Dissipation of Parathion and Related Compounds from Field-Sprayed Spinach

Thomas E. Archer

Parathion in the form of Thiophos Parathion 4 E.C. was applied at two rates, 1 and 0.5 lb/acre of active ingredient, to spinach in the field 14 days preharvest. Sampling was at daily intervals from time of application through harvest and the plant material was extracted immediately after sampling for residue analysis. The samples were analyzed for levels of parathion, paraoxon, aminoparathion, *S*-ethylparathion, *S*-phenylparathion, *p*-nitrophenol, *O,O*-diethyl phosphate, and *O,O*-diethyl phosphorothioate. All residues declined from application through harvest. The total initial residues were approximately 58 and 25 ppm and at harvest 0.5 and 0.3 ppm for the 1 and 0.5 lb/acre of active ingredient, respectively. The percentage of paraoxon, *p*-nitrophenol, and diethyl phosphate in the total residue increased from application to harvest while parathion percentage decreased for both applications.

Parathion applications have been recommended for the control of aphids and other insects on spinach at the rate of 0.5 lb of actual material per acre not less than 14 days before harvest (*Calif. Agr. Exp. Sta. Bull.*, 1973). Previously, investigators have studied the residue levels of parathion on field-treated spinach and the effect of frozen storage on these residues (Beckman and Thornburg, 1965) as well as the removal of parathion from the spinach by home and commercial preparative procedures (Lamb *et al.*, 1968). Although parathion levels were determined, most of the other possible related compounds were not determined. The purpose of the present investigations was to determine quantitatively the fate of the levels of parathion and possible related compounds in the environment on field-sprayed spinach sampled daily from application through harvest.

EXPERIMENTAL SECTION

Spinach Plots. In June in Yolo County, Calif., rows of spinach 44 in. wide and totaling 150 ft in length were divided into 50-ft sections. A 50-ft section (plot A) upwind from the other 50-ft sections was established as a control plot. A second plot (plot B) was established for spraying with the recommended rate of parathion at 0.5 lb of active ingredient per acre, and a third plot (plot C) was sprayed with double the recommended rate of parathion or 1 lb/acre. The spinach was at the stage of maturity 2 weeks before harvest. The first sampling of the control plot was taken before spraying the other plots. Each of the other plots was sampled immediately after spraying, and all plots were randomly sampled (approximately 500 g) thereafter at the same time of day during the experiment's duration and extracted for analysis. At the harvest sampling all the spinach from each plot was completely harvested and composited, and a subsample was immediately extracted for analysis. Samples were taken daily from time of pesticide application to time of harvest 14 days after pesticide application. The sprayed plots were duplicated and the analytical data represent average values for the duplicate plots.

Spray Application. The spinach was sprayed with Thiophos 4 lb E.C. using a Hudson Climax 6335 Simplex Sprayer, 8.5-l. capacity equipped with a Hudson 149-403 spray control valve and a nozzle extension. A 0.95-cm i.d. flexible neoprene, Teflon-lined rubber tubing for chemical inertness was attached between the pressure tank and the Roto-Spray Nozzle.

Parathion Applied. The parathion applied was in the formulation of American Cyanamide Co. designated as Thiophos Parathion 4 E.C. (1 gal contains 4 lb of parathion). Active ingredients included: parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate), 46.7%; xylene-range aromatic hydrocarbon solvent, 47.6%; inert ingredients, 5.7%.

Plot B (0.5 lb of active parathion/acre) was sprayed as evenly as possible with 1 gal of water containing 0.971 g of parathion using the equipment described above, and plot C (1 lb of active parathion/acre) was evenly sprayed with 1 gal of water containing 1.94 g of parathion.

The formulation was found to contain by analysis and calculated as the parathion active ingredient equivalent 95.4% parathion, 3.88% aminoparathion, 0.12% paraoxon, 0.11% *O,S*-diethyl *O*-*p*-nitrophenyl phosphorothiolate, 0.05% *O,O*-diethyl *S*-*p*-nitrophenyl phosphorothiolate, 0.43% *p*-nitrophenol, and 0.01% *O,O*-diethyl phosphorothioate.

