

Studies with Radioactive Phosphine-³²P in SugarcaneH. Wayne Hilton* and John M. L. Mee¹

Because zinc phosphide (Zn_3P_2), used as a rodenticide in sugarcane fields, would be expected to release phosphine (PH_3), studies were undertaken to determine the behavior and fate of PH_3 in a number of related substrates. Radioactive phosphine-³²P was applied to sugarcane leaves, to roots of sugarcane plants growing in nutrient culture, to chopped

sugarcane to be analyzed for residues, to soil, to sugarcane juice, and to oat groats used for rodent bait. From 30 to 60% of the applied material formed nonvolatile phosphorus compounds. The reaction products were largely water-soluble and appeared to be oxyacids of phosphorus.

Zinc phosphide (Zn_3P_2), used on crimped oat groats as an air-broadcast rodenticide in sugarcane fields, might be expected to liberate phosphine gas, PH_3 , as the result of hydrolytic conditions in soil, on the plant surfaces, or on the deteriorated grain kernels. Assuming that some PH_3 would be generated, we desired to study its interaction with the sugarcane plant, soil, water, and bait material.

Two other subsequent studies will be the subjects of separate papers: the residues of Zn_3P_2 and PH_3 found in sugarcane from the aircraft applications (Robison and Hilton, 1971), and the fate and longevity of Zn_3P_2 and PH_3 residues in contact with sugarcane soils and in water (Hilton and Robison, 1972). In both of these papers PH_3 was measured directly and specifically by gas chromatography.

For the current experiments we employed radioactive phosphine-³²P available in sealed ampoules from Amersham/Searle Corp., Des Plaines, Ill. To our knowledge no study has been made of the interaction of PH_3 with growing plants, or of its facile oxidation in contact with plant and mineral substrates.

Andreev *et al.* (1959) and Curry *et al.* (1959) used Zn_3P_2 labeled with ³²P (and ⁶⁵Zn in one experiment) to study the liberation and distribution of PH_3 (and zinc) in the rat. Phosphine gas, used as a fumigant for dry food products, reacts readily, in varying quantities, with many vegetable and mineral substrates to form nonvolatile substances (Berck, 1968; Berck and Gunther, 1970). Although Dieterich *et al.* (1967) disputed some of the earlier evidence, there seems little doubt that PH_3 reacts chemically and irreversibly in contact with the substrate rather than being adsorbed physically or adsorbed in the form of an insoluble phosphide. None of the authors suggest that PH_3 may be oxidized by the substrate to the phosphorus oxyacids; the identity of the "chemisorbed" product was not investigated except that it did not generate PH_3 when treated with acid.

Phosphine is a reactive gas, combining with oxygen to form oxyacids of phosphorus. However, there seems to be a lack of information on its interaction at ambient conditions and in dilute form with plant, water, and soil substrates (Van Wazer, 1958), in spite of its long history of use.

METHODS AND RESULTS

Phosphine gas, as ³²PH₃, was received in sealed ampoules containing 1 mCi per ampoule. The specific activity of the

gas was given by the manufacturer as 20.2 mCi/mM; each ampoule then contained 0.05 mM or 1.7 mg of ³²PH₃.

Individual ampoules were cooled in a freezer and attached, after breaking the inner seal, to a 1-l. round-bottomed flask flushed with N₂ and containing 500 ml of cold 95% ethanol. Since PH_3 is the only volatile compound to be expected, stability of solutions could be checked by vaporizing the ³²PH₃ and measuring residual radioactivity which might result from reaction to other nonvolatile forms of phosphorus. Ethanolic solutions of ³²PH₃ were reasonably stable. They could be added to water without vapor loss of radioactivity, but the aqueous solutions were chemically less stable, resulting in conversion of the ³²P to nonvolatile forms over a period of time.

As prepared, the ethanolic solutions contained about 1.4×10^6 cpm per milliliter. Radioactive counting was performed by liquid scintillation in a Beckman model 150 spectrometer in dioxane containing 100 g of naphthalene and 5 g of PPO (2,5-diphenyloxazole) per liter. Since no standard source of ³²P was available to determine the counting efficiency, experiments could only be corrected for radioactive decay and for background counts of 68 to 80 per minute. ³²PH₃ placed in a sealed vial with the dioxane scintillation mixture and counted for 35 days showed a reduction in radioactivity corresponding exactly with the theoretical rate of decay of ³²P. There was thus no loss of volatile ³²P.

