case. Systox which is a mixture of two isomers, was a crude product and contained a large portion of relatively volatile components that were eluted with the solvent peak. The other compounds were 95% pure or better. There was some evidence that the column required conditioning with a given pesticide before good recoveries could be obtained. This was especially true for parathion, *p,p'*-DDT, and lindane.

Because of this problem, recoveries may be poorer at the microgram level available in actual residue samples than at the milligram level used in the present study. On the other hand, the conditions for elution and elution times can probably be accurately predicted at the microgram level from data taken for milligram quantities. Work is going forward, to be reported later, on the development of sensitive and specific detectors necessary for the detection of microgram quantities of the various pesticides. The present thermal conductivity detectors have a sensitivity limit of approximately 200 γ.

Studies on the improvement of gas chromatographic columns and other conditions are being continued. As soon as sufficient background information on the gas chromatography and measurement of microquantities of pesticides is developed, the practical application of this method as a systematic approach to residue analysis in foods will be undertaken.

Projected Applications

Using the thermal conductivity type of detector, which is relatively insensitive, direct analysis of pesticide preparations and formulations may be made. With residues of known pesticides gas chromatography will undoubtedly find wide usage as a combined cleanup and separation method, to be followed by conventional measurement techniques. Its major advantage for this purpose is its speed. In many cases, such a procedure could be completed within minutes. Finally, as suitably sensitive, specific direct detection methods are developed, complete residue analyses may be performed by gas chromatography. Where spray histories of the food are known, only those components known to be present need be looked for. Materials with unknown spray histories, but with the possibility of only certain residues being present, such as those included in this study, can be identified by the gas chromatographic elution time, and the amount present by the magnitude of the detector signal.

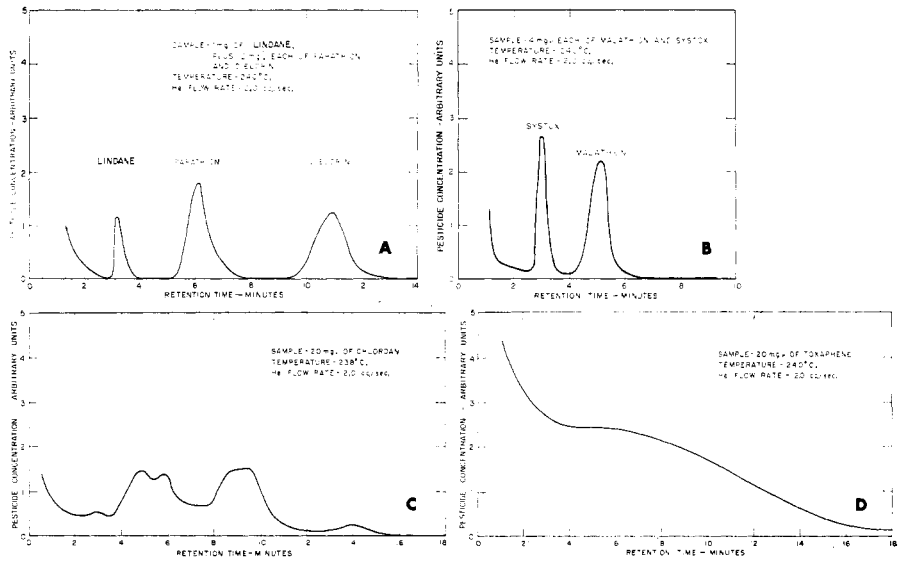

Figure 2. Gas chromatograms on mixed and unmixed pesticides

A. Mixture of lindane, parathion, and dieldrin. B. Mixture of malathion and Systox. C. Chlordan. D. Toxaphene

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ORGANOPHOSPHORUS INSECTICIDES

Some New Organophosphorus Compounds with Insecticidal Properties

DAVID G. COE,[1] HENRY HURTIG,[2] B. JOSEPH PERRY, and EDWARD S. SHERLOCK

Suffield Experimental Station, Canadian Defence Research Board, Ralston, Alberta, Canada

