Simple Apparatus for Combustion of Samples Containing C^{14}-Labeled Pesticides for Residue Analysis

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A simple apparatus is described for the combustion of biological samples containing C^{14}-labeled pesticides. The apparatus can be used to trap the liberated radioactive carbon dioxide as barium carbonate or in ethanolamine for scintillation counting. The apparatus is rapid, simple, safe to operate, and can be used with any type of biological sample.

Numerous methods have been described for the combustion of organic compounds for the micro determination of their carbon-14 content (*1-4, 6-14*). From a pesticide standpoint, all these methods have one or more of the following disadvantages. Most of the procedures were designed for the combustion of small samples (1 to 25 mg.) of homogeneous material. In pesticide analysis, large samples (1 to 2 grams) must be used to minimize sample error. Most of the equipment used in the liquid combustions must be continuously attended, and since generally no provisions are made to prevent the trapping solution from being drawn backwards into the combustion liquid, explosions result. The dry combustion procedures were generally too time-consuming for routine analysis, and automatic equipment is quite expensive. In many cases, the procedures were unsatisfactory when easily combustible materials (fat) were employed.

To develop a satisfactory method for combusting and counting samples containing carbon-14, the more promising procedures were tested (*1-4, 6-14*). Generally, the dry combustion procedures were too time-consuming even with automatic equipment. One to 2 hours were required for adequate combustion.

The authors concluded that a wet combustion would be most satisfactory if a suitable apparatus could be designed for combusting large samples. In addition, the apparatus should be versatile so that the radioactive carbon dioxide could be either counted as a gas or as barium carbonate, or trapped in a liquid for scintillation counting.

The present paper describes a procedure and apparatus developed several years ago and employed in several Dow laboratories for routine pesticide investigations involving carbon-14.

Apparatus

The combustion apparatus shown in Figure 1 consists of four main parts—a carbon dioxide scrubber (Corning No. 440170), a combustion unit, iodine trap, and carbon dioxide absorber.