The ³²PH₃ gas as received was reasonably pure and stable. Polymerization of two ampoules to a yellow water-insoluble solid occurred only in the gaseous state, not after the gas was diluted in a solvent. It is possible that the radioactive gas is less stable to dissociation to hydrogen and polymerizable phosphinyl groups than is the unlabeled PH_3 (Van Wazer, 1958).

Volatility and Stability. Ten milliliters of freshly prepared ethanolic ³²PH₃ lost 99.86% of the ³²P when evaporated to dryness with a stream of N₂. Dry N₂ bubbled through a solution of 1 ml of ethanolic ³²PH₃ in 50 ml of water removed 95% of the ³²P. Gentle boiling of a similar mixture removed 91% of the ³²P in an hour, and heating a sample to dryness removed 88.3%.

Aqueous ³²PH₃ stored 3 days in a refrigerator was placed in a counting vial and heated in a furnace from room temperature to 550° C. Counting in scintillation liquid after cooling showed a loss of 60% ³²P from a 0.1-ml aliquot and 56% from 0.5 ml.

Alcoholic ³²PH₃ added in varying amounts to finely chopped sugarcane (5 g) in counting vials, and ashed overnight in a furnace at 550° C showed losses of ³²P as follows: 60% from 0.1 ml; 58% from 0.2 ml; 59% from 0.3 ml; 57% from 0.4 ml; and 55% from 0.5 ml.

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Residue Recovery of ³²PH₃ from Water and Sugarcane. It was desirable to investigate the recovery of PH₃ both in the form of ³²P and H₃PO₄, when ³²PH₃ was used in the procedure to determine analytical residues (Hazleton Labs, 1968). Freshly prepared alcoholic ³²PH₃, 100 ml (about 150 μg of PH₃), was added to 1000 ml of distilled water in the 5-l. flask of the residue apparatus. Sulfuric acid, 2000 ml of 1.5% v/v, was slowly introduced using N₂ pressure. The flask was heated 2 hr with N₂ sweeping the vapors through two gas washing bottles in series, each containing bromine water and 10 ml of excess Br₂ over that needed for saturation of the water. The Br₂ solutions, containing H₃³²PO₄ from the oxidation of ³²PH₃, were reduced in volume to remove Br₂ and for counting. The recovery of ³²P from the Br₂ water was 67.4%, with 32.6% left behind in the aqueous acid. Aliquots from 2 different ampoules of ³²PH₃ gave the same results. All recoveries were measured by scintillation counting.

An aqueous solution of ³²PH₃ stored 3 days before use in the same procedure (with water and acid only) gave an apparent 62.5% recovery from the Br₂ water, based on scintillation counting. The aqueous acid contained 40.3% of the ³²P.

Alcoholic ³²PH₃, 100 ml, added to 1000 g of chopped sugarcane and 1000 ml of distilled water, was treated with aqueous H₂SO₄ and the residue recovery carried out as before. Results showed: recovery from Br₂ water, 61.2%; retained in aqueous acid, 29.8%; and retained in fiber after washing, 9.9%.

Aqueous ³²PH₃, stored 3 days and used in the same procedure with 1000 g of chopped sugarcane, gave a recovery of 46.9% from the Br₂ water, with 47.6% retained in the acid in nonvolatile form and 9.8% retained by the fiber in an insoluble form.

A portion of the aqueous acid, after distillation of the ³²PH₃, was treated with the molybdate reagent used to determine phosphate ion. A bluish color resulted with two absorbance peaks at 785 and 630 mμ. The same reaction product with H₃³²PO₄ from the distillate gave only one peak at 820 mμ. Oxidation of the aqueous acid solution with Br₂ produced the single 820 mμ peak.

Sugarcane, 500 g containing ³²P from experiments (detailed below) in which ³²PH₃ was added in nutrient solution to roots of growing plants, was analyzed for ³²PH₃ residue by the same procedure. Results were:

	Sample 1	Sample 2
Colorimetric H ₃ PO ₄	0.007 ppm	0.003 ppm
Radioactivity measurements in sugarcane	2064 cpm/g	2010 cpm/g
in Br ₂ water	12 cpm/g	12 cpm/g
Recovery based on ³² P	0.6%	0.6%

The colorimetric blanks may be as low as 0.002 ppm if extraneous phosphate has been carefully excluded.

