The Fate of Parathion in a Model Ecosystem

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The gradual termination of the use of persistent chemicals, such as DDT, for insect control has been initiated by public concern for an environment free from insecticide residues. The ubiquitous presence of DDE, the primary contaminant resultant from the widespread use of DDT over the past 25 years, demonstrates the necessity for prior screening of pesticide chemicals before they are approved for general use in agricultural and public health entomology. The primary replacements for the persistent insecticides are derivatives of phosphoric or carbamic acid. While these compounds do not possess the long-term stability that is associated with the chlorinated hydrocarbon insecticides, several members of these two classes of insecticides are acutely toxic to some organisms and must be used with caution. Recently a terrestrial-aquatic model ecosystem has been developed (METCALF *et al.* 1971) which provides useful information about the persistence and accumulation of pesticides and organic chemicals. As a continuing program to evaluate the fates of pesticides in aquatic organisms, ^{14}C parathion has been examined in the model ecosystem.

MATERIALS AND METHODS

The procedures outlined by METCALF *et al.* (1971) for the program of the ecosystem and work-up of the system were followed except that, since parathion is quite toxic to mosquito larvae, the experiment was extended to 38 days instead of the usual 33 days. In addition, the organisms at the experiment's conclusion were extracted with acetone instead of acetonitrile. The acetone unextractable radioactivity remaining in the organisms was treated with 0.2N hydrochloric acid (25 ml) at 70°C for 1 hour to release conjugated products. These were then extracted, after evaporation of the hydrochloric acid, with acetone. Finally, any radioactivity remaining in the residues was solubilized with Protosol® (New England Nuclear) for liquid scintillation counting in Aquasol® (New England Nuclear). The separation, identification, and quantitation of metabolites was carried out by spotting the extracts of the organisms along with model compounds on tlc

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plates (Silica Gel-254, Brinkmann) for development in a solvent system of diethyl ether-*n*-hexane, 7:3 v/v. Radioautograms were prepared (No-screen X-ray film, Eastman Kodak) to facilitate the location of metabolites and degradation products. The metabolite distribution was determined by scraping the spots from the plate for liquid scintillation counting in a dioxane-based scintillation fluid containing 7 g PPO, 0.05 g POPOP, and 120 g napthalene in one liter of dioxane. An external quench correction method was employed.

In addition to the regular one ml water samples specified by the original procedures (METCALF *et al.* 1971), 10-ml samples of water were taken periodically, the pH was adjusted to pH 2 with dilute hydrochloric acid, and then extracted three times with diethyl ether. This added procedure should give some insight into the degradation of parathion in the water.

The model ecosystem experiments were run in duplicate with 2,6 ^{14}C ring labeled parathion having a specific activity of 2.84 mCi/mM, (ICN Nuclear Chemicals). The parathion was purified to >99% purity (assayed by tlc and radioautography) by preparative tlc using a solvent system of ether-*n*-hexane 7:3 v/v. The insecticide (5 mg) was applied to sorghum leaves in an acetone solution (0.5 ml). The ecosystems were housed in a Hotpack Environmental Chamber held at 27 $\pm$ 1°C with a 12-hr photoperiod. The aquatic portion of each system was aerated with a small aquarium pump.

RESULTS AND DISCUSSION

Tables I and II show the data for the distribution of metabolites and degradation products of parathion in the various components and organisms of the ecosystem. The data depicted in Figure I describe the degradation of parathion in the water.

Table I shows the distribution of parathion, metabolites and the degradation of products in the various organisms of the model ecosystem. Except for the fish, which contained an average of 0.1006 ppm of parathion, none of the organisms contained parathion or paraoxon, the toxic species of this organophosphate pesticide. Furthermore, while most of the organisms contained radioactivity (0.40 to 3.8 ppm), an average of 65% of the radioactivity in the algae, *Daphnia*, mosquito larvae and snails was unextractable, and in the fish only 19.7% was unextractable. The greater proportion of extractable residues from the fish as compared to the other organisms may be the result of

TABLE I

Concentrations (ppm) of parathion, metabolites, and degradation products in organisms of a model ecosystem

