Water Translocation of Diazinon-C14 and Parathion-S35 off a Model Cranberry Bog and Subsequent Occurrence in Fish and Mussels[1]

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ABSTRACT

Transportation of diazinon-C14 and parathion-S35 off a model cranberry bog following a simulated frost protection flood was shown 24 hr after application of the chemicals. Fish (*Fundulus heteroclitus* L.) and freshwater mussels (*Elliptio complanatus* Solander) were exposed to these contaminated waters and analyzed periodically. The majority of the chemicals disappeared from the water after 144 hr. During this time, no labeled metabolites of diazinon were detected; however, three labeled parathion degradation products were encountered. Accumulation of both pesticides occurred in the fish and mussels, often to levels far in excess of that present in the waters. The fish appear able to metabolize these accumulated chemicals at a faster rate than the mussels.

INTRODUCTION

It has been established that a major portion of the surface waters in America today are polluted with chemicals to some degree (Faust, 1961; Middleton and Lichtenberg, 1960). Agricultural chemicals constitute a significant segment of these pollutants and their use has aroused concern among both scientists and the general public. Pesticides enter surface waters as a result of direct application, either planned or accidental, aerial drift, leaching, or surface runoff.

The cranberry industry is dependent upon an adequate freshwater supply. Bogs may be flooded with 6 inches of water for 3 to 4 months to protect the vines from winter injury. It is a common practice to flood bogs in the evening and draw off the water the following morning at frequent intervals during the growing season to protect against frost damage. In some areas of the country the bogs are flooded to facilitate picking, and in others the bogs are picked dry and flooded afterwards to remove accumulated debris. The waters used in this operation may be returned to the original reservoir for re-use, or emptied into a runoff stream and carried away. Since a variety of pesticides are used for various purposes in cranberry culturing, it is important to know to what extent these chemicals are removed in the floodwater and to determine the ultimate fate of these compounds.

MATERIALS AND METHODS

Experimental design

To facilitate study of this problem, a model bog 2 ft × 1 ft × 1½ ft was constructed in the greenhouse to closely approximate natural conditions. A section of cranberry bog, thickly covered with vines, was dug to a depth of 4 inches and fitted into the model atop an 8-inch layer of sand–peat mixture (4 : 1). Cranberries can be transplanted in the same manner as turf, thus this operation was accomplished without excessive disturbance of the root system.

Drainage holes were cut in the ends of the model at the soil level and 1 inch up from the bottom. These were fitted with glass tubing and sealed in place. Rubber tubing was affixed to the glass and closed with pinch clamps. With this arrangement, floodwaters which remained on the bog and those which leached downwards could be collected separately.

Diazinon (O.O-diethyl O-(2 isopropyl-4-methyl-6-pryimidinyl)phosphorothioate) was applied to the model bog at a rate corresponding to 3 lb/A (62.8 mg containing 2.8 mg diazinon-C14, specific activity 3.21 μc/mM, labeled with C14 alpha carbon atoms on the ethoxy groups of the molecule). The model was flooded 1 hr after application and the water left on for 4 hr. In a second experiment, the model was flooded 24 hr after application.

Parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate) was applied in a third experiment at a 1 lb/A rate (20 mg containing

10 mg parathion-S^{35}, specific activity 2.23 $\mu c/mM$). A standard curve was constructed based on the decay constant of S^{35}, which allowed interpolation of activity counts in terms of relative occurrence of parathion and its metabolites. Flooding was done 24 hr after application, as outlined for diazinon.

The waters which remained on the bog after 4 hr were drained off and the total volume determined. A 1-liter subsample was taken for analysis and the remainder added to an aquarium containing a known volume of natural pond water. Prior to addition of the floodwater, freshwater mussels (*Elliptio complanatus* Solander) and estuarine fish (*Fundulus heteroclitus* L.) were placed in the aquarium. The leach water from the bottom of the bog was also collected, the volume determined, and 1 liter used for analysis. The remainder was discarded.