Uptake of ³²PH₃ through Sugarcane Roots via Nutrient Solution. Each of eight individual sugarcane plants, variety H50-7209, growing in 3000 ml of aerated nutrient solution, was treated with 50 ml of fresh alcoholic ³²PH₃. Aeration was suspended for the first 18 hr, then resumed for the remainder of the 1-month experiment. Water was added as needed to maintain volume. Each container received 69 × 10⁶ cpm of ³²P or about 170 μg of PH₃. The glazed porcelain containers were covered to exclude light; radioactivity did not accumulate on the containers. Plants were removed at intervals, the roots washed, dried in front of a fan, and then sectioned for radioactive counting. The plant parts were finely chopped; samples of 0.5 to 1.0 g were counted directly and were ashed at 550° C. Table I shows the data. The ³²P was not lost by ashing and the ashed samples gave more reproducible results without quenching or chemiluminescence.

Table I. Uptake of ³²P in Sugarcane from ³²PH₃ Added to Roots via Nutrient

Sample	Time interval, days	Green wt, g	Recovery of ³² P as % of added cpm ^a	
			Fresh	Ashed
Green leaves	1	193	2.3	2.2
	10	226	7.0	9.2
	20	147	5.4	7.2
	28	243	20.0	13.1
Stalk	1	140	2.2	1.7
	10	230	7.9	6.4
	20	436	8.2	9.0
	28	552	10.1	11.3
Suckers	1	6	5.8	6.2
	10	55	3.6	3.0
	20	None		
	28	37	1.6	1.5
Dry leaf trash	1	2.5	0.04	0.06
	10	10	0.71	0.76
	20	33	1.6	3.8
	28	14	0.66	0.48
Roots	1	14	15.2	14.3
	10	42	31.3	33.9
	20	48	15.8	26.7
	28	93	26.2	22.4
Total plants as sum of plants	1	356	25.7	25.2
	10	563	50.6	53.3
	20	664	31.0	46.8
	28	939	58.4	48.8
Nutrient solution	1		76.5	77.3
	10		44.8	45.0
	20		20.9	20.9
	28		7.2	7.2
Unaccounted losses ^b	1		None	None
	10		4.6	1.7
	20		48.1	32.3
	28		34.4	44.0

^a Corrected for decay. ^b By difference.

Chromatographic assay of ³²P extracts from leaves and roots, using techniques employed for uptake of H₃³²PO₄, indicated that 92% of the ³²P in the leaves was in the form of phosphate compounds, while only 35% of the root extract ³²P could be so identified. The remainder consisted of non-volatile unknowns.

As shown in the table, ³²P was rapidly absorbed through the roots and distributed throughout the sugarcane plant. About half of the total ³²P was absorbed by the plants. Of the ³²P absorbed, about half remained in the roots. The total count of the green leaves and stalk increased with time from 16 to 24% of the initial ³²P added, while the roots showed corresponding decreases from 34 to 22%. The lack of accumulation of radioactivity in the older dry leaves is evidence of active transport in the phloem, rather than xylem storage. This mechanism corresponds to that of H₃³²PO₄.

The ³²P in the nutrient solution decreased with time to 7% of the initial count, with no corresponding increase in the plants. Since the solutions, almost from the beginning, could be ashed without loss of ³²P, it was found that the ³²P in a nonvolatile form was removed physically as an aerosol by the bubbling of air through the medium. It is suggested that ³²PH₃ converted rapidly to oxyacids of phosphorus in the nutrient medium or on the roots, and that H₃³²PO₄ was the only substance actively transported in the plant.

Addition of ³²PH₃ to Sugarcane Leaves and to Soil. Ethanollic ³²PH₃, 0.2 ml, was added by pipette to single leaf axils (dewlap regions) of several sugarcane stalks. Radioactivity was found after 14 days in all the above-ground parts of the plants. Essentially all the ³²P was retained after ashing (Table II).

Table II. Plant Residue of ^{32}P 14 Days after Leaf Sheath Applications of $^{32}\text{PH}_3$

Plant part	Recovery, cpm/g ^a	
	Fresh	Ashed
Meristem	128	142
Leaves		
Above treated leaf	100	57
Treated leaf	1417	2358
Below treated leaf	202	144
Stalk	100	50

^a Corrected for background. Applied 379,428 cpm per stalk weighing approx. 2000 g or about 190 cpm/g. Stalk weight about 75% of total weight.

