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

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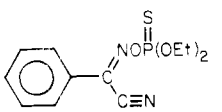
Degradation Products of Phoxim (Bay 77488) on Stored Wheat

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.

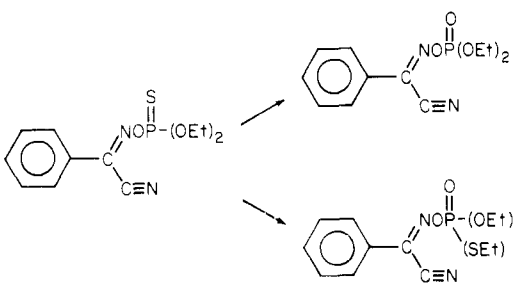


Phoxim

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

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Table I. TLC R_f Values

Solvent system	Phoxim	Oxygen analog	Thiol isomer	Diethyl thiochloro-phosphate	Sulfo-tepp	Oxime	
						Anti	Syn
C_6H_{12}-EtOAc, 1:1 (lined)	0.57	0.31	0.40	[a]	[a]		
C_6H_{12}-EtOAc, 1:1 (unlined)	0.61	0.20		[a]	[a]		
C_6H_{12}-EtOAc, 9:1	0.45	0.13		0.55	0.52		
C_6H_{12}-EtOAc-DMF, 4:1:1	0.45	0.35		0.57	0.54	0.32	0.13
MeC_6H_{12}-DMF (immobile solvent)	0.24	0.07	0.16	0.47	0.36		

[a] Spot diffused beyond determination limits.

setting obtained using a calibrated film strip.

Eastman polyester backed silica gel and S&S 4 in. × 8 in. glass backed plates were used, 250 μm thick.

Chemicals used included: developing solvents—(1) cyclohexane–ethyl acetate, 1:1 (v/v); (2) methylcyclohexane–dimethylformamide–diethyl ether; extracting solvents—(1) benzene; (2) acetone; (3) pentane; cleanup materials—(1) Celite 545; (2) silica gel, grade 950, 60–200 mesh; chromogenic sprays—(1) 2,6-dibromo-*N*-chloro-*p*-benzoquinone imine (DCQ) 0.5% in distilled acetone (must be prepared fresh daily); (2) *N*-2,6-trichloro-*p*-benzoquinone imine (TCQ) 0.5% in distilled acetone (must be prepared fresh daily); (3) 4-(*p*-nitrobenzyl)pyridine, 2% in distilled acetone; tetraethylpentamine, 10% in distilled acetone (prepared fresh daily); (4) tetrabromophenolphthalein ethyl ester, 0.2% in acetone; $AgNO_3$, 0.5% in 25 ml of H_2O, then diluted to 100 ml with distilled acetone; citric acid, 0.5 g in 50 ml of H_2O, then diluted to 100 ml with distilled acetone; (5) horse serum cholinesterase, Worthington Biochemical: 50 mg in 100 ml of 0.25 *M* THAM; indoxyl acetate, 50 mg in 25 ml of 95% ethanol (these solutions must be stored cold); diethyl phosphorochloridothionate, prepared by the method of Stevens (1963), yield 79%; diethyl phosphorochloridate, prepared in the manner of diethyl phosphorochloridothionate, using 48.5 g of $POCl_3$, yield 93%; phenylacetonitrile, prepared by the method of Vogel (1962), yield 73%; phenyl glyoxylonitrile oxime, prepared by the method of Schrader and Muhlmann (1952), yield 52%; phoxim, prepared as described by Mason and Meloan (1973); phoxim, oxygen analog, prepared as phoxim except diethyl phosphorochloridate was used in place of the diethyl phosphorochloridothionate; the product distilled at 138° (10^{-3} Torr), yield 84%; tetraethyl dithiopyrophosphate, prepared by the method of Weisler and Helmkamp (1945), yield 45%; ^{32}P-labeled Phoxim, described previously by Mason and Meloan (1973); ^{14}C-labeled (cyanide position) Phoxim, previously described by Mason and Meloan (1973).

Procedures. *Sample Spiking.* Five-hundred-gram samples of hard red winter wheat, supplied by the Midwest Grain Investigation Laboratory, Manhattan, Kan., and maintained at 10–14% moisture, were placed in half-gallon fruit jars, then treated to contain 10 or 20 ppm of Phoxim by coating with either 10 or 20 ml of a 1 mg/ml toluene solution of Phoxim. Care was taken to ensure the wheat was covered and not the container walls. The sample was rotated for 10 min and then set in a hood for 30 min, with periodic rotation, to allow all of the solvent to evaporate. The jars were then sealed with their normal lids.

