Synerholm, M. E., Zimmerman, P. W., *Contrib. Boyce Thompson Inst.* 14, 369 (1947).
Wain, R. L., *J. Agric. Food Chem.* 3, 128 (1955a).
Wain, R. L., *Ann. Appl. Biol.* 42, 151 (1955b).
Walters, H. J., Caviness, C. E., *Plant Dis. Rep.* 52, 355 (1968).

Received for review August 1, 1975. Accepted November 3, 1975.

Interaction of Pesticide Chemicals. Effect of Eptam and Its Antidote on the Uptake and Metabolism of [^{14}C]Phorate in Corn Plants

Ken R. Schulz, Tom W. Fuhremann, and E. Paul Lichtenstein*

The effects of soil treatments with the herbicide Eptam (EPTC) and its corn antidote (Stauffer, R-25788) on the translocation and metabolism of [^{14}C]phorate in corn seedlings were investigated. While at concentrations of 5 ppm of Eptam in a [^{14}C]phorate treated (2 ppm) Plainfield sand no visible damage to the growing corn plants was noticeable, the amounts of ^{14}C compounds in corn greens from herbicide-treated soil were significantly increased by a factor of 1.8. In particular, the amount of unextractable ^{14}C-labeled residues was higher in plants grown in [^{14}C]phorate plus Eptam-treated soil. After 18 days soils contained phorate, phorate sulfoxide, and phorate sulfone, while in greens only the metabolites phorate sulfoxide, phorate sulfone, and phoratoxon sulfoxide could be detected. Due to the presence of Eptam in the [^{14}C]phorate-treated soil, the amounts of both phorate sulfoxide and phorate sulfone were significantly increased by a factor of 1.6 and those of phoratoxon sulfoxide by a factor of 2.4. Addition of the antidote (at 1 ppm) to [^{14}C]phorate-treated soil had no effect on the translocation and metabolism of insecticide in corn greens, yet counteracted the effects of Eptam. Thus, analyses of corn greens grown in [^{14}C]phorate-treated soil containing also Eptam and its antidote gave results similar to those grown in soil which had only been treated with [^{14}C]phorate. These findings further point to the problem of potential interactions of environmental chemicals in biological systems.

Modern agricultural practices have made extensive use of insecticides and plant growth regulators to increase food production. As a consequence, many soils contain residues of the various chemicals and/or their degradation products. The effects of these residues on subsequent pesticide applications as well as the effects of simultaneous applications of mixtures of agricultural chemicals on their metabolism in soil and their potential uptake by plants must be investigated. While the fate of individually applied agricultural chemicals has been extensively studied, relatively few reports on the interaction of herbicides and insecticides are available. Arle (1968), Hacskaylo et al. (1964), Nash (1967), and Parks et al. (1972) have all reported that phorate and other systemic insecticides increase the uptake of various herbicides and can alter their toxicity toward plants. However, the number of reports concerning the effects of herbicides on insecticides is relatively small. This laboratory has previously reported on the interactions and synergistic action of herbicides in combination with insecticides (Lichtenstein et al., 1973b; Liang and Lichtenstein, 1974). Krueger and Mason (1974) tested 45 plant growth regulators for their effects on the uptake and metabolism of phorate and aldicarb in soybeans but they found no significant differences. Chang et al. (1971) demonstrated that certain herbicides may alter the metabolism of carbaryl, dyfonate, and malathion in bean and tomato leaves.

This study was initiated to investigate the effects of the herbicide Eptam (*S*-ethyl dipropylthiocarbamate) on the persistence and metabolism of phorate in soil and on its uptake by corn seedlings. Eptam is an effective preemergence herbicide commonly used for the control of grasses and broadleafed weeds but its phytotoxicity toward certain varieties of corn has necessitated the introduction of antidotes to minimize that effect. The compound *N*,*N*-diallyl-2,2-dichloroacetamide (Stauffer R-25788) is one of these antidotes. Lay et al. (1975) have shown that the antidote raises the glutathione (GSH) and GSH *S*-transferase levels in corn, resulting in rapid detoxication of the translocated Eptam sulfoxide, which is the presumed active growth regulator (Casida et al. 1974).