Ethanollic $^{32}\text{PH}_3$, 50 ml, was added to several 1 ft² areas of soil at the base of sugarcane plants. Insignificant amounts of ^{32}P were found in plant parts at the end of 14 days. Soil residues were high and not decreased by ashing (Table III).

Clarification of Raw Sugarcane Juice Containing $^{32}\text{PH}_3$. Raw sugarcane juice was mixed with ethanollic $^{32}\text{PH}_3$ (16,115 cpm per g of juice). The juice was treated with $\text{Ca}(\text{OH})_2$ slurry to pH 10 and heated to boiling over a 40-min period. The coagulated solids were filtered; recoveries of ^{32}P were as follows: after heating, 45.5%, of which 39.3% remained in the clear filtered juice and 6.4% was contained in the filtered solids.

Sorption and Weathering of $^{32}\text{PH}_3$ Applied to Oat Groats. Crimped oat groats, 200 g, retained 65% of the ^{32}P 16 hr after being mixed with ethanollic $^{32}\text{PH}_3$ and allowed to dry uncovered. There was no further loss of ^{32}P after 6 days, after 30 days in freezer storage, or on ashing in a furnace.

Dry oat groats containing ^{32}P from $^{32}\text{PH}_3$ were placed in screen boxes: in the open, exposed to daily sprinkling; in the open, but partially protected; in a 1-year-old sugarcane field on top of the mat of dry leaves; and in the sugarcane field on the ground under the leaf mat. During a 3-week period of exposure the ^{32}P decreased according to the amount of direct exposure to rainfall (Table IV).

DISCUSSION

Radioactive phosphine- ^{32}P proved useful for the investigation of a variety of interactions of the gas with plant and mineral substances, especially in predominantly aqueous media. From the experimental evidence we have concluded that in the natural environment of air, water, soil, and growing sugarcane with which we are concerned, adsorption or absorption of PH_3 released from Zn_3P_2 and not immediately dissipated in air would result in the irreversible formation of heat-stable, nonvolatile phosphorus compounds. While the identity of the nonvolatile compounds was not fully established, they were for the most part water-soluble—probably the oxyacids: hypophosphorous, phosphorous, or phosphoric, depending on the oxidation state. In the presence of inorganic constituents, especially in moist soil, insoluble relatively inert nonvolatile compounds formed. It is suggested by this and other work to be reported separately that these are the insoluble metal salts of the phosphorus oxyacids. Physical adsorption, by which the PH_3 molecule might be held in soil or plants but would be released with heat or on treatment with acid, did not appear likely.

These views are consistent with those of Berck (1968) and Berck and Gunther (1970) from their experiments with the irreversible reaction of a portion of the PH_3 gas passed through various more or less dry food products and a few

Table III. Residues of ^{32}P 14 Days after Application of $^{32}\text{PH}_3$ Beneath Sugarcane Plants

Plant part	Recovery, cpm/g ^a	
	Fresh	Ashed
Meristem	0	0
Leaves		
3 ft above ground	0	8
1 ft above ground	5	55
Stalk	100	10
Soil (surface layer only)	20,994	30,902

^a Corrected for background.

Table IV. Weathering of ^{32}P from $^{32}\text{PH}_3$ Sorbed on Oats

Days of exposure	Radioactivity remaining on oats, % ^a			
	Sprinkler area		Sugarcane field	
	Exposed	Under racks	Above mat	Under mat
7	9	59	35	100
14	7	19	11	36
21	2	13	4	47

^a Rainfall in the sugarcane field was about 6 in. in 21 days.

soils. Since our experiments were carried out largely with moist or aqueous media, the ease and extent of PH_3 reaction with water or with dissolved oxygen or mineral constituents, as well as the effect of water on the reaction of PH_3 with organic substrates, may have altered the composition of the final products. The use of the radioactive ^{32}P nuclide may also have altered the reactivity of the PH_3 molecule; however, the results obtained here are consistent with those of our direct gas chromatographic procedure with PH_3 .

Berck observed that sorption of PH_3 increased with increasing moisture content in certain of his samples; it seems probable that reaction of PH_3 with most substrates is facilitated both by an increase in moisture and by the time of physical contact.

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