Recovery. Several of the methods present in the literature were tried. While they all worked well for removing Phoxim immediately after spiking, only the 10% acetone in benzene worked well (88%) after the Phoxim had been on the wheat several days.

Samples (50 or 100 g) of the treated wheat were weighed, then blended for 5 min in a Waring high speed explosion proof blender with 100 ml of 10% acetone in benzene. The solvent was carefully decanted into a fluted filter paper containing 1–2 g of Celite 545 with about 15 g of anhydrous Na_2SO_4 placed on top. The residue was blended again for 5 min with 100 ml of the solvent and filtered and the filter paper washed with the solvent. The volume was reduced under vacuum to about 20 ml. This solution was then passed through a 25 × 200 mm stopcockless chromatographic column containing a 1-in. plug of silica gel 950, that had been activated for 30 min at 200°C, and about 12 g of anhydrous Na_2SO_4. The collected solution was then reduced in volume in a Kuderna-Danish concentrator to less than 1 ml and then diluted to exactly 1 ml with the solvent. Very small glass chips were used as boiling stones in the concentrator.

Na_2SO_4 was used to remove water which would separate later and make further sample preparation difficult. The silica gel was to remove as much of the plant extract as possible.

TLC Plate Development. Several solvent systems were used to develop the TLC plates and the R_f values are given for several compounds in Table I.

Probably the best system for separation utilized methylcyclohexane as the eluting solvent and dimethylformamide (DMF) as an immobile phase. The immobile solvent was a 20% solution of DMF in diethyl ether. The spotted plate was immersed upside down just to the spots in a dipping tank containing the immobile solvent. The plate was removed immediately, allowed to stand until the ether evaporated, which only required a few seconds, and then the plate was placed in a lined developing tank containing methylcyclohexane.

Chromogenic Spray Reagents. The spraying sequence for all of the reagents has been described (Duggan et al., 1968).

A general reagent which is easy to use and reacts with all P=O and P=S compounds is 4-(*p*-nitrobenzyl)-pyridine. The reagent (Getz and Watts, 1964) was sprayed on the eluted plate and then the plate was heated at 110°C for 5 min. The plate was then oversprayed with tetraethylenepentamine. The developed spot was deep blue to purple on a white background.

DCQ (Duggan et al., 1968) was also very easy to use and could distinguish between P=O and P=S bonds and sometimes would indicate the S—P=O group. The sprayed plates are heated at 110°C for 7 min. TCQ has been used for the same purpose and has the added advantage that it is not necessary to store it in the cold. A grayish color was observed after spraying and heating with P=O containing compounds and a dull orange color was observed with P=S containing compounds.

Bromocresol green–$AgNO_3$ sprays (Getz and Watts, 1964) will only react with compounds containing either a S=P—S or O—P=S configuration. A tetrabromophenolphthalein–$AgNO_3$–citric acid detection system (Getz and Watts, 1964) is also selective for thiol or thiono organophosphorus compounds. The $AgNO_3$ is an overspray as is the citric acid. The colored background limits quantitative analysis.

