etc.) results in its rapid metabolism.

The method was applied to the analysis of field-treated samples. McIntosh apple trees from an orchard in Lagrangeville, N. Y., were treated seven times at regular intervals between June 6 and August 16, 1963, with 1 pound of 50% wettable Sevin per 100 gallons of water. Replicate apple samples harvested on September 17 showed residues of 1.5, 1.2, 1.5, and 1.4 p.p.m. of Sevin. Untreated apples showed less than 0.02 p.p.m. of Sevin and recovery of Sevin at the 0.1-p.p.m. level was 94%.

On May 31, 1963, in Cambridge, N. Y., an airplane application of 1.25 pounds of Sevin per acre (formulation 80 W) was made on a 590-acre woodlot and pasture in which bee hives were located. Dead bees taken 14 and 24 hours after treatment showed, respectively, 0.3 and 0.5 p.p.m. of Sevin. Separate bee samples taken from two different hives after 48 hours showed 0.4 and 0.6 p.p.m. Control bees showed less than 0.02 p.p.m. and recovery of 0.5 p.p.m. of Sevin was 92%.

Acknowledgment

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PESTICIDE RESIDUE METHODOLOGY

Gas Chromatographic Determination of Flash Heater–Modified Pesticides

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The possibility of direct chemical modification of a number of the more common chlorinated pesticides in the flash heater of a gas chromatograph with electron capture detection is explored. The action of sodium carbonate, cupric oxide, cadmium chloride, aluminum chloride, and potassium dichromate at 240° C. on BHC isomers, DDD, DDE, DDT, methoxychlor, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, chlordan, toxaphene, and methyl parathion is described in terms of aldrin retention ratios and specific aldrin area ratios of the maxima produced. Mild and severe modifications are noted. A system utilizing multiple-injection modifiers ahead of a single column and electron capture detector is suggested to provide more positive identification of specific pesticides in a residue extract and to eliminate interferences normally occurring on silicone columns.

SINCE the introduction by Goodwin, Goulden, Richardson, and Reynolds (*8*) of electron capture gas chromatography (EC/GC) for the determination of pesticide residues, the method has been applied widely by a number of investigators (*2, 3, 7, 11, 12, 16, 17, 19, 22, 24, 28, 29*). The high sensitivity of the electron-capture detector for molecules containing electronegative substituents such as halogens and aromatic nitro groups has uniquely fitted it to the problem of detecting trace quantities of many pesticides which contain such groups.

Nevertheless, extracts of plant and animal origin can contain electron-capturing materials other than the sought-after chlorinated or thiophosphoryl pesticides. Incorrect identifications of chromatographic maxima based solely on retention times or ratios can occur for this reason. Interferences also occur between certain of the commonly occurring pesticides chromatographed on the usual variants of silicone greases that have been employed. Examples of this latter are the nearly identical aldrin retention ratios as defined by Burke and Johnson (*6*) and the present authors (*24*) for aldrin and a chlordan component, aldrin and methyl parathion, *p,p'*-DDD and *o,p*-DDT, *p,p'*-DDE and dieldrin, endrin and *p,p'*-DDD, and a methoxychlor degradation product, endrin, and *p,p'*-DDT. The positive identification of a particular pesticide residue in a biological product is considered to be a major problem by these and other investigators (*9, 27*), particularly if the extract is not well cleaned up before chromatography.

Several methods have been employed to minimize these difficulties. Extensive cleanup of the extracts prior to gas chromatography is helpful in preventing the occurrence of many natural electron-capturing contaminants in the final mixture, and is recommended by most groups utilizing the gas chromatographic technique.

Chemical modification of the residues followed by EC/GC is a generally useful technique for confirmatory identification. Klein, Watts, and Daminco (*17*) confirmed the presence of DDT and DDD in butter by chromatographing extracts before and after treatment with NaOH to convert the pesticides to the dehydrochlorinated ethylenic derivatives. Gutenmann and Lisk have shown that pesticides insensitive to electron capture may be converted to appropriately sensitive forms by bromination (*13, 15*) or the Zeisel alkoxy method (*14*), where the alkyl iodides formed are detected. A similar approach was employed by Beckman and Berkenkotter (*1*), who utilized gas chromatography with flame ionization detection to determine residues before and after reduction with sodium in anhydrous ammonia. All of this modification work was carried out on a macro or semimicro scale in separate reaction vessels prior to sampling and chromatography.

Use of well developed paper chromatographic methods (*20, 21, 23*) as well as recently advanced thin layer chromatographic techniques (*18, 31*) as a supplemental identification is an obvious technique.