SINCE THE DISCOVERY of bis-*N*-dimethyl phosphorodiamidic fluoride and octamethylpyrophosphoramide by Schrader, numerous compounds have been prepared and tested as systemic insecticides. The majority of systemic phosphorus compounds are not inherently powerful anticholinesterases, but are metabolized to active compounds either in the plant or after ingestion by the insect. Some general insecticides also act through a labile metabolite, but many of them are active inhibitors *per se*. In the case of octamethylpyrophosphoramide (schradan), which has been extensively investigated by numerous workers, the insecticide can be metabolized in vivo or in vitro by liver preparations of certain mammals or converted by chemical oxidation with potassium permanganate to an extremely active anticholinesterase (*4, 9, 13, 24*). This metabolite was regarded as being either an amine oxide or an *N*-methylol compound, both of which would be very labile and readily decomposed (*4, 19, 24*). Spencer *et al.* (*21, 23*) have recently demonstrated that it is not the *N*-oxide and have advanced fresh evidence for the

[1] Present address, Jackson Laboratory, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

[2] Present address, Division of Entomology, Science Service, Canada Department of Agriculture, Ottawa, Ont.

The insecticidal properties of a new series of organophosphorus compounds are described. These substances, which are alkyl *N*-dialkyl alkylphosphonamidates, are readily prepared and have a very low mammalian toxicity. A number of them exhibit a systemic toxicity to aphids comparable to that of octamethylpyrophosphoramide. In addition, it has been shown that some dialkyl alkylphosphonates have short term systemic properties and will also act as cholinesterase inhibitors. Two bis-*N*-dialkyl methylphosphonic diamides were also tested and found to be phytotoxic.

N-methylol hypothesis. In the equally well investigated cases of the systemic *O,O*-dialkyl-*S*-alkylthioalkyl phosphorothioates and dithioates–e.g., demeton and Thimet (I) (*5*, *14*, *16*)–it has been established (*2*, *11*, *12*, *17*) that the sulfinyl (II) and sulfonyl (III) derivatives are the active metabolites and are formed by an oxidation mechanism as is the metabolite from schradan:

$(C_2H_5O)_2P(S)SCH_2SC_2H_5 \rightarrow$

I

$(C_2H_5O)_2P(S)SCH_2SC_2H_5 \rightarrow$ (O)

II

$(C_2H_5O)_2P(S)SCH_2\overset{O}{\overset{\uparrow}{S}}\overset{O}{\downarrow}C_2H_5$

III

The compounds described in this paper fall into three classes which can be logically related to one another:

$CH_3P(O)(NR'_2)_2$ $CH_3P(O)(NR'_2)(OR'')$ $CH_3P(O)(OR'')_2$

IV V VI

The parent compound may be regarded as bis-*N*-dialkyl methylphosphonic diamide (IV), which on replacement of its dialkylamido groups leads successively to alkyl *N*-dialkyl methylphosphonamidates (V), a class of compounds not previously described, and then to dialkyl methylphosphonates (VI). After completion of this work some compounds of this type were described by Razumov *et al.* (*20*), including Compound No. 8 ($R' = R'' = C_2H_5$). In a number of cases the dialkylamido group is heterocyclic when a piperidido- or morpholido- radical is introduced.

Methods and Materials

Compounds of Types IV and VI were obtained by standard procedures as outlined schematically below. The alkyl *N*-dialkyl methylphosphonamidates (V) (*7*), were obtained from the corresponding dialkyl methylphosphonate (VI) by "phosgenation" to give the methylphosphonochloridate (*6*), which was treated with dialkylamine to yield the desired compound [details of the preparation and physical properties of these compounds will be published (*7*)].

