Table II. Valencia Oranges Given Stem-End Treatment of 2-Dimethylamino-1-p-menthanol (III)

III, Concn., %	Decay, %			
	1 wk.	2 wk.	3 wk.	4 wk.
0.0 (check)	4	8	10	14
0.0001	4	10	10	16
0.001	2	4	6	14
0.01	0	6	8	16
0.1	0	4	4	8
1.0	0	0	2	6

is provided after 3 weeks' storage by the emulsion containing only 0.001% of III. The emulsion containing 1.0% of III provides very effective decay control (80%) after 3 weeks' storage at 21° and moderate control (57%) after 4 weeks' storage. Decay control refers to per cent reduction of decay based on the untreated check. This decay control is of the same order of magnitude as that provided by citrus fruit fungicides such as Dowicide A. However, results comparing the effectiveness of compounds III with Dowicide A are not complete. It is likely that compounds I and IV will show a similar degree of decay control, but they have not yet been tested on citrus fruits.

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PESTICIDE RESIDUE ANALYSIS

Microcoulometric Gas Chromatography of Pesticides

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A new rapid screening method of pesticide residue analysis has been developed. Quantitative analysis for several pesticides, such as γ-BHC, aldrin, dieldrin, DDT, chlordan, endrin, toxaphene, and other chlorinated organic pesticides, can be made in a single determination requiring only 1 hour. The method, based on gas chromatography and coulometric detection, can also be used for thiophosphates.

THE PROBLEM of identifying and quantitatively measuring pesticide residues on agricultural products has become increasingly difficult owing to the wide variety of chemicals used for controlling pests. The number of chemicals in use increases annually, which continually increases the number of combinations of pesticides and plant materials. In order to make the task of the analyst somewhat less difficult, a program of development of rapid screening tests for pesticide residues on fresh vegetables was undertaken. The main objective was to develop a rapid, yet sensitive, technique that would be useful for a wide variety of plant materials. In addition to good sensitivity, the method must be reasonably simple to perform, and yet it should give quantitative information concerning a wide variety of materials. At the beginning of this study, a complete survey of available equipment and methods was made, and it was decided that a new and fresh approach to the problem was needed. Such techniques as paper chromatography were considered, along with column chromatography and various classical colorimetric methods. At that time there was no published work on gas chromatography as a tool for rapid screening pesticide residue analyses. After a cursory laboratory evaluation of the potentiality of gas chromatography, it was decided that this technique, if properly developed, showed great promise (*3*). Commercially available equipment was surveyed and found to be lacking in certain necessary features for this particular problem. Therefore, a method and instrument development program was undertaken. This resulted in development of new rapid screening procedures for pesticide residues on leafy vegetables and other food products.

In order to accomplish a rapid screening procedure for a variety of pesticides, it was necessary to develop new equipment. This new equipment had to be consistent with the concept that a single catchall type of extraction would be used, followed by a rapid step in which the extract would be concentrated to a small volume, with or without additional cleanup procedures. If a cleanup procedure was necessary, it could be relatively crude.

Equipment

Figure 1 shows a block diagram demonstrating the principles on which the new instrument operates. The instrument consists of three major modules and a titration cell. The first module contains a gas chromatographic column and microcombustion furnace. The second, not shown in Figure 1, is a power supply for the gas chromatograph. The third module is the coulometer.

It was found that such chlorinated pesticides as lindane (γ-BHC), aldrin, dieldrin, endrin, DDT, toxaphene, chlordan, gamma-chlordan, heptachlor, heptachlor epoxide, ronnel, and DD could be successfully gas chromatographed, only if materials of construction were carefully selected. After considerable research it was found that a $^1/_4$-inch in outside diameter by 6-feet long aluminum column packed with 30-to-60-mesh Chromosorb (Johns-Manville Co.) coated with 15 to 30% by weight of Dow-Corning high vacuum silicone grease is a satisfactory gas chromatographic column. In order to avoid chemical effects it was necessary to acid-wash the Chromosorb with hot 6*N* hydrochloric acid, followed by a thorough