The possibility of finding other stationary phases suitable for gas chromatog-

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Table I. Composition of Standards I and II

I			II		
Component	Concn., ng./μl.	Std. inject. mass, ng.	Component	Concn., ng./μl.	Std. inject. mass, ng.
γ-BHC	0.32	1.6	Heptachlor	0.2	1.0
Aldrin	0.2	1.0	Heptachlor epoxide	0.2	1.0
p,p'-DDD	1.0	5.0			
p,p'-DDT	0.2	1.0	Dieldrin	0.2	1.0
Methoxychlor	0.88	4.4	Endrin	1.0	5.0

raphy column packings which would yield separation patterns different from those usually obtained on the nonpolar silicones such as SE-30, SE-96, or high vacuum silicone grease has occurred to a number of investigators. Robinson and Richardson (*27*) describe four column packings useful for pesticide work, several of which have distinctly different elution patterns. Goulden, Goodwin, and Davies (*9*) have described five column packings, which they have incorporated into a parallel column instrument utilizing a single detector and recorder to sense the multiple output of the sample as it is successively eluted from the five columns onto which it had been simultaneously loaded from a splitter behind the single flash heater. The resulting "spectrochromatogram" is characteristic for each pesticide, but yields a complicated pattern difficult to interpret if more than one or two pesticides are present in the sample. Further, the life of the system is limited somewhat by the bleeding of several of the stationary phases, altering the spectrochromatograms with time.

Goulden, Goodwin, and Davies offered a seemingly more promising attack (*10*), utilizing a halogen-sensitive detector with detection characteristics different from a normal electron capture cell. Tandem operation of this new detector with an electron capture cell yields a dual trace chromatogram, the retention time and the ratio of peak areas for each maximum in the two traces being characteristic of the pesticide.

Beroza and Sarmiento (*4, 5*) and Ralls (*26*) have utilized high temperature chemical modifications of compounds at the inlet of gas chromatography equipment to improve identification of maxima.

We noted earlier (*24*) the ease of degradation of nanogram quantities of pesticides in the chromatograph, and felt that a virtue might be made of a defect. Direct modification of residues on the surface of reactive solids or nonvolatile liquids in the flash heater seemed a distinct possibility. This work is a study of several such possible modification reagents.

Experimental

Apparatus. The gas chromatograph utilized has been described (*24*), except that the modified flash heater was replaced by an improved, simpler version (Figure 1, *A*) which allowed replaceable borosilicate glass inserts to extend through the rear of the block. Connections between the flash heater insert and the column, and between the column and the cell were made with $^1/_{16}$-inch Teflon tubing (Barber-Colman Co., Rockford, Ill.). Silicone through-hole septums bridging over the Teflon to glass joint provided good seals usable to 30 p.s.i.g.

Preliminary work indicated that the $^1/_4$ inch × 4-foot aluminum tubing used previously was complicating the degradation patterns produced in the flash heater. Borosilicate glass columns were employed thereafter, of 0.6 cm. O.D. × 121 cm. tubing coiled in a 15-cm.-diameter helix packed except for 6 cm. at both ends, which were pulled out to 0.15-cm. capillaries after packing.

Flash heater inserts of 6-mm. borosilicate glass tubing (Figure 1*B*) were filled with approximately 12 cm. of granulated reagent between two borosilicate glass wool plugs.

A 10-μl. Hamilton syringe with Chaney adapter set to deliver 5-μl. samples was used for sampling.

A Keuffel & Esser adjustable arm polar compensating planimeter was used to measure peak areas.

Instrumental Operating Conditions. Column packing, 5% SE-30 on 60/80 mesh Chromosorb W, conditioned 36 hours at 245° C. with N_2 flow. Flow rate, 100 ml. per minute measured with soap bubble flowmeter. Inlet pressure, 18 to 20 p.s.i.g., as needed to maintain aldrin retention time constant. Temperatures, flash heater 240° C., column 195° C., cell bath 290° C. Cell potential, 25 volts. Electrometer input, positive. Gain, 1000 (full scale recorder deflection 1×10^{-10} ampere).

Reagents. Modification reagents used were:

Na_2CO_3, Mallinckrodt granular primary standard, heated in insert at 240° C. for 30 minutes with gas flow through it before connecting to column.

Aluminum, granulated.

$AlCl_3$ on surface of granulated aluminum, produced by injecting 10 μl. of concentrated HCl into the insert packed with aluminum while in the flash heater at 240° C. with gas flow through insert.

CuO, Mallinckrodt wire form, analytical reagent.

$CdCl_2 \cdot 2^1/_2$ H_2O, J. T. Baker, ACS grade, heated in furnace at 300° C. for 1.5 hours before packing in insert.

$K_2Cr_2O_7$, Fisher Scientific Co., C.P.

$CuSO_4 \cdot 5$ H_2O, Baker and Adamson ACS grade, heated at 300° C. for 1.5 hours before packing in insert.

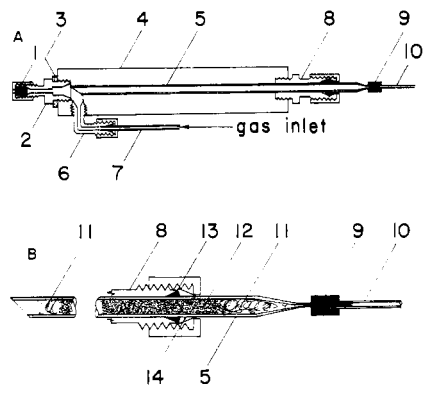

Figure 1. Axial section through flash heater and detail of insert

1. Silicone rubber septum
2. $^1/_4 \times ^1/_8$-inch Swagelok reducing union, stainless steel
3. Silicone rubber gasket
4. 1 × 5-inch aluminum rod
5. 6 mm. O.D. borosilicate glass insert
6. $^1/_8$-inch Swagelok aluminum ell
7. $^1/_8$-inch × 2-foot aluminum tubing wrapped on heater body, covered with asbestos sheet and wrapped with resistance wire
8. $^1/_4$-inch Swagelok aluminum male connector drilled through to $^1/_4$-inch bore
9. Silicone rubber through hole septum
10. $^1/_{16}$-inch Teflon tubing
11. Borosilicate glass wool
12. Modification reagent
13. Silicone gasket
14. $^1/_4$-inch Swagelok front ferrule, reversed