EXPERIMENTAL SECTION

Materials. Phorate, [S-methylene-^{14}C]phorate (sp act. 9.7 mCi/mmol), phorate sulfoxide and sulfone, and phoratoxon and its sulfoxide and sulfone were obtained through the courtesy of the American Cyanamid Co. Eptam and its corn antidote (R-25788) were provided by the Stauffer Chemical Co. The solvents used were anhydrous methanol, redistilled acetone, benzene, and acetonitrile, and reagent grades nitromethane and toluene.

Preliminary experiments in this laboratory demonstrated that the growth of corn seedlings was greatly stunted when 75 ml of water containing 10 mg of Eptam was applied to the surface of 1 kg of a Plainfield sand previously planted with germinated corn seeds. No reduction in growth of corn plants occurred when 75 ml of water containing 10 mg of Eptam plus 1 mg of the antidote had been applied as described. The concentrations of Eptam and its antidote in the 1 kg of soil would have been 10 and 1 ppm, respectively, if the chemicals had been mixed throughout the soil. However, the effective concentration in the soil surrounding the developing corn roots was probably considerably larger, since the herbicide had

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

Table I. Effects of Eptam on the Translocation of [^{14}C]Phorate from Plainfield Sand into Corn Plants

		^{14}C recovered[a] in % of applied from plants and soil 18 days after soil treatment;[b] soil treated with [^{14}C]phorate (2 ppm) plus:						
	Extraction phases	None (Ck) % ^{14}C	Eptam (E), 5 ppm % ^{14}C	Eptam (E), 5 ppm % Ck[c]	Antidote (A), 1 ppm % ^{14}C	Antidote (A), 1 ppm % Ck	E + A, 5 + 1 ppm % ^{14}C	E + A, 5 + 1 ppm % Ck
Corn greens (G)	Benzene	2.13 ± 0.39	3.58 ± 0.22	168[f]	2.41 ± 0.90	113	2.46 ± 0.22	116
	Water	1.23 ± 0.06	2.18 ± 0.28	177[f]	1.29 ± 0.28	105	1.46 ± 0.06	118[f]
	Bound[d]	0.62 ± 0.17	1.46 ± 0.11	236[f]	0.84 ± 0.11	136	1.01 ± 0.11	164[g]
	Total	3.98 ± 0.62	7.22 ± 0.62	182[f]	4.54 ± 1.29	114	4.93 ± 0.22	124[h]
Corn roots (R)	Benzene	0.70 ± 0.26	0.61 ± 0.09	88	0.70 ± 0.09	100	0.70 ± 0.09	100
	Water	0.70 ± 0.09	0.70 ± 0.09	100	0.70 ± 0.00	100	0.87 ± 0.09	125[h]
	Bound[d]	0.44 ± 0.00	0.44 ± 0.09	100	0.52 ± 0.00	120	0.61 ± 0.17	140
	Total	1.84 ± 0.35	1.75 ± 0.09	87	1.92 ± 0.09	105	2.18 ± 0.35	109
Soil (S)	Benzene	68.32 ± 1.12	65.99 ± 1.56	97[g]	59.55 ± 5.64	87[h]	67.33 ± 1.68	99
	Water	0.27 ± 0.01	0.54 ± 0.03	200[e]	0.75 ± 0.52	278	0.56 ± 0.37	207
	Bound[d]	2.82 ± 0.57	2.18 ± 0.67	77	3.08 ± 0.65	109	2.19 ± 0.45	78
	Total	71.41 ± 1.47	68.71 ± 1.34	104[h]	63.38 ± 5.24	92[h]	70.08 ± 1.97	102
Total (G + R + S)		77.23	77.68		69.84		77.19	

[a] Recovered from total green or root material and from soils. Data are expressed as mean ± standard deviation for three replicates except for corn greens (two replicates) grown in soil treated with phorate plus antidote only. [b] [^{14}C]Phorate (9 μCi) applied to soil at 2 ppm. [c] Percent Ck, data are expressed in percent of control soils, treated only with phorate. [d] Bound = unextractable residues determined by combustion. [e-h] Values are significantly different from control (None) at: (e) 0.1%, (f) 1%, (g) 5%, or (h) 10% level as determined by Student's *t* test.

been applied to the soil surface.