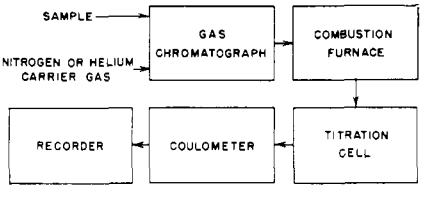

Figure 1. Block diagram of pesticide analyzer

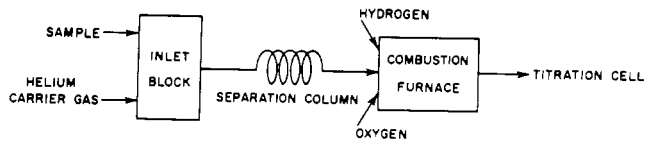

Figure 2. Block diagram of gas chromatograph

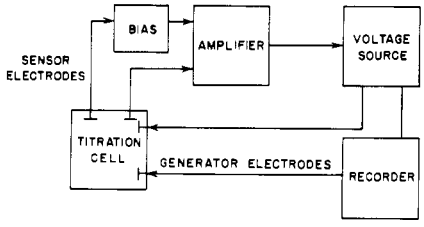

Figure 3. Block diagram of coulometer

washing with distilled water. This solid packing material is relatively inert to various chlorinated hydrocarbons, and participates in gas chromatography primarily as a solid support medium for the liquid phase. However, there is some evidence of adsorption by Chromosorb itself.

The stationary liquid phase was prepared by extracting Dow-Corning high vacuum silicone grease with ethyl acetate to remove some of the more volatile components. Complete solvent removal at this state is not necessary since solvent removal occurs during the final drying of the column packing. Some of the other liquid phases that were tested, and found to be inferior to high vacuum silicone grease, were polyethylene, several silanes, several Apiezon greases, and paraffin oil. The sample introduction block and separation column, in the column oven, and the combustion furnace (Figure 2) are combined into one module.

A coulometric detection system is used to follow the elution of the pesticides from the separation column. This system has been described by Coulson and Cavanagh (*2*). It is based on the continuous titration of chloride and sulfide with silver ions that are electrically generated in the titration cell. The success of this coulometric method of analysis was dependent on the design of a suitable titration cell. A four-electrode system is used, including two sensor electrodes and two generator electrodes. The gases eluted from the separation column are mixed with oxygen in the furnace and burned to form carbon dioxide, water, hydrogen chloride, and other inorganic substances. The technique of burning organic chlorinated pesticides in a tube furnace was reported by Agazzi, Peters, and Brooks (*1*) and by Peters, Rounds, and Agazzi (*5*). A similar combustion technique is used for the decomposition of the pesticides in the combustion furnace. These gases are then bubbled into the titration cell. Under these conditions the coulometric detector is specific for chloride (bromide and iodide would also be detected). If the combustion is followed by catalytic hydrogenation in the high temperature furnace, sulfur is converted to hydrogen sulfide and both chloride and sulfide are detected. Thus the sulfur-containing constituents may be determined by the difference between samples thermally degraded oxidatively and reductively. This makes possible the detection of both chlorine- and sulfur-containing materials by means of the coulometric system.

The coulometer is based on a continuously balancing system, in which the titrating agent present in the titration cell is kept at a fixed concentration throughout the titration. Thus, the environment within the titration cell remains constant even during intervals when sample constituents are entering the titration cell. Figure 3 is a block diagram of the coulometer portion of the pesticide analyzer. The silver ion concentration may be varied as a result of changing the bias voltage in the sensor electrode circuit. It was found that a bias voltage of +250 mv. *vs.* a saturated silver acetate electrode gives maximum sensitivity with an electrolyte in the titration cell composed of 15% water in glacial acetic acid. Under these conditions, the titration curve, a current-time curve on the strip chart recorder, follows the elution of pesticides from the column very precisely.

The electrical current used to maintain a constant silver ion concentration in the titration cell is recorded on a strip chart recorder as a function of time. The type of pesticide present may be determined by the elution time from the gas chromatograph. Calibrations may be made by measuring elution times for known pesticides. The quantity of the pesticide represented by an elution peak is determined by a theoretical calculation based on the area under the titration curve and the per cent of chlorine in the molecule. The area under the titration curve is proportional to the amount of silver ion generated. The relation between the area under an elution peak and the amount of a chlorinated organic pesticide is given by Equation 1.

The power supply module permits the temperature in the gas chromatograph to be varied over a broad range. Column temperatures may be varied from room temperature to as high as 400° C. For the common pesticides, a column temperature of 220° to 250° C. has been found satisfactory. The combustion furnace, containing a platinum gauze catalyst, is normally maintained at 800° C., but may be operated at temperatures as high as 1000° C. The power supply module also houses pyrometers for continuous indication of temperature at various critical points in the gas chromatograph and combustion furnace.

