Phosphine Residues from Phostoxin-Treated Grain

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An analytical method sensitive to less than 0.005 p.p.m. has been developed for the determination of phosphine residues on grain. Results from the laboratory and field treatment of grain with Phostoxin indicate that the phosphine residues rapidly disappear. Data are included to show that no residues are to be expected in bread baked with Phostoxin-treated grain or flour.

PHOSTOXIN, a tablet formulation composed of aluminum phosphide and ammonium carbamate, decomposes slowly on exposure to the atmosphere to liberate phosphine. Phosphine has been used successfully in this form for the control of insect pests in stored grain in several countries for a number of years (*5*) and has recently been introduced commercially in the United States. The objectives of these studies were: to investigate methods for the determination of phosphine residues; to determine residues that might occur on grain treated in the laboratory under simulated field conditions; and to determine actual residues that do occur on grain fumigated in the field. In addition, investigations were made concerning the possible residue of phosphine in bread made from flour of Phostoxin-treated grain.

Analytical Methods

The analytical methods studied for the determination of Phostoxin in grain are based on modifications of White and Bushey's procedures (*7*), as described in a report from Diemair (*2*). In general, aluminum phosphide, the active constituent of Phostoxin, is hydrolyzed in the presence of dilute sulfuric acid to form phosphine. The liberated phosphine is driven out of the sample with nitrogen gas into scrubbers which collect the phosphine. The contents of the scrubbers are then analyzed.

The first method considered was that used by Diemair, which depends upon the reaction of phosphine with mercuric chloride to liberate hydrochloric acid, which is then titrated with standard base. The second method involves the oxidation of phosphine by bromine to form phosphoric acid, which is then determined colorimetrically.

Each of these methods has proved satisfactory for the determination of phosphine. The mercuric chloride method is more specific, but requires a large sample of grain to obtain the sensitivity necessary for determining residues. The second method, involving oxidation with bromine water, has been used in this laboratory and is described below in detail. Specificity is given to this method by separation of phosphine from the grain under nitrogen. Few other phosphorus compounds would be liberated by this procedure and undergo oxidation to phosphate with bromine water. However, as noted below, some material is liberated from bread which interferes with this reaction.

Materials

The all-glass aeration apparatus consisted of a 5-liter round-bottomed flask immersed in a water bath and two gas scrubbers with medium-porosity, sintered-glass disks. The 5-liter flask contained the sample and the two scrubbers were filled with saturated bromine water. Hydrazine sulfate, 0.15% (w./v.). Ammonium molybdate tetrahydrate, 2.5% (w./v.). DK-2 spectrophotometer.

All reagents, with the exception of the 10% sulfuric acid, were prepared from water obtained from an all-glass distillation apparatus. This water contained less than 0.0005 p.p.m. of phosphorus.

Method

The sample of fumigated material was transferred to a 5-liter, standard-taper, round-bottomed flask that had been weighed to the nearest gram. The flask was immediately stoppered and reweighed to determine the weight of sample present. This flask was then attached to the aeration apparatus; a volume of 10% (v./v.) sulfuric acid equal to the weight of the sample was quickly added and nitrogen was bubbled through the apparatus for 30 minutes. The water bath was then heated until it began boiling and aeration was continued for 2 hours. At the end of aeration, the two bromine-water scrubbers were removed and their contents transferred quantitatively to a 600-ml. beaker. The solution was then concentrated on a hot plate to remove bromine, then diluted to either 100 or 50 ml. in a volumetric flask. Aliquots of 5 ml., or less, were taken for phosphate determination.

The method used for phosphate determination (*4*) is a modification of the well known Fiske and Subbarow (*3*) method. The method was further modified by using 0.5 ml. of 0.15% hydrazine sulfate instead of the 1 ml. of 1-amino-2-naphthol-4-sulfonic acid reagent (*1*). This method proved satisfactory over the range of 0.80 to 12.0 μg. of phosphorus. The specific absorbance of 858, expressed as 1.0 gram per liter in 1-cm. cells, checks well with the value of 860 found by the original authors (*4*).

