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Parathion: Persistence on Cotton and Identification of Its Photoalteration Products

Ronald L. Joiner*[1] and Karl P. Baetcke[2]

Parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) was found to be at least 7× more persistent than previously reported on cotton. [^{14}C]Parathion was applied four times in 3 successive weeks to cotton in four environmental situations: environmental growth chamber; greenhouse; controlled-exposure field; and open field. After 28 days, 11.2 to 15.4% of the total radioactivity applied was recovered by methanol extraction and was found to be 58 to 68% unchanged [^{14}C]parathion. There was a constant increase in photoalteration products, coupled with a consistent decrease in [^{14}C]parathion with time. Photoalteration products of parathion present included *S*-ethyl parathion, *S*-phenyl parathion, paraoxon, and *p*-nitrophenol. No previously unreported metabolites were found on cotton foliage. Procedures for the extraction, purification, identification, and quantitation of ^{14}C-radioactivity on cotton are discussed.

Ethyl parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate) has been utilized effectively in the past in insect control. The effectiveness of ethyl parathion is due chiefly to characteristics which are typical of most organophosphorus chemicals: it is highly toxic to target organisms and easily applied as aerosols, emulsifiable concentrates, ultralow volume sprays, and formulated sprays. In addition, ethyl parathion is quick acting, inexpensive, possesses relatively low residual activity, and is a broad spectrum pesticide. An undesirable feature of ethyl parathion is that it is equally toxic to nontarget and target organisms. However, due to the continuing controversy that has developed concerning the widespread usage of persistent organochlorine insecticides, ethyl parathion has come to be regarded as a more desirable chemical for utilization in insect control.

A serious drawback to the replacement of chlorinated compounds with organophosphorus compounds is their high mammalian toxicity. As will be shown later, several incorrect assumptions have been made in the past, including that ethyl parathion can be applied to crops with little subsequent danger to man. Recent evidence, based on a number of poisoning incidents, indicates that some statements concerning the safety of parathion were incorrect.

Kalkat *et al.* (1961) showed that high temperature and high humidity decreased the field half-life of parathion, but increased its toxicity. Lichtenstein and Schultz (1964) reported that one-half of the paraoxon applied to a soil sample had disappeared 5.5 hr after soil treatment (as determined by a colorimetric method) and that parathion, under the same conditions, lost one-half of its residue in just over 6 days. Over this 6-day period, the amount of paraoxon steadily increased, indicating direct conversion of parathion to paraoxon. Coffin (1966) reported a 19-fold decrease in parathion residues in 4 days when sprayed on lettuce, and Hoelscher *et al.* (1968) demonstrated a 15-fold decrease in parathion residue on cabbage before the end of 4 days. El-Rafai and Hopkins (1966) found a half-life for parathion on glass and leaves of slightly more than 1 day, but when concentrations of parathion decreased, paraoxon and *S*-ethyl parathion (isoparathion) increased. Two other degradation products were unidentified.

Light has been implicated as a factor responsible for the breakdown of parathion. Cook and Pugh (1957) reported that exposure of parathion to light results in the formation of cholinesterase inhibitors chromatographically different from parathion. Frawley *et al.* (1958) treated parathion with ultraviolet light and found the resulting compounds to be a mixture of parathion, paraoxon, and other oxidation and degradation products. Studies by Gar and Kipiani (1956) and by Koivistoinen (1963) showed that ultraviolet light clearly accelerated the disappearance of parathion from plants.

Despite the information cited above, which suggests that parathion is short-lived, a number of unexplainable poisonings have occurred in recent years. Quinby and Lemmon (1958) summarized 11 episodes of poisoning from contact with parathion residues involving a total of more than 70 workers who were employed in harvesting, thinning, cultivating, and irrigating such crops as apples, grapes, citrus, and hops. Six of the outbreaks occurred within 2 days of parathion application; however, in five episodes, the poisonings occurred from 8 to 33 days after application. More recently, Milby *et al.* (1964) reported parathion poisonings from 16 separate orchards in California in which there was a mean of 23 days between the last application and the poisonings. These studies, as well as similar reports by West (1964), Holmes (1964), and Durham (1964), when viewed in conjunction with reports already presented on the longevity of parathion residues, indicate that some product other than parathion could be responsible for the poisonings or that the reported longevity of parathion is in error.

