Determination of Phosalone and Its Oxygen Analog in Citrus Crops

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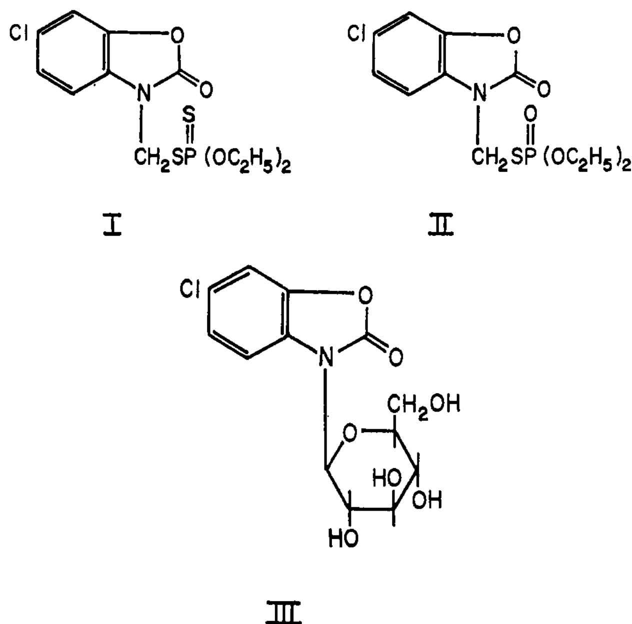
A method for determining residues of phosalone [*O*,*O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)-methyl phosphorodithioate] a new low toxicity broad spectrum pesticide and its oxygen analog metabolite [*O*,*O*-diethyl *O*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorothioate] in citrus crops is presented. The pentane and/or ethyl acetate extractives of lemons, grapefruit, or oranges were subjected to liquid partition followed by Florisil column chromatography. The metabolite is eluted from the same column as the parent compound by use of a more polar solvent system. The phosalone fraction is analyzed by gas chromatography (glc) using an Ni^{63} electron-capture detector and the metabolite by thin-layer chromatography (tlc). Recoveries of phosalone were generally better than 90%, and those of the oxygen analog were 80 to 100%. The sensitivity of the glc procedure was about 0.05 ppm for phosalone and about 0.1 ppm for the metabolite by tlc.

Phosalone (Zolone, Rhodia Inc., Chipman Division), a new insecticidal product, is *O*,*O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorodithioate (I). It provides good control of a variety of insect pests which may attack crops such as grapes, apples, pears, oranges, and grapefruit. Of particular importance is the use of phosalone as a miticide at the suggested rates of 0.25 to 0.50 lb in 100 gal of water to afford a total of about 5 lb per acre by a complete coverage spray. To determine the fate of phosalone and its metabolite in processed citrus, oranges treated at 1.5 times the recommended rate and at 14 days from application were processed at the Florida Citrus Experiment Station, Lake Alfred, Fla., to obtain samples for residue analyses. Residues may consists of the parent insecticide (I), the oxygen analog (II), and the atoxic glucopyranose derivative (III).



The metabolism of phosalone has been previously explored (Colinese and Terry, 1968; Bureau of Sport Fisheries and Wildlife, 1969). Phosalone degrades in plants *via* oxidation and hydrolysis. The oxygen analog obtained is much less stable than its parent compound and consequently degrades much more rapidly than phosalone, so that the quantity of the oxygen analog is usually between 1 to 10% of the phosalone residue. Both compounds are easily hydrolyzed to afford diethyl phosphorodithiolate and diethyl phosphorothiolate, respectively. The latter acids are rapidly degraded further to dilute phosphoric acid (phosphates). The benzoxazolone moiety reacts with plant carbohydrates to give the atoxic glucopyranose derivative.

The high sensitivity of electron-capture detectors for chlorinated and certain organophosphate pesticides has led to their acceptance for the gas chromatographic analysis of pesticide residues at the nanogram and picogram level (Burke, 1963; Burke and Giuffrida, 1964; U.S. Dept. of HEW, 1964).