The lower limit that can be determined is 0.80 μg. of phosphorus corresponding to 0.878 μg. of phosphine. With a 1000-gram sample of wheat and a dilution of the phosphate solution of 50.0 ml., 0.009 p.p.m. of phosphine could be determined. Smaller amounts of 0.0009 p.p.m. could satisfactorily be determined by evaporating the phosphate solution almost to dryness, and transferring the total sample to the centrifuge tube for analysis.

Recoveries

Recoveries of phosphine from grain were carried out in two ways: by adding known amounts of Phostoxin; and by adding, for the lower range of recoveries, a carbon disulfide solution of phosphine to grain. Each Phostoxin tablet weighs 3 grams and liberates 1 gram of phosphine gas under proper conditions. To run recoveries in the higher range, the tablets had to be divided into smaller parts. This was accomplished by transferring a tablet to a clean, cold, dry weighing bottle and placing in the deep freeze for approximately 2 hours, then, while still cold, shaking in order to pulverize the tablet. After this process had been repeated several times, the tablet was reduced to a fine powder, which then was stored in a desiccator. Samples of this powder were rapidly weighed by difference for analysis.

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Table I. Recoveries of Phosphine Added to Control Wheat as Phostoxin

Added, P.P.M.	Found, P.P.M.	Recovery, %
48.2	45.9	98.6
38.8	37.1	95.6
7.3	6.4	94.5
5.8	4.6	79.3
5.0	4.7	94.0
2.7	2.7	100.0
2.4	1.9	79.2
0.48	0.32	66.7
0.020	0.0192	96.2
0.010	0.0101	101.0
0.005	0.0048	96.0
0.005	0.0045	90.0
0[a]	0	
	Av.	90.9

[a] Results of eight control samples.

Table II. Analyses of Common California Red Wheat[a]

Days after Treatment	Phosphine, P.P.M. Expt. 1	Expt. 2	Expt. 3	Expt. 4
1	...	0.143	...	...
2	...	0.208	1.08	...
3	...	0.131	...	...
5	...	0.065	...	0.167
7	...	0.032	0.088	...
9	0.132	0.005	...	0.019
12	...	...	0.005	...
13	0.009	0.001	...	...

[a] Fumigated with four tablets of Phostoxin per 200 pounds, equivalent to 40 tablets per ton of grain.

Table III. Analyses of Grain Fumigated with Phostoxin under Various Conditions in the Laboratory

Expt.	Grain	Conditions: Dosage, tablets/100 lb.	Conditions: Temp., °C.	Conditions: Other	Days after Treatment	Phosphine Found, P.P.M.
5	Common California red wheat	0.5	Room	...	5	0.035
					12	0.061
13	Common California red wheat	0.5	Room	...	5	0.031
					8	0.12
					11	0.062
					12	0.018
6	Common California red wheat	2.0	Room	Before aeration	6	0.27
				After aeration	6	0.015
7	Common California red wheat	2.0	Room	Cracked	3	0.28
					5	0.11
					7	0.014
					10	0.0015
8	Common California red wheat	2.86	Room	Sealed container	4	3.03
				After exposure to air 7 days		0.0041
9	Hard Idaho winter wheat	2.0	Room	...	2	0.71
					7	0.015
10	Common California red wheat	2.0	7	...	2	0.088
					5	0.18
					8	0.12
11	Common California red wheat	2.0	Room	Refumigation of Expt. 4	2	2.28
					6	0.20
					10	0.013
12	Common California red wheat	2.0	43	...	2	0
					7	0.21
					9	0.0020
14	Corn	2.0	Room	...	3	0.25
					12	0.014
15	Milo	0.5	Room	...	3	0.037
					7	0.17
					11	0.43
					16	0.031
16	Milo	0.5	Room	...	7	0.29
				Turned	11	0.085

The procedure of running recoveries was to place 1000 grams of wheat, 1000 ml. of 10% sulfuric acid, and the sample of Phostoxin in a 5-liter flask and carry this through the procedure as described above. Phosphine recoveries in the range of 0.48 to 48.2 p.p.m. were run in this manner (Table I). These recoveries varied from 66.7 to 100%. The amount recovered did not appear to vary consistently with the amount added. Considering that phosphine is liberated to some extent in transferring the Phostoxin powder, as is evidenced by the odor, the results appear to be excellent. The results of the analysis of eight control samples of wheat indicated that values for blanks were less than 0.0003 p.p.m. (95% transmittance). Such values were considered insignificant.