Analytical Standards. The analytical standards were obtained commercially or synthesized in our laboratory.

Sample Extraction. Twenty-five grams of spinach was extracted by refluxing in 250 ml of a solvent mixture containing 5% isopropyl alcohol–95% benzene (v/v) and 2 ml of 0.1 *N* HCl for 30 min. The 0.1 *N* HCl increased the extraction efficiency for the diethyl phosphate and *O,O*-diethyl phosphorothioate without interfering with the extraction efficiency of the other compounds. The refluxed sample was cooled in an ice bath and the solvent was filtered through Whatman No. 1 filter paper. The extraction was repeated for a total of three refluxes and the filtered solvent was pooled and stored for cleanup and analysis. The extracted plant material was discarded. All solvents used in the experiment were reagent grade freshly distilled prior to use. Extraction efficiencies were studied with fortified control samples at 1.0–0.1 ppm fortification.

Diazomethane Preparation and Methylation of the Sample Extract. The laboratory preparation of diazomethane is detailed on the label of the Diazald reagent bottle obtained from the Aldrich Chemical Co., Inc., Milwaukee, Wis.

The extractives from 10 g of spinach (300 ml of solvent extract) were concentrated *in vacuo* at 50–60° to approximately 1 ml and the volume was quantitatively adjusted to 5 ml with *n*-hexane. The extractives equivalent to 5 g of spinach were added to 5 ml of an ethereal alcoholic solution of diazomethane at room temperature and allowed to stand for at least 15 min prior to sample cleanup.

Sample Cleanup. A glass column 2.54 × 25.0 cm with a solvent reservoir at the top was packed with a plug of glass wool, 10 g of anhydrous Na_2SO_4, 20 g of Florisil (activated at 270° for 3 hr), and 10 g of anhydrous Na_2SO_4. *N*-Pentane (100 ml) was allowed to flow to the column top by gravity flow, and the solvent was discarded after the column was washed. The methylated sample was added to

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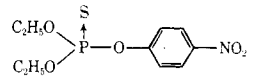
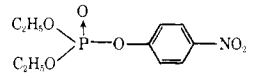
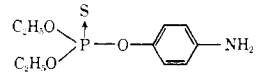
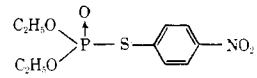
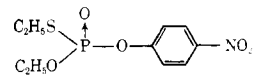
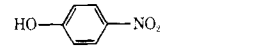
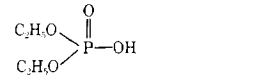
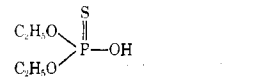
Table I. Retention Times (Glc) and R_f Values (Tlc) of Compounds Studied

Compound	Column temp, °C (glc)	Retention time, min (glc)	Detectable glc sensitivity, ppm	R_f (tlc) value[c]	Clearly detectable (tlc) amts, μg
Parathion[a]	210	7.0	0.001	0.40	10–25
Aminoparathion[a]	210	4.5	0.001	0.12	10–25
Paraoxon[a]	210	8.2	0.002	0.12	10–25
Diethyl phosphate[a] (methylated)	150	2.0	0.001	0–0.24	10–25
O,O-Diethyl *S*-*p*-nitrophenyl phosphorothiolate[a]	210	11.4	0.010	0.42	10–25
O,S-Diethyl *O*-*p*-nitrophenyl phosphorothiolate[a]	210	12.5	0.010	0.18	10–25
p-Nitrophenol[b] (methylated)	140	2.0	0.001	0.34	10–25
O,O-Diethyl phosphorothioate[a] (methylated)	150	1.6	0.001	0.49	10–25

[a] Column 8 ft glass packed with 5% DC 200 and 7.5% QF-1 on 60–80 mesh Gas-Chrom Q. [b] Column 6 ft glass packed with 5% SE-30 and 5% Dow 710 on acid washed and silylated 60–80 mesh Chromosorb W. [c] Silica gel H 250 μ thickness containing 1% zinc silicate with 20% acetone–80% *n*-hexane as developing solvent.