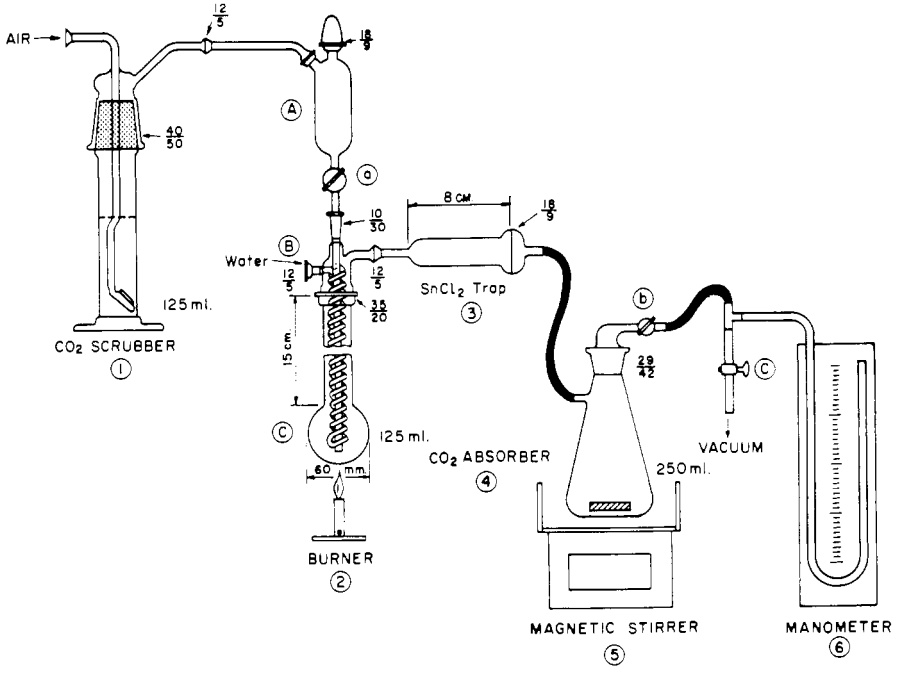

Figure 1. Combustion apparatus for scintillation counting procedure

The combustion unit is constructed so that the stem of the reservoir (*A*) extends through the double-coiled condenser (*B*) to within 5 cm. of the bottom of the combustion flask. The condenser and the stem of the reservoir nearly fill the neck of the combustion flask. The water inlet for the condenser is shown in Figure 1, while the water outlet is behind the plane of the drawing and is not shown.

The bottom of the absorption flask is blown out slightly to form a well which tends to collect the absorbing solution at the bottom and permits use of minimum volumes of the absorbing liquid.

Reagents

Ethanolamine solution. Mix 7 volumes of methylcellosolve with 3 volumes of ethanolamine. This solution should be prepared fresh daily and kept in a closed container.

Scintillation solution. Dissolve 8.25 grams of 2,5-diphenyloxazole (PPO) in 1 liter of redistilled toluene.

Van Slyke General Combustion Reagents (*14*).

Stannous chloride. (Stannochlor; Metal and Thermit Corporation, Rahway, N. J.).

Sample Preparation

For combustion, all biological samples can be grouped into four classes—homogenates, tissue slices, extracts, and dried powders. Each of these required special handling.

Whole organs from small animals are ground in a glass homogenizer (Corning No. 7725) with an equal weight of water. Aliquots of the homogenate are weighed into tared glass cups 18 mm. in diameter, and 18 mm. deep. The weighed samples are stored in the frozen state until combusted. The frozen samples are added to the combustion flask. Organs from large animals, such as dogs, cattle, or sheep, are homogenized by repeatedly (seven to 10 times) grinding them in an electric meat grinder. The subsamples are prepared from the homogenate.

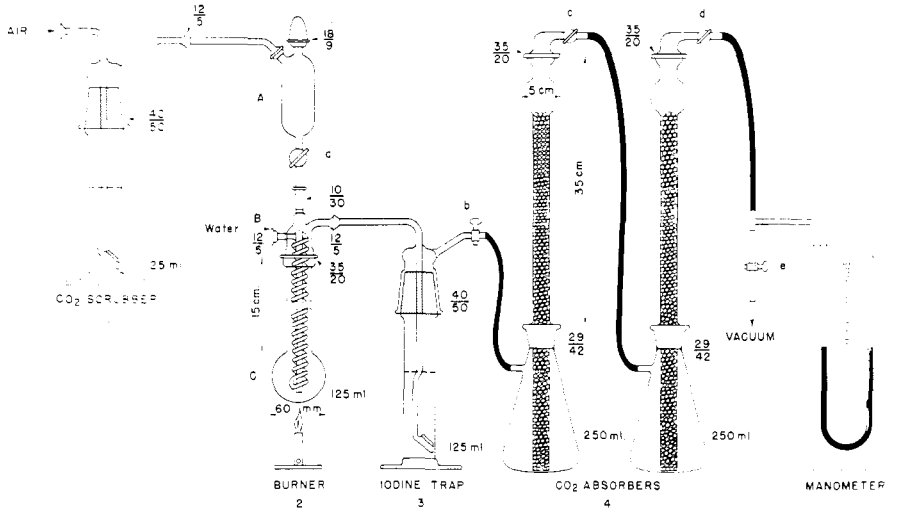

Figure 2. Combustion apparatus for $BaCO_3$ counting procedure

Subsamples of muscle, fat, etc., can be obtained by sectioning with a scalpel and combining random sections. Tissue slices placed directly into the combustion flask tended to float on top of the combustion liquid, resulting in incomplete combustion. This difficulty was alleviated by placing the tissue slices in short sections of glass tubing (35- × 8-mm. o.d.). This technique submerges the sample, retards the rate of combustion, and thus gives more reliable results. Sample weight should be determined before freezing as the tissue will dehydrate during storage.