Department of Biochemistry, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi 39762.
[1] Present address: Department of Entomology, Texas A&M University, College Station, Texas 77843.
[2] Present address: National Center for Toxicological Research, Jefferson, Arkansas 72079.

MATERIALS

Solvents. All solvents used in this study were redistilled before use. One gallon of each solvent was concentrated to 1 ml and chromatographed by gas chromatography (glc) to determine if interfering impurities were present. Redistillation and rechromatography were continued until all major interferences were removed.

Standards. [^{14}C]Parathion (2,6-ring-labeled) was purchased from Volk Radiochemical Company. Radiochemical purity was confirmed by thin-layer chromatography (tlc) in 9:1 hexane–acetone (v/v) and scanning by a radioscanner to locate areas of activity.

Commercially available photoalteration products of parathion were obtained and purity was confirmed by tlc in an 80:15:5 petroleum ether–diethyl ether–glacial acetic acid (v/v/v) solvent system. Paraoxon (diethyl *p*-nitrophenyl phosphate), DEPP (diethyl phenyl phosphate), DEPT (*O,O*-diethyl *O*-phenyl phosphorothioate), EBNPT [*O*-ethyl *O,O*-bis(*p*-nitrophenyl) phosphorothioate], EBNPP [ethyl bis(*p*-nitrophenyl)phosphate], SEP (*O,S*-diethyl *O*-*p*-nitrophenyl phosphate), SPP (*O,O*-diethyl *S*-*p*-nitrophenyl phosphate), and PNP (*p*-nitrophenol) were all found to be of 93+% purity. PAP (*p*-aminophenol), DEP (diethyl phosphate), and MEP (monoethyl phosphate) were found to contain impurities but not of sufficient quantity to warrant purification. PA (phosphoric acid) was certified to be 86+% by elemental analysis.

Experimental Plots. Four environmental situations were utilized in the study.

Environmental Growth Chamber (EC). Several acid-delinted cottonseeds (*Gossypium hirsutum*, Stoneville 213 variety) were potted in Mississippi Sharkey Clay (buckshot soil) in plastic pots. At the age of 2 weeks, the plants were thinned to one plant per pot and placed in a Percival Walk-In environmental chamber to become accustomed to the constant temperature (75 ± 2°F), relative humidity (55–60%), and 14-hr photoperiod. Each plant received adequate fertilizer and distilled water to maintain optimum growth.

Open Field (OF). Acid-delinted cottonseeds (Stoneville 7A variety) were planted in 120-ft rows of Mississippi Sharkey Clay. Plantings on the 40-in. rows were hill-dropped on 20-in. centers at the rate of 20 lb of seed per acre.

Greenhouse (GH). This was the same as for environmental growth chamber except for temperature (average: high, 104°F, low, 74°F), relative humidity (average: 75%), and photoperiod (average: 13.5 hr).

Controlled Environment Field (CF). Greenhouse seedlings were transferred to the field plot after 3 weeks. Motorized coverings were opened daily at 6:00 a.m. and closed daily at 7:00 p.m. Precipitation caused the coverings to close automatically.

EXPERIMENTAL PROCEDURE

Application of Radioactivity. [^{14}C]Parathion was diluted to approximately 0.5 μCi/100 μl by the addition of absolute methanol. Three 100-μl samples were obtained for liquid scintillation spectroscopy to determine the exact concentration (15 ml of 500:458:42 Triton X-100, toluene, and Nuclear Chicago Spectrafluor). Scintillation counting was allowed to progress for a period of time that would give a standard error of less than ±5%. Appropriate corrections for background counts were determined by counting blanks with each sample series. Efficiency was determined by a channel-ratio method based on an accurately calibrated [^{14}C]benzoic acid standard.