Table II. Chemical Structures and Names of Parathion and Related Compounds Investigated

Structure	Name
	*O,O*-Diethyl *O*-*p*-nitrophenyl phosphorothioate (parathion)
	*O,O*-Diethyl *O*-*p*-nitrophenyl phosphate (paraoxon)
	*O,O*-Diethyl *O*-*p*-aminophenyl phosphorothioate (aminoparathion)
	*O,O*-Diethyl *S*-*p*-nitrophenyl phosphorothiolate (*S*-phenylparathion)
	*O,S*-Diethyl *O*-*p*-nitrophenyl phosphorothiolate (*S*-ethylparathion)
	*p*-Nitrophenol
	*O,O*-Diethyl phosphate
	*O,O*-Diethyl phosphorothioate

the column in 25 ml of pentane followed by two 25-ml aliquots of pentane washes. The sample was eluted from the column with 390 ml of 30% diethyl ether–70% pentane (v/v) and with 390 ml of 25% methanol–75% benzene (v/v) after changing the column receiver between solvents. The eluates were collected and analyzed separately by gas–liquid chromatography (glc) or in combination with thin-layer chromatography (tlc) after concentration *in vacuo* at 50–60° to approximately 1 ml, and transferred and adjusted to appropriate volumes in 6.5-ml MacKay-Shevky-Stafford sedimentation tubes. Parathion, aminoparathion, *S*-phenylparathion, methylated *p*-nitrophenol, and methylated *O,O*-diethyl phosphorothioate were eluted from the column with the ether–pentane solvent. Paraoxon and the methylated diethyl phosphate were eluted with the methanol–benzene solvent. A separate spinach extract aliquot was cleaned up on the Florisil column which was eluted only with the 25% methanol–75% benzene solvent (v/v) for the detection of the *S*-ethylparathion. All recoveries of samples cleaned up were quantitative ranging from 70 to 90%. Samples were analyzed both with and without cleanup. The cleanup procedure facilitated analysis by glc using the electron capture detector as well as tlc. An additional advantage of cleanup was the separation of the compounds into groups with the separate eluting solvents as described above.

Gas–Liquid Chromatography (Glc). The gas chromatograph (Aerograph Model 200) was equipped with a thermionic phosphorus detector with a cesium bromide pellet. The glass coiled 8-ft column was packed with an equal mixture of 10% DC 200 and 15% QF-1 on 60–80 mesh Gas-Chrom Q column support. The carrier gas flow (N_2) was 20 ml/min. The detector and injector temperatures were 200°. All parathion and related compounds were quantitated on this column with the exception of *p*-nitrophenol. The column temperatures at which the following compounds were detected are: (1) parathion, 210°; (2) aminoparathion, 210°; (3) paraoxon, 210°; (4) diethyl phosphate, 150°; (5) diethyl phosphorothioate, 150°; (6) *S*-phenylparathion, 210°; and (7) *S*-ethylparathion, 210°. Diethyl phosphorothioate and diethyl phosphate were detected as the methylated compounds by glc. A Varian-Aerograph Model 1200 gas chromatograph equipped with an electron capture detector, a 6-ft glass column packed with 5% SE-30 and 5% Dow 710 fluid on 60–80 mesh Chromosorb W acid washed and silylated, a carrier gas flow (N_2) of 30 ml/min, and a column temperature of 140° were utilized to quantitate the *p*-nitrophenol as the methylated compound. The detector and injector temperatures were 200°. Quantitation of all compounds was accomplished by measurement of peak areas with a polar planimeter and compared with reference standards. The parts per million data are based on the spinach fresh weight calculations rather than a dry weight basis since the per cent moisture of the spinach samples over the 14-day sampling period ranged from 86.5 to 89.2 with a mean of 88.0 ± 0.8, and this slight per cent moisture variation would not significantly affect the residue data.