Spot	R_f [a]	Algae	Daphnia	Fish	Mosquito	Snails
I [b]	0.97	0.0356 (0.0384, 0.0327) [c]	--	--	--	--
Parathion	0.90	[d]	--	0.1006 (0.1408, 0.0604)	--	--
A [b]	0.55	--	--	0.0086 (0, 0.0171)	--	--
III	0.33	--	--	0.0222 (0, 0.0443)	--	--
Origin	0.00	0.3613 (0.2081, 0.5144)	0.2987 (0.2178, 0.3796)	0.0621 (0.0477, 0.0765)	0.2031 (0.0817, 0.3244)	0.2701 (0.3259, 0.2142)
Acid Hydrolysis		0.2625 (0.464, 0.0609)	--	0.1262 (0.0423, 0.2100)	0.0356 (0, 0.0712)	0.0094 (0.0188, 0)
Unextractable		2.3659 (2.3364, 2.3953)	0.3126 (0.2877, 0.3375)	0.0793 (0.12, 0.0385)	0.4329 (0.2173, 0.6485)	0.5724 (0.9752, 0.1696)
Total		3.0251 (3.0469, 3.0033)	0.6114 (0.5055, 0.7172)	0.3988 (0.3508, 0.4468)	0.6716 (0.2990, 1.0441)	0.8518 (1,3198, 0.3837)

a/ Silica Gel GF-254, diethyl ether-*n*-hexane, 7:3 v/v.

b/ Roman numerals - chemical structure unknown.

c/ Values in parentheses indicate Tanks I and II, respectively.

d/ None detected.

A *p*-nitrophenol

TABLE II

Concentration (ppm) of parathion, metabolites and degradation products in unhydrolyzed and hydrolyzed water

Spot	R_f [a]	Tank I			Tank II		
		U-H_2O [c]	H-H_2O [d]	Total	U-H_2O	H-H_2O	Total
I [b]	0.97	0.00029	[e]	0.00029	0.00010	--	0.00010
Parathion	0.90	0.00040	--	0.00040	0.00020	--	0.00020
II	0.73	0.00012	--	0.00012	--	--	--
A	0.55	0.00087	0.00080	0.00167	0.00060	0.00043	0.00103
III	0.33	0.00015	0.00004	0.00019	0.00021	0.00010	0.00031
B	0.25	0.00024	--	0.00024	0.00037	0.00032	0.00069
IV	0.13	0.00040	--	0.00040	0.00057	--	0.00057
V	0.09	0.00130	0.00074	0.00204	0.00171	0.00171	0.00342
Origin	0.00	0.00111	0.00301	0.00412	0.00333	0.00456	0.00789
Unextractable		0.01081	0.00621	0.00621	0.01799	0.01086	0.01086
Total ^{14}C				0.01569			0.02507

a/ Silica Gel GF-254, diethyl ether-*n*-hexane, 7:3 v/v.

b/ Roman numerals - chemical structure unknown.

c/ Water extracted with diethyl ether.

d/ The aqueous phase of U-H_2O after ether extraction was treated with 0.025 N HCl^2 (25 ml) at 70°C for 20 hours and then extracted with diethyl ether.

e/ None detected.

A *p*-nitrophenol

B paraoxon

the low titer of mixed function oxidase enzymes in the fish. In support of this, a hepatic microsomal preparation from female *Gambusia affinis*, was shown to contain a low level of aldrin epoxidase activity (KRIEGER and LEE 1973). A comparison of parathion with dieldrin in this model ecosystem (SANBORN and YU 1973) clearly demonstrates the greater susceptibility of parathion to undergo degradation as compared with dieldrin. The data from the examination of dieldrin in the model ecosystem indicated that every organism except the mosquito larvae contained dieldrin and that average of 97% of the extractable radioactivity was dieldrin. In this ecosystem experiment parathion constituted only 31.5% of the radioactivity isolated from the fish. This decrease in the percentage of parathion in the extractable radioactivity as compared to the data obtained for dieldrin is reasonable, as this organophosphate insecticide has numerous points susceptible to degradative processes, while dieldrin lacks sites for further degradation.

The amount of parathion isolated from the fish, ∿0.1 ppm, is about 335 x the concentration extracted from the water, ∿0.3 ppb (Table II). The uptake of parathion by the fish is much less than the approximately 6000 x concentration of dieldrin by the fish (SANBORN and YU 1973). The approximate hundredfold difference in water solubilities of 25 ppm for parathion (GUNTHER *et al.* 1968) and 0.25 ppm for dieldrin (FARMER and JENSEN 1970) may be an explanation for the lower uptake of parathion as compared to the uptake of dieldrin by the fish. In a recent publication of a similar relationship between uptake by fish and water solubility (METCALF *et al.* 1973) has been delineated for several chlorinated hydrocarbons.

Since the parathion utilized in this study was labeled in the 2,6 positions of the aromatic ring of the *p*-nitrophenol, it was possible to examine the fate of this molecule after it was released from the parent compound. In one fish in one of the ecosystems, small amounts (8.6 ppb) of *p*-nitrophenol were found. This appears to be the first quantitative demonstration of the "environmental" fate of the aromatic portion of a phosphate insecticide. If a complete picture of the deposition of pesticides in the environment is desired, it is necessary to determine for organophosphorus insecticides the ultimate fate of both the aromatic moiety, for example *p*-nitrophenol for parathion, and the phosphoryl moiety.