In many biochemical investigations, individual tissue components are isolated from the tissues by solvent extractions. In many cases, it is desirable to know the amount of radioactivity in each phase of the extraction system. Under these conditions, the tissue component is generally present in a relatively large volume of combustible solvent which must be removed prior to combustion. The solvent is removed by evaporating the solution on strips of filter paper.

Many tissue components are isolated as dried powders. These powders tend to float on the surface of the combustion liquid and incomplete combustion results. To correct this, the cups containing the sample were wrapped with glass wool. Most ground plant samples are handled in this manner.

Procedure

Into the absorption flask is pipetted 10 ml. of the ethanolamine solution. The flask is placed in an ice and $CaCl_2$ (−5° C.) bath on a magnetic stirrer regulated to turn slowly without splashing. The flask and contents are allowed to cool while the rest of the apparatus is set up. The tared sample is placed in the combustion flask (*C*), and 8 grams of dry digestion reagent are added. The apparatus is assembled using 85% phosphoric acid to lubricate all the joints. Stopcock (*a*) is closed and 50 ml. of the liquid digestion reagent is introduced into the dropping funnel (*A*). The water is started flowing through the condenser, and the system is evacuated to 10 mm. Hg or less. Stopcock (*c*) is closed, the system is checked for leaks, and if airtight, stopcock (*b*) is closed.

The liquid digestion reagent is allowed to run slowly into the combustion flask, and stopcock (*a*) is closed while a small quantity of the liquid remains in the funnel. The liquid is allowed to react with the sample for 5 minutes before combustion. The combustion flask is then heated slowly until the liquid starts to boil. Boiling is then continued vigorously for 2 to 3 minutes.

After the sample has cooled for 5 minutes, the vacuum is slowly released by opening stopcock (*a*). The sample is allowed to stand 15 minutes to ensure complete absorption of the carbon dioxide. The absorption flask is disconnected, and with a pipet, the sides of the flask are rinsed with 20 ml. of methylcellosolve.

The contents of the flask are mixed by swirling and allowed to warm to room temperature. Ten milliliters of this mixture are pipetted into a scintillation vial containing 10 ml. of the toluene scintillation solution. The sample is mixed and, after precooling, counted in the scintillation counter.

A reference standard is prepared by mixing 10 ml. of scintillation solution, 8 ml. of methylcellosolve, 1 ml. of ethanolamine, and 1 ml. of a methylcellosolve solution of the radioactive compound having a known specific activity. A scintillation blank is prepared by mixing 10 ml. of the scintillation solution with 9 ml. of methylcellosolve and 1 ml. of ethanolamine. The quantity of the radioactive compound can then be calculated from the net c.p.m. of the standard

If the apparatus is to be used in trapping the carbon dioxide as barium carbonate, two modifications shown in Figure 2 are used. The iodine trap in Figure 1 is replaced with a gas-washing bottle (Corning No. 440170) which is about half filled with anhydrous crystalline stannous chloride. Two carbon dioxide absorption towers (Figure 2, No. 4) are used in place of the single absorption flask. The absorption towers (2.5 cm. in diameter) extend to within 2 to 3 mm. of the bottom of the flask and are filled with 5-mm. glass beads. Approximately 1 cm. above the bottom of the tower are four finger indentations to support the glass beads.

The combustion procedure is the same as the one previously described except that 2*N* NaOH (20 ml.) is used in the first absorber and 1*N* NaOH (20 ml.) is used in the second absorber instead of the ethanolamine solution. The system is evacuated, with stopcocks open, to 10 mm. Hg or less, and then stopcocks (*b*), (*c*), and (*d*) are closed. When the combustion is completed and the flask has cooled for 5 minutes, stopcock (*b*) is opened slowly. After a static system is obtained, stopcock (*c*) is opened, and finally stopcock (*a*). A slow stream of air is drawn through the apparatus by means of a vacuum for 10 minutes.