Thin-Layer Chromatography (Tlc). Glass plates 20 × 20 cm were coated 250 μ in thickness with silica gel H containing 1% zinc silicate in a water slurry. The plates were air dried and activated in a warm air oven at 100° for 30 min and stored in a dessicator prior to use. After spotting, the samples were developed for a 15-cm solvent travel in 20% acetone–80% *n*-hexane (v/v). The solvent was air dried and the spots visualized under ultraviolet light at 254 nm. The compounds in the detected spots were extracted from the plate in 3 ml of 25% methanol–75% benzene (v/v) in a 10-ml volumetric flask with shaking for 5 min. The sample extracts were adjusted to appropriate volumes and aliquots were injected into the glc for quantitation. The compounds were taken from the plates for extraction into the solvent in two strips. The first strip ranged in R_f from 0 to 0.24 (0–3.9 cm) and the second strip ranged in R_f from 0.24 to 0.60 (3.9–9.0 cm). The glc procedure was able to resolve the compounds in these extracts efficiently. The glc retention times and tlc R_f values are shown for the various compounds in Table I as obtained by the experimental conditions described.

Table III. Levels[a] of Parathion and Related Compounds Applied to Field Spinach in an Emulsifiable Concentrate Formulation

Sample day	Parathion, ppm[b]	Amino-parathion, ppm[a]	Paraoxon, ppm[c]	*S*-Ethyl-parathion, ppm[d]	*S*-Phenyl-parathion, ppm[d]	Diethyl phos-phate, ppm[b]	*p*-Nitro-phenol, ppm[b]	Total residue, ppm
1	56.3	0.905	0.252	0.432	0.042	0.039	0.453	58.4
2	25.7	0.301	0.188	0.190	<0.010	0.038	0.305	26.7
3	15.1	0.176	0.180	0.057	<0.010	0.036	0.240	15.7
4	9.68	0.060	0.192	0.019	<0.010	0.035	0.188	10.1
5	6.20	0.084	0.190	0.016	<0.010	0.035	0.136	6.66
6	4.30	0.042	0.160	0.010	<0.010	0.024	0.216	4.75
7	3.71	0.034	0.111	<0.010	<0.010	0.022	0.117	3.99
8	1.61	0.026	0.107	<0.010	<0.010	0.020	0.018	1.78
9	1.01	0.005	0.173	<0.010	<0.010	0.019	0.019	1.23
10	0.961	0.009	0.108	<0.010	<0.010	0.015	0.018	1.11
11	0.890	0.009	0.152	<0.010	<0.010	0.016	0.018	1.08
12	0.810	0.012	0.094	<0.010	<0.010	0.015	0.022	0.953
13	0.566	0.005	0.081	<0.010	<0.010	0.001	0.016	0.669
14	0.365	0.006	0.082	<0.010	<0.010	0.002	0.019	0.474

[a] Parts per million calculated on a fresh weight basis; 1 lb of active ingredient/acre applied. Per cent moisture ranged from 86.4 to 89.2 with a mean of 88.0 ± 0.8. [b] Ppm method sensitivity, 0.001. [c] Ppm method sensitivity, 0.002. [d] Ppm method sensitivity, 0.010.

Table IV. Levels[a] of Parathion and Related Compounds Applied to Field Spinach in an Emulsifiable Concentrate Formulation

Sample day	Parathion, ppm[c]	Amino-parathion, ppm[c]	Paraoxon, ppm[d]	*S*-Ethyl parathion, ppm[e]	Diethyl phosphate, ppm[c]	*p*-Nitrophenol, ppm[c]	Total residue, ppm
1	25.2	0.410	0.186	0.182	0.006	0.172	26.2
2	13.9	0.290	0.140	<0.010	0.004	0.088	14.4
3	6.92	0.147	0.153	<0.010	0.004	0.073	7.30
4	3.82	0.151	0.119	<0.010	0.004	0.076	4.17
5	2.13	0.089	0.108	<0.010	0.004	0.073	2.40
6	1.44	0.041	0.124	<0.010	0.007	0.072	1.68
7	1.21	0.038	0.101	<0.010	0.007	0.035	1.39
8	0.920	0.016	0.102	<0.010	0.004	0.040	1.08
9	0.768	0.009	0.104	<0.010	0.003	0.034	0.918
10	0.364	0.008	0.026	<0.010	0.008	0.034	0.440
11	0.412	0.002	0.022	<0.010	0.004	0.031	0.471
12	0.425	<0.001	0.032	<0.010	0.001	0.038	0.496
13	0.234	<0.001	0.015	<0.010	0.001	0.028	0.278
14	0.206	<0.001	0.021	<0.010	0.001	0.033	0.264

