Determination of Zinc Phosphide in Range Vegetation by Gas Chromatography

by IWAO OKUNO, RICHARD A. WILSON, and ROBERT E. WHITE
U.S. Fish and Wildlife Service
Wildlife Research Center
Denver, Colo.

INTRODUCTION

Zinc phosphide, a compound that hydrolyzes to phosphine gas, has long been used as a rodenticide. Since it is relatively free of secondary hazards, the U.S. Bureau of Sport Fisheries and Wildlife is attempting to maintain or expand its registration as a bait for control of certain field rodents. Part of this registration program requires the assessment of environmental contamination resulting from baiting. In one experiment, 2% zinc phosphide baits were hand broadcast at three different rates in grassland plots to assess residue levels in vegetation at various intervals after treatment. To determine the levels present, this laboratory developed a procedure that can measure as little as 0.01 µg of zinc phosphide in 1-g samples of shortgrass range vegetation (foliage of grass and small forbs).

The gas chromatographic procedure of ROBISON AND HILTON (1971) for measuring zinc phosphide residue in sugarcane was found unsuitable for range vegetation. The sensitivity was borderline and, with foliage, the required 50-g sample size resulted in an impractically large volume. Consequently, it was necessary to develop a procedure that would provide greater sensitivity with a substantially smaller sample.

EXPERIMENTAL

Summary of Method

A 1-g sample of foliage is sealed in a flask and acidified to hydrolyze zinc phosphide to phosphine gas. This gas is sampled from the headspace and measured by gas chromatography with a flame photometric detector.

Reagents

The zinc phosphide was commercial grade (94% pure), sieved with a nylon mesh monofilament screen, mesh opening 37 µ (Small Parts Inc. #CMN-37*).

* Reference to trade names does not imply Government endorsement of commercial products.

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Equipment

The gas chromatograph used was a Micro-Tek MT-220 equipped with a flame photometric detector (phosphorous filter, 526 nm) and an 18- x 1/4-in. O.D. aluminum column packed with Chromosorb 102. Column and inlet were at ambient temperature. The flow rate of the nitrogen carrier gas was 150 ml/min. The detector was operated at 175° C. Gas flow rates to the detector were: hydrogen, 150 ml/min; air, 25 ml/min; and oxygen, 20 ml/min.

Procedure

Foliage samples were cut with hand scissors in to about 1-cm lengths. To minimize losses of zinc phosphide from the surface of the foliage and to avoid cross contamination through excessive handling, samples were not mechanically homogenized but were thoroughly mixed by hand. One gram of sample was weighed into a clean 50-ml Erlenmeyer flask (previously soaked overnight in 10% sulfuric acid and rinsed twice each with tap and distilled water). The flask was capped with a skirted rubber stopper (16-mm plug, 19-mm sleeve length), which was wetted with distilled water to ensure a tight seal, and 15 ml of air was withdrawn from the flask with a gas-tight syringe. With another syringe, 10 ml of 1.2 N HCl was added. The flask was swirled to thoroughly wet the foliage and then tapped to wash most of it to the bottom. Between 1.5 and 5 hr was allowed for the zinc phosphide to hydrolyze and the phosphine gas to equilibrate. Then the desired volume of headspace gas, which depended on the estimated concentration, was withdrawn from the headspace with a gas-tight syringe and injected into the chromatograph.

To obtain nanograms of injected phosphine, the sample response was compared with a standard curve prepared from the primary phosphine gas reference standards. The response for 0.1 ng of phosphine was usually about 5% of full scale at an attenuation of 64×10^3 and was linear in the normal working range of 0.1 to 2 µg. The retention time of phosphine was 1.5 min.

Preparation of Primary Gas Reference Standards

Pure phosphine was prepared in a gas generator similar to that described by ROBISON and HILTON (1971). An aliquot of the pure gas was diluted with argon to the desired concentration in a rubber-stoppered 500-ml flask.

Preparation of Zinc Phosphide-Water Secondary Standards for Determining Recoveries

Exactly 40 mg of zinc phosphide and 20 ml of distilled water were put into a 20- x 125-mm culture tube and capped with a skirted rubber stopper (13-mm plug, 14-mm sleeve length). The mixture was shaken vigorously for 2 min, allowed to stand 15 min, shaken for 2 min, again allowed to stand for 15 min, and finally

shaken for 2 min, immediately filtered through a Whatman #3 filter paper into another 20- x 125-mm culture tube, and capped with a skirted rubber stopper. The mixture was shaken vigorously for 30 sec each time before an aliquot was withdrawn with a hypodermic syringe.

Each secondary standard was standardized by the same hydrolysis procedure used for foliage samples: an aliquot was injected and hydrolyzed in a sealed, air-evacuated, 50-ml Erlenmeyer flask. After the 1.5-hr hydrolysis period, a measured amount of the headspace gas was analyzed by gas chromatography. The flame photometric response for phosphine was compared with that obtained from known amounts of the primary gas standard.

Recovery levels were measured by injecting and hydrolyzing two equal aliquots of a fresh, standardized secondary standard in sealed, evacuated 50-ml Erlenmeyer flasks, one empty and one containing a 1-g sample of untreated foliage. Percent recovery for the sample was determined by comparing the concentration of phosphine in the headspace of the two flasks.