All pesticides used were analytical standards (>98% purity) obtained from appropriate commercial producers, except for the methoxychlor, which contained 12% of γ-BHC. All methoxychlor values of relative sensitivity were suitably corrected. Redistilled Skellysolve B was employed as solvent in the standard pesticide solutions.

Single standard solutions were made up by accurately weighing 100 mg. of the pesticide and diluting to 100 ml. These stock solutions were diluted 1 to 100, and from this dilutions of 1 to 50 and 5 to 50 were prepared. Final solutions contained 0.2, 1.0, and 10.0 ng. per μl. of standard, allowing 1.0-, 5.0-, and 50.0-ng. injections.

Procedure. Packed inserts were conditioned at 240° C. in the flash heater of the instrument for 1 to 8 hours after being put into operation until instrument sensitivity increased to approximately normal levels. Multiple injections of single standards were made at the 1-, 5-, and 50-ng. level. Aldrin retention ratios for the maxima produced were calculated as previously described (*6, 24*) and peak areas were planimetered in duplicate using a base line across the minimum points on both sides of each maximum. The specific area for each maximum was calculated by dividing the average area by the sample injection mass in nanograms. Each value thus obtained was divided by the measured area produced by 1 ng. of aldrin under identical conditions. The specific aldrin area ratio, A_a, so obtained for that maximum was tabulated along with the aldrin retention ratio, R_a. Two standard mixtures were prepared as indicated in Table I, and 5 μl. of each were injected after running all single standards.

Results and Discussion

Rotating the cell body with respect to the anode gas inlet of the Barber-Colman Sr^{90} detector cell (Model A-4150) produced variable sensitivity. Maximum sensitivity at optimum angular orientation was greater by a factor of 2 than that previously obtained. This effect is presumed to be associated with alteration of the nominally cylindrical geometry of the cell. The beta-emitting foil cylinder around the axial gas flow has a slot where the two edges join, and may not be exactly cylindrical. Any off-axial flow component of the entering gas might then produce an effective electrode spacing change as the relative anode-cathode orientation is changed. The cell was adjusted to optimum sensitivity, necessitating a gain setting of 1000 at the electrometer to give on scale deflections for 1 ng. of γ-BHC.

Column performance varied somewhat during its use. Immediately after preparation and conditioning of a new column, sensitivity for DDT, methoxychlor, and endrin was low. This was improved considerably by injecting up to 100 μl. (1200 μg. of solids) of apple peel extract as recommended by Shuman and Collie (*30*). All peaks were sharpened and sensitivity was improved for *p,p'*-DDT, methoxychlor, and endrin (R_a = 2.07) maxima, presumably because of coating of reactive sites in the system with polar acids and waxes from the apple peel. After the various modifiers described had been used, sensitivity decreased for these same maxima and shifts in the positions of the remaining endrin maxima occurred. Figure 2, *A* and *F*, shows the effect on endrin. These changes occurred principally after using the $K_2Cr_2O_7$ modifier, which was darkened by use in the instrument. The degrading effect of dichromate on the standards apparently reintroduced reactive sites onto the column, which altered the normal degradation pattern of the column for endrin.

Values computed for A_a of a given maximum were not constant, with injected mass of material giving rise to the maximum. A logarithmic relationship between A_a and mass seems to exist, although this has not been explored fully. The change in A_a with mass of injection was small as long as the maximum peak height was less than approximately 50% full scale deflection—i.e., <25% of cell standing current. The A_a values tabulated were averaged only for the 1-ng. injections, unless larger injections gave less than half-scale maxima. The small maxima appearing in the 50-ng. injections usually fell in this category, and tabulated values of A_a <0.01 arose in this manner. Because of the inconstancy of the A_a values with respect to injected mass, they should be considered only as semiquantitative descriptions of relative areas of maxima. Application of these data to other instrumental arrangements must be accompanied by standardization and calculation of R_a and A_a values for that system.

Although approximate, the A_a values do give some estimate of relative peak areas. The measured area of the 1-ng. aldrin maximum varied with the insert used from 250 to 420 sq. mm., sensitivity increasing in the order $AlCl_3$, CuO, $CdCl_2$, unmodified, $K_2Cr_2O_7$, and Na_2CO_3. It is estimated that the smallest detectable maximum would be about 2 mm. high by 2-mm. base width, with A_a between 0.008 and 0.005. Thus maxima with A_a <0.006 would not be seen at the 1-ng. injection level, but would appear at higher injection masses. In practice, no area was planimetered unless it was about four times the minimum described, since base line variations could produce maxima 2 × 4 mm., height times base. Sharp, narrow maxima approaching this practical limiting area were more reproducible and consequently measured.