[a] Ppm calculated on a fresh weight basis; 0.5 lb of active ingredient/acre applied. Per cent moisture ranged from 86.5 to 89.2 with a mean of 88.0 ± 0.8. [b] *S*-Phenylparathion was nondetectable (<0.010 ppm) in all samples. [c] Ppm method sensitivity, 0.001. [d] Ppm method sensitivity, 0.002. [e] Ppm method sensitivity, 0.010.

RESULTS AND DISCUSSION

The chemical structures and names of the compounds investigated are presented in Table II. The tolerance level for parathion on spinach at harvest is presently 1 ppm. Application recommendations are not less than 14 days before harvest at the rate of 0.5 lb of active ingredient/acre.

Table III shows the levels of parathion and related compounds applied to field spinach at the rate of 1 lb of active ingredient/acre in the emulsifiable concentrate formulation 14 days prior to harvest and sampled daily through harvest. The data represent average values for the duplicate plots. Unsprayed spinach control samples taken prior to any spray treatments showed trace parts per million residues such as parathion, 0.034; aminoparathion, <0.001; paraoxon, 0.002; *S*-ethylparathion, <0.010; *S*-phenylparathion, <0.010; diethyl phosphate, 0.005; *p*-nitrophenol, 0.095; and total parathion residue, as the sum of the individual residues, 0.136. *O,O*-Diethyl phosphorothiolate was not detected in either the controls or sprayed spinach. As shown, parathion steadily dropped from approximately 56 ppm initially to <1.0 ppm by the tenth day and to 0.365 ppm at harvest. Aminoparathion, present in the Thiophos, dropped to <0.1 ppm by day 4. Paraoxon and *p*-nitrophenol decreased slowly from 0.252 and 0.453, respectively, to 0.082 and 0.019 at harvest. Both *S*-ethylparathion and *S*-phenylparathion declined to very low values in the early spinach samples. Diethyl phosphate levels were low initially and remained fairly constant throughout the experiment with 0.002 ppm at harvest. Table IV shows the dissipation of the parathion and related compounds levels on the spinach which had been treated with the recommended rate of 0.5 lb of active ingredient/acre and the data represent average values for the duplicate plots. Similar dissipation results were obtained as for the 1 lb of active ingredient/acre application and the total residues declined from approximately 26 ppm to 0.264 ppm at harvest.

The dissipation of parathion and related compounds from the spinach at the two treatment levels is compared in Tables V and VI (see paragraph at end of paper regarding supplementary material). The values were obtained from Tables III and IV by considering the initial sampling as 1.00 and relating it to each successive sampling. The values after the first samplings were generally less than 1.00 indicating that either there was no production of related products or that they were rapidly dissipated from the plant material and not detected during the sampling intervals.

The percentage of each compound as compared with the individual and total parts per million values obtained mathematically from Tables III and IV is available in Tables VII and VIII (supplementary material). Parathion dropped from 96.4 to 77.0% of the total residue at harvest on the 1-lb application and from 96.3 to 78.0% on the 0.5 lb application. The percentage of paraoxon, diethyl phos-

phate, and *p*-nitrophenol in the total residue increased from the initial sample at both rates of application to the harvest sample although the opposite occurred in the parts per million values obtained in the samplings. Aminoparathion, *S*-ethylparathion, and *S*-phenylparathion either did not increase in percentage of the harvest residues or were not detected as being present.

