be enzymatically reduced by more than 90% within 24 h.

Other applications of this pesticide hydrolyzing activity which have been examined are the use of enzyme immobilized onto glass for continuous detoxification of pesticide waste streams (Munnecke, 1979) and the use of nylon-immobilized enzyme for possible use in blood detoxification systems or in analytical procedures (Sundaram and Munnecke, 1979).

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Degradation of Triphenyltin Hydroxide in Water

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The behavior of triphenyltin hydroxide (Ph_3SnOH) in dilute aqueous solution has been examined. The solubility in water was 1.2 mg/L at pH 7–9 and 6.6 mg/L at pH 4.2. Solutions at pH 5–10 were stable in the dark even at 32 °C and did not lose Ph_3SnOH by volatilization. However, aqueous Ph_3SnOH was readily degraded by homolytic cleavage of the tin–carbon bond to diphenyltin oxide when exposed to sunlight or to UV light in a laboratory photoreactor, and the photolysis rate was markedly increased in the presence of acetone. While neither tetraphenyltin, monophenyltin species, nor inorganic tin were detected as products, the formation of a water-soluble, nonextractable organotin polymer was indicated. A similar product distribution was observed for the decomposition of aqueous diphenyl- and monophenyltin species, even in the absence of light.

Triphenyltin hydroxide (Ph_3SnOH, Du-Ter) belongs to the organotin class of agricultural chemicals which includes tricyclohexyltin hydroxide (Plictran) and triphenyltin acetate (Ph_3SnOAc, Brestan). Du-Ter is currently under investigation in California as a fungicide for use on rice. As part of a study of the fate of chemicals used in rice culture (Soderquist and Crosby, 1975; Soderquist et al., 1977), we are investigating the environmental chemistry of Ph_3SnOH.

The widespread use of Ph_3SnOAc in Europe has resulted in a number of reports on its degradation in the environment (Cardarelli, 1977). While Ph_3SnOAc and Ph_3SnOH are chemically very similar, extrapolation from these previous studies is limited since they were conducted on substances such as soil and leaf surfaces, while rice culture involves application of Ph_3SnOH directly to the water in the flooded field. Using radiolabeled materials, earlier workers generally concluded that Ph_3SnOAc degrades through di- and monophenyltins to inorganic tin—a terminal product of no toxicological significance (Evans, 1974). Our study began with development of procedures suitable to routine analysis for these potential degradation products in water without need for radiolabels (Soderquist and Crosby, 1978).

The purpose of the work reported here was to examine the environmental fate of Ph_3SnOH in dilute aqueous solution, including aqueous solubility, aqueous reactivity (hydrolysis reactions), volatilization rate from water, and the effects of sunlight (photodegradation).

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For brevity, each of the phenyltin species is referred to here as though it existed only in cationic form (e.g., Ph_2Sn^{2+}); this formalism is not meant to imply exact identities for these species in dilute aqueous solution.

EXPERIMENTAL SECTION

Reagents. Dichloromethane and hexane were nanograde or equivalent and carbon disulfide and diethyl ether were analytical reagent grade (Mallinckrodt). Water was distilled and passed through Amberlite XAD-4 resin (Rohm and Haas) unless otherwise specified; the resin was purified as described elsewhere (Woodrow and Seiber, 1978). All other chemicals were used as received. Buffered solutions employed the Carmody system, with final concentrations in the following ranges: boric acid, 2–0.2 mM; citric acid, 0.5–0.05 mM; trisodium phosphate, 1–0.1 mM. All glassware was cleaned by soaking in 2 M hydrochloric acid followed by copious rinses with distilled water.

Standard Tin Compounds. Organotin chromatographic and fortification standards were prepared at the milligram/milliliter level in dichloromethane (DCM) with Ph_3Sn^+ [as either triphenyltin chloride or triphenyltin hydroxide (Alpha Ventron)]; Ph_2Sn^{2+} as diphenyltin dichloride (Research Chem. Corp.) or diphenyltin oxide (Thompson–Hayward, dissolved in DCM/acetic acid, 95:5); $PhSn^{3+}$ as phenyltin trichloride (Research Chem. Corp.); and Ph_4Sn (Aldrich). Du-Ter (Thompson–Hayward), a wettable-power formulation containing 47.5% Ph_3SnOH, was used as received. An inorganic tin standard was prepared by dissolving 0.250 g of tin metal in 150 mL of concentrated hydrochloric acid and diluting to 0.50 L; further dilutions were made with a 5% sulfuric acid/2.5% citric acid (w/v) mixture. Uniformly ring-labeled ^{14}C diphenyltin dichloride was provided by Thompson–Hayward. Each of the standard compounds was judged to be

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