The same test was then repeated except that Eptam and the antidote were distributed uniformly throughout the soil resulting in actual concentrations of 10 ppm of Eptam and 1 ppm of antidote. Under these conditions growth differences due to the Eptam treatment were no longer observed. To ensure that no apparent damage to the seedlings would occur, actual concentrations of 5 ppm of Eptam and 1 ppm of antidote were used in the following experiment. In these studies we attempted to ascertain, both qualitatively and quantitatively, the effects of Eptam on the translocation and metabolism of [^{14}C]phorate in corn plants, after applying the herbicide at a concentration which did not cause visible plant injury. In addition, the interaction of Eptam and its antidote with respect to insecticide uptake and metabolism was also investigated.

The experimental set-up consisted of 12 cylindrical ice cream cartons each containing 925 g of Plainfield sand (0.6% organic matter; 94% sand; 3% silt; 3% clay). Three of these cartons contained soil treated with 2 ppm of [^{14}C]phorate only (9 μCi per 925 g of soil), three with 2 ppm of [^{14}C]phorate and 5 ppm of Eptam, three with 2 ppm of [^{14}C]phorate and 1 ppm of antidote, and three with 2 ppm of [^{14}C]phorate, 5 ppm of Eptam, and 1 ppm of the antidote.

Immediately after soil treatment, eight germinated corn seeds were planted in each of the 12 test soils. After watering, the weight of each container was determined and maintained throughout the experiment by daily additions of distilled water. The containers were held for 18 days at 21–24°C under a bank of Gro-Lux lamps (Sylvania Electric Products, Inc.) on a 12-h photoperiod.

Plant Harvest and Sample Preparation. Eighteen days after planting, corn greens had grown to a length of 25–29 cm. No visible plant injury could be observed. Leaves were cut 1 cm above the soil surface and the total weight of the greens from each replicate was determined. The containers were then dismantled. The roots were carefully removed from the soil and rinsed with tap water to remove any adhering soil particles. The total weight of the roots from each replicate was also determined.

No differences in the growth, weight, or general appearance of the corn seedlings, due to the various soil treatments, were observed. The average maximum length and combined weight of the greens from the eight plants in each of the three replicates treated with phorate only were 28.9 ± 2.3 cm and 5.8 ± 1.1 g, respectively; with phorate and Eptam, 26.0 ± 0.6 cm and 5.6 ± 0.5 g, respectively; with phorate and the antidote, 26.5 ± 1.9 cm and 6.0 ± 0.6 g, respectively; and with phorate, Eptam, and the antidote, 24.8 ± 2.4 cm and 5.0 ± 0.9 g, respectively. The weights of the roots from 8 plants per container varied between 8.0 and 10.5 g.

Extraction and Analyses. The soils and plant material were extracted twice with a 1:1 mixture of methanol and acetone, followed by a third extraction with a 1:1:1 mixture of benzene–methanol–acetone, as described previously (Lichtenstein et al., 1973a). The extracts were ultimately partitioned into benzene and water-soluble fractions and the residual soils and plant material were retained for determination of unextracted (bound, Tables I and II) products.

Liquid scintillation analyses of all benzene and water phases and gas and thin-layer chromatographic analyses of the benzene solutions were performed as previously described (Lichtenstein et al., 1973a). Unextractable products in the soil and plant material were determined by oxidation to $^{14}CO_2$ and subsequent liquid-scintillation analyses, as described by Flashinski and Lichtenstein (1974).