Analytical procedures

The purity of the labeled compounds was determined by thin-layer chromatography (TLC) using plates coated with Silica gel G and developing in 0.7% ethanol in chloroform. The plates were exposed to Eastman No-Screen X-ray film and autoradiographs made. The parathion and diazinon fractions were localized, the gel scraped from the plate, and the chemicals eluted with acetone. The concentration of each was determined on a basis of counts per minute (CPM) per mg, using a Nuclear-Chicago Model 1312 low background gas counting system. Counting efficiency of this instrument was calculated at 19% by use of a C^{14} barium carbonate standard. All data reported herein have been corrected on the basis of this value and all samples were statistically significant above background at the 5% level.

Extraction of the chemicals from water was by the method of Teasley and Cox (1963). Using this method, recoveries of 84% were obtained for both compounds.

Fish and mussels were extracted using the acetonitrile method as outlined by Margot and Stammbach (1964). Recovery from fortified samples were 91% for diazinon and 89% for parathion.

Plant material was ground in a Waring Blendor with 50 ml acetonitrile, filtered and re-extracted with 25 ml acetonitrile. The acetonitrile fractions were combined and excess water removed azotropically. Final dilutions were made with acetone. Recovery of fortified samples using this method was 92% for diazinon and 90% parathion.

Extraction of 100-g soil samples were made by slurrying with 20 ml distilled water and shaking on a wrist action shaker for 30 min with 150 ml hexane-isopropyl alcohol (2 : 1 - v : v). The extract was filtered into a separatory funnel and washed three times with 50 ml distilled water to remove the alcohol. The hexane fraction was then filtered through sodium sulfate, evaporated to incipient dryness and brought to desired volume with acetone. Recovery of diazinon fortified samples was 98%. Parathion was extracted in a similar manner using benzene-isopropyl alcohol (2 : 1 v : v) and yielded 93% recovery from fortified samples. All data reported herein have been corrected for recovery.

The percentage recovery for the extraction procedures was checked on an Aerograph model 204 gas chromatograph equipped with an electron capture detector. In analysis of experimental materials, the quantity of labeled material was determined on the gas flow counter. Since the labeled material represents a fixed percentage of the total applied, the total amount of chemical present could be calculated by the CPM of each sample.

All samples were processed by TLC and autoradiographs made, in the manner previously described, to localize the pesticide and possible labeled metabolites. Where labeled metabolites occurred, their ratio to each other and to the parent pesticide molecule was determined by eluting the spots from the plate and counting each separately.

Labeled diazinon and parathion standards were spotted on all plates for reference. In addition, nonlabeled standards for paraoxon, aminoparathion, p-nitrophenol, and p-aminophenol were also spotted. Visual identification of these compounds for comparison with labeled and nonlabeled metabolites was achieved by spraying with palladium chloride (0.5 g palladium chloride and 2 ml conc HCl in 98 ml distilled water) for parathion and aminoparathion. Counterspraying with 5 N

sodium hydroxide resolved paraoxon, p-aminophenol, and p-nitrophenol.

RESULTS

Diazinon

In this series of experiments autoradiographs of all sampled material never revealed any labeled compounds except the original diazinon molecule. A check on that portion of the extracting solution normally discarded never yielded significant amounts of activity. It is assumed that the labeled portion of the molecule was split off and metabolized to $C^{14}O_2$. The following results, therefore, refer only to the intact diazinon molecule.

Flooding the bog 1 hr after application of the chemical resulted in the floodwater transporting 17% (0.95 ppm) of the total applied diazinon off the bog. No chemical was detected in the leach water.

After 3 hr, fish within the aquarium to which the collected floodwater was added became disorientated, and all were dead after 24 hr. Analysis of the fish at that time revealed a diazinon content of 10.1 ppm, a value 10 times greater than that of the surrounding water. The mussels had a diazinon residue of 1.77 ppm after 48 hr, indicating they also were accumulating large amounts of the chemical.

When sampled after 120 hr, the residue in the water had decreased to 0.09 ppm whereas that in the mussels remained quite high at 1.35 ppm. Additional fish were added to the water at this time with no mortality or symptoms of poisoning occurring for a period of 216 hr. After this exposure time the fish had accumulated 0.05 ppm of the chemical.