Results and Discussion

A gas chromatogram on a mixture of γ-BHC, aldrin, dieldrin, and DDT is given in Figure 4. This was based on 2 mg. each of the first three compounds and 4 mg. of DDT, using a thermal conductivity type detector. It was recognized from the outset that thermal conductivity detection would probably not be sensitive enough for the detection of pesticide residues. Consequently, research was undertaken to develop a much more sensitive and selective type detector for use in a pesticide residue gas chromatograph. For this purpose an electrochemical technique was developed that is sensitive only to chloride or chloride and sulfide, depending on the procedure of operation.

In this equipment, the gas chromatographic column operates in the conventional manner. The effluent gases from it are oxidatively decomposed and bubbled through the solution in a coulometric titration cell. The resulting inorganic chloride is coulometrically titrated, quantitatively with an automatic titrator. The coulometric method requires no empirical calibration factors for quantitative work. It is, however, necessary to determine empirically the time required for each of the pesticides to pass through the gas chromatographic separation column in order to identify the pesticide present.

Figure 5 shows a microcoulometric gas chromatographic determination of a mixture of γ-BHC, aldrin, dieldrin, and DDT. In this case, the sample consisted of 10 μl. of a xylene solution that contained 0.02% of γ-BHC, 0.04% of aldrin and of dieldrin, and 0.08% of DDT. These quantities are smaller by a factor of 1000 than necessary for the ordinary gas chromatographic procedure. The peaks are considerably sharper than those in Figure 4, indicating that with smaller

$$\text{Micrograms} = \frac{\left(\frac{\text{peak}}{\text{area}}\right) \times \left(\frac{\text{recorder}}{\text{sensitivity}}\right) \times 35.5\ \frac{\text{grams}}{\text{equivalent}} \times 60\ \frac{\text{second}}{\text{minute}} \times 10^{6}\ \frac{\gamma}{\text{grams}} \times 10^{-3}\ \frac{\text{volts}}{\text{mv.}} \times 10^{2}}{\left(\frac{\text{recorder input}}{\text{resistance, ohms}}\right) \times (\%\ \text{chlorine in pesticide}) \times 96{,}500\ \frac{\text{coulombs}}{\text{equivalent}}} \quad (1)$$

