

oxidized after formation or that the hydrolysis of the P—S—C bond in dimethoate always follows a preliminary phosphorothionate oxidation.

Hydrolysis at the alkoxy group occurred mainly inside leaves of corn, cotton, and potato, indicating an enzymatic action at this site. Degradation at the alkoxy group has been reported for mammals (5, 7, 9, 10), but this is the first similar observation in plants.

Hydrolysis in and on the surface of mature pea plants was greatly different from the other plants in that phosphoric acid was the major derivative formed in peas but was absent in corn, cotton, and potatoes. The explanation of this striking difference with peas is not known.

When dimethoate was administered to mammals, the major metabolite excreted was the thio-carboxy derivative (5). As none of this derivative was found in or on plants, the formation of the oxy-carboxy derivative probably proceeded by oxidation of dimethoate to its oxygen analog first and then by hydrolysis of the carbamoyl group. As the oxy-carboxy derivative was found in largest amount on the surface, the carbamoyl bond cleavage was probably nonenzymatic and it is unnecessary to postulate an enzyme hydrolyzing the C—N bond of this compound in plants.

In a previous study on dimethoate metabolism in mammals (5), no oxygen analog was isolated although there was

indirect evidence for its formation. The plant studies reported here were made with a phosphorus-32 dimethoate sample of five times greater specific activity, which may partially account for the ability to detect the oxygen analog in plants but not mammals. An unidentified dimethoate metabolite reported in the mammalian study and designated as "unknown A" (5) has the same properties as the oxy-carboxy derivative found in plants.

The pathway of dimethoate degradation in plants is summarized in Figure 3.

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PESTICIDE RESIDUES

Rapid Combustion and Determination of Residues of Chlorinated Pesticides Using a Modified Schöniger Method

CHLORINE has been removed from chlorinated pesticides prior to their determination as residues, by combustion and sodium reduction. Schöniger (2, 3) used an oxygen-filled flask to burn samples in a rapid combustion method for the microdetermination of halogens and sulfur in organic compounds. In the work reported, an oxygen-filled flask with a balloon attached for pressure control is used for combustion of residues of chlorinated pesticides.

A benzene extract of alfalfa containing the pesticide is evaporated in a cone of cellulose acetate. The cone and contents are burned in the flask and the hydrogen chloride gas is absorbed in dilute sodium hydroxide. A specially designed platinum holder permits complete combustion of the residue with no carbon for-

mation. Chloride is determined spectrophotometrically by an adaptation of the method of Bergmann and Sanik (1) involving displacement of thiocyanate by chloride in the presence of ferric ion.

Description of Combustion Flask

The combustion flask and platinum holder are illustrated in Figure 1 (A and B).

A 1-liter borosilicate glass Erlenmeyer flask with a 34/28 standard-taper, female ground joint sealed on the neck is used. A side arm (1.5 cm in outside diameter, 7 cm. long) is sealed to the flask at the base of the shoulder as shown. A rubber balloon about 7 cm. long is secured to the side arm with string. A small rubber band is placed on the arm as shown.

DONALD J. LISK

Pesticide Residue Laboratory, Department of Entomology, New York State College of Agriculture, Cornell University, Ithaca, N. Y.

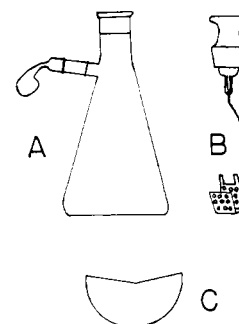


Figure 1. Combustion apparatus

- Flask
- Platinum holder
- Plastic cone template

The platinum holder is constructed by sealing a 13-cm. length of No. 16 B and S gage platinum wire onto a 34/28

A method is described for the rapid combustion and determination of residues of chlorinated pesticides. Organic solvent extracts of plant samples are evaporated in a cone of cellulose acetate, which is burned in an oxygen-filled (Schöniger) flask and the hydrogen chloride gas is absorbed in dilute sodium hydroxide. The platinum holder has been specially designed and a balloon attached to the flask to permit complete combustion of the cone and its contents safely. The spectrophotometric determination of chloride is based on its displacement of thiocyanate in the presence of ferric ion. The time required for combustion and determination of chloride is about 20 minutes per sample. The recovery of DDT, Thiodan, and lindane from alfalfa by the method is described.

standard-taper, male ground joint which has been drawn out 4 cm. below the ground portion. At the lower end, 2 cm. of wire are bent to form a right angle. A piece of Style 4, perforated platinum sheet (J. Bishop and Co. Platinum Works, Malvern, Pa.), 2 cm. wide and 4 cm. long, and bent as shown is electrically welded to the wire at the lower end. The two platinum tabs (4 by 12 mm.) may be welded on or cut out as part of the sheet.