The [^{14}C]parathion was applied to the upper surface of three leaves from each plant with a 100-μl pipette equipped with a microliter pipette control. Each dosed leaf was labeled with radioactive tape to distinguish it from other leaves on the plant. The closely surrounding nontreated leaves were clipped to prevent accidental contact and removal of the [^{14}C]parathion.

All plants were initially dosed on day 1 of a predetermined schedule. Samples were taken on days 8, 15, 22, and 29. Subsequent applications were made to the plants remaining on days 8, 15, and 22.

One-hundred-microliter samples of the [^{14}C]parathion were taken after dosing the first plant, during the middle of the dosing, and after dosing the last plant on each application day. These samples were counted in a liquid scintillation spectrometer and their average was used as the amount of radioactivity applied per leaf for that particular application day (Table I).

Collection and Storage of Samples. Each treated leaf was collected individually, placed in a plastic bag, and transferred to an ice chest for transport to the laboratory. Leaves from each experiment were grouped into large plastic bags and stored at −20° for future analyses.

Extraction of Samples. Samples were divided initially into two fractions according to the extraction procedure

Table I. [^{14}C]Parathion Applied per Week per Leaf of Cotton[a]

		[^{14}C]Parathion applied per week (DPM)	Total [^{14}C]parathion applied (DPM)	Total μCi [^{14}C]parathion applied
Growth chamber (EC)	1st week	970,622	970,622	0.4412
	2nd week	982,753	1,953,375	0.8880
	3rd week	994,884	2,948,259	1.3401
Open field (OF)	1st week	749,073	749,073	0.3405
	2nd week	817,725	1,566,798	0.7122
	3rd week	886,377	2,453,175	1.1151
Greenhouse (GH)	1st week	1,058,926	1,058,926	0.4813
	2nd week	1,119,963	2,178,889	0.9904
	3rd week	1,144,911	3,323,800	1.5108
	4th week	1,157,706	4,481,506	2.0370
Controlled field (CF)	1st week	899,738	899,738	0.4090
	2nd week	1,398,248	2,297,986	1.0445
	3rd week	1,452,814	3,750,800	1.7049
	4th week	1,659,057	5,409,857	2.4590

[a] Average of three 100-μl samples collected during application of [^{14}C]parathion to cotton leaves.

used. One leaf was homogenized in a Virtis 23 homogenizer with 50 ml of absolute methanol for 15 min. The blender was washed three times with absolute methanol and the total volume was filtered through glass wool to remove particulate matter. The funnel was rinsed three times with absolute methanol to ensure total recovery and a 1-ml sample was obtained for liquid scintillation counting. This procedure was used to determine the total amount of radioactivity remaining in each sample after the appropriate time period and number of applications.

A second leaf from each sample was homogenized with 50 ml of 50% methylene chloride–Skelly F petroleum ether (1:1). The blender was washed three times with the solvent and the total volume was filtered through glass wool. The funnel was washed three times, and the remaining debris was rehomogenized with absolute methanol. The blender was washed in a like manner with absolute methanol and its contents were filtered through glass wool. One-milliliter samples were taken from each solvent for liquid scintillation counting, and the remaining 50% methylene chloride fraction was stored for column chromatographic separations. The methanol rinse was conducted to ensure that total recovery of the ^{14}C-radioactivity was obtained from the 50% methylene chloride fraction.