Table I. General Toxicity to Adult Grain Weevils (*Sitophilus granarius*)

Candidate Compound				% Mortality of Concn. Stated, % W./W.		
Type	No.	-NR_2	R'	0.32	0.16	0.01
IV	1	-NMe_2		68	..	0
	2	-NEt_2		1	3	..
V	3	-NMe_2	Et-	100	..	16
	4	-NMe_2	*n*-Pr-	100	100	94
	5	-NMe_2	Iso-Pr-	100	85	0
	6	-NMe_2	*n*-Bu-	100	100	22
	7	-NMe_2	*n*-Am-	100	100	57
	8	-NEt_2	Et-	4	3	
	9	-NC_5H_{10}	$(EtO)_2$-	100	100	50
	10	-NC_5H_{10}	Iso-Pr-	100	90	17
	11	-NC_4H_8O	Iso-Pr-	100	97	17
VI	12		Et-	86	26	10
	13		*n*-Pr-	74	34	26
	14		Iso-Pr-	53	26	2
	15		*n*-Bu-	100	98	26
	16		*n*-Am-	100	97	53
Schradan				36	18	5
				98	96	90
Acetone control				0–9		

Table II. Residual Fumigant Toxicity to Green Peach Aphids (*Myzus persicae*)

Candidate Compound				Mortality, %	Concn., Vol. %		
Type	No.	-NR_2	R'-	0.5	0.1	0.05	0.01
IV	1	-NMe_2		0	0	0	..
	2	-NEt_2		..	4	7	
V	3	-NMe_2	Et-	10–15	1–30	..	..
	4	-NMe_2	*n*-Pr-	..	1–30	..	..
	5	-NMe_2	Iso-Pr-	..	1–30	..	0
	6	-NMe_2	*n*-Bu-	..	60–70	..	..
	7	-NMe_2	*n*-Am-	..	100	..	70–80
	8	-NEt_2	Et-	..	3	..	..
	9	-NC_5H_{10}	$(EtO)_2$-	..	0	..	..
	10	-NC_5H_{10}	Iso-Pr-	..	80–90	..	80–90
	11	-NC_4H_8O	Iso-Pr-	..	10–15	..	10–15
VI	12		Et-	5–10	..	..	..
	13		*n*-Pr-	..	0	..	..
	14		Iso-Pr-	..	1–30	..	0
	15		*n*-Bu-	..	30–60	..	0
	16		*n*-Am-	..	50–60	..	10–15
Schradan				1–30	0	0	0
Parathion				100	..	100	..
Controls, H_2O				0			

$CH_3P(O)Cl_2 + 4R'_2NH \rightarrow CH_3P(O)(NR'_2)_2 + 2R'_2NH\cdot HCl$

IV

$CH_3P(O)Cl_2 + 2R''OH + 2(C_2H_5)_3N \rightarrow CH_3P(O)(OR'')_2 + 2(C_2H_5)_3N\cdot HCl$

VI

$VI + COCl_2 \rightarrow CH_3P(O)(OR'')Cl + R''Cl + CO_2$

$CH_3P(O)(OR'')Cl + 2R'_2NH \rightarrow CH_3P(O)(OR'')(NR'_2) + R'_2NH\cdot HCl$

V

Data on one compound of a somewhat similar nature have been included: diethyl phosphoropiperidate (No. 9), which was prepared from diethyl phosphorochloridate and piperidine.

Before testing, all the compounds were purified by fractional distillation. The purity of known compounds was checked from the refractive index.

All of the compounds prepared were first screened for mammalian toxicity at a level of 250 mg. per kg. (intraperitoneal injection of female mice). Only one compound, isopropyl methylphos-

phonomorpholidate (No. 11), proved to be toxic at this level (LD_{50} 60 mg. per kg.). Because none of the other compounds showed any marked toxic effects at this level, they were regarded as virtually nontoxic.

Screening for Insecticidal Properties

In this investigation the methods employed have not been designed to provide precise bioassay or dosage-mortality data. The procedures developed and utilized have been intended to allow the rapid screening of a large number of compounds with a view to selecting those possessing potential value as insecticides, for further detailed examination. In the screening process, simple methods have been used for elucidating contact, fumigant, and systemic toxicity. Observations on the phytotoxicity of candidate compounds were also made in order to eliminate from further evaluation, substances exhibiting this undesirable property.