Preparation of Plastic Cones

Prepare the cones from S-600, 1-mil cellulose acetate film (Celanese Corp. of America). Wearing cloth gloves, cut the plastic using a cardboard template having the shape (4-cm. radius) shown in Figure 1, C. Wash each piece of plastic by dipping for 10 seconds (use forceps) in 6*N* sodium hydroxide warmed to about 60° C. in a 1-liter beaker. Rinse thoroughly in running distilled water and hang in a spring clamp, allowing excess water to drip off. When most of the water has drained, remove the plastic from the clamp using clean forceps and place on a clean sheet of aluminum foil. Remove the plastic from the foil and manually join the two edges to form a

cone. Wear rubber or polyethylene gloves which have been cleaned by immersing in warm 6*N* sodium hydroxide, rinsed, and hung to dry. Heat-seal the joined edges to form a seam about 5 mm. wide. Immerse the seam for 10 seconds to about one half its width in acetone contained in a watch glass. Remove and immediately dry in the warm air stream of a hair dryer. Place each cone in a clean 65-mm., 60° funnel.

Combustion and Determination of Residues

Procedure for Combustion and Determination of Chloride. Pipet 5 ml. of the solvent extract of the sample into the cone. Evaporate the solvent using a warm air stream. Wearing rubber gloves, remove the cone and its dry contents from the funnel. Fold the cone over and up to form a small packet about 1 sq. cm. in area. Compress and place in the center of the platinum holder, which is supported from a ring stand. Fold the tabs over to hold the packet tightly between the metal surfaces. Insert a fuse (3 mm. wide and 5 cm. long, cut from Whatman No. 42 filter paper) into the top of the holder between the folds of the packet. Pipet 30 ml. of 0.08*N* sodium hydroxide into the combustion flask. Thoroughly purge the flask with oxygen. Light the fuse and place the holder in the flask immediately after about one half of the fuse has burned away. Hold the top on until combustion is complete. Keep the flask upright throughout the combustion. As soon as the balloon deflates, fasten the end of it to the side arm with the rubber band. This prevents the balloon from being drawn into the flask by the partial vacuum created. Shake the flask vigorously for 3 minutes. Remove the holder and slide the rubber band back off the balloon. Invert the flask several times so that the balloon is rinsed through the side arm by the solution in the flask.

Pipet a 15-ml. aliquot of the solution into a 50-ml. Erlenmeyer flask. Add 5 ml. of a 0.25*M* ferric ammonium sulfate solution in 9*M* nitric acid, followed by 5 ml. of a saturated solution of mercuric

thiocyanate in absolute ethyl alcohol. Mix the solution and allow it to stand for 10 minutes. Using a Beckman DU spectrophotometer, measure the absorbance of the solution at 460 m μ in a 2-cm. cell with distilled water in the reference cell. To minimize chloride absorption from the air, make the measurement promptly (7).

The calibration curve (0 to 100 γ of chloride) is prepared as follows:

Pipet 0, 2, 4, 6, 8, and 10 ml. of a standard sodium chloride solution (10 γ of chloride per ml.) into a series of 50-ml. Erlenmeyer flasks. Make up to 15 ml. with distilled water and add 5 ml. of each of the above reagents. Determine the absorbance as described above.

Results and Discussion

The method was used to recover DDT, Thiodan, and lindane from alfalfa. Samples of alfalfa to which the insecticides were added in benzene were extracted by tumbling with benzene for 20 minutes (2 ml. of benzene per gram of crop). The benzene strip solutions were filtered (Whatman No. 1) and evaporated. A 5-ml. aliquot of each sample was placed in a plastic cone and evaporated. The recoveries obtained after combustion and evaporation of chloride are given in Table I.

In those samples fortified at 1.74 p.p.m. plant extractives representing a 28.64-gram sample were burned in each cone. In those fortified at all higher levels, extractives were burned representing 14.32 grams of alfalfa. The check value of the alfalfa was about 0.60 p.p.m. as chloride.

The cone weighed about 85 mg. and the cone plus contents of the 28.64-gram sample weighed about 150 mg. The flask and platinum holder effected complete combustion of the sample with no carbon formation. Cellulose acetate burns more vigorously than paper (2, 3) and the balloon was used to allow for expansion of gases. About 200 combustions have been safely conducted in the flask. Carbon forms if the flame touches glass or the platinum wire. Therefore, the platinum holder was positioned low in the flask and the wire bent so that the flame did not contact cool surfaces.