In the second experiment, the model bog was not flooded until 24 hr after application of diazinon. A marked reduction in the amount of chemical occurring in the drained floodwater was noted (Table 1) and this figure (0.32 ppm) represents 4.6% of the applied total. Residue in the bog soil to a depth of 4 inches (0.11 ppm) and in the vines (7.33 ppm) represents 3.4% of the original dosage, indicating only 8% of the diazinon could be accounted for. It is believed that the remaining 92% was lost as a result of either volatilization or loss of the C^{14}

Table 1.—Persistence and translocation of Diazinon-C14 following application to a model cranberry bog

Time of sample (hr)	Bog soil	Bog vines	Flood-water	Aquarium water	Fish	Mussels
0[a]	0.11[b]	7.23	0.32	0.12[c]		
24					0.11	0.59
48				0.02		0.14
120					0.04	
144				0.01		0.02

[a] The samples were collected after the floodwaters were drained. The elapsed period of time between application and sampling was 28 hr. The 0 time is used for clarity in subsequent sampling of the aquarium, fish, and mussels.
[b] Data presented as ppm.
[c] Collected floodwaters added to aquarium water resulting in dilution.

label from the molecule via breakdown. No diazinon could be detected in the leach water removed from the bottom of the model, indicating downward migration of the chemical in the water did not occur.

Samples of the aquarium waters taken after 48 and 144 hr revealed a diazinon content of 0.02 and 0.01 ppm, respectively. These results correlate quite closely with those of the first experiment in demonstrating the rapid disappearance of the chemical in the water.

In the second experiment the fish did not die or become disorientated, indicating a sublethal, nontoxic level of chemical. After a 24-hr period, the fish had a residue of 0.11 ppm which decreased after 120 hr to 0.04 ppm. Again accumulation of the chemical could be noted.

The diazinon concentration in the mussels after 24 hr was 0.59 ppm, a value almost twice that of the water. This level decreased after 48 hr to 0.14 ppm and was constant throughout the remainder of the test.

Parathion

A greater quantity of parathion was recovered, as compared with diazinon, following the elapsed 24-hr period between application and flooding. The residue in the vines (12.0 ppm) represented 16.27% of the applied material, that in the soil (0.12 ppm) equaled 2.2%, and that in the water (0.12 ppm) corresponded to 6.5%. Combined, these values represent 24.9% of the applied dosage.

Subsequent addition of the water to the aquarium resulted in dilution which lowered the concentration (Table 2). In contrast to diazinon, a number of labeled degradation

TABLE 2.—*Persistence and translocation of parathion-S³⁵ following application to a model cranberry bog*

Time of sample (hr)	Bog soil	Bog vines	Flood-water	Aquarium water	Fish	Mus-sels	Aquarium soil
0	0.12ᵃ	12.0	0.12	0.07ᵇ	—	—	—
24	--	--	--	0.02	1.68	0.99	0.020
48	--	--	--	0.01	2.11	0.03	0.004
96	--	--	--	0.009	0.21	0.14	0.006
144	--	--	--	0.002	0.22	0.04	0.007

ᵃ Data presented as ppm.
ᵇ Collected floodwaters added to aquarium water resulting in dilution.

products were encountered in extraction of the various samples. Of these labeled metabolites, only one chromatographed similarly to a known standard and it corresponded to aminoparathion. No other attempts were made to identify the metabolites.

The concentration of parathion in the aquarium dropped to 0.02 ppm after 24 hr. At this time the first evidence of a labeled metabolite, hereafter referred to as unknown A, was observed which had an activity approximately 10% that of the parathion. After 48 hr, labeled parathion and unknown A were still present and a second labeled metabolite appeared. This second metabolite corresponded to aminoparathion by TLC. Parathion concentration at this time was calculated at 0.01 ppm. The aminoparathion metabolite and unknown A both had activities almost identical to the parent compound.

At the end of 96 hr, a third labeled metabolite, hereafter referred to as unknown B, occurred. Concentration of parathion was now determined at 0.009 ppm. The activity of aminoparathion and unknown A were approximately 70% of the parent compound, and unknown B was approximately 50%. By 144 hr, the parathion concentration was calculated at 0.002 ppm. Both aminoparathion and unknown A were equally as intense as parathion, however unknown B was now five times more active than any of the above compounds. It would appear that during the experiment, a selective group or groups of microorganisms had developed in the aquarium which could utilize the parathion and possibly aminoparathion and unknown A as a food source. This could account for the rapid increase in the amounts of unknown B which occurred.