The apparatus is disassembled, and each absorption tower is washed with 80 ml. of warm water and allowed to drain directly into the flask. Each tower is then washed with 10 ml. of water containing phenolphthalein indicator to ensure that all the sodium hydroxide has been removed. Seven milliliters of 40% barium chloride solution is added to the first flask and 3 ml. to the second. While still warm, each flask is titrated with 1*N* HCl until a pink color persists (*5*).

The quantity of barium carbonate formed can then be calculated from the titration. The volume of the barium carbonate suspension is adjusted to 300 ml. and made slightly basic with a few drops of 1*N* NaOH and placed in a 500-ml., glass-stoppered Erlenmeyer flask. A stirring bar is inserted and the suspension heated to incipient boiling and maintained at this temperature for about 1 hour. The suspension is cooled overnight to room temperature. Aliquots of the barium carbonate suspension containing 80 mg. of barium carbonate can be plated using Tracerlab E-29 filter tower apparatus. The precipitate is rinsed successively with water, acetone, and ether. While the precipitate is still wet, the tower is disassembled, and the paper disk and precipitate are transferred to a Tracerlab E-7B sample holder and air-dried.

The samples can then be counted and the amount of radioactivity in the sample determined by comparison with standard samples of barium carbonate.

Table I. Comparison of Carbon Recoveries Obtained with Biological Samples Combusted by Wet and Dry Combustion

Sample Wt., Mg.	Carbon, % Wet combustion		Dry combustion[a]
Benzoic Acid			
96.9	68.4		68.8
121.8	68.6		68.7
95.8	69.0		68.7
77.6	68.7	Av.	68.7
50.2	67.6		
53.0	68.6		
	Av. 68.5		
Starch			
116.6	41.6		41.9
141.6	41.2		41.8
115.4	41.0		41.8
99.4	41.4	Av.	41.8
104.8	41.6		
103.1	41.6		
	Av. 41.4		
Egg Albumin			
101.5	46.2		46.9
106.9	46.3		46.8
100.6	46.2		47.0
104.6	46.6	Av.	46.9
124.2	46.5		
120.8	46.6		
	Av. 46.4		
Sucrose			
100.1	42.4		42.3
107.3	42.2		42.4
99.7	42.5		42.4
88.8	42.6	Av.	42.4
93.6	42.3		
51.0	42.7		
	Av. 42.4		
Beef Fat			
126.6	72.8		74.3[b]
134.9	71.8		75.4
130.4	72.3		72.4
129.1	71.6		73.2
	Av. 72.1		71.4
		Av.	73.3

[a] Microcombustion using 5-mg. samples. [b] Macrocombustion using 1-gram samples.

Table II. Results of Duplicate Combustions of Animal Tissues

Sample No.	Wt. Tissue Combusted, Mg.	Wt. Carbon, Recovered, Mg.	Carbon in Fresh Tissue, %
Liver Homogenates			
1	791.0	107.9	13.6
	794.8	108.0	13.5
2	537.4	77.0	14.3
	578.8	80.4	13.9
3	537.5	66.7	12.4
	499.8	63.1	12.6
4	510.1	78.4	15.4
	508.0	76.1	15.0
5	551.0	74.4	13.5
	522.9	72.8	13.9
6	537.4	74.0	13.8
	513.0	68.1	13.3
7	539.1	79.3	14.7
	505.8	72.8	14.4
Chicken Breast Muscle			
1	695.3	87.2	12.5
	684.9	85.2	12.4
2	765.0	102.9	13.4
	663.2	88.5	13.3
3	650.8	86.5	13.3
	623.0	84.1	13.5
4	716.6	89.3	12.5
	720.7	90.7	12.6
5	723.1	94.3	13.0
	662.6	89.2	13.5
6	724.7	91.3	12.6
	756.1	93.8	12.4
7	759.7	96.8	12.7
	565.5	72.7	12.8
8	774.7	98.4	12.7
	686.0	86.1	12.6