Tables II and III list R_a and A_a values obtained with the pesticides and modifying agents investigated. Figures 2 to 5 are comparative chromatograms for a given pesticide or mixture as modified by the reagents employed.

The action of the modifying reagents on the injected materials is probably a combination of chemical change and adsorption on the solid. The packed insert is essentially a short gas-solid chromatographic column preceding the gas-liquid chromatographic column. Relative shifts in maxima may occur due to GSC action, and maxima may disappear because of irreversible binding of the original or altered materials.

Reagents other than those listed in Tables II and III were tested. No modification occurred on the aluminum until it was treated with 10 μl. of concentrated HCl to produce a surface film of $AlCl_3$. The resulting modifier then gave results as tabulated. Anhydrous cupric sulfate completely removed all maxima of all pesticides tested, and was thus eliminated from consideration. Silver nitrate, 20% (w./w.) on 30/60-mesh Chromasorb W, gave promising first results but was not consistent and caused a gradual over-all sensitivity decrease with time.

Individual Action of Modifying Agents. Na_2CO_3. Removal of all BHC isomers, heptachlor, heptachlor epoxide, endrin, and methyl parathion maxima is a valuable characteristic of this modifier (Figures 2*D* and 3*D*). Removal is probably associated with quantitative dehydrochlorination by this base, or nucleophilic cleavage of the ester linkage in the case of methyl parathion. Parathion would be expected to react in a similar manner. The major maximum (R_a = 1.89) produced by *p,p'*-DDT may be *p,p'*-DDE (R_a = 1.86) formed by essentially quantitative dehydrochlorination of the side chain. Both DDE and DDT form a minor product, R_a = 1.45, which may be the same as the major product of *p,p'*-DDD at

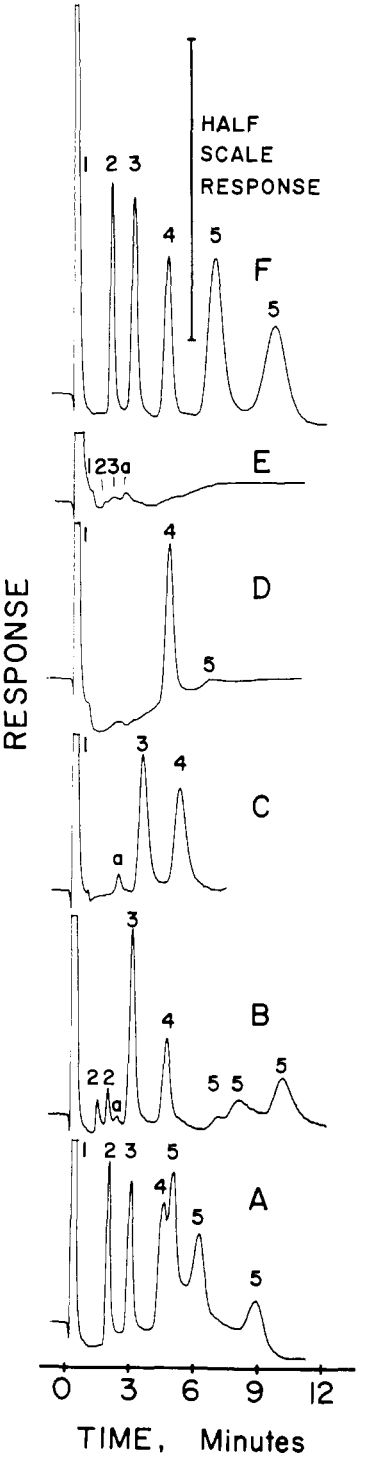

Figure 2. Chromatograms of standard II as modified in flash heater

A. No modifier
B. CuO
C. $K_2Cr_2O_7$
D. Na_2CO_3
E. $CdCl_2$
F. No modifier, old column

Maxima
1. Solvent
2. Heptachlor
3. Heptachlor epoxide
4. Dieldrin
5. Endrin
a. Artifact