A few plates were sprayed with a solution of horse serum

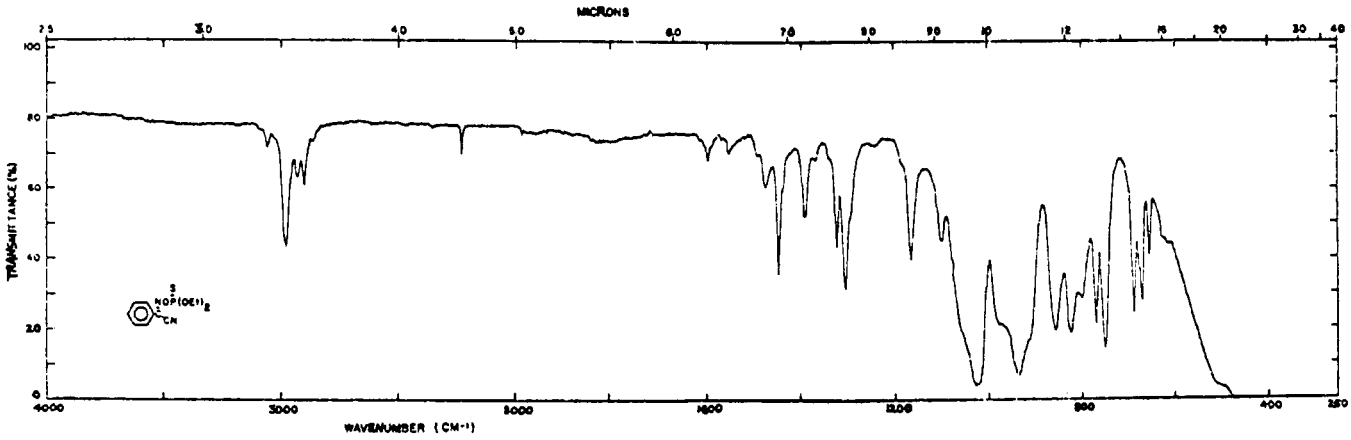


Figure 1. Infrared spectrum of Phoxim (Bay 77488).

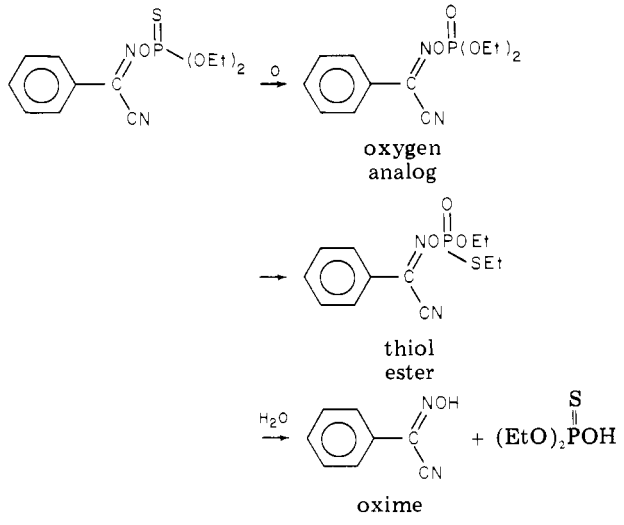
cholinesterase, incubated at 40°C for 5 min, sprayed with indoxyl acetate solution, and then viewed under uv light. The spots were white on a light blue background.

RESULTS AND DISCUSSION

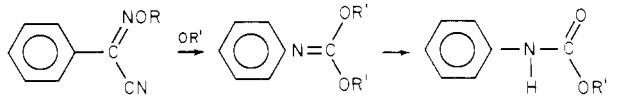
Types of Compounds Expected. It is necessary to mention the environment in which the pesticide will exist, in this case on wheat. It is possible for different grains or other crops to produce different degradation products from the same pesticide. Factors to be considered would be moisture content, fat content, and the enzymes present.

The ideal moisture content for stored wheat is 12% (Hlynka, 1964) and these samples were held at that value by controlled humidity. The fat content for most wheat is about 2%. Although amylase and lipase are present in the largest quantities in wheat, hexosediphosphatase and pyrophosphatase are probably the most important in this case since they are able to cause hydrolysis of a number of phosphate esters.

The literature was surveyed to find what products other organophosphorus compounds had produced (Burchfield et al., 1965; Duggan et al., 1968; Gunther and Gunther, 1962–1971; O'Brien, 1960; Thornburg, 1971; Thornburg and Beckman, 1969; Weisler and Helmkamp, 1945). In some respects Parathion is similar to Phoxim and should produce similar degradation products. These are listed below.



Another potential product is the anti isomer of the parent compound. In addition to the above reaction, there are a number of products that might be formed from the oxime. The oxime will hydrolyze to form benzoic acid and under certain conditions substituted oximes will undergo a Beckman rearrangement (Schrader and Muhlmann, 1952; Stevens, 1967) to form a *N*-arylimidocarbamate converted to the corresponding *N*-arylurethan:



Hydrolysis to form HCN and the formation of tetraethyl dithiopyrophosphate, $(EtO)_2P(=S)OP(=S)(OEt)_2$, are also possible.

There are many other possibilities that can be imagined and we tried to keep an open mind when looking for the products although the analytical procedures were designed to be sure to detect the more likely products if they were formed.

To this end all suspected products were prepared and analytical methods developed. In addition, ^{32}P was incorporated into the original molecule and ^{14}C was added at the CN position in another series of experiments.