Sample Purification. Samples homogenized in 50% methylene chloride were purified and separated into component fractions by Florisil column chromatography. Glass wool filters were placed in the bottom of 20 mm (i.d.) by 300 mm chromatographic columns equipped with a coarse fritted glass disk and a Teflon stopcock. Fifteen grams of 5% hydrated Florisil PR was wetpacked in 20% methylene chloride (in Skelly F petroleum ether, 1:4). Each column was topped with 5 g of anhydrous sodium sulfate and then capped with a glass wool plug. The columns were washed with 200 ml of 20% methylene chloride (in petroleum ether, 1:4) followed by 200 ml of 40% methylene chloride (in petroleum ether, 2:3) and 250 ml of 50% methylene chloride (in petroleum ether, 1:1).

Each sample was allowed to adsorb slowly onto the column, and the column was then washed with 100–150 ml of 50% methylene chloride. As the 50% methylene chloride fraction reached the top of the column packing, 200 ml of acetone was added to the column. The acetone was followed in like manner by 200 ml of methanol and 200 ml of 1% HCl (in methanol, 1:99). All 200-ml fractions were collected in ground-glass flasks, stoppered, and refrigerated for further analyses. One-milliliter aliquots were obtained from each fraction for liquid scintillation counting.

Thin-Layer Chromatography. Samples recovered from the Florisil column were pooled according to eluent and type of sample. Composite samples were then evaporated to a volume of 1 ml in a Kuderna-Danish concentrator.

The concentrated samples were divided into two groups: 0.9 ml was retained for tlc and 0.1 ml was removed for glc. The tlc samples were spotted on Brinkmann pre-prepared silica gel F_{254} tlc plates, along with authentic standards in order to identify the components of each column fraction as described previously (Joiner, 1971).

In samples in which pigmentation obscured direct visual comparisons of sample components with standards, the components were identified by scanning the tlc plates with a Varian Aerograph tlc scanner and plotting coordinates of the radioactive spots. The coordinates were then located on the tlc plate, the R_f value was measured, and identification was made by comparison to tabular R_f values and the standards present on the tlc plate (Table II).

Quantitation of the ^{14}C-radioactivity present in the component spots of each sample was accomplished by a procedure similar to that for quantitative determination of the obscured spots. Each tlc plate was scanned at 1.0-cm intervals and the radioactive spots were determined and identified as above. Quantitation was accomplished by calibrating the tlc scanner with a ^{14}C-radioactive standard calibration plate. By comparing the sample peak height to the standard peak height, the amount of ^{14}C-radioactivity present in each spot could be determined. This comparison had several drawbacks, the most serious being that this procedure quantitated only the surface radioactivity escaping the tlc plate. It did not possess the 4π geometry needed for accurate quantitation of all radioactivity present in a three-dimensional system nor the degree of accuracy afforded by liquid scintillation spectrometry. Despite these limitations, tlc scanning was very useful in "semi-quantitating" and locating the radioactive spots.

RESULTS AND DISCUSSION

Extraction. Methanol was chosen for determining the total concentration of ^{14}C-radioactivity remaining on each leaf sample after the appropriate treatment period due to its ease of handling and the high percentage recovery. Data from preliminary experiments of parathion and photoalteration products with this solvent indicated that methanol extraction recovered 99.7% of the ^{14}C-radioactivity present on cotton leaves. Samples were also dried and processed for the oxygen combustion technique for determining total ^{14}C-radioactivity as a $^{14}CO_2$ combustion product (Davidson and Oliverio, 1967). Experimental data from these tests indicated that the methanol extraction procedure was more efficient (99.7 *vs.* 93.5%), less time-consuming (25 *vs.* 50 min), easier to conduct, and less dangerous than the oxygen combustion technique (Joiner and Baetcke, 1972). Total radioactivity was determined by removing 0.1 and 1.0-ml samples for liquid scintillation counting.

The averaged percent recoveries of ^{14}C-radioactivity from each application-treatment extraction procedure and the percent recoveries of each procedure to the total [^{14}C]parathion applied are presented in Table III. In all cases there was a significant amount of radioactivity found on leaves after each treatment, as indicated by the methanol extraction procedure for determining total radioactivity. However, Table III also shows there was a decrease or, at most, only a slight increase in the ratio of the radioactivity remaining on each leaf to the total [^{14}C]parathion applied per leaf.