General Toxicity. In this screening program, the first test applied, designated as the "general toxicity" test, is a combination of contact, fumigant, and ingestion toxicities, due to the nature of the test. However, because of the procedure adopted, contact toxicity is probably the predominant factor except in the case of highly volatile compounds.

Granary weevils, *Sitophilus granarius* (L), reared on hard spring wheat were used as the test insect. The procedure was carried out and observed at 70° to 72° F. and a relative humidity of 55 to 65% using hard spring wheat as the test medium. The candidate compound was dissolved in 10 ml. of acetone and added to 30 grams of wheat in 4-ounce jars fitted with screen lids. The acetone was used as a volatile carrier in order to obtain uniform distribution of the compound throughout the wheat. The jars were then spun on a rolling machine until the acetone had completely evaporated; the concentration being expressed as weight per cent of the wheat. Control samples of wheat were treated with acetone alone. The contaminated sample of wheat was divided into two equal portions, so that each concentration was tested in duplicate. Twenty-five insects of a random sex ratio were added to each jar and mortality counts made at the end of a 96-hour period.

Residual Fumigant Toxicity. The candidate compound was made up initially in two concentrations in distilled water, 0.1 and 0.01% by volume. Distilled water was used as a control. Filter papers were treated with 1 ml. of the test solution, allowed to air-dry thoroughly, and then were placed in the bottom section of a Petri dish. A small cabbage leaf infested with a minimum of 25 normally established aphids, *Myzus persicae* (Sulz.), was securely taped to the inside of the upper section of the dish. A taut section of cheesecloth between the two sections prevented the aphids from coming into contact with the treated surface. The top and bottom of the Petri dish were taped together. Each concentration was tested in triplicate. At the end of 18 hours the dishes were opened and the per cent change in living aphids was noted, and then corrected for the change observed in the control groups, because aphids reproduce at a very high rate, and some organic fluorine compounds have been observed to increase the rate of reproduction significantly.

Systemic Toxicity. Because variations of temperature, humidity, and lighting significantly affect the rate of translocation and transpiration in plants, these tests were all carried out in a room at a temperature of 75° to 80° F. (relative humidity 70 to 75%) illuminated by fluorescent lighting.

Aphids, *Myzus persicae* (Sulz)., were used as the test insect. Four-week-old cabbage plants were carefully washed and placed in individual Erlenmeyer flasks containing distilled water. After being infested with a bulk population of not less than 50 aphids, the plants were set aside for 24 hours to allow the insects to establish themselves.

The infested plants were transferred to blackened flasks containing an aqueous solution of the compound under test. With each group of tests, distilled water controls in triplicate and a "schradan control" to check the uniformity of response of the aphids were also carried out. At the end of 48 hours, counts were made of the remaining aphids, which were then removed; the roots of the plant were washed in tap water and the plant was then re-established in tap water. A new group of aphids was established on the plant and left for a further 48 hours before making the final count. All mortality data were corrected for change from the original establishment count, based on observations of normal change in the control population. Phytotoxic effects were also noted during this test.

Cholinesterase Inhibition. The candidate compounds were all evaluated for their ability to inhibit insect cholinesterase (ChE). Female fly heads were used as the source of ChE, the inhibition of which was determined essentially by the method of Metcalf and March (*18*).