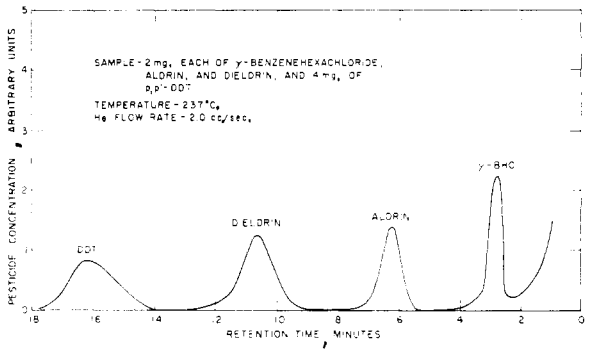

Figure 4. Gas chromatogram on a mixture of chlorinated hydrocarbons

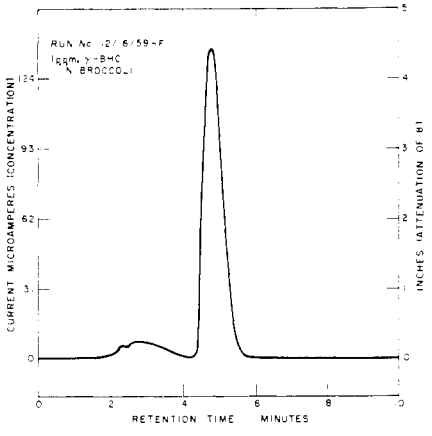

Figure 6. Gas chromatogram on broccoli extract containing γ-BHC

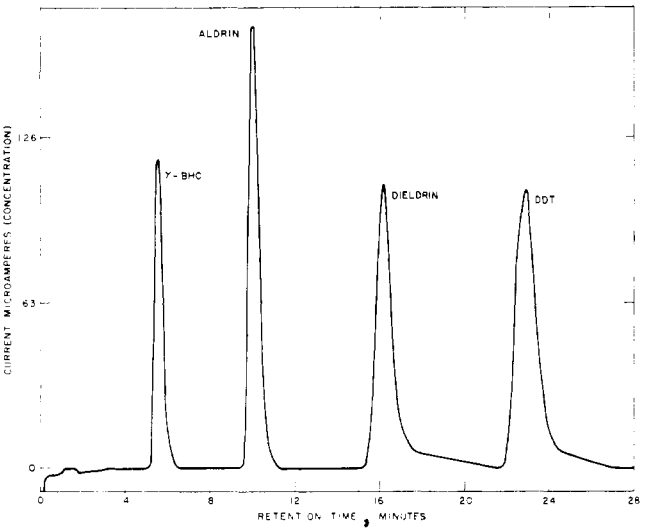

Figure 5. Microcoulometric gas chromatogram on a pesticide mixture

Temperature: 252° C.
Helium flow rate: 1 ml./second
Column: 6 feet × ¼ inch in outside diameter, 20% silicone grease on 40/60-mesh Chromosorb
Recorder: 10 mv. ordinate, 0.5 inch/minute; abscissa attenuation factor of 8

quantities of pesticide the gas chromatographic behavior improved.

Figure 6 is a gas chromatographic determination on broccoli fortified with γ-BHC. In this case, the sample introduced into the gas chromatograph represented 1 gram of broccoli and the peak shown on the figure represents 1 γ of γ-BHC. The quantity of γ-BHC represented by this peak can be calculated from theoretical factors, including Faraday's constant and the per cent of chlorine in γ-BHC. Quantitative recovery was obtained in this case. The only factor that is empirical in this analysis is the retention time. Under the conditions of operation of the gas chromatograph used for this experiment, aldrin would be eluted in 10 minutes, dieldrin in 16 minutes, and DDT in approximately 23 minutes.

Earlier laboratory models of the equipment gave somewhat less than quantitative recoveries, oftentimes 50% or lower. As the equipment was improved and the problems became somewhat better understood, recoveries were improved to the point where it is now possible to get virtually quantitative recoveries for the common chlorinated hydrocarbon-type pesticides.

Table I gives results for γ-BHC, aldrin, and dieldrin standards. In each case the pesticide concentration was 0.10% in xylene. Two types of problems resulted in losses during preliminary experiments not reported here. One cause of low results was the complexity of the gas chromatographic system which contained a number of components coupled together mechanically. This problem was overcome by designing a complete unit with the gas chromatographic oven combined with the combustion furnace. A second cause for losses was decomposition of certain pesticides in the gas chromatographic system. Aldrin has little tendency to decompose during gas chromatography under the conditions used in these experiments. The most serious decomposition problems were observed with DDT, while γ-BHC gave some difficulty. In these cases, it was necessary to precondition the gas chromatographic system with preliminary samples before good recoveries could be obtained. This problem was solved in part by developing an improved column packing material.

Table II contains typical results for aldrin residues in lettuce and broccoli extracts. In every case, these extractions were made, after the addition of the pesticide directly to the chopped material, with either a tetrahydrofuran-hexane or an acetonitrile-hexane mixture. These results, therefore, do not represent studies of extraction efficiency, but they can be used to demonstrate the usefulness of the gas chromatographic, microcoulometric technique on crude extracts of vegetables without previous cleanup procedures. A complete determination may be completed within 1 to 2 hours from the time the experiment is undertaken until the final result is calculated. The procedure involves chopping the vegetable, followed by a Waring Blendor-type extraction, removal of 95 to 99% of the solvent, followed by gas chromatographic, microcoulometric analysis of the concentrate. These extracts were dark green and contained large quantities of plant pigments. Most residue methods require rather elaborate cleanup procedures, prior to the measurement step. In the case of microcoulometric gas chromatography, the cleanup and measurement steps have been combined. The gas chromatographic column performs the cleanup and the titration cell and coulometer measure the amount of each pesticide. For the purposes of detecting the presence of any one of several unknown pesticides on a plant material, it is desirable to minimize the amount of cleanup preceding the measurement step in order to avoid partial or complete losses of pesticides during the cleanup. If, after having established the presence of a particular pesticide, it is desirable to make a more sensitive measurement, it then would be appropriate to perform a suitable cleanup procedure prior to subjecting the sample to microcoulometric gas chromatography. In this case, an even greater degree of sensitivity can be reached due to additional sample concentration that can be effected.