Table II. R_f Values of Photoalteration Products of Parathion for Four Thin-Layer Chromatography Systems

	R_f values[b]			
Component[a]	System I[c]	System II[d]	System III[e]	System IV[f]
DEPT	0.888	0.975	0.872	0.799
Parathion	0.708	0.900	0.897	0.738
SPP	0.584	0.550	0.918	0.640
EBNPT	0.462	0.879	0.873	0.616
DEPP	0.332	0.624	0.890	0.603
PNP	0.316	0.735	0.677	0.174
SEP	0.298	0.661	0.904	0.545
Paraoxon	0.216	0.518	0.894	0.715
EBNPP	0.142	0.503	0.875	0.414
PAP	0.000	0.009	0.535	0.045
DEP	0.000	0.000	0.020	0.027
MEP	0.000	0.000	0.007	0.015
PA	0.000	0.000	0.000	0.001

[a] See text for abbreviations used (Materials: Standards). [b] Average of 15 runs for each component in each system. [c] System I: petroleum ether–diethyl ether–glacial acetic acid, 80:15:5 (v/v/v). [d] System II: petroleum ether–diethyl ether–glacial acetic acid, 50:45:5 (v/v/v). [e] System III: chloroform–methanol–10% ammonium hydroxide, 75:25:3.5 (v/v/v). [f] System IV: hexane–chloroform–methanol, 70:20:10 (v/v/v).

Table III. ^{14}C-Radioactivity Recovered from Total [^{14}C]Parathion Applied by Extraction and Comparisons of the Extraction Procedures

	Recovery of total ^{14}C applied, %						
	Extraction procedure				Comparative recovery, %		
	Leaf 1	Leaf 2					
Treatment[a]	Methanol	Methylene chloride	Methanol re-extraction	Total[b] recovery	Methylene chloride–methanol	Methanol re-extraction–methanol	Total[b]–methanol
EC1W	7.49	7.05	NT[c]	7.05	94.11	NT	94.11
EC2W	5.85	5.65	NT	5.65	96.60	NT	96.60
EC3W	5.25	4.46	NT	4.46	84.63	NT	84.63
Average					91.78		91.78
OF1W	20.54	8.51	12.02	20.53	41.42	58.50	99.92
OF2W	15.94	5.60	8.94	14.54	35.12	56.08	91.20
OF3W	13.58	4.79	6.64	11.43	35.26	48.89	84.15
Average					37.27	54.59	91.76
GH1W	10.06	6.88	2.81	9.69	68.40	27.96	96.36
GH2W	10.63	6.69	3.36	10.05	62.89	31.63	94.52
GH3W	10.74	6.24	2.74	8.98	58.09	25.52	93.61
GH4W	11.24	5.84	4.37	10.21	51.93	38.90	90.83
Average					60.33	31.00	91.33
CF1W	10.82	3.17	7.14	10.31	29.34	65.97	95.31
CF2W	14.36	3.64	11.24	14.88	25.35	78.29	103.64
CF3W	15.56	3.80	9.92	13.72	24.44	63.78	88.22
CF4W	15.42	2.61	10.77	13.38	16.94	69.80	86.74
Average					24.02	69.46	93.48

[a] See text for abbreviations used (Materials: Experimental Plots). [b] Total radioactivity recovered from leaf 2 by extraction (sum of the two extraction procedures). [c] NT = not taken.

Treatment-application means of the total radioactivity recovered (methanol extraction) from each application within an experimental design were analyzed by the "Studentized-t" test of mean comparison (Snedecor, 1962; Steel and Torrie, 1960). Results of these statistical analyses are presented in Table IV. Highly significant differences were obtained between the mean levels of total ^{14}C-radioactivity recovered from each treatment-application within each experimental design (with the exception that only a significant difference existed between the environmental growth chamber 2W and 3W treatment-applications). These data indicate a significant buildup of ^{14}C-radioactivity with each new application of [^{14}C]parathion and a significant quantity of ^{14}C-radioactivity being retained on each leaf from previous applications.