RESULTS AND DISCUSSION

Several revisions of the ROBISON and HILTON method (1971) were necessary to extend the detection limit to 0.01 ppm of zinc phosphide in 1-g samples of range vegetation. Modifications included preparing zinc phosphide secondary standards in water instead of ground sugar, using hydrochloric in place of sulfuric acid for the hydrolysis of zinc phosphide to phosphine, and collecting phosphine as a gas in the headspace instead of in toluene for subsequent analysis by gas chromatography. These changes significantly improved recoveries and precision.

The use of finely ground sugar as the solvent matrix for the zinc phosphide secondary standard was discontinued after repeated standardization tests showed that a homogeneous mixture of zinc phosphide in sugar could not be prepared. Since fortifying 1 g of grass with 10 ppb of zinc phosphide required as little as 10 mg of a mixture of 1 ppm zinc phosphide in sugar, uneven distribution within this secondary standard made recovery data unreliable.

A homogeneous secondary standard was prepared by suspending the zinc phosphide in water instead of mixing it with sugar. These secondary standards were standardized by hyrdolyzing a series of aliquots and comparing the amount of phosphine gas generated with a primary standard curve obtained with pure phosphine gas. Table 1 shows the results obtained when several aliquots, ranging from 0.01 to 1.0 ml (0.01-3.0 µg of zinc phosphide), were taken from seven fresh secondary standard mixtures and standardized against the primary gas standard. The coefficient of variation averaged 13% and did not exceed 23%.

TABLE 1

Variarion in secondary standards (mixtures of zinc phosphide in water): concentrations in aliquots of seven fresh mixtures, determined by comparison with pure phosphine gas

Mixture	Aliquots N	Aliquots Volume range, ml	Concentration, μg Zn_3P_2/ml H_2O Mean	Concentration, μg Zn_3P_2/ml H_2O S.D.	Coefficient of variation, %
A	4	0.04-1.0	0.63	0.13	21
B	5	0.02-1.0	0.93	0.21	23
C	5	0.02-1.0	0.92	0.065	7
D	3	0.02-0.08	0.94	0.13	14
E	3	4.0	0.71	0.035	5
F	6	0.01-1.0	2.43	0.09	4
G	5	0.01-1.0	0.80	0.15	19

The use of hydrochloric acid in place of sulfuric acid improved recoveries of phosphine from foliage samples fortified with zinc phosphide. Hydrolysis with sulfuric acid produced erratic results, possibly because of partial oxidation or other intermediate reactions that prevented the quantitative conversion of zinc phosphide to phosphine. These interferences were not apparent with hydrochloric acid. The loss of phosphine gas due to its solubility in hydrochloric acid was found to be minimal. When five different amounts of a 0.1-ppm primary gas standard, ranging from 60 to 200 μl, were injected and bubbled through 10 ml of the acid solution contained in a sealed 50-ml Erlenmeyer flask, the average recovery in the headspace was 99.6% (range, 85%-126%).

Another factor influencing recovery was the internal pressure of the sample container during hydrolysis. Foliage samples generated gasses other than phosphine (primarily carbon dioxide), as evidenced by a pressure build-up in the sealed containers after addition of the acid. Internal pressures greater than atmospheric pressure caused erroneously low results because a portion of the gas sample expanded out of the gas syringe during the headspace sampling. This loss was minimized by withdrawing a volume of air from the container before adding the acid so that the pressure after hydrolysis was near atmospheric pressure.

Recoveries from three different samples fortified with 0.01 to 1.6 μg zinc phosphide ranged from 55% to 100% (Table 2). Recoveries of zinc phosphide from fortified foliage samples varied with different samples as well as with the condition (at least, dryness) of the samples. With sample A (grass foliage), the mean recovery was 107% when the sample was fresh, but only 66% after the sample was allowed to air dry overnight before fortification.

TABLE 2

Recoveries of zinc phosphide from three grass samples fortified when freshly cut or 1 day later

Grass sample	Fortification, µg	N	Recovery, % Mean	S.D.	Coefficient of variation, %
A (fresh)	0.01-0.63	5	107	13	12
(1 day)	0.02-0.93	10	66	14	21
B (fresh)	0.02-1.6	6	79	10	12
(1 day)	0.01-0.41	5	56	11	20
C (frozen, 1 day)	0.5	3	55	3	6

With sample B (grass foliage similar to A), average recoveries were 79% fresh and 56% after overnight drying. Recoveries from dry foliage may be lower because of increased surface activity, which could result in irreversible adsorption of zinc phosphide and/or phosphine. Sample C (mixed grass and forb foliage), which was stored overnight in a freezer and thawed before fortification, showed a mean recovery of 55%.

Differences in recoveries between various samples might have been expected, in view of the literature. BERCK (1968) and BERCK and GUNTHER (1970) showed that the degree of chemisorption of phosphine varies with different types of cereal products. ROBISON and HILTON (1971) reported that sugarcane irreversibly absorbs about 42% of the total phosphine in an acid medium. Because of these variations, laboratory recovery data for a particular sample of foliage are not necessarily applicable to all such samples, and recovery levels should be determined separately for each sample type. Fortification of treated field samples with zinc phosphide was found to be unreliable for determining recoveries because the residual zinc phosphide was not distributed uniformly in the subsamples. Therefore, recoveries were determined with control samples obtained from each test plot before zinc phosphide baits were applied. Although range vegetation might differ in composition or condition from vegetation cut on the same plots 30 or 60 days later, such variations should be slight. Reasonable care in handling the pre- and post-treatment samples in the same way should ensure adequately reliable recovery data from pre-treatment samples.

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