To determine the recovery of phosphine in the ranges found as wheat residues, it was necessary to prepare a solution of phosphine in carbon disulfide. Phosphine was generated from a Phostoxin tablet and the gas passed through carbon disulfide. The resulting solution was cooled in a deep freeze and, after coming to constant temperature, was analyzed. Two solutions were prepared from this concentrated solution to contain 2.5 and 20.0 mg. per ml.

The carbon disulfide solution of phosphine was added to the grain sample by inserting a pipet all the way to the bottom of the flask, giving assurance that the phosphine would penetrate completely throughout the grain-acid mixture.

In the case of the powdered tablet, the powder was added to the grain-acid mixture. The flask was then stoppered and shaken vigorously to ensure mixing of the powder throughout the sample.

Aliquots of these solutions were added to 1.0-kg. samples of wheat to give concentrations of 0.005, 0.010, or 0.020 p.p.m. of phosphine. These samples were analyzed and the results are shown in Table I.

Laboratory Experiments

A series of 16 experiments to investigate the residues that might be found under a variety of conditions was conducted using the maximum recommended fumigation rate of ten tablets of Phostoxin per ton of grain and with four times this rate of application. Experiments were also made to determine the effects of high and low temperatures, aeration of fumigated grain, fumigation of cracked grain, fumigation in sealed containers, and refumigation of grain previously treated with Phostoxin.

In general, grain was treated with Phostoxin by placing a known weight of the grain in a 60-gallon fiber drum that had a metal cover and bottom. The cover, unless otherwise specified, was loosely placed on top during the experiment. The Phostoxin tablets were placed approximately 5 inches from the bottom of the drum by use of a metal tube slightly larger in diameter than the tablets.

Samples of treated grain were taken with a metal tube, 3 feet long, sealed at the bottom, and with holes opening along the side of the tube at 2-inch intervals. The tube was inserted at the bottom of the drum of grain and rotated 10 to 12 turns. This permitted the grain to run into the tube from various levels. By inserting the tube at five different places in the drum, a uniform sample, weighing from 900 to 1300 grams, was collected. Samples were taken rapidly to minimize exposure to the air.

Table IV. Phosphine Residues on Wheat Fumigated under Field Conditions

Sample No.	Dosage, Tablets/ Ton	Interval Fumigation, Days	Phosphine Residue, P.P.M.
37A	6.1	1	0.004
77A	10.0	1	0.0
77B	10.0	2	0.002
35C	5.9	3	0.002
36C	6.1	3	Not run
38C	6.3	3	0.016
39C	6.4	3	0.0
72C	10.0	3	0.008
73C	2.9	3	0.007
74C	4.1	3	0.0
75C	5.0	3	0.003
37D	6.1	4	0.002
77D	10.0	4	0.0
37G	6.1	7	0.0
77G	10.0	7	0.004
35L	5.9	12	0.0
36L	6.1	12	0.0
38L	6.3	12	0.0
39L	6.4	12	0.029
72L	10.0	12	0.017
73L	2.9	12	0.0
74L	4.1	12	0.0
75L	5.0	12	0.0
37N	6.1	14	0.016

Unusually high results would be expected if a sample were taken which included a portion of the Phostoxin tablet. This could easily happen under the conditions of these experiments and may explain some unexpectedly high results obtained (Table IV). The results of these experiments (not corrected for percentage recovery) and the conditions under which they were carried out are shown in Tables II and III. Table II shows the rate of formation and disappearance of phosphine in wheat after treatment. It is apparent from these results that the residue had almost completely disappeared 12 days after treatment.

Table III shows the results of fumigating certain grains under various conditions.

