Table . Mass Spectrograph Analysis, Mole %

Sample No.	C_3H_6	C_2H_2	C_4H_8	Iso-C_4H_{10}	n-C_4H_{10}	Total Olefin	$HgSO_4$ Analysis, Mole % Total Olefin
1	14.0	0.5	0.3	85.2	0.0	14.3	14.2
2	13.9	0.3	0.3	85.5	0.0	14.2	14.3
3	13.3	0.5	0.1	86.0	0.1	13.4	13.3
4	7.3	0.6	0.0	91.5	0.0	7.9	8.1

D. Effect of Hydrogen and Carbon Monoxide. Samples of pure hydrogen and carbon monoxide and duplicate samples of a mixture of 51% carbon monoxide and 49% ethylene were analyzed with the mercuric sulfate solution in a pipet packed with vertical tubes, leaving the sample in the pipet 30 seconds between passes:

Passes	Pure H_2	Pure CO	Mixture (49% C_2H_4)	
	Ml.	*Ml.*	*Ml.*	*Ml.*
0	100.0	100.0	100.0	100.0
1	100.0	100.0	82.0	84.3
2	100.0	100.0	71.4	73.6
3	100.0	100.0	63.9	65.6
4	100.0	100.0	56.2	58.8
5	100.0	100.0	52.3	53.6
6	100.0	100.0	51.1	51.1
7	100.0	100.0	50.9	50.9
10	100.0	100.0	50.9	50.9

E. Commercial Samples. Twenty-nine samples of experimental gases which varied in total olefin content from 2 to 30% were analyzed both by the mass spectrograph (*8*) and by absorption into the mercuric sulfate reagent. Results by the two methods differed by an average of 0.7%, which is the order of uncertainty of the former method. Analyses of four of these samples, which happened to be free of ethylene but contained higher olefins, are indicated in Table I.

ACKNOWLEDGMENT

The authors are indebted to W. A. Stover of this laboratory for making these 29 analyses available to them.

LITERATURE CITED

(1) Adams, R., Roman, F. L., and Sperry, W. N., *J. Am. Chem. Soc.*, 44, 1781 (1922).
(2) Cuneo, J. F., and Switzer, R. L., Ind. Eng. Chem., Anal. Ed., 15, 508 (1943).
(3) Eberl, J. J., *Ibid.*, 14, 853 (1942).
(4) Gooderham, W. J., *J. Soc. Chem. Ind.*, 57, 390T (1938).
(5) Hoitsema, C., *Z. phys. Chem.*, 17, 664 (1895).
(6) Hugel, G., and Hibou, J., *Chimie et Industrie*, Special No. 296 (Feb., 1929).
(7) Matuszak, M. P., Ind. Eng. Chem., Anal. Ed., 10, 354 (1938).
(8) Washburn, H. W., Wiley, H. F., and Rock, S. M., *Ibid.*, 15, 541 (1943).
(9) Winbladh, R., *Ing. Vetenskaps. Akad. Handl.*, No. 138, (1936).

Presented before the Division of Analytical Chemistry at the Spring Meeting of the Philadelphia Section, American Chemical Society, June 13, 1945, and the Division of Petroleum Chemistry at the Meeting-in-Print of the American Chemical Society, September, 1945.

Colorimetric Determination of DDT

Color Test for Related Compounds

MILTON S. SCHECHTER, S. B. SOLOWAY, ROBERT A. HAYES, AND H. L. HALLER
Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.

A colorimetric method has been developed for the estimation of small amounts of DDT down to about 10 micrograms. The method involves intensive nitration and the production of colors by the nitrated products in benzene plus methanolic sodium methylate. This color reaction can also be used as a test for degradation products of DDT and some compounds related to it.

THE extraordinary development of the insecticide commonly known as DDT (*1*, *7*) has made the need for a sensitive method of detection and determination rather urgent. A method which could detect small amounts of DDT would find application in such fields of study as spray-residue determinations, water analyses, and pharmacological investigations. Much of the analytical work on DDT has depended on chlorine determinations. Either the "labile" chlorine split out on dehydrochlorination by alcoholic alkali can be determined, as recommended by Neal *et al.* (*21*) and by Gunther (*9*), or else the total chlorine can be determined by some method such as the Parr bomb, Carius, or Umhoefer (*26*), or by a modification of the Winter method proposed by Hall *et al.* (*12*).

The labile-chlorine method determines only 1 chlorine atom per molecule of DDT, whereas the total-chlorine methods determine 5 chlorine atoms per molecule. If DDT completely decomposes to dehydrochlorinated DDT, the former method would yield no chlorine while the latter group would determine 4 chlorine atoms per molecule. If a total-chlorine method is used as the sole method of determination, no measure of decomposition of the DDT can be obtained. Both labile and total organic chlorine must