Examination of the data in Figure I and Table II for the degradation of parathion in water reveals several interesting points. In contrast to the organisms, the water contains many more metabolites, including paraoxon, which was not found in the fish. The average concentration values for the two ecosystems at the end of the 38-day experiment for parathion, paraoxon, and *p*-nitrophenol were 0.30, 0.47, and 1.4 ppb respectively. The level of 0.30 ppb is about 1/10 of the value suggested as an acceptable residue level for water (LEVINE 1962, 1963).

In addition to establishing the final concentrations of metabolites or degradation products in the water at the end of the experimental period, it is of interest to look at the dynamics of the degradation of parathion in the water over the entire experiment. To provide this type of information 10-ml samples of water were taken and extracted with diethyl ether after the pH was adjusted to pH 2. The tlc data for these extracts is given in Figure I, which shows the time dependence of the degradation of parathion in water. The points in Figure I are averages for the two ecosystem experiments. The degradation of parathion in water is slow, with a half-life of about 15-16 days. Simultaneous to the gradual disappearance of parathion from the water of the ecosystem is the gradual increase of polar material at the origin, reaching about 45% of the total extractable material at the termination of the experiment. Both *p*-nitrophenol and paraoxon constituted very little of the extractable material, with paraoxon remaining at less than 10% of the extracts and *p*-nitrophenol becoming about 15% of the extract on the 34th day of the experiment. The pattern of parathion may be typical for an organic molecule, such as parathion, which undergoes degradation in the water. While the amount of polar material at the origin may be largely dependent on the solvent system used to develop the tlc plate, a better measure of susceptibility to degradation may reside in the change in ether-unextractable radioactivity over the experimental period. Over the experimental period the unextractable material gradually increases until it reaches 45% of the radioactivity in the water. The increase in unextractable material indicates that parathion degrades to water soluble materials, perhaps conjugates, that become unextractable by an organic solvent.

CONCLUSION

From the data presented in this paper, it is concluded that parathion does not accumulate to any appreciable extent in any of the organisms of this

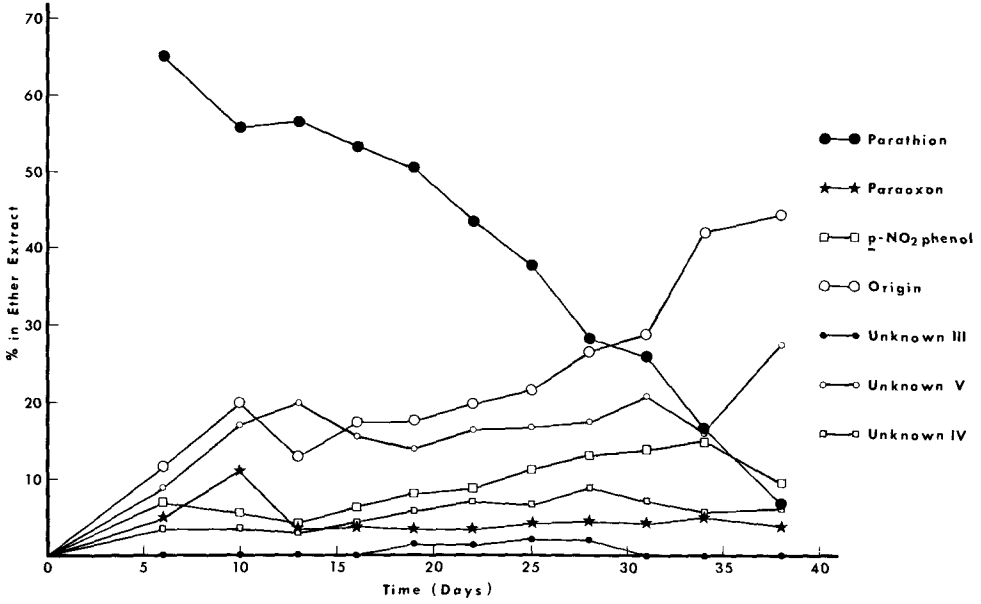

Figure I. Degradation of ^{14}C parathion in the water of a model ecosystem.

data to environmental situations is reasonable, as there has not been any indication of accumulation of organophosphate insecticides by upper food chain organisms such as fish. The chemical properties of parathion, such as the susceptibility to degradation by physical, chemical, or biological means to polar products, readily account for the low accumulation of parathion by the aquatic organisms of this ecosystem. It appears that the continued use of parathion for insect control will not result in the ubiquitous environmental contamination which has occurred with the use of chlorinated hydrocarbons, such as DDT.

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