A number of papers have also been published on the thin-layer chromatographic analyses of organophosphorus pesticides (Abbott *et al.*, 1967; Bunyan, 1964; Ragab, 1967a,b; Salame, 1964; Stanley, 1964; Walker and Beroza, 1963). A methodology for the determination of phosalone and its oxygen analog is presented.

EXPERIMENTAL

Solvents. All solvents were nanograde quality (Mallinckrodt Chemical Works, Saint Louis, Mo.)

Gas Chromatography. A Micro-Tek Model MT 220 (Tracor Analytical Instruments, Austin, Texas) gas chromatograph equipped with an Ni^{63} electron-capture detector was used for the determination of phosalone residues. This instrument was fitted with either a 4 ft × 6 mm o.d. glass column packed with 5% OV 210 (Applied Science Laboratories, Inc., State College, Pa.) on 80/100 mesh Gas Chrom Q, or a 4 ft × 6 mm o.d. glass column packed with 3.8% UCW-98 on 80/100 mesh Gas Chrom Q (Applied Science Laboratories).

The following operating parameters were generally used: carrier gas, 5% methane in argon at 70 ml/min with 40 psi pressure; column oven temperature, 245° C; injection port, 260° C; and detector temperature, 275° C. After conditioning the column for 2 days at 260° C, 200-ng samples of phosalone were injected until tests with 1–2 ng injections afforded a constant response (Bowman and Beroza, 1965).

The amount of phosalone residue sample was determined by injecting 1–2 μl of a benzene solution and comparing the peak heights with those of reference standards located in the linear range of the instrument response. The typical retention time of phosalone was about 3 min.

Thin-Layer Chromatography (tlc). Tlc was performed on precoated Merck silica gel F-254 plates (Brinkmann Instruments, Inc., Westbury, N.Y.). Absorbent thickness was

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250 μ. The plates were heated to 130° C for 1 hr and then cooled to room temperature prior to use. A developing mixture of either hexane–acetone 70:30 or benzene–ethyl acetate 80:20 was used.

PROCEDURE. A stock solution of phosalone and its oxygen analog in acetone was prepared so that the concentration was 1 mg/ml and then 1, 3, 5, and 10 μl spotted. Concurrently, samples of the crop extracts under investigation were spotted. The plate was developed so that the solvent front had migrated about 15 cm from the origin. After the plate had been air-dried, it was sprayed uniformly with a solution of 1 *N* sodium hydroxide in ethanol and then heated for 3 to 5 min at 130° C in an oven. The plate was then air-cooled and sprayed with a 0.5% solution of 2,6-dibromo-*N*-chloro-*p*-benzoquinoneimine (Menn *et al.*, 1957) in cyclohexane (Eastman Organic Chemicals, Rochester, N.Y.). The maximum blue coloration appeared in 4 to 5 min. The concentration of the crop residue was estimated by visually comparing the spot size and intensity with those of the reference standards. The R_f's of phosalone and its oxygen analog were, respectively, 0.60 and 0.36 (hexane–acetone), and 0.83 and 0.31 (benzene–ethyl acetate).

Crop Sampling. All samples were stored at −20° C ± 5° C until ready for analysis. A representative portion of whole citrus fruits, washed and unwashed, was quartered and sliced to afford two 100- or 200-g samples. The pulp was subjected to a similar treatment. A representative portion of peel was removed to afford a 50- or 200-g sample.

Extraction and Cleanup. A 50-, 100-, or 200-g citrus sample in 200–300 ml of pentane and/or ethyl acetate was homogenized for 2–3 min at high speed in a Waring blender. The extract was then filtered under vacuum suction through a layer of glass wool, Super-Cell, and a filter paper contained on a Buchner funnel.

The filter cake was washed with 100–200 ml of the homogenization solvent and the solvent then removed in a flash evaporator (Buchner Instruments, Fort Lee, N.J.). The residue so obtained was transferred into a 500-ml separatory funnel by washing the evaporating flask alternately with two volumes of acetonitrile and one volume of hexane. The hexane fraction was separated and discarded. The acetonitrile layer was further extracted three to five times with hexane. The hexane fractions were again discarded. Enough aqueous sodium sulfate solution (10 g/l.) was added to the acetonitrile to afford a minimum 3:1 ratio of water–acetonitrile. The pesticide residues were then extracted from the aqueous acetonitrile with three 80–100 ml portions of hexane. The hexane extracts were then filtered through anhydrous sodium sulfate and evaporated to near dryness in a flash evaporator.