RESULTS AND DISCUSSION

Results of the radiocarbon assays for soils and corn plants are presented in Table I. After 18 days of incubation, the soils still contained between 63 and 71% of the radioactivity applied as [^{14}C]phorate. Most of the radiolabeled compounds in soil were benzene soluble and only small amounts of water-soluble and unextractable compounds were present. Water phases of the soil extracts from the Eptam-treated soils contained more ^{14}C than did the extracts from soil treated only with phorate. Qualitative and quantitative analyses by gas–liquid chromatography (GLC) of the benzene extraction phases of these soils (Table II) showed that phorate and phorate sulfoxide were the major compounds present and accounted for over 92% of the detectable benzene-soluble materials; the

Table II. Effects of Eptam on the Translocation and Metabolism of [^{14}C]Phorate from Plainfield Sand into Corn Plants

	Ppm recovered[a] in benzene extracts of plants and soil 18 days after soil treatment;[b] soil treated with [^{14}C]phorate (2 ppm) plus:			
	None (Ck)	Eptam (E), 5 ppm	Antidote (A), 1 ppm	E + A, 5 + 1 ppm
Corn greens				
PS, S[c]	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.01
PS, SO	3.44 ± 0.80	5.74 ± 0.95[f]	3.96 ± 1.25	4.55 ± 0.55
PS, SO_2	1.76 ± 0.17	2.75 ± 0.33[e]	1.78 ± 0.49	2.09 ± 0.21
PO, SO	0.54 ± 0.08	1.31 ± 0.04[d]	0.41 ± 0.26	0.51 ± 0.14
Total	5.74 ± 0.87	9.80 ± 1.32[e]	6.18 ± 2.02	7.16 ± 0.83
Soil				
PS, S	0.55 ± 0.02	0.57 ± 0.02	0.55 ± 0.09	0.56 ± 0.02
PS, SO	0.62 ± 0.04	0.55 ± 0.05	0.48 ± 0.00[e]	0.59 ± 0.01
PS, SO_2	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
Total	1.25 ± 0.02	1.17 ± 0.04[g]	1.10 ± 0.07[f]	1.24 ± 0.02

[a,b] Footnotes as in Table I. [c] Phorate (PS, S), phorate sulfoxide (PS, SO), phorate sulfone (PS, SO_2), phorate oxygen analogue sulfoxide (PO, SO). [d-g] Values are significantly different from control (None) at (d) 0.1%, (e) 1%, (f) 5%, or (g) 10% level as determined by Student's t test.

remainder was found to be phorate sulfone. No phoratoxon, phoratoxon sulfoxide, or phoratoxon sulfone were detected in any of the soils. Since there was little difference in the amounts and distribution of the phorate residues recovered and in the total radioactivity present in the soils of various treatments, it appears that phorate plus Eptam and/or its antidote did not interact significantly in this soil.

Analyses of the corn roots for their radiocarbon content showed that only small amounts of ^{14}C material (ca. 2% of applied) were present (Table I). No significant differences in the total radioactive content between roots from the various soil treatments were noticeable. Because of the low radiocarbon concentrations and the absence of measurable differences between the various treatments, no GLC analyses of root extracts were conducted.

Striking differences in the amounts of translocated radioactive compounds, however, were observed with the corn greens grown in [^{14}C]phorate and Eptam-treated soils when compared with control plants grown in soil treated with [^{14}C]phorate only (Table I). Approximately 1.7 times more (significantly different at the 1% level) phorate-derived ^{14}C-labeled products were found in the benzene and water extraction phases of the corn greens than in comparable extraction phases of the greens grown in control soils. In addition, the amount of unextracted radiolabeled material was 2.4 times greater in plants grown in Eptam-treated soil. It is possible that Eptam affected the plant in such a way that ^{14}C-labeled residues were more bound and, therefore, unextractable.

Incorporation of the antidote (R-25788) into the soil at 1 ppm had no effect on the translocation of radiocarbon into corn greens (Table I, A) but counteracted the effects of Eptam (Table I, E + A). The amounts of benzene-soluble radiocarbon were similar to those in control corn greens although some effect of Eptam on the amounts of water-soluble and unextractable radiocarbon was still noticeable. This was indicated by the slightly higher amounts of ^{14}C present.