Table III. Results Obtained by Combusting Various Plant and Animal Tissues Containing C^{14}-Labeled Compounds

Tissue Analyzed	Wt. of Tissue, Grams	Net C.P.M. Per Sample	P.P.M. Compound in Tissue
Scintillation Counting Background, 46 ±1 c.p.m.			
Coccidiostat, 1 mc. per mmole			
Muscle	0.795	28	0.03
	0.496	18	0.03
Spleen	0.498	36	0.06
	0.517	39	0.06
Liver	0.472	149	0.27
	0.504	135	0.23
Kidney	0.491	29	0.05
	0.538	32	0.05
Lung	0.521	70	0.12
	0.536	73	0.12
Skin	0.307	35	0.10
	0.359	43	0.10
Fat	0.273	11	0.03
	0.274	12	0.03
Red cells	0.549	210	0.33
	0.539	188	0.30
Herbicide, 0.98 mc. per mmole			
Roots	1.000	30	0.004
	1.050	32	0.004
Stems	0.546	177	0.04
	0.550	180	0.04
Leaves	0.171	97	0.07
	0.183	100	0.07
Barium Carbonate Background, 16 ± 1 c.p.m.			
Coccidiostat, 3.45 mc. per mmole			
Lung	0.568	149	1.89
	0.623	151	1.88
Muscle	0.820	6	0.12
	0.814	6	0.12
Blood	0.777	75	0.69
	0.714	72	0.72
Gizzard	0.746	11	0.16
	0.715	11	0.15
Liver	0.133	10	0.13
	0.162	10	0.12
Fat	0.181	11	0.78
	0.185	12	0.77
Skin	0.210	35	1.24
	0.220	30	1.22
Herbicide 1.0 mc. per mmole			
Roots	0.407	4	0.03
	0.330	5	0.04
Stems	1.147	4	0.12
	0.941	3	0.11
Leaves	1.180	13	0.04
	1.432	15	0.05

Discussion

In some of the procedures described in the literature, a small amount of radioactivity is retained in the equipment, and it is necessary to precondition the equipment and to limit samples to those containing approximately the same quantity of radioactivity.

To ascertain whether radioactivity was retained in the equipment, samples of radioactive sodium bicarbonate were prepared on the filter paper and subjected to the combustion procedure. The concentration of sodium bicarbonate used was kept constant, while the specific activity was varied over a 100-fold range. This was accomplished by mixing cold sodium bicarbonate with radioactive sodium bicarbonate.

When barium carbonate counts were made, average recovery was 98.6 ± 0.3% in eight determinations, but when scintillation counting was done, average recovery in 10 determinations was 98.9 ± 0.8%. Similar combustions were carried out using the same amount of filter paper, but the radioactive sodium bicarbonate was added to the absorption flask. Barium carbonate counts in this case gave an average recovery of 98.2 ± 2% in eight determinations, and scintillation counting indicated an average recovery of 99.4 ± 0.4% in 10 determinations. Results indicated that no significant retention of radioactivity occurred in either modification of the combustion apparatus, nor was it necessary to precondition the apparatus or restrict the level of radioactivity in the sample.

Most procedures for the combustion of samples containing C^{14} are checked for efficiency by combusting samples of benzoic acid-C^{14} or similar nonvolatile slowly oxidized compounds. The assumption is made that if benzoic acid can be combusted completely any biological sample can be combusted satisfactorily. This is an erroneous assumption. When many of the combustion procedures described in the literature were tested with biological samples, poor recovery of carbon was obtained. Several factors were involved. With fats and other lipids, the compounds were volatilized before being combusted. With carbohydrates such as starch and sucrose, the samples floated on top and were incompletely combusted to carbon monoxide.