The residue was dissolved in 10 ml of benzene and the flask rinsed with 3–5 ml portions of benzene. The combined benzene extract and washings were then subjected to Florisil chromatography on a 2.5 × 50 cm column. The phosalone was eluted by successively passing 80 ml of benzene, and then 200 ml of 5% ethyl acetate in benzene. Further elution with 300 ml of 5% methanol in benzene afforded the oxygen analog. The two fractions were separately evaporated *in vacuo* to near dryness on the flash evaporator, while being warmed by a water bath at 50° to 60° C.

Fortifications. To determine recovery data for the various crop substrates, samples were spiked (Figure 1) with phosalone and analyzed as previously described.

$$\% \text{ Recovery} = \frac{\text{ppm found (fortified analysis} - \text{untreated analysis)} \times 100}{\text{ppm fortification}}$$

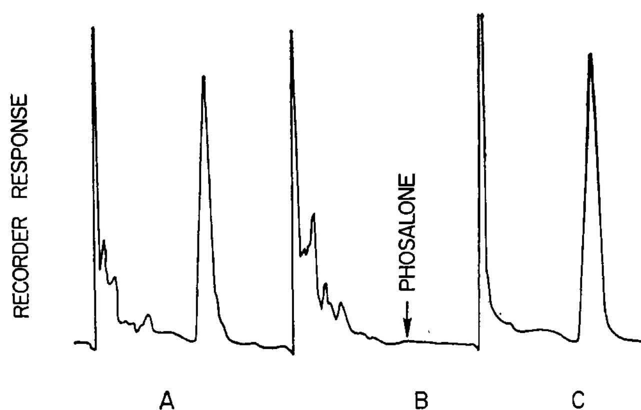

Figure 1. Typical phosalone recovery from whole oranges. A. Fortification of control sample at 2 ppm; recovery = 92.5%. B. Control. C. Phosalone standard equivalent to 1 μg/ml

In all cases the interference from untreated crop material was less than the detectable limit (0.05 ppm) for phosalone.

RESULTS AND DISCUSSION

When used at a rate of 4.5 lb of active ingredient per acre (1.5× the recommended rate) with a preharvest interval of 14 days, 1.50 ppm of phosalone was detected on whole unwashed oranges and 7.12 ppm was detected on the peels (Table I). Since oranges are composed of about 20% peel, the residue on the whole unwashed orange can be calculated as 1.42 ppm, which is in good agreement with that experimentally determined. At 1.5× the recommended rate, the phosalone residue in ground dry citrus pulp was 4.23 ppm. This fraction may normally be considered for use as an animal feed. The fairly high residues present in the orange oil from processed oranges, which had also been treated at 1.5× the recommended rate, is not unusual. However, orange oil is a relatively minor commercial product, normally used at levels of less than 0.1% in a very limited number of foods. The phosalone oxygen analog could not be found in the citrus peels, where the residues usually are highest, or in the ground

Table I. Phosalone Residues in Valencia Oranges

Crop Substrate	14 Days after Application of 4.5 lb of Phosalone per Acre	Spiked Control Samples	
	Phosalone Found, ppm	Fortification, ppm	Recovery, %
Before processing			
Whole washed fruit	0.88	1.00	87.1
Whole unwashed fruit	1.50	0.50	88.3
Peel washed fruit	1.56	1.00	90.7
Peel unwashed fruit	7.12	...	...
Pulp	<0.05	0.25	90.4
Controls (all substrates)	<0.05	...	...
After commercial processing			
Ground dry citrus pulp	4.23	0.25	80.0
Juice single strength	<0.05	0.50	97.0
Water phase emulsion	<0.05	0.50	90.8
Press liquor	1.95	0.25	97.5
Molasses	1.46	0.50	90.9
Juice sac extracted (finished)	<0.05	0.50	93.0
Chopped residue (peel)	0.87	0.50	89.5
Orange oil	34.0	...	...
Controls (all substrates except oil)	<0.05	...	...