Table III. 48-Hour Systemic Toxicity to Green Peach Aphids (*Myzus persicae*)[a]

Candidate Compound				Mortality, %, at Concn. Vol. %			
Type	No.	-NR-	R'	0.1	0.01	0.001	Control, HIO
IV	1	$-NMe_2$		P	..	..	
	2	$-NEt_2$		P	..	..	
V	3	$-NMe_2$	Et-	84	28	13	+24
	4	$-NMe_2$	*n*-Pr-	93	40	27	+14
	5	$-NMe_2$	Iso-Pr-	98	72	38	+14
	6	$-NMe_2$	*n*-Bu-	91	60	25	+2
	7	$-NMe_2$	*n*-Am-	100	88	41	12
	8	$-NEt_2$	Et-	13	..	..	+49
	9	$-NC_5H_{10}$	$(EtO)_2$-	43	16	0	12
	10	$-NC_5H_{10}$	Iso-Pr-	98	16	13	12
	11	$-NC_4H_8O$	Iso-Pr-	100	67	43	12
VI	12		Et-	92	36	34	+37
	13		*n*-Pr-	67	45	33	+2
	14		Iso-Pr-	57	53	50	+14
	15		*n*-Bu-	+5	+7	+1	+2
	16		*n*-Am-	100	51	+13	12
Schradan, records of max. variation in all schradan tests				100–97	61–59	35–2	
Controls, H_2O, max. variation 12 to +66.							

[a] Plant roots in aqueous solution of candidate compounds for 48 hours.

Results and Discussion

The general toxicity of the compounds evaluated is given in Table I, the residual fumigant toxicity in Table II, the systemic toxicity (48 and 96 hours) in Tables III and IIIa, respectively, and the anticholinesterase properties in Table IV.

In a screening test such as has been used with these compounds, the results will not be sufficiently accurate to form as precise a picture of the dose-mortality response as would be required to establish a relationship between the toxicity and molecular structure.

The compounds of the bis-*N*-dialkyl methylphosphonic diamide type (IV) proved to be of little interest because of their low insect toxicity combined with a marked phytotoxicity.

The alkyl *N*-dialkyl methylphosphonamidates (V) possessed a pronounced systemic activity; in particular isopropyl methylphosphonomorpholidate (No. 11) and *n*-amyl *N*-dimethyl methylphosphonamidate (No. 7). The latter

also exhibited high general and fumigant toxicities. These two compounds were the strongest cholinesterase inhibitors evaluated with IN_{50} 1×10^{-7} and 4×10^{-6}, respectively; the former compound had some degree of mammalian toxicity (LD_{50} 60 mg. per kg.). This suggests that possibly these compounds act by virtue of their being inhibitors *per se* rather than giving rise to active metabolites. Others—e.g., Nos. 4 and 9—were as potent when the insect ingested them directly. This may be due to the latter pair's more ready decomposition in the plant. If the alkyl *N*-dialkyl methylphosphonamidates do act via a metabolite, the most likely mechanism would be similar to that proposed for schradan by Spencer (*21*, *23*) and Fish *et al.* (*10*) and are converted by oxidation and rearrangement to *N*-alkylol derivatives. However, the analogy with schradan cannot be carried further, because these compounds do not contain an anhydride linkage which would be capable of phosphorylating ChE, as is the case with schradan.

The occurrence of insecticidal activity in the dialkyl methylphosphonates was very surprising in view of the length of time these compounds have been known, in particular, diethyl methylphosphonate (No. 12) which is completely nontoxic to mammals. Taking into account the low in vitro anti-ChE activity of these compounds, there are several possible explanations for their insecticidal properties: They may be converted to active metabolites by the insect while being rapidly destroyed by mammals as is the case with malathion (*8*); a metabolic process may occur in insects which cannot occur in mammals; or they may act by some entirely different route not dependent on inhibition of ChE.

Casida (*3*) has collected all the available information on the metabolism of organophosphorus compounds from inactive to active inhibitors. None of the previously reported routes appears to be open to the dialkyl methylphosphonates. The most comparable compound reported is Dipterex (*O,O*-dimethyl 1-hydroxy-2-trichloroethylphosphonate) which although it undergoes facile rearrangement and dihydrochlorination in vitro to yield the very potent inhibitor dimethyl-2,2-dichlorovinyl phosphate, does not in fact act by the same mechanism in vivo (*1*).