Table IV. Treatment-Application Means of the Total Radioactivity Recovered from Each Application within an Experimental Design Using the Studentized-t Test of Mean Comparison

Treatment[a]	Sample size (N)	Mean ($\bar{X}$)	Standard error ($s_{\bar{x}}$)	t-test (t)
EC1W	10	73652	±5906	
EC2W	15	114979	±4656	−5.51[c]
EC3W	12	154656	±15321	−2.73[b]
OF1W	20	153902	±8625	
OF2W	20	249684	±13262	−6.05[c]
OF3W	17	333158	±10293	−4.84[c]
GH1W	15	106526	±5674	
GH2W	15	231961	±15143	−7.76[c]
GH3W	15	357079	±15601	−5.75[c]
GH4W	15	503745	±17907	−6.18[c]
CF1W	20	97346	±8223	
CF2W	10	329918	±15263	−15.07[c]
CF3W	15	583464	±27192	−7.09[c]
CF4W	15	834421	±36853	−5.48[c]

[a] See text for abbreviations used (Materials: Experimental Plots). [b] Significant differences between any two means at the 95% level of probability ($t \geq 0.05$). [c] Highly significant differences between any two means at the 99% level of probability ($t \geq 0.01$).

Table III shows that the recovery of radioactivity from cotton leaves by the 50% methylene chloride extraction procedure was considerably greater from leaf samples housed in artificial chambers (environmental growth chamber and greenhouse) than those from the field (controlled and open field). In addition, the average recovery from environmental growth chamber samples (91.78%) was greater than that from greenhouse samples (60.33%), while open field sample recovery (37.27%) exceeded that of controlled field samples (24.02%). However, there were no significant differences between the average recovery of each treatment obtained by considering the 50% methylene chloride extraction and the methanol re-extraction together as representative of the total radioactivity recovered by extraction from these leaves (environmental growth chamber samples, 91.78%; greenhouse samples, 91.33%; controlled field samples, 93.48%; and open field samples, 91.76%).

In the case of the 50% methylene chloride-extracted material, there was an increase in the quantity of ^{14}C-radioactivity extracted in every treatment except the last application of the controlled field experiment (Table V, CF4W). However, when expressed as a percent of the total applied, methylene chloride extraction led to a recovery of generally decreasing amounts of radioactivity within treatments (Table III, column 3).

Table V also presents data obtained from samples homogenized in 50% methylene chloride following Florisil column chromatography. Recovery of ^{14}C-radioactivity from the Florisil column was 97.31, 94.31, 98.02, and

Table V. Average ^{14}C-Radioactivity of 5% Hydrate Florisil PR Column Eluates from Treatment-Applications Extracted with 50% Methylene Chloride in Skelly F Petroleum Ether[a]

Treatment[b]	50% methylene chloride[c] extraction (DPM)	Column eluents: 50% methylene chloride,[c] %	Acetone, %	Methanol, %	1% HCl,[d] %
EC1W	69,315	50.73	38.70	9.60	0.97
EC2W	111,065	68.49	14.97	15.97	0.57
EC3W	130,881	69.60	15.62	13.55	1.22
OF1W	63,749	60.81	21.15	18.04	0.0
OF2W	87,690	39.99	29.16	22.29	8.77
OF3W	117,465	36.52	25.28	29.17	1.22
GH1W	72,860	43.84	40.41	23.32	2.11
GH2W	145,709	38.61	29.85	28.08	4.34
GH3W	207,415	36.20	32.34	24.22	0.0
GH4W	261,591	49.21	23.44	26.03	1.23
CF1W	28,560	60.16	29.02	29.78	3.79
CF2W	83,645	22.99	39.37	24.47	8.79
CF3W	142,653	22.24	30.64	35.91	6.43
CF4W	141,381	15.57	35.11	42.53	7.15

[a] Average of 10-20 samples per mean. [b] See text for abbreviations used (Materials: Standards). [c] In Skelly F petroleum ether, 1:1 (v/v). [d] In methanol, 1:99 (v/v).