Nonphosphine Phosphorus Residues

As the phosphine residues obtained in these studies were much lower than might be expected if all the phosphorus were converted to phosphine, it was of interest to determine the nature of any phosphorus-containing materials other than phosphine remaining on the wheat. The theoretical amount of phosphorus that would occur following the recommended dosage level of Phostoxin or with four times this recommendation would be extremely difficult to detect in the presence of the normal phosphorus content of wheat. The extent to which nonphosphine phosphorus residues occur on wheat following Phostoxin fumigation was determined by adding one tablet of Phostoxin to 200 grams of wheat in a 250-ml. graduated cylinder. This dosage, approximately 450 times the recommended rate of treatment, was necessary to bring the analytical method within the range of significance. The wheat was allowed to stand for 2 weeks, after which time it was spread out in a thin layer and aerated under a hood overnight. The odor of phosphine after aeration was barely detectable. The treated wheat was ground in a Wiley mill and samples were taken for total phosphorus determination. A control sample of wheat was prepared in the same manner. Total phosphorus was determined after wet-ashing with nitric and sulfuric acids. Water-soluble phosphorus was determined by analyzing aqueous extracts of the wheat.

The results of this analysis gave an increase of approximately 0.03% total phosphorus in the treated sample over the amount found in the control.

Table V. Phosphine in Wheat Samples Fumigated under Field Conditions and Treated by Turning, Aeration, or Washing

Sample	Conditions	Phosphine Found, P.P.M.
1	Two tablets/ton; 5 days after fumigation; turned	0.004
2	Two tablets/ton; 5 days after fumigation; turned and aerated	0.006
3	Sample 2 after washing	0.003
4	Three tablets/ton; turned	0.046
5	Three tablets/ton; 4 days after fumigation; turned and aerated	0.006
6	Sample 5 after washing	0.0
7	Four tablets/ton; 6 days after fumigation; turned only; uncleaned	0.017
8	Four tablets/ton; 6 days after fumigation; cleaned	0.012
9	Sample 8 after washing	0.003
10	Six tablets/ton; 5 days after fumigation; turned	0.013
11	Six tablets/ton; turned and aspirated	0.012
12	Sample 11 after washing	0.0

Field Residue Determinations

Samples of wheat taken from fumigated bins located in Glasco, Kan., and Beloit, Kan., were submitted for analysis for phosphine residues (*6*). The samples represented a composite of grain from all four quadrants and multilevels of grain in the bin and were immediately placed in polyethylene freezer bags and cooled with dry ice until stored in a deep freezer. Groups of samples were shipped by Air Express with sufficient dry ice to ensure their remaining at a low temperature. Immediately upon receipt at this laboratory, the samples were unpacked, recorded, and placed in a deep freezer at −20° F., until they could be analyzed.

Residue analysis values ranged from a maximum of 0.023 p.p.m. in one sample (39-L) to a low of less than 0.001 p.p.m. (Table V). Values of less than 0.001 p.p.m. are reported as 0.0 p.p.m. All residues of phosphine on the grain were less than 0.03 p.p.m. during the study and most values were below this maximum by a factor of 10^{-1}. There was no clear evidence to suggest that higher dosage rates result in higher residues of phosphine.

Sample 37-N, which was taken from a bin of wheat being turned by augers on the 14th day after fumigation, contained a residue of 0.016 p.p.m. of phosphine. A sample of grain from this same bin showed less than 0.001 p.p.m. of phosphine at 7 days. The apparent residue appears to be due to particles of disintegrated pellets of Phostoxin coating the grain kernels, and is a source of contamination resulting from the movement of the grain with the attendant dust disturbance.

A second group of wheat (Table V) was fumigated with Phostoxin under field conditions and shipped to this laboratory for analysis. The samples, according to each label, were treated with two, three, four, or six tablets per ton of grain. Following fumigation, they were turned, or turned and aerated. These samples were received in duplicate. One of each of the samples of turned and aerated wheat was washed twice with water by decantation, following receipt at this laboratory.

The results of the analyses are shown in Table II. The highest residue was found in sample 4, which was treated at three tablets per ton and turned. After aeration, the phosphine content had dropped from 0.046 to 0.006 p.p.m. The residues of the remaining samples that were only turned, varied from 0.017 to 0.004 p.p.m. Residues on the samples that were turned and aerated varied from 0.012 to 0.006 p.p.m., indicating little difference from those that were only turned. After washing, the residues on all wheat samples dropped to less than 0.005 p.p.m.

Baking Studies

To determine whether any phosphine residues might occur in a final baked product from Phostoxin-treated wheat, bread was baked from flour prepared from sample 39-L of the Kansas study and from flour prepared from control wheat.