To determine the efficiency of the present combustion procedure, a series of biological samples was analyzed and the carbon content of each sample determined from the titration procedure. Similar samples were then analyzed by a modification of the Pregl method (*7*) used for the routine determination of the carbon content of organic compounds. The results shown in Table I indicate good agreement between both methods.

Typical results obtained when a series of duplicate combustions were run on tissues from individual animals are shown in Table II. The individual duplicates checked very closely while there was about a ±6% variation in the overall results obtained with the liver samples from various birds and about a ±3% variation with the muscle samples.

After several years of experience with this combustion method, a general guide has been established as to the optimum sample size of various tissues which can be combusted. The optimum sample size for most animal tissues is about 0.5 to 0.7 gram, but skin or dried blood samples should be about half this size. Plant samples should be 1 to 2 grams.

Typical results obtained when various tissues containing labeled compounds were combusted are shown in Table III. A background sample and standard were counted with each set of samples. The background counts shown in Table III are the average for a 6-month period. In general, the background count with both types of equipment varied less than ±1 c.p.m.

In general, scintillation counting gives the best results because of the large aliquot counted and the increased sensitivity of the method. In general, with agricultural chemicals having a specific activity of 1 mc. of C^{14} per mmole, it is easily possible to detect residues present in the tissues in concentrations of 0.02 p.p m. With barium carbonate counting, the limit of sensitivity is about 0.1 p.p.m. if 1-inch diameter plates are counted. If the level of radioactivity is too low to be detected by this method, the sample of barium carbonate can be placed in a 4.5-inch pan of Dynacon or can be converted to carbon dioxide and counted as a gas in the Dynacon.

The method described has several advantages over methods previously employed in that it permits the rapid combustion of samples containing as much as 100 mg. of carbon in an apparatus which is simple to operate and requires a minimum amount of watching. Since the samples can vary considerably in composition and weight, the operator need not know the history of the sample before combusting it. Because of the simplicity of the method, scientifically trained personnel are not needed to carry out routine combustion. Using two combustion trains, an average operator can do 16 to 20 combustions per day.

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INSECTICIDE RESIDUES

Determination of Residues of *O,O*-Dimethyl-*S*-(*N*-methylcarbamoylmethyl) Phosphorodithioate (Dimethoate) in Fruits and Forage

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An analytical method originally described for the determination of diazinon was adapted for the analysis of dimethoate residues on alfalfa, apples, grapes, and cherries. The method is based on the extraction of the insecticide from a hexane solution with hydrobromic acid, followed by hydrolysis and determination of the evolved hydrogen sulfide as methylene blue.

THE INSECTICIDE dimethoate [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] is not only effective for the control of aphids and mites on a variety of fruits and vegetables (*1, 5, 9, 11, 12*) but also shows promise as a systemic insecticide for the control of grub on cattle (*3*).

The increasing importance of this insecticide necessitated the development of a rapid, sensitive method for the analysis of residues on agricultural crops. Existing methods were investigated and found to lack the desired sensitivity of 0.1 p.p.m. for the crops under investigation (*1, 8, 10*). Chilwell and Beecham (*2*) have recently reported a method for the residue analysis of dimethoate which appears to have broad application. The authors have described a semiquantitative method for dimethoate in milk (*4*), but there remained a need for a general method for plants which would have the desired sensitivity.

The method of Suter *et al.* (*13*) for the analysis of residues of *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate (diazinon) is relatively simple and is highly sensitive, and it was reasoned that this method could be adapted to the determination of microgram quantities of dimethoate, since both compounds yield H_2S when boiled in an acid solution.

The method as modified consists of extracting the plant material with either chloroform or aqueous acetone and then, after a suitable cleanup procedure, re-extracting the dimethoate from *n*-hexane with hydrobromic acid. Upon boiling the acid solution, the dimethoate sulfur is converted to hydrogen sulfide