Of the fish present in the aquarium only 12, from a total population of 60, were alive after the first 24 hr. Analysis of the dead specimens indicated a parathion concentration of 1.68 ppm, an amount 80 times greater than that in the water at the same period. In addition, an unknown metabolite, hereafter referred to as unknown C, was detected. This unknown was approximately 30% as active as the labeled parathion. It was observed that unknown C had an rf similar to unknown A, and that the radioactive intensity of each was similar. It is not, however, possible to state that they are chemically similar without further analysis.

After 48 hr, three of the remaining fish showed symptoms of poisoning. Analysis of these specimens showed an increased accumulation of parathion to 2.11 ppm. At the same time, the intensity of unknown C had increased to 75% that of the parathion. In sampling the fish after 96 hr, it was found that unknown C had greater activity than the parathion; however, after 144 hr the parathion concentration remained the same whereas activity of unknown C had decreased.

Accumulation of parathion also occurred in the mussels; however, the residue value varied during the periods of the test. It is suspected that this is a reflection in sampling error. Many of the clams burrowed deep into the soil, and in such a position would not be directly exposed to the pollutant. It is also not certain that these individuals were actively pumping water through their bodies, since it was not possible to observe if their valves were open or closed. Incorporation of such individuals in the sample would result in a lower residue value.

DISCUSSION

It has been established experimentally that parathion and diazinon could be removed in floodwaters drained from a cranberry bog. Whether the chemical was present in solution or in suspension adsorbed onto fine soil particles cannot be stated with any degree of certainty; however, it is certain that the chemicals were rapidly lost from the water. These results are similar to those of Sato and Kubo (1965) who could find only trace amounts of parathion in irrigation waters 7

days after application to a rice paddy. These authors also state that the presence of algae greatly accelerated the decomposition of parathion. Ahmed and Cassida (1958) and Gunner et al. (1966) have also shown organophosphate insecticides are readily metabolized by microorganisms. In addition to breakdown by hydrolysis and loss by evaporation, it is believed that the development of microflora in the aquarium is the major factor in governing the loss of the chemicals in the experiment. The accumulation in the body tissues of the fish and clams would also account for loss of the chemical. In a check on the inhibition of acetyecholinesterase by organophosphates, Weiss (1959) found a decrease in enzyme activity in fish following exposure to diazinon. When the fish were transferred to uncontaminated water, the enzyme activity levels rapidly returned to normal. Parathion, however, produced a continual effect on the enzyme when the fish were removed from the contaminated area. The results of the experiment reported herein closely parallel the above findings, in that an initially high residue accumulation occurred in the fish which decreased as the level of contamination in the waters decreased. It appears that the fish are quite capable of metabolizing both compounds, the presence of unknown C is evidence of this fact, and apparently diazinon is more rapidly degraded than is parathion.

The ability of oysters and mussels to concentrate pesticidal compounds at levels far above that present in the surrounding waters has been shown to be characteristic of the mollusks (U. S. Fish and Wildlife, 1964). In both experiments, the mussels accumulated the chemical at levels far above that of the surrounding water. The decrease in residue in the mussels is not as sharply indicated as in the fish, suggesting a much slower mechanism of metabolism. Indeed the limited amount of labeled metabolic products is quite surprising considering the many parathion breakdown products in the fish.

Since many aquatic organisms are susceptible to organophosphates at a concentra-

tion of 1 ppb, the transportation of these compounds into associated waters is of concern, especially in relation to the food chain sequence and the persistence in the organism involved. The high degree of accumulation encountered in the mussels and the degree of persistence is evidence of this point. The results of this investigation form a basis for further study of diazinon and parathion in natural conditions peculiar to cranberry culturing. Certainly, field conditions do not approach those which are controlled in the laboratory. Experiments are presently in progress to compare the data reported herein with normal field operations.

ACKNOWLEDGMENTS

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