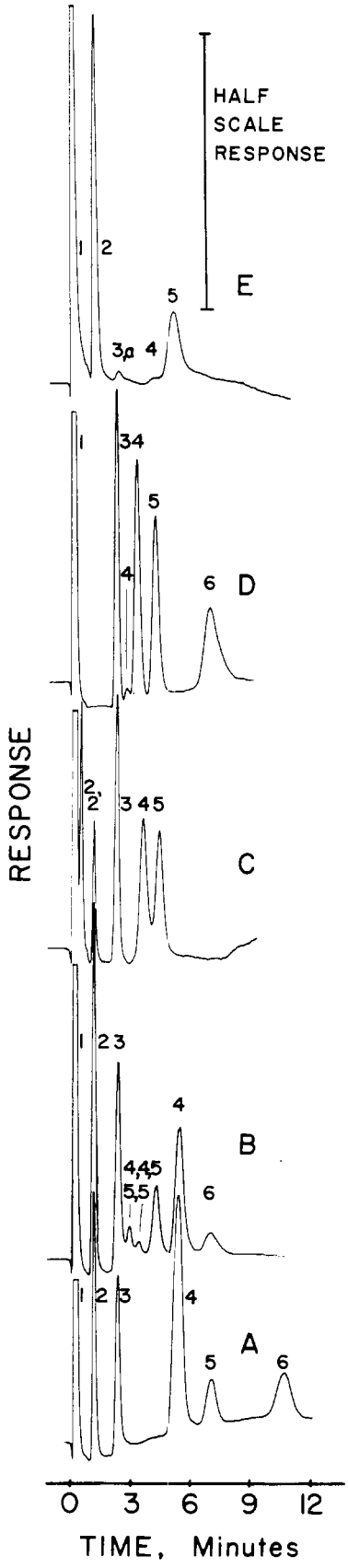

Figure 3. Chromatograms of standard I as modified in flash heater

A.	No modifier	Maxima	
B.	CuO	1.	Solvent
C.	$K_2Cr_2O_7$	2.	γ-BHC
D.	Na_2CO_3	3.	Aldrin
E.	$CdCl_2$	4.	DDD
		5.	DDT
		6.	Methoxychlor

R_a = 1.49. By analogy with the proposed mechanism this would be 1-chloro-2,2-bis-[*p*-chlorophenyl] ethylene, MDE, and would involve a reduction of DDE followed by dehydrochlorination of the side chain. Degradation by thermal cracking in any of the compounds could produce the hydrogen necessary for reduction. Regardless of the possible mechanisms or end products formed, the reproducibility of the normal and modified chromatograms allows distinction between *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD by this reagent.

Methoxychlor is attacked to produce a lower boiling component with greater sensitivity for electron-capture detection than the parent compound. This is consistent with the mechanism of dehydrochlorination of the ethane chain to produce the dichloroethylenic structure similar to DDE. The DDT R_a = 1.89 maximum is also more sensitive than the unmodified R_a = 3.06 one, even though 1 ng. of DDT would produce only 0.9 ng. of DDE at 100% yield. Conjugation of the side chain with the rings in these compounds apparently enhances the electron-capturing ability of the side chain chlorines, as would be expected theoretically.

Aldrin appears to be unaffected by this reagent, dieldrin is attacked moderately, presumably losing sensitivity by producing some hydrocarbons or other EC-insensitive materials by dehydrochlorination, while endrin is thoroughly degraded. Since endrin is easily decomposed thermally (*22*, *25*), it is not surprising that these products are further attacked by base.

Another outstanding feature of this reagent is its effect on toxaphene and chlordan (Figures 4*D* and 5*B*). Although the total area response for both pesticides is decreased, the resulting chromatograms are much sharper and well resolved, while the mixture is eluted in less time.