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The technical assistance of James D. Stokes and Clayton Reece in aspects of this work is gratefully acknowledged.

Supplementary Material Available. Tables V-VIII will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-74-974.

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Fumigant Residues of Carbon Tetrachloride, Ethylene Dichloride, and Ethylene Dibromide in Wheat, Flour, Bran, Middlings, and Bread

Ben Berck

Dowfume EB-5, consisting of carbon tetrachloride, ethylene dichloride, and ethylene dibromide (CT, EDC, and EDB) in 63:30:7 w/w proportions, was applied to 1000 bu (27.3 metric tons) of wheat stored in a paper laminate bin. The CT-EDC-EDB distribution-persistence patterns were monitored at 16 bin locations over a 14-day period by gc and hydrogen flame ionization detection. CT gas concentrations were greatest at the bottom, the descending order of magnitude being bottom, middle, top, headspace. EDB gas concentrations, and those of EDC to a lesser extent, were greatest in the headspace-top interface, the descending order of magnitude being the reverse of CT. Fumigant residues in wheat, in the flour, bran, and middlings derived from wheat, and in bread baked from the flour, were determined over a 7-week period of fumigant exposure by gc with EC detection. Amounts of unchanged CT and EDB as small as 0.01 ng could be detected. EDC residues could not be satisfactorily removed or determined. CT and EDB residues of the wheat varied, depending on the bin location and contact time, and ranged from 3.2 to 72.6 ppm of CT, and from 0.0 to 3.3 ppm of EDB. CT and EDB residues of bran and middlings were greater than those of flour, and ranged from 0.2 to 2.23 ppm of CT and 0 to 0.4 ppm of EDB. No EDB residues were found in any portion of the bread tested. CT residues in bread ranged from 0 to 0.04 ppm.

Fumigants are gaseous pesticides used to control infestations of insects, mites, rodents, and, to a lesser extent, bacteria, yeasts, and molds in stored foodstuffs. All commercial fumigants are physically or chemically sorbed, the amount depending on the nature and amount of fumigant used, gas-air concentrations, nature of the substrate, temperature, moisture content, period of exposure, etc. (Berck, 1966, 1971).

At least 14 factors (nature of the fumigant; applied dosage; method and conditions of application; nature of the substrate; dockage content (foreign matter); moisture content; absolute humidity; vapor pressure; temperature; diffusion and atmospheric pressure; interstitial atmospheric composition; chemical and physical sorption affinities of the fumigants; chromatographic properties of the cereal substrates; and air movement patterns affected by temperature gradients) influence the distribution and persistence (concentration-space-time) relationships of fumigant gases of volatile liquids applied to grain piles (Lindgren and Vincent, 1962; Berck, 1964, 1965c). The behavior of stored wheat as a chromatographic column toward fumigant gas mixtures applied to the surface of a wheat pile, with concomitant effects on migration patterns of the components, has been described (Berck, 1956, 1965a; Berck and Solomon, 1962). A range of analytical techniques was developed to show comparative sorption and chromatographic behavior of fumigants toward diverse food products, soils, and other substrates (Berck, 1960, 1961, 1962, 1965b, 1968a,b; Berck and Solomon, 1962; Berck *et al.*, 1970; Berck and Gunther, 1970). The scientific literature through April, 1970 on analytical methods for the determination of fumigants was reviewed by Malone (1971). A multidetection scheme for the gas chromatography of solvent extracts of fumigant residues in cereal and other foodstuffs was developed by Heuser and Scudamore (1969). Methods and data pertaining to determination of unchanged residues after application of carbon tetrachloride (CT), ethylene dichloride (EDC), and ethylene dibromide (EDB) in admixture were reported by Lynn and Vorhes (1957), Mapes and Shrader (1957), Conroy *et al.* (1957), Lindgren *et al.* (1968), Malone (1969, 1970), and McMahon (1971).

While data on gas concentrations and sorption affinities of different fumigants toward a wide range of products are available, more facts are needed on the nature and amount of unchanged residues that persist after applica-

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