Table I. Recovery of DDT, Thiodan, and Lindane from Alfalfa

Added, P.P.M.	DDT Found, P.P.M.	Recovery, %
1.74	1.67	96.0
3.49	3.80	109.0
6.98	6.53	93.6
6.98	6.11	87.6
6.98	6.67	95.6
13.96	14.21	101.8
13.96	12.95	92.8
	Thiodan	
1.74	1.43	82.0
1.74	1.09	62.8
1.74	1.47	84.7
	Lindane	
1.74	1.47	84.2
1.74	1.66	95.5
1.74	1.66	95.5

Also, with the flask remaining upright during a combustion, the platinum holder formed a bottom beneath the sample. The last fragment of the sample burned away upon it rather than falling into the solution. The perforations in the holder were adequate for admitting oxygen and still allowing for sufficient contact between the metal and sample. If the sample was not compressed in the platinum holder, a flame large enough to contact the glass or metal resulted and carbon was formed. Such samples were discarded. Normally after absorption of gases, the solution in the flask (without color reagents) showed an absorbance of 0.005 to 0.02 in a 2-cm. cell with distilled water as the reference.

The burning characteristics, solvent resistance, sealability, and chlorine content of about 20 plastic films were investigated. Cellulose acetate (S-600) was

the most satisfactory. It is resistant to the solvents commonly used for extraction of residues with the exception of acetone which dissolves it. The blank value of the cone and fuse was about 11 γ as chloride. The cones did not leak when made according to the procedure. Heat-sealing was necessary to form a tight seam in order that the solvent seal would be leak-proof. The capacity of the cone is 5 ml.

The flask, platinum holder, and balloon were rinsed thoroughly with distilled water after each combustion. The balloon was replaced after about 25 combustions. The standard curve follows Beer's law as long as the mercuric thiocyanate solution remains saturated. This solution is best prepared by heating, with agitation, an excess of powdered mercuric thiocyanate in absolute ethyl alcohol and allowing it to settle for 48 hours before use. The clear solution and solid

mercuric thiocyanate should be mixed, heated, and allowed to settle once every 2 weeks during use. The method should be applicable to the determination of residues of other chlorinated pesticides.

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HERBICIDE RESIDUES

Determination of Small Amounts of Arsenic in Potatoes. Extraction and Reduction of Molybdoarsenic Acid

DONALD J. LISK

Pesticide Residue Laboratory,
New York State College of
Agriculture, Cornell University,
Ithaca, N. Y.

A method is presented for the determination of small amounts of arsenic in potatoes. Potato tissue is ashed with magnesium nitrate and the ash dissolved in acid. Acid-molybdate is added, followed by extraction with a 1-butanol-chloroform mixture to remove interfering phosphorus as molybdophosphoric acid. The aqueous solution is extracted with 1-butanol to remove arsenic as molybdoarsenic acid. A simple procedure is then used for adjusting acidity and reducing to the heteropoly blue with stannous chloride in ethyl alcohol. The effect of varying the concentration of acid and reducing agent on the blue color is shown. Silicon causes some interference. Silicon interference can be reduced by use of a 1-butanol-ethyl acetate mixture for extraction of molybdoarsenic acid. The method, used to determine possible traces of arsenic in potatoes resulting from the application of sodium arsenite to kill potato vines and weeds, yields an average recovery of 94.1%.

A MODIFICATION of the procedure of Akkseev (7) is described by Wadelin and Mellon (9) for the removal of phosphorus prior to determining arsenic. Sodium molybdate in hydrochloric acid is added to a solution containing phosphate and arsenate ions to form the respective molybdic acids. Successive extractions of the solution with a 1-butanol-chloroform mixture remove interfering phosphorus as molybdophosphoric acid.

Arsenic is then removed as molybdoarsenic acid by extraction with butanol. It is determined by ultraviolet absorptiometric measurement of 12-molybdoarsenic acid. The method is not quite sensitive enough for determining traces of arsenic in biological material.

In this paper, the method of Wadelin and Mellon is used to separate phosphorus and arsenic contained in an acid solution of potato tissue-magnesium

oxide ash. A sulfuric acid-ethyl alcohol solution is then added to the 1-butanol followed by a solution of stannous chloride in ethyl alcohol which reduces molybdoarsenic acid to the heteropoly blue. Spectrophotometric measurement is then made of the blue color at 740 μ .

Method

Preparation of Standard Curve. (0 to 50 γ of arsenic.) Pipet 0-, 2-, 4-, 6-,