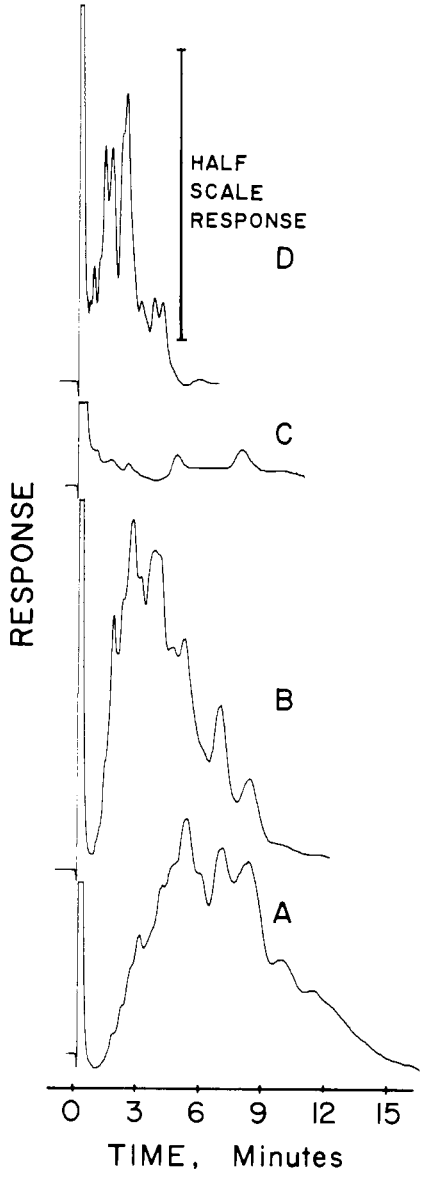

Figure 4. Chromatograms of toxaphene (50 ng.) as modified in flash heater

A.	No modifier	C.	$CdCl_2$
B.	CuO	D.	Na_2CO_3

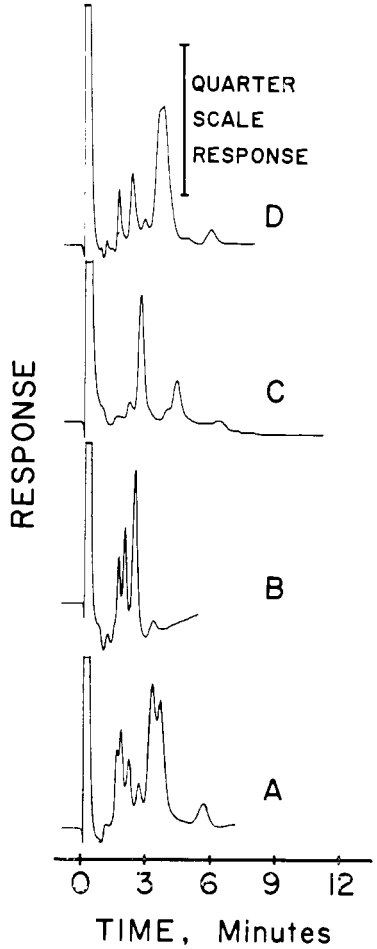

Figure 5. Chromatograms of chlordan (5 ng.) as modified in flash heater

A.	No modifier	C.	$CdCl_2$
B.	Na_2CO_3	D.	$K_2Cr_2O_7$

Table II. Characteristic Modifications of Standard Components by Selected Reagents[a]

Pesticide	Unmodified		Na_2CO_3		$AlCl_3/Al$		CuO		$CdCl_2$		$K_2Cr_2O_7$	
	R_a	A_a	R_a	A_a	R_a	A_a	R_a	A_a	R_a[b]	A_a[c]	R_a	A_a
Standard I												
γ-BHC	0.48	0.8	...	...	0.48	0.9	0.48	0.8	0.24	0.004	0.21	0.2
									0.52	0.8	0.51	0.3
Aldrin	1.00	1.0	1.00	1.0	1.00	1.0	1.00	1.0	0.76	0.0005	1.00	1.0
									1.04, 1.30	0.002		
									2.00	0.002		
p,p'-DDD	2.34	0.8	1.24	0.02	1.58	0.02	1.28, 1.46, 1.65		1.72	0.004	1.60	0.3
			1.49	0.1	2.49	0.8		0.06			2.52	0.01
			1.82	0.0006			1.97	0.0008				
							2.33	0.2				
p,p'-DDT	3.06	0.5	1.45	0.0009	1.33	0.007	1.22, 1.42	0.02	2.15	0.6	1.95	0.9
			1.89	0.7	1.93	0.06			2.52	0.04	3.21	0.05
					2.52	0.06	1.82	0.3				
					3.22	0.2	2.28	0.008				
							3.02	0.006				
Methoxychlor	4.68	0.2	3.13 (TT)	0.2	3.24 (T)	0.08	3.01	0.06	...	...	4.32 (TT)	0.07
							3.81	0.01				
					4.28	0.06						
							4.16	0.004				
					4.96	0.002						
Standard II												
Dieldrin	1.92	1.1	1.99	0.5	1.98	0.7	1.96	0.5	...	...	2.30	0.8
	New Column											
Endrin	2.07	0.3	0.30	0.002	1.17	0.002	1.16	0.002	2.70 (TT)	0.1	1.20	0.001
	2.61	0.1	1.19	0.002	3.28 (T)	0.1	1.96	0.002			2.24	0.0005
	3.77	0.1	2.87	0.01	4.43 (T)	0.1	2.92	0.01			3.25, 3.80	0.03
	Old Column						3.41	0.04				
	2.98	0.4										
							4.27	0.1			4.49	0.02
	4.25	0.3										
							5.67	0.004				
Heptachlor	0.79	0.7	...	...	0.81	0.7	0.57	0.1	0.61	0.01	1.50	0.006
							0.78	0.1				
							1.10 (TT)	0.08				
							2.70	0.003				
Heptachlor epoxide	1.26	0.9	0.67	0.0003	1.27	1.0	1.26	0.9	0.25	0.003	1.45	1.0
			1.28	0.002					0.77	0.1	2.36	0.002

[a] R_a = aldrin retention ratio; A_a = specific aldrin area ratio; T = tails; TT = tails badly. [b] Calculated on basis of assumed value of aldrin retention time equal to that in unmodified system. [c] Calculated on basis of assumed value of 0.8 for γ-BHC in absence of aldrin maximum.

This is especially true for toxaphene, which might be quantitated on the basis of several of the major maxima.

$AlCl_3/Al$. This reagent is a typical Lewis acid, well known for its catalytic activity. Modifications produced are essentially those described previously (*24*), with DDT, endrin, and methoxychlor undergoing considerable alteration. Methyl parathion is much less sensitive with this modifier. The use of aluminum systems or even aluminum columns is not recommended if degradation of these compounds is to be avoided.

CuO. This reagent was chosen as a mild oxidant with basic character. Figures 2*B* and 3*B* illustrate its action on standards II and I, respectively. Endrin, heptachlor, *p,p'*-DDD, *p,p'*-DDT, methoxychlor, and methyl parathion are severely attacked. As with Na_2CO_3, DDT appears to convert to DDE, and the degradation patterns for DDT and DDD seem to include several common products. Toxaphene (Figure 4*B*) is somewhat altered to lower boiling compounds, while chlordan is essentially unchanged.