The most significant fact arising from this work is that these compounds, while exhibiting insecticidal properties comparable with schradan in some cases, are nontoxic to mammals. Extension of the chemistry utilized in the preparation of these compounds could lead to compounds of greater insecticidal activity, but displaying the low mammalian toxicity which is desirable in insecticides.

Table IIIa. 96-Hour Systemic Toxicity[a]

Candidate Compound				Mortality, % Concn., Vol. %			
Type	No.	-NR_2	R'	0.1	0.01	0.001	Control, H_2O
V	3	-NMe_2	Et-	100	58	7	+8
	4	-NMe_2	*n*-Pr-	100	89	1	+49
	5	-NMe_2	Iso-Pr-	100	96	30	+4
	6	-NMe_2	*n*-Bu-	100	83	10	+49
	7	-NMe_2	*n*-Am-	100	91	61	+27
	8	-NMe_2	Et-	P			
	9	-NC_5H_{10}	$(EtO)_2$-	P			
	10	-NC_5H_{10}	Iso-Pr-	100	26	26	+27
	11	-NC_4H_{10}	Iso-Pr-	100	49	46	+27
VI	12		Et-	96	67	40	8
	13		*n*-Pr-	96	65	17	+19
	14		Iso-Pr-	46	18	8	+4
	15		*n*-Bu-	..	..	..	..
	16		*n*-Am-	P	28	5	+27
Schradan, max. variation				100	99–98	74–72	
Controls, H_2O, max. variation				+27–+55			

[a] Plant roots in water for 48 hours following 48-hour immersion of roots in water solution of test compound. New aphid population established at end of first 48 hours.

P. Marked phytotoxicity precluded further evaluation; or marked phytotoxicity at this concentration.

+ %. Increase above initial established population.

Table IV. Anticholinesterase Properties of Candidate Compounds

Candidate Compounds				Inhibition of Fly Brain ChE, / Head's Molar Concn.			
Type	No.	NR_2	R'	Substrate[a]	Substrate	Inhibitor ChE	Inhibition, %
III	1	-NMe_2		AchBr	0.4	2×10^{-5}	4
				Mech	0.5	4×10^{-5}	9
	2	-NEt_2		AchBr	0.4	2×10^{-5}	3
IV	3	-NMe_2	Et-	AchBr	0.4	2×10^{-4}	93
				AchBr	0.4	2×10^{-5}	23
				Mech	0.5	4×10^{-5}	62
	4	-NMe_2	*n*-Pr-	AchBr	0.4	2×10^{-4}	32
				AchBr	0.4	2×10^{-8}	1
	5	-NMe_2	Iso-Pr-	AchBr	0.4	2×10^{-4}	51
				AchBr	0.4	2×10^{-8}	7
	6	-NMe_2	*n*-Bu-	AchBr	0.4	2×10^{-4}	98
				AchBr	0.4	2×10^{-5}	74
				AchBr	0.4	2×10^{-6}	14
				AchBr	0.4	2×10^{-8}	2
	7	-NMe_2	*n*-Am-	AchBr	0.4	4×10^{-6}	50
	8	-NEt_2	Et-	AchBr	0.4	2×10^{-4}	9
				AchBr	0.4	2×10^{-8}	5
	9	-NC_5H_{10}	$(EtO)_2$-	AchBr	0.4	2×10^{-3}	50
	10	-NC_5H_{10}	Iso-Pr-	AchBr	0.4	2×10^{-6}	50
	11	-NC_4H_8O	Iso-Pr-	AchBr	0.4	1×10^{-7}	50
V	12		Et-	AchBr	0.4	2×10^{-4}	12
					0.4	2×10^{-8}	5
	13		*n*-Pr-	AchBr	0.4	2×10^{-5}	13
					0.4	2×10^{-6}	4
				Mech	0.5	1.2×10^{-3}	9
	14		Iso-Pr-	AchBr	0.4	2×10^{-4}	11
					0.4	2×10^{-8}	6
	15		*n*-Bu-	AchBr	0.4	2×10^{-5}	3
					0.4	2×10^{-6}	4
				Mech	0.5	1.2×10^{-3}	48
	16		*n*-Am-	AchBr	0.4	2×10^{-5}	50
		Paraoxon		AchBr	0.4	2×10^{-8}	98
					0.4	2×10^{-9}	50–55
					0.4	2×10^{-10}	8–9
		Schradan		AchBr	0.4	2×10^{-3}	60
				Mech	0.5	1×10^{-2}	81
					0.5	1.2×10^{-3}	18
					0.5	4×10^{-5}	9

[a] AchBr. Acetylcholine bromide.
Mech. Methacholine chloride.