To date this research has involved samples of uncontaminated vegetables to which known quantities of pesticides were added. It is now felt that the techniques have been developed to the point where practical problems can be successfully undertaken. This has been done to a limited extent. The gas

chromatographic, microcoulometric procedure was employed on several samples of potatoes grown under controlled conditions in which dieldrin (Shell Development Co.) had been applied in known quantities to the soil. In one sample, to which dieldrin had been applied at the 0.1 p.p.m. level in the soil before the potatoes were planted, approximately 0.06 p.p.m. of dieldrin was found when the potatoes were harvested 109 days after planting. A somewhat smaller quantity of dieldrin was found to be present in potatoes harvested 116 days after planting. It was convenient to extract the potatoes with acetonitrile, hexane, and water, with the pesticides partitioning into the hexane phase. Sucrose was used to prevent stable emulsions. The hexane phase was then evaporated to a small volume in a Kuderna-Danish evaporator. An aliquot of the concentrate, representing approximately 20 grams of potatoes, was injected into the gas chromatograph.

The new microcoulometric, gas chromatographic procedure was also employed in the analysis of several hay and silage samples. The results of the gas chromatographic analyses on Skellysolve B extracts are given in Table III.

Samples number 2 and 8 were pea hay. Samples number 10 through 13 were alfalfa hay, and the others were pea vine silage. As a check on the gas chromatographic, microcoulometric procedure samples number 2 and 6 were analyzed by the classical nitration, colorimetric method (*6*). The results were 122 and 9 p.p.m., respectively. This excellent check with the gas chromatographic procedure indicates that the rapid gas chromatographic microcoulometric procedure is capable of yielding quantitative results for DDT on hay and silage.

In these experiments, the total elapsed time from beginning to end can be as little as 1 hour. The instrument time per sample may be from 5 to 20 minutes, depending on the time required for the pesticide present to go through the gas chromatographic column. As soon as one sample is completed, a second sample may be injected into the gas chromatograph without changing the solution in the titration cell or making any other modifications of the equipment. In the case of excessively oily plant materials, and meat and dairy products, it may be necessary to carry out a separation procedure prior to subjecting the sample to microcoulometric gas chromatography. Certain chlorinated organic pesticides can be separated almost quantitatively from butter by a simple liquid-liquid partition procedure using acetonitrile and hexane as the partitioning solvents. This procedure is commonly used in the preparation of butter samples for paper chromatography (*4*).

It is also possible to measure sulfur-containing materials such as thiophosphates by the same method, with a minor modification in operating procedure. In this case, after gas chromatographic separation, the pesticides are decomposed under reducing conditions; this results in the formation of hydrogen sulfide which is then titrated in the coulometric titration detector. Malathion, parathion, and Systox have been successfully determined by this procedure. Under these conditions, both classes of pesticides, the chlorinated organic compounds and the thiophosphates, are detected and quantitatively determined.

Although most of the research to date has been on pesticides, the new coulometric, gas chromatographic apparatus will certainly find broad application in the fields of food additives, flavor constituents, petroleum chemistry, health research, and other problems dealing with volatile organic materials.

Research on specific detection, in gas chromatography, of elements other than sulfur and chlorine is in progress. It now appears that specific and theoretically quantitative detection of hydrogen and carbon is possible. With the coulometric method the specific detection of functional groups such as amines and aldehydes is also possible.

Table I. Pesticide Standards by Gas Chromatography

No.	γ Taken	γ Found	Recovery, %
	Lindane		
95	10	0.6	6
96	10	3.0	30
98	10	5.7	57
100	10	7.1	71
12-3-59C[a]	5	5.2	104
12-15-59H[a]	2	1.9	95
12-16-59B[a]	1	1.1	110
	Aldrin		
170	10	9.4	94
37	5	4.2	84
81	5	5.5	110
61	2.5	2.1	84
12-15-59M[a]	4	4.2	105
12-15-59N[a]	3	3.8	93
	Dieldrin		
45	20	13.8	69
88	10	5.3	53
62	5	3.8	76
12-15-59J[a]	8	7.4	93
12-29-59A[a]	4	4.1	102
12-29-59B[a]	4	4.1	102

[a] Results obtained with prototype equipment.

Table II. Aldrin in Vegetable Extracts

No.	P.P.M. Taken	P.P.M. Found	Recovery, %	Vegetable
74	5.0	6.4	128	Lettuce
108	1.0	0.94	94	Lettuce
159	0.25	0.36	144	Lettuce
13[a]	10	10.0	100	Broccoli
14[a]	5.0	4.8	96	Broccoli

[a] Results obtained with prototype equipment.

Table III. Gas Chromatographic Results for Hay and Silage

No.	DDT, P.P.M. Gas Chromatography	DDT, P.P.M. Schechter-Haller (6)
1	90	...
2	130	122
3	0	...
4	3	...
5	6	...
6	10	9
7	1	...
8	0.5	...
9	5	...
10	0.3	...
11	0.6	...
12	0	...
13	0	...

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