96.29% of that applied to the column for the environmental growth chamber, open field, greenhouse, and controlled field samples, respectively.

The 50% methylene chloride extract was brilliant green in color due to the extraction of plant pigments with the labelled compounds. Cleanup of the 50% methylene chloride extract by Florisil column chromatography accomplished a twofold purpose: it removed plant pigments from the 50% methylene chloride eluent, and allowed subsequent tlc analysis to be made without interference in visual identification. Further, it separated the ^{14}C-radioactivity into three fractions, each containing different components. Of 214 samples, only 11 of the 50% methylene chloride eluates contained plant pigments. These pigmented fractions may have been due to small quantities of acetic acid in one shipment of Skelly F petroleum ether, which was not detected until after several samples had been processed. The pigments were adsorbed onto the Florisil column and did not accompany the 50% methylene chloride eluate in 203 of the 214 samples. In seven of the samples, traces of a yellow pigment passed through the column, while in the remaining four samples heavy pigmentation passed through the column in the 50% methylene chloride eluent. In all samples, intense pigmentation accompanied the acetone fraction and only slight pigmentation was eluted by the methanol fraction. The 1% HCl fraction removed the remaining pigments from the column with ranges in color from faint yellow to light green to light pink. The total ^{14}C-radioactivity recovered from the Florisil cleanup column averaged 96.44 ± 1.69% of that originally administered to the column in the form of the 50% methylene chloride extract.

The eluates for each solvent from each treatment-application were pooled and reduced in volume by a Kuderna-Danish (KD) concentrator to less than 1.0 ml. Samples were reconstituted to 1.0 ml by adding the appropriate solvent and stored at −20° for analyses.

Qualitative and Quantitative Analyses. The components of each treatment-application, as determined by tlc and quantitated by liquid scintillation counting of an aliquot from the Florisil column eluants, are presented in Table VI. These data indicate that approximately 70–75% of the total radioactivity extracted with methylene chloride after the appropriate treatment-applications and elapsed time periods was parathion. Paraoxon was present in each instance and constituted approximately 23.7% of the total radioactivity in the extract. There was a slight decrease in the parathion concentration with time and repeated application, and an almost constant amount of paraoxon was present regardless of time and repeated applications. PNP was present in each treatment-application sample, increasing with time and number of applications in both artificial systems and remaining fairly constant in both field systems.

SEP appeared in later samples of each treatment at a fairly small concentration and increased slightly with time

Table VI. Identification and Quantitation of the Eluant Components of the 50% Methylene Chloride in Skelly F Petroleum Ether Extract Following Florisil Column Chromatography[a]

Treatment[b]	Components, %[c]: Parathion	Paraoxon	PNP	SEP	SPP
EC1W	79.3	18.1	2.6		
EC2W	76.1	19.3	3.9	0.7	
EC3W	70.9	21.6	6.2	1.3	
OF1W	71.4	24.1	4.7		
OF2W	69.9	23.1	6.0	1.0	
OF3W	68.1	23.9	5.9	2.1	
GH1W	81.7	17.5	0.8		
GH2W	74.8	22.7	2.5		
GH3W	71.2	24.0	3.9	0.9	
GH4W	67.7	23.9	6.2	1.7	0.5
CF1W	67.1	27.7	5.2		
CF2W	65.6	26.9	3.8	3.7	
CF3W	61.0	27.9	6.3	4.1	0.7
CF4W	57.8	30.3	5.3	5.3	1.3

[a] Average of three replications. [b] See text for abbreviations used (Materials: Experimental Plots). [c] Percentages based on the radioactivity of each component to the total radioactivity present in each sample, as determined after tlc separation and scintillation counting.

and repeated applications. SPP was present only in the greenhouse and controlled field experiments, appearing after the third and fourth applications.