Table II. Oxygen Analog Residues in Unwashed Orange Peels and Ground Dry Citrus Pulp

Substrate	14 Days after Application of 4.5 lb of Phosalone per Acre — Oxygen Analog Found, ppm	Spiked Control Samples — Fortification, ppm	Recovery, %
Peel	<0.1	...	...
Peel, control	<0.1	0.5	~80
Ground dry citrus pulp	<0.1	...	...
Ground dry citrus pulp, control	<0.1	0.25	~80–90

Table III. Phosalone Residues in Eureka Lemons

Substrate	Days after Last Application of 28 lb of Phosalone per Acre	Phosalone Found, ppm	Spiked Samples — Fortification, ppm	Recovery, %
Whole unwashed fruit	0	4.12		
	15	...		
	31	3.80		
	73 (H)[a]	1.15		
Whole (unwashed fruit) controls		<0.05	5.0	90.0
			1.0	98.0
			0.25	87.5
Peel (unwashed fruit)	0	8.51		
	15	7.79		
	31	6.22		
	73 (H)	2.38		
Peel (unwashed fruit) controls		<0.05	5.0	97.0
			2.5	87.0
			0.25	94.5
Pulp (unwashed fruit)	0	<0.05		
	15	<0.05		
	31	<0.05		
	73 (H)	<0.05		
Pulp (unwashed fruit) controls		<0.05	1.00	92.5
			0.25	96.0
			0.25	94.0

[a] (H) = Harvest.

dry citrus pulp (Table II). Whole unwashed lemons which have been treated with 28 lb of active ingredient per acre (about 2× recommended rate) followed by a preharvest interval of 31 days afforded a 3.80 ppm residue (Table III), and a 6.22 ppm unwashed peel residue. A residue of only 0.24 ppm (Table IV) was found on whole unwashed grapefruit at a 10-day preharvest interval, when the crop had been treated with the maximum recommended rate of 1.5 lb of active ingredient per acre. No phosalone residues were found in the above tested critus edible pulp by the glc method, which is sensitive to 0.05 ppm.

The data suggest that the maximum expected residue, when using the recommended rate, would not exceed 5 ppm in whole unwashed oranges, lemons, and grapefruit. The residues found in the edible portions were consistently less than 0.05 on the same day or 3 days after application at the highest application rate. The amount of phosalone residue found on dried citrus pulp animal feed does not exceed 5 ppm,

Table IV. Phosalone Residues in Ruby Blush and Marsh White Grapefruits

Substrate	Days after Last Application of 1.5 lb of Phosalone per Acre	Phosalone Found, ppm	Spiked Samples — Fortification, ppm	Recovery, %
Whole unwashed fruit	0	0.84		
	10	0.24		
	30	0.27		
	60	<0.05		
	100 (H)[a]	<0.05		
Whole (unwashed fruit) controls		<0.05	2.5	91.5
Peels (unwashed fruit)	0	1.44		
	10	0.66		
	30	...		
	60	<0.05		
	100 (H)	<0.05		
Peel (unwashed fruit) controls		<0.05	1.0	90.0
Pulp (unwashed fruit)	0	<0.05		
	10	<0.05		
	30	<0.05		
	60	<0.05		
	100 (H)	<0.05		
Pulp (unwashed fruit) controls		<0.05	0.5	94.5

[a] (H) = Harvest.

even at the maximum rates applied. No metabolite could be detected. Citrus pulp commonly used for feed usually constitutes 10 to 15% of the animal diet. A maximum amount would be 35%. Even if the pulp constituted 50% of the diet with a 5-ppm phosalone residue, the total residue would be only 2.5 ppm.

ACKNOWLEDGMENT

The authors acknowledge the research and development conducted by J. Metivier, J. Desmoras, M. Laurent, and their research team at the Rhone-Poulenc Research Center "Nicolas Grillet" in Vitry, France, and at their Agricultural Experiment Station in Ermenonville, France. The authors also express appreciation to Bernard Buchner and Donald T. Lillie for their helpful suggestions.

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Received for review December 7, 1970. Accepted February 4, 1971.