$CdCl_2$. Interesting changes occur with many of the pesticides when this modifier is used. It removes methoxychlor, DDD, aldrin, dieldrin, heptachlor, methyl parathion, and toxaphene maxima at low mass levels, (Figures 2*E*, and 3*E*). An artifactual response as yet unexplained appeared (R_a = 1.04) in all the single standard chromatograms with this reagent, CuO, and $K_2Cr_2O_7$. This maximum is labeled *a* in Figures 2 and 3.

Since cadmium chloride removes the aldrin maximum, it is necessary to use some other maximum as an area standard to obtain A_a values. Accordingly, γ-BHC was assigned its unmodified A_a value of 0.8 and all other A_a values were calculated therefrom. Aldrin retention ratios were calculated assuming the unmodified aldrin retention time under standard gas pressure and flow conditions. Such assumptions introduce an uncertainty into the R_a and A_a values as tabulated for $CdCl_2$ in comparison to those for other modifiers, although they are self-consistent. The major justification for choosing γ-BHC as an area standard is that it appears to be the least changed of all pesticides used, having a peak area about equal to its unmodified value.

On this basis it appears that α-, β-, and δ-BHC are improved as electron capturers by $CdCl_2$. The maximum at R_a = 0.28 in the α and δ chromatograms agrees with the value for HCl, and dehydrochlorination of these materials

Table III. Characteristic Modifications of Pesticides by Selected Reagents

Pesticide	Unmodified		Na_2CO_3		$AlCl_3Al$		CuO		$CdCl_2$		$K_2Cr_2O_7$	
	R_a	A_a	R_a	A_a	R_a	A_a	R_a	A_a	R_a[a]	A_a[b]	R_a	A_a
Miscellaneous Pesticides												
p,p'-DDE	1.85	1.0	1.45	0.001	1.86	1.0	1.81	1.0	1.67	0.0004	1.54	0.0006
			1.86	1.0					2.14	0.9	1.93	1.0
α-BHC	0.40	0.3	...	...	0.41	0.4	0.40	0.4	0.29	0.003	0.28	0.02
									0.42	0.8	0.43	0.4
β-BHC	0.42	0.08	...	...	0.50	0.06	0.45	0.05	0.48	0.2	0.32	0.002
											0.57	0.06
δ-BHC	0.49	0.3	...	...	0.28	0.03	0.54	0.5	0.28	0.02	0.28	0.08
					0.58	0.5			0.56	0.9		
Methyl parathion	0.75	0.4	...	...	0.95	0.05	0.92	0.009	...	...	...	...
Chlordan and Toxaphene												
Chlordan	0.71	0.06	0.48	0.008	0.70	0.06	0.45	0.005	0.63	0.004	0.47	0.01
	0.78		0.69	0.02	0.77		0.56		0.87	0.005	0.56	
	0.95	0.02	0.81	0.03	0.94	0.03	0.69	0.04	1.10	0.08	0.72	0.03
	1.15	0.01	1.01	0.09	1.16	0.02	0.91	0.03	1.60	0.04	1.00	0.03
	1.43	0.2	1.40	0.007	1.42	0.2	1.12	0.01	1.77		1.24	0.01
	1.59		1.85	0.0003	1.57		1.39	0.2	2.60	0.009	1.60	0.2
	2.50	0.03			2.50	0.03	1.54		3.16	0.001	2.10	0.004
							2.40	0.02			2.60	0.02
											3.12	0.0008
											3.56	0.0008
Toxaphene	1.29	0.6	0.15	0.1	0.80	0.5	0.75	0.4	0.37	0.0003	1.23	0.4
	1.79		0.26		1.31		0.96		0.67	0.001	1.45	
	2.03		0.33		1.80		1.16		1.98	0.002	1.71	
	2.28		0.55		2.05		1.28		3.27	0.002	1.86	
	2.56		0.71		2.31		1.63				2.14	
	3.05		0.99		3.09		1.97				2.31	
	3.62		1.32		3.72		2.23				3.17	
	4.34		1.62				3.01				3.82	
	5.02		1.78				3.65					

[a] Calculated on basis of assumed value of aldrin retention time equal to that in unmodified system. [b] Calculated on basis of assumed value of 0.8 for γ-BHC in absence of aldrin maximum.

would produce an unsaturated chlorinated ring which might well be more sensitive to electron capture detection even though total chlorine content is decreased.

Figures 4*C* and 5*C* show typical modifications produced on toxaphene and chlordan, respectively.

$K_2Cr_2O_7$. This acid oxidizer produces marked changes in γ- and δ-BHC, *p,p'*-DDT, *p,p'*-DDD, methoxychlor, endrin, heptachlor, dieldrin, and methyl parathion. Figures 2*C* and 3*C*, illustrate modifications on standards I and II. Chlordan is modified slightly (Figure 5*D*) and toxaphene is changed somewhat while still retaining the typical broad, diffuse maximum. This reagent alters dieldrin in a manner that removes its interference from the *p,p'*-DDE maximum, a valuable characteristic. However, it tends to cause deterioration of column performance, and may not be of great utility for this reason.

Conclusions

Selective removal, shifts, and enhancement of many pesticide maxima can be achieved by utilization of several of the described modifiers in parallel ahead of a single column and detector. Sequential injection of an extract into the various modifiers would produce normal and modified chromatograms. Probability of definite identifications of individual pesticides in cleaned-up plant extracts would be increased severalfold, allowing determination of several combinations interfering in the unmodified state. The savings in time as compared to carrying out such modifications on a macro scale external to the instrument prior to chromatography is evident.