EBNPT, EBNPP, DEPT, and DEPP were not detected in any of the treatments. There were two unidentified spots present on the tlc plates developed in the petroleum ether–diethyl ether–glacial acetic acid (80:15:5, v/v/v) system. Neither spot was radioactive and therefore did not contain a ^{14}C-labeled ring system derived from parathion.

One of the major objectives of this investigation was to develop methodology for the extraction and identification of parathion and its photoalteration products from plant material. Results were not completely satisfactory in this endeavor, but a number of improvements were made in previously reported techniques for the analysis of parathion and its metabolites. For example, the extraction of plant samples with methanol to obtain total ^{14}C-radioactivity afforded a more efficient and safer method of quantitation than the oxygen combustion technique. Methanol extraction is less expensive and easier to conduct than oxygen combustion, samples need not be dried or processed in any way before extraction, and the ^{14}C moiety is not destroyed in the procedure but can be utilized for further experimentation.

Large quantities of pigments were eluted from the Florisil column by the acetone and methanol fractions. These fractions presented a problem in identification and therefore accurate quantitation. In part, this problem was solved by using the tlc radioscanner and basing the identification of each component on the coordinates obtained. In several instances radioactivity appeared continuously from the origin to near the solvent front. Koivistoinen and Meriläinen (1963) reported a similar phenomenon in their attempts to identify the photoalteration products of parathion. They proposed as a possible explanation, saying that many rearrangement products were present which formed a continuous streak of ^{14}C-radioactivity.

A problem encountered in the determination of parathion and its metabolites present in plant material was the inability to develop an extraction mixture which would effectively remove all ^{14}C material in a form suitable for further cleanup. Although methanol extraction recovered essentially all ^{14}C compounds, no method was found to remove pigments from such preparations, which would have permitted further analyses for metabolites of parathion. However, analyses of the methylene chloride extracted material alone provided evidence that parathion is more persistent than previously stated. Based on El-Rafai and Hopkins' (1966) reported half-life of 26 hr for parathion in the field, one would expect to find approximately 0.85% of the parathion applied remaining after 1 week. In the current study, the total ^{14}C-radioactivity present in the methylene chloride extract of leaves from open field plants was found to consist of 71% parathion 1 week after application (Table VI). Since the methylene chloride extract obtained from the 1-week samples contained 41% of a total of 21% ^{14}C activity present (Table III), then the least amount of parathion present was 6% of the amount originally applied (41% × 21% × 71% = 6%). This represents a residue of approximately 7× that reported by El-Rafai and Hopkins.

No evidence was obtained suggestive of the formation of previously unreported toxic metabolites of parathion on plant material. In growth chamber material, essentially all radioactivity was recovered in methylene chloride, and this fraction was found to contain predominantly parathion and paraoxon. Recovery of radioactivity from plants grown under other conditions was much less satisfactory, and it is possible that additional metabolites were present which were not extracted. However, since methylene chloride apparently successfully extracted compounds quite different in polarity (parathion, paraoxon, and PNP), an *a priori* assumption can be made that perhaps factors such as cuticle thickness prevented extraction of all radioactivity and that additional metabolites were in fact not present.

The experimental designs utilized in this study were selected to conform with current agricultural practices; therefore comparisons of treatment-application means within designs are valid, but those between designs are not, since environmental conditions varied between treatments. All successive treatment-applications of total radioactivity within a design are significantly different from the previous mean. Also, there is an observable increase in the ^{14}C-radioactivity extracted by the 50% methylene chloride with successive treatment-applications within an experimental design. These data indicate an accumulation of total ^{14}C-radioactivity (and subsequently total [^{14}C]parathion) with each application.

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