Results obtained after qualitative and quantitative analyses of the benzene extraction phases of corn greens by GLC are presented in Table II and Figure 1. The presence of Eptam in soil caused a significant increase in the appearance of phorate metabolites. While the total residues recovered and the amounts of both phorate sulfoxide and phorate sulfone were approximately 1.6 times larger than controls due to soil treatment with Eptam, the amount of phoratoxon sulfoxide was 2.4 times larger in corn greens grown in phorate plus Eptam treated soil. As previously reported with corn grown in phorate-treated soil

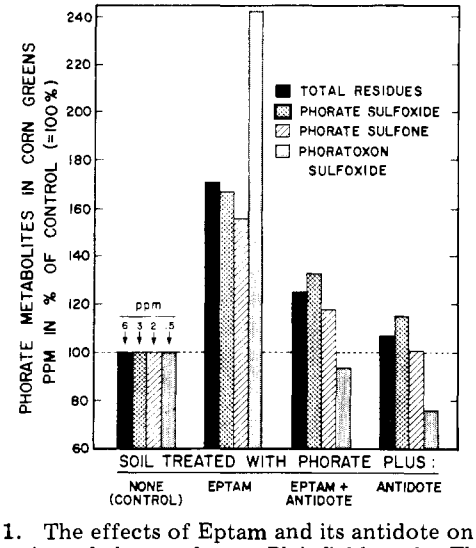


Figure 1. The effects of Eptam and its antidote on the translocation of phorate from a Plainfield sand. The soil had been treated with the insecticide at 2 ppm (control), with the insecticide and Eptam (at 5 ppm), with both plus the antidote (at 1 ppm), and with the antidote alone. Actual concentrations of insecticide metabolites in greens grown in soils containing only phorate (control) are indicated (parts per million).

(Lichtenstein et al., 1974) phoratoxon sulfoxide and phoratoxon sulfone were only found in corn greens, while neither of these metabolites could be detected in the soil or corn roots. In this study identical results were obtained, except that phoratoxon sulfone could not be detected in greens. Thin-layer chromatography of the benzene extraction phases from corn greens and subsequent autoradiography confirmed that phorate sulfoxide, phorate sulfone, and phoratoxon sulfoxide were the only radioactive products present.

The increased translocation and/or metabolism of phorate-derived products into and in the greens of corn grown in Eptam-treated soil could have been the result of an effect of Eptam on the plant's transport system and/or on the metabolism of insecticide-derived compounds within the plant tissues. Talekar and Lichtenstein (1972) demonstrated that some metabolic inhibitors can affect the translocation of lindane in peas. A similar effect is possible through the application of Eptam to the soil.

The increases in phoratoxon sulfoxide and unextractable radiolabeled products in the greens point to an increase in the degradation rate in the greens of corn seedlings grown in Eptam-treated soil. This report provides further

information relative to the interaction of environmental chemicals in biological systems.

LITERATURE CITED

Arle, F., *Weed Sci.* **16**, 430 (1968).
Casida, J. E., Gray, R. A., Tilles, H., *Science* **184**, 573 (1974).
Chang, F. Y., Stephenson, G. R., Smith, L. W., *J. Agric. Food Chem.* **19**, 1187 (1971).
Flashinski, S. J., Lichtenstein, E. P., *Can. J. Microbiol.* **20**, 399 (1974).
Hacskaylo, J. J., Walker, J. K., Jr., Pires, E. C., *Weeds* **12**, 288 (1964).
Krueger, H. R., Mason, J. F., *J. Agric. Food Chem.* **22**, 338 (1974).
Lay, M. M., Hubbell, J. P., Casida, J. E., *Science* **189**, 287 (1975).
Liang, T. T., Lichtenstein, E. P., *Science* **186**, 1128 (1974).
Lichtenstein, E. P., Fuhremann, T. W., Schulz, K. R., *J. Agric. Food Chem.* **22**, 991 (1974).
Lichtenstein, E. P., Fuhremann, T. W., Schulz, K. R., Liang, T. T., *J. Econ. Entomol.* **66**, 863 (1973a).
Lichtenstein, E. P., Liang, T. T., Anderegg, B. N., *Science* **181**, 847 (1973b).
Nash, R. G., *Agron, J.* **59**, 227 (1967).
Parks, J. P., Truelove, B., Buchanan, G. A., *Weed Sci.* **20**, 89 (1972).
Talekar, N. S., Lichtenstein, E. P., *J. Agric. Food Chem.* **20**, 1260 (1972).