The technique of direct modification of injected compounds utilizing acidic, basic, oxidizing, and reducing reagents should not be limited to this particular class of compounds. Beroza and Sarmiento (*4, 5*) have shown that catalytic hydrogenation is useful in determining structure of a number of different classes of compounds. Coupling of the two techniques in a multiple injection port, multiple column, and multiple detector system could yield a wealth of information concerning structure and class type of the compounds investigated. Work is under way in this direction.

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INSECTICIDE IDENTIFICATION

Hydriodic Acid as a New Selective Reagent for Detection of Rotenone in Chromatography

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Hydriodic acid (1 volume of 5*N* potassium iodide solution in 15 volumes of 85% phosphoric acid) produces a characteristic light blue color with rotenone useful in spot tests and in paper chromatography. After application of the reagent, the color develops within 15 minutes at room temperature. On filter paper the lower limit of detection is 4 μg. per sq. cm. Elliptone gives a pink or violet color: sumatrol, isorotenol, deguelin, dihydrodeguelin, dehydrodeguelin, tephrosin, and toxicarol are faintly visible only after several hours. Except for rotenone and elliptone, none of the materials present in crude extracts of *Derris elliptica* (Wall.) Benth. roots or *Tephrosia vogelii* Hook. f. leaves gave a color with the new reagent.

IN RECENT years there has been renewed interest in rotenone as an insecticide because of its low toxicity (*1*) to warm-blooded animals. Although rotenone has been investigated for many years, no selective reagents have been developed for its detection on paper chromatograms. Both alkaline permanganate and alkaline fluorescein followed by bromine vapor were used by Chen and Tsai (*3*) to locate rotenone on paper chromatograms, but these reagents also react with compounds other than rotenone and the rotenoids.

The chemical procedures for the determination of rotenone include extraction and crystallization (*2*), ultraviolet (*14*) or infrared (*11*) absorption, titration of the mercuric acetate adduct (*8*) or the dichloroacetic acid solvate (*9*), or reaction with sulfuric acid and sodium nitrite to produce a red color (*5, 13*), with vanillin and sulfuric acid to produce a blue color (*6*), with heavy metal oxides in sulfuric acid followed by ammonia to produce various colors (*15, 17*), or with nitric acid followed by ammonia to produce a green or a blue color (*10*). None of these procedures appears to be readily adaptable as a spray reagent for paper, and none is specific for rotenone.

During a study of the reaction of rotenone with various reagents which split ether linkages in an attempt to produce a characteristic chromophore, it was found that rotenone gives a blue precipitate with hydriodic acid (orthophosphoric acid plus potassium iodide) at room temperature. This reagent is a sensitive and selective means of detecting rotenone on paper chromatograms or in evaporated eluates from chromatographic columns.

Materials and Methods

Spray Reagent. One volume of 5*N* potassium iodide is mixed with 15 volumes of 85% orthophosphoric acid just before use. The iodide solution is stable several weeks in a refrigerator and is discarded upon turning yellow. The ratio of the two reagents is not critical; however, reducing the amount of potassium iodide solution to less than 1 to 20 makes the rotenone spots develop more slowly and fade more quickly, whereas increasing the amount to more than 1 to 10 produces a yellow discoloration of the paper.

The spray is applied to the paper until the paper appears slightly damp (approximately 1 ml. per 100 sq. cm. with Whatman No. 1 paper). The color is developed at room temperature; heating the chromatogram discolors the paper.

Purification of Rotenoid Standards. Commercial rotenone (K and K Laboratories, Jamaica, N. Y.) was recrystallized twice from carbon tetrachloride and twice from 95% ethyl alcohol. The other rotenoids were recrystallized once from each of these solvents. All compounds were dried 2 hours at 70° C. in a vacuum oven and stored over silica gel. Their purity and identity were checked by their melting points with a Fisher-Johns apparatus and noncorrosive glass cover slips.

Extraction of Plant Material. Leaves of *Tephrosia vogelii* Hook f. were collected from approximately 5-month-old flowering plants, and roots of *Derris elliptica* (Wall.) Benth. were collected from 5-year-old plants. Both materials were dried at 70° to 80° C. and ground to pass a 60-mesh screen. The rotenoids were extracted for 16 hours with 25 ml. of acetone per gram of dry plant powder. The extracts were filtered and used for paper chromatography without further purification. The amount of aliquot per chromatogram was equivalent to 4 mg. of dried plant material.

Paper Chromatography. Strips of Whatman No. 1 filter paper 5 cm. wide by 45 cm. long were cut with a bridge 0.5 cm. wide toward the bottom, according to the method of Matthias (*12*). The material to be chromatographed was spotted on the bridge and 5:1:1 methanol–benzene–acetic acid (*3*) was used for ascending development.

Results and Discussion

The hydriodic acid reagent is a sensitive indicator of the presence of rotenone on paper chromatograms, giving a characteristic blue color which increases in intensity for 15 to 30 minutes