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ACARICIDE RESIDUES

Persistence of Residues of 2, 4, 5, 4′-Tetrachlorodiphenylsulfone in Florida Citrus Fruits

J. J. McBRIDE, Jr.

University of Florida, Citrus Experiment Station, Lake Alfred, Fla.

Residues of 2,4,5,4′-tetrachlorodiphenylsulfone, a compound effective in controlling the citrus red mite [*Metatetranychus citri* (McG.)] have been determined in all major varieties of Florida citrus fruits at varying times after application. Tedion is absorbed into the peel of citrus fruits and persists in the peel for longer than three months after application. Absorption into the pulp and juice, when it occurs, is extremely low.

THE COMPOUND 2, 4, 5, 4′-tetrachlorodiphenylsulfone (Tedion) has been found to be an effective acaricide for the citrus red mite [*Metatetranychus citri* (McG.)] in Florida (*3*). A project has been carried out in these laboratories to determine the amounts of Tedion residues at various times after application in the major varieties of citrus fruits grown in Florida and furnished in the fresh-fruit market.

Analytical Procedure

Tedion residues were determined by the method of Fullmer and Cassil (*2*), except that benzene was used throughout in place of chloroform and no keeper was used (it was found unnecessary).

Tedion was extracted from the samples by first thoroughly macerating the peel or pulp-juice portion of the fruit in a Waring Blendor and then tumbling the macerate with benzene for one hour. For peel samples 150 grams of peel and 200 ml. of benzene were used. For pulp-juice samples 200 grams of pulp-juice, 200 ml. of benzene, and 60 grams of anhydrous sodium sulfate were used. Aliquots taken for analysis varied from 20 to 50 ml., depending on the Tedion content.

Table I. Recovery of Tedion from Citrus Fruits

Variety	Tedion, γ Added	Tedion, γ Found	Recovery, %
Valencia orange			
Peel	8.0	6.8	85
	24.0	20.0	83
	37.0	36.7	99
Pulp-juice	8.0	7.9	99
	24.0	26.3	109
	40.0	35.4	89
Grapefruit			
Peel	8.0	7.5	94
	16.0	17.6	110
	32.0	35.9	112
Pulp-juice	8.0	7.8	98
	20.0	20.4	102
	36.0	32.9	91
Tangerine			
Peel	8.0	8.2	102
	16.0	18.3	114
	32.0	33.9	106
Pulp-juice	8.0	7.7	96
	16.0	14.3	89
	24.0	25.5	106

Recoveries. Recoveries were good from both the peel and the pulp-juice of all varieties. Recovery samples were prepared by adding a standard solution of Tedion in chloroform to the macerate prior to extraction with benzene. Table I shows some representative recoveries.

Residues. Sprays were applied with a hand pressure sprayer to the point of runoff which is a more thorough coverage than is generally obtained in commercial applications. Fruit was sampled at intervals after application until the Tedion content became constant or decreased. All fruit was washed with a detergent by thorough hand brushing, using a stiff-bristled brush to remove surface deposits. Table II shows Tedion residues in major varieties of citrus fruits at varying intervals between application and harvest.

Discussion

Inspection of Table II shows that Tedion is absorbed into the peel of citrus fruits in significant amounts and is extremely persistent. No attempt was made to determine the distribution of the Tedion residues in the peel. Varietal differences are slight. Tangerine peel shows slightly higher Tedion content than other varieties. In gen-