Ir, NMR, and mass spectroscopy were used to verify the correctness of the compounds synthesized. Representative spectra of these are shown in Figures 1–5.

The infrared spectra allowed an identification of most of the compounds, especially the distinction between the parent compound and the oxygen analog. In Phoxim, the P=S stretch was observed at 750 cm^{-1} and in the oxygen analog, the P=O stretch was observed at 1290 cm^{-1}. The positions of these peaks were determined by exposing the compound to bromine vapor to oxidize the thiono compound to the oxygen analogue. It was interesting to note in the bulletin "Active Ingredient Bay 77488 in Product 5864" that the 1025-cm^{-1} band was used to determine the active ingredients in a Phoxim formulation. The 1025-cm^{-1} absorption is a P—O—C stretch which occurs in the oxygen analog and the thiol ester as well as the parent compound. Since all of these are active, the method is

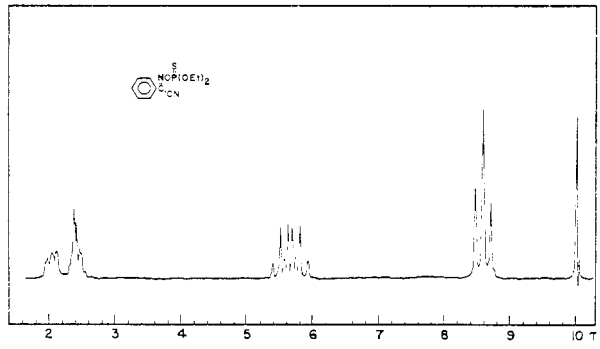


Figure 2. NMR spectrum of Phoxim (Bay 77488).

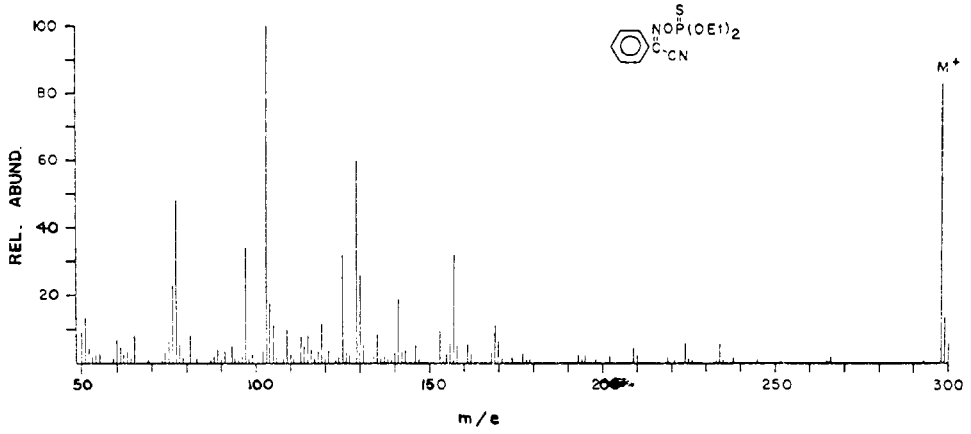


Figure 3. Mass spectrum of Phoxim (Bay 77488); probe temperature 20°C.