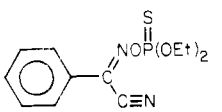
Received for review September 15, 1975. Accepted November 19, 1975. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by a grant from the National Science Foundation (GB-3502). Contribution by Project 1387 from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Regional Cooperative Research Project 96, entitled "Environmental Implications of Pesticide Usage".

Degradation Products of Phoxim (Bay 77488) on Stored Wheat

Walter A. Mason[1] and Clifton E. Meloan*

The pesticide Phoxim (Bay 77488) (glyoxylonitrile phenyl oxime *O,O*-diethyl phosphorothioate) was found to form two extractable products when placed on stored wheat—the oxygen analog and the *S*-ethyl isomer. The extraction solvent is 10% acetone in benzene and provided 88% recovery. The degradation products plus the original compound accounted for 85% of the radioactive tracer with about 15% not being able to be extracted from the wheat surface. A TLC method providing for the detection of 0.1 μg of Phoxim qualitatively and 1 μg quantitatively was developed using methylcyclohexane as the eluting solvent and dimethylformamide as the stationary phase on silica gel plates. The chromogenic sprays were 2,6-dibromo-*N*-chloro-*p*-quinone imine, trichloro-*p*-quinone imine, or 4-(*p*-nitrobenzyl)pyridine.

Phoxim (Bay 77488) or, more correctly, glyoxylonitrile phenyl oxime *O,O*-diethyl phosphorothioate is an experimental pesticide whose most promising use is on stored grains. Pesticides used on stored grains must either degrade fairly rapidly into nontoxic compounds or have a low mammalian toxicity. The mammalian toxicity (rats) as shown by Chemagro (1969) was LD_{50} oral 8500 mg/kg, dermal 1000 mg/kg.

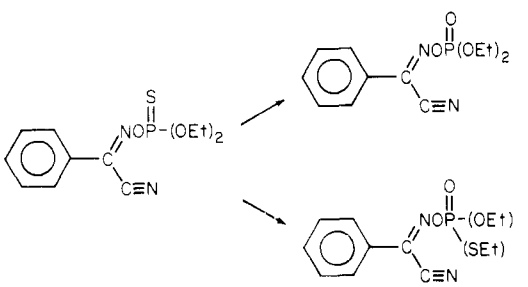


Phoxim

The purpose of this research was to determine how long Phoxim would exist on stored wheat and what the major degradation products were.

Wheat, maintained at 10–14% moisture, was treated with 20 ppm of Phoxim. Every few days a sample was extracted with 10% acetone in benzene.

Column chromatography was used for the sample cleanup. Thin-layer chromatography (TLC) was found to be the most effective analytical method and ^{32}P and ^{14}C containing compounds were used to track and aid in the identification. About 85% of the radioactive compounds could be extracted from the wheat and these were found to be the original compound, the oxygen analog, and the *S*-ethyl ester, as shown below.



EXPERIMENTAL SECTION

Equipment used included: Perkin-Elmer 457 grating infrared spectrometer; Varian T-60 NMR spectrometer; AEI-MS-902 mass spectrometer; F&M 609 gas chromatograph.

Radioisotope labeled compounds were detected using a Baird-Atomic RSC-54 chromatogram scanner, a Model CS-210 Pre-amp, a Model CS-400 ratemeter, a Model 432 ratemeter as the high voltage source, and the corresponding Baird-Atomic (Texas Instrument) recorder. Optimum flow rates for the counter gases were 42 ml/min for helium and 2.8 ml/min for methane. The optimum voltage was 2300 V.

The TLC scanner system utilized a photovolt 530 TLC densitometer, a 520-A photometer, and a Honeywell Electronik-19 recorder operating on the 1-V scale. The "1" range was used on the photometer, the instrument zeroed with a blank unsprayed TLC plate, and the infinite density

Department of Chemistry, Kansas State University, Manhattan, Kansas 66506.

[1]Present address: Armour Pharmaceutical Co., Kankakee, Ill.