The results of this study indicated that flour from the control wheat contained 0.049 p.p.m. of apparent phosphine while the treated wheat flour contains only 0.018 p.p.m. The resulting breads from the control and treated wheat were found

to have 0.021 and 0.004 p.p.m. of phosphine present, respectively.

A second baking experiment was carried out using a 3-kg. sample of commercial flour to which 120.4 mg. of powdered Phostoxin tablet had been added. This, theoretically, represents 13.4 p.p.m. of phosphine, but analysis showed only 4.95 p.p.m. This is to be expected because of losses of phosphine during mixing. Two loaves of bread were made, and analysis indicated that 0.029 p.p.m. of apparent phosphine was present. On comparison with the control values obtained above, however, no phosphine appeared.

Discussion

The analytical method as described above is extremely sensitive. The limiting factor, as is true in most residue analyses, is the extent of separation of the compound of interest from interfering materials. In the present case, this refers to the phosphorus content of the reagents, especially the water, used in the reactions. To determine quantities in the range of parts per billion, the concentration of phosphorus in the reagents must be below this range. Reference should also be made to the results obtained in the baking studies with control flour and bread. The cause of this apparent phosphine in the control samples is not known, though it may be due to particles of flour being carried over from the reaction flask, as flour is very difficult to wet. However, this is not true of the bread and an appreciable blank value was present in this case also. These results point out the necessity of always running control or blank samples with any analysis.

The results of experiments with grain indicate the residues that may be expected on fumigation with Phostoxin under a wide variety or circumstances. The original recommendation by the manufacturer to treat with ten tablets of Phostoxin per ton of grain has since been changed to six tablets per ton.

The rate of application in laboratory experiments was considerably in excess of the recommended rates, and the results of these studies should be interpreted in terms of maximum residues to be expected. The field experiments were carried out using the now recommended dosages of Phostoxin. The maximum residue found was 0.046 p.p.m. of phosphine and this decreased to 0.006 p.p.m. after aerating.

Aeration proved very effective in removing any residues present, as shown in the results of both laboratory and field experiments. Laboratory experiment 8 indicates this effect very clearly with a decrease in phosphine content from 3.03 to 0.004 p.p.m. after simple exposure to air and is confirmed in other laboratory and field experiments.

No consistent relation was found between dosage and residue in field experiments, nor with time after exposure, as there appears to be under laboratory conditions. All residues in samples received from field conditions were very small, possibly because of sampling conditions, although every precaution was taken to prevent loss of phosphine.

The results of the baking studies indicate that no phosphine should be expected in baked products from Phostoxin-treated wheat; even when Phostoxin was added to flour immediately before baking, no residue was found.

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SOIL FUMIGANTS

Diffusion and Pest Control by Methyl Bromide and Chloropicrin Applied to Covered Soil

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METHYL BROMIDE and chloropicrin have frequently been used to fumigate soils covered with plastic film for the control of weed seeds, fungi, and nematodes. The influence of temperature, moisture, air space, organic matter, depth of injection, and other factors on depth of control by these fumigants is not sufficiently understood and is the subject of this investigation.

Materials and Methods

The soil used was a Hanford fine sandy loam from Orange County, California. It was composed of 58.0% sand, 27.0% silt, and 15.0% clay (*1*). The pH was 8.1 and the organic carbon content 0.2 % (*6*). Moisture content was determined by drying soil in an oven at 221° F. and the porosity of soil was considered to be the per cent of its volume occupied by gases (*2*).

Infestation of the soil with seeds was accomplished by mixing commercial oats (*Avena* sp.) with soil that had been previously fumigated with methyl bromide to eliminate damping-off organisms and then thoroughly aerated. Per cent reduction in germination of seeds was recorded for each experiment and was considered to be an index of relative pest control.

Two experimental methods were used. Elimination of rate of diffusion as a factor influencing control was accomplished by injecting the fumigants into the center of 650-ml. capacity Ball freezer jars completely filled with loosely packed soil containing seed. Methyl bromide was applied as a gas and chloropicrin as a liquid dissolved in acetone. The amounts of acetone used (less than 0.2 ml. per jar) did not influence the apparent toxicity of chloropicrin to the seeds. The jars were tightly sealed, incubated for the required period of time, and uncapped, and germination in comparison to check treatments was determined.

The toxicity of the two fumigants to the oats in the above-mentioned type of test was determined for temperatures of