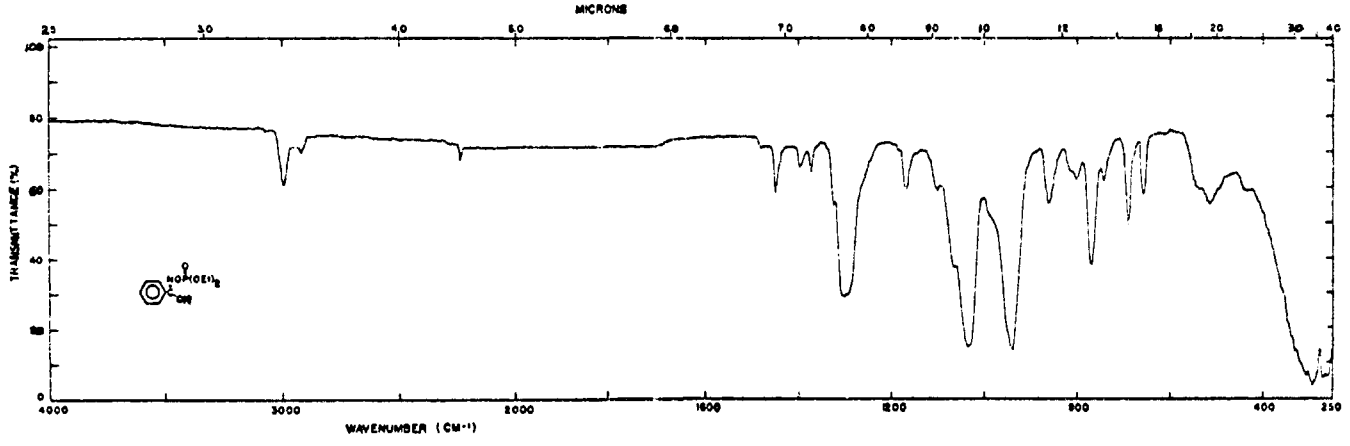


Figure 4. Infrared spectrum of the O-analog of Phoxim.

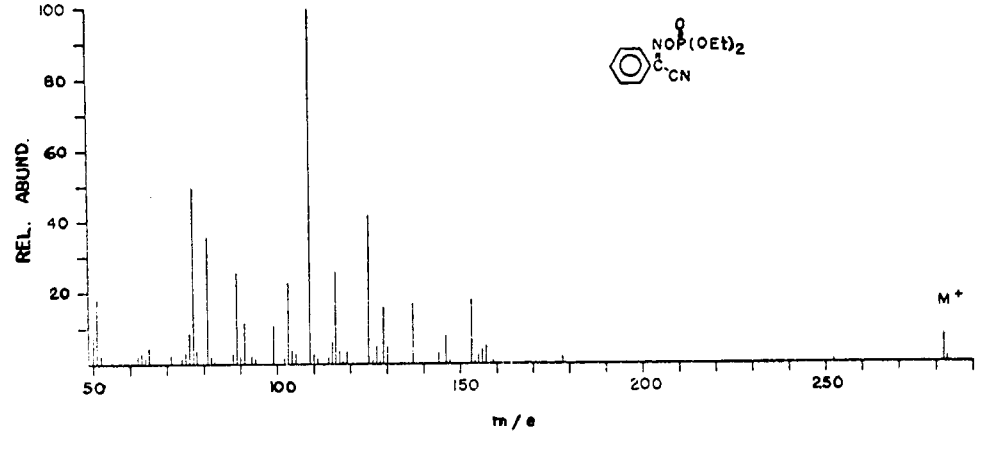


Figure 5. Mass spectrum of the O-analog of Phoxim.

valid for its intended use but is not a measure of the concentration of the parent compound.

NMR spectra are as expected and if doubts exist as to the presence of phosphorus in the compound, the J_{P-H} = 12 Hz split was easily observed on the methylene group present. The spectra of the oxygen analog and Phoxim were identical.

A mass spectrometer identification of tetraethyl pyrothiophosphate was not found in a second sample obtained from Chemagro nor was it detected in the synthesized product. A possible explanation could have been that different methods of synthesis were used for the two samples.

It was hoped that mass spectra would be useful in identifying the degradation products of Phoxim extracted from the wheat. Due to the difficulty of cleanup the technique was not useful in this respect; however, it was very useful in establishing the identity of the products from

Table II. Extraction Efficiencies of ^{32}P-Labeled Phoxim (5 μg)

Solvent	% recovery
Pentane	87
Benzene	90
10% methanol in benzene	94

Table III. Cleanup Recoveries of ^{32}P-Labeled Phoxim (5 μg)

Solvent	Florisil, %	Silica gel, %
Pentane	78	83
Benzene	82	88
10% methanol in benzene	87	94

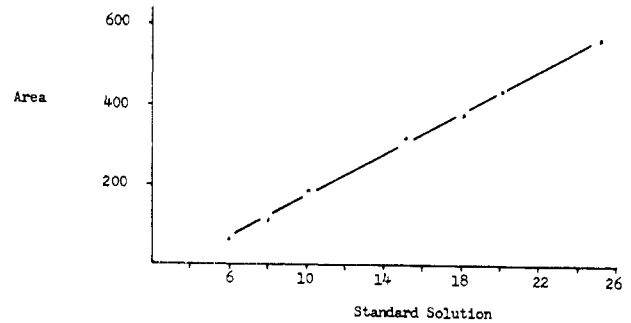


Figure 6. Final working curves for ^{32}P-labeled Phoxim.

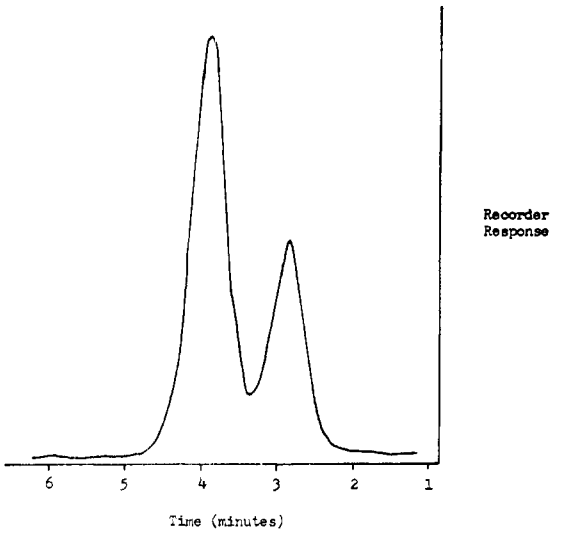


Figure 7. Resolution of a 10- and a 5-μl ^{32}P-labeled sample placed 2 cm apart.

the various syntheses (see Tables II and III).

Analytical Methods. TLC was found to be the best technique used for the analysis of Phoxim, even though the method is more tedious and normally not as accurate as gas chromatography. It was not possible to use gas chromatography because decomposition of the parent compound occurred with all columns used. Mass spectrometric identification of the eluted peaks indicated the compound emerging was not the same one injected and in many cases it appeared to have reacted with the stationary phase.

The detection systems used for the TLC analysis utilized a radioisotope scanner for the labeled compounds and a scanning densitometer for the samples sprayed with a chromogenic reagent.

The ^{32}P-labeled compounds were separated on Eastman polyester backed silica gel TLC plates. The flexible back plates were required because it was necessary to cut the plates in 1.5-in. strips and curve the plates to fit them into the scanner.

Working curves were prepared by spotting a plate with different volumes of a standard solution. The volume of the 0.092 mg/ml solution used for spotting varied between 1 and 10 μl. Duplicate plates were spotted for the working curve, and the plates were then eluted with the same solvent system used for the extracted samples. Figure 6 was the working curve for the initial extraction of Phoxim from wheat. A new working curve was prepared each time the analyses were conducted. This was done for two reasons. As the ^{32}P decayed, the slope of the working curve changed because of the decreased detector response. Also, by making a working curve from the same reference solution each time, no decay correction was necessary.

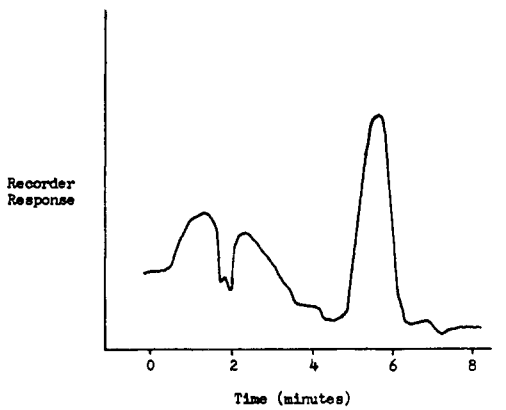


Figure 8. Mixture of Phoxim, O-analog, and the residue extracted from wheat.

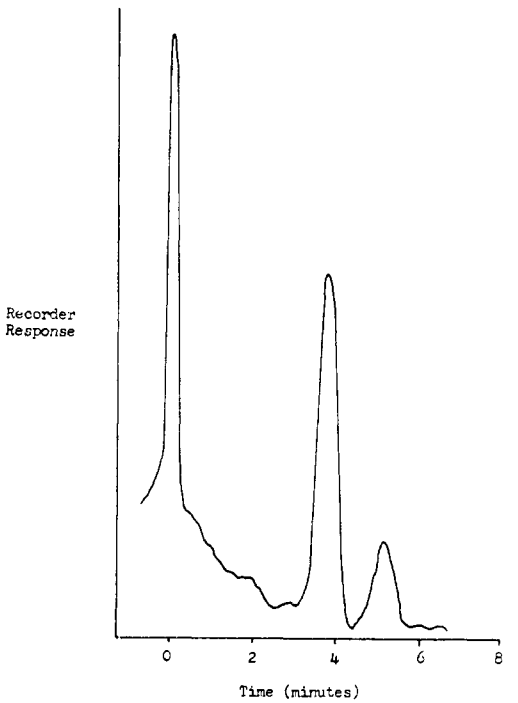


Figure 9. Mixture of Phoxim, thiol isomer, and the residue extracted from wheat.

Table IV. Summary of Recovery of ^{32}P-Labeled Phoxim (Closed Container)

	% of total applied	Phoxim, %	Other, %	Recovered, μg/50 g	Equivalent, ppm
Applied		100		460	9.2
Initial	95	95		380	8.7
1 week	88	88	Trace	360	8.1
2 weeks	74	74	Trace	300	6.8
1 month	54	42	12	155	3.9
2 months	18		18	70	1.6

Figure 7 gives an indication of the separation required to obtain adequate results using the radioisotope scanner. The chromatogram was obtained by spotting a TLC plate with 10 and 5 μl of the standard Phoxim solution. The two applications made were 2 cm apart. After drying, the plate was eluted and then scanned from side to side rather than the usual scan mode of scanning from the origin to the solvent front.

Figures 8 and 9 are actual scans of Phoxim extracted from wheat.

Tables IV and V summarize the recovery results obtained with the ^{32}P-labeled Phoxim. The data presented are an average of duplicate determinations corrected for

Table V. Summary of Recovery of ^{32}P-Labeled Phoxim (Open Container)

	% of total applied	Phoxim, %	Other, %	Recovered, μg/50 g	Equivalent, ppm
Applied		100		460	9.2
Initial	96	96		390	8.8
1 week	80	80		320	7.4
2 weeks	61	57	4	250	5.6
1 month	25		25	100	2.3

an 88% extraction and column cleanup efficiency. Because of the 14-day half-life of ^{32}P, the activity of the samples could not be measured after a period of 5 half-lives.

The sample measured after 1 month was not well resolved and, based on the R_f value, appeared to be an unresolved mixture of the oxygen analog and the thio isomer. The sample was not collected for mass spectrometric analysis because the sample was radioactive. The samples were eluted using cyclohexane, DMF, and ethyl acetate, or methylcyclohexane and DMF solvent systems. In both cases the "other" most common appears to be an unresolved mixture of the oxygen analog and the thiol ester. The high concentration of the plant extract compared with the components of interest changed the R_f values to such an extent that the identity of the compound or compounds is questionable. It was observed that mixtures of the two compounds behaved in the same manner when applied to the plate from a dilute solution which contained plant extract.

Residual activity was noted in the wheat sample after extraction, but combined shaking and further blending did not increase the amount of labeled compound extracted. Attempts to extract further residue or degradation products with a 1:1 acetone–water mixture were also unsuccessful. This area requires extensive study to elucidate the state of Phoxim on the wheat.

Recovery results from wheat spiked with ^{32}P labeled Phoxim stored in open jars in a hood are summarized in Table V. As indicated, the compound was only detectable up to 1 month. The significant loss of Phoxim was probably due to evaporation of the compound.

The same general procedures were used with the ^{14}C-labeled compound, except the slope of the standard curve did not change because there was no measurable decrease in activity because of the long half-life of ^{14}C.

The results of the ^{14}C-labeled Phoxim are presented in Figure 6. Duplicate samples were analyzed and it is noted that the results are the same as those obtained with the ^{32}P-labeled compound. The close agreement between the results of the ^{32}P and ^{14}C-labeled compounds indicates that the extracted portions of the compound remain unaltered.

The results obtained with the scanning desitometer are summarized in Figure 7. The results were consistently higher than those obtained with the labeled compound. A contributing factor to the high results would be the difficulty in obtaining a uniform layer of chromogenic reagent on the TLC plate. Another factor was the difficulty in removing all plant extract in the cleanup procedure. On several occasions interfering plant extract necessitated repeating the extraction and cleanup.

No evidence of the presence of the oxime or a salt of the oxime was found. The oxime or its salt would be formed upon hydrolysis of Phoxim.

Bowman and Leuck (1972) used a GLC procedure to determine Phoxim residue from field applications. They found no evidence of the oxygen analog and no evidence of the parent compound after 21 days. The results of the open container ^{32}P spike samples are in general agreement with the work of Bowman and Leuck (1972).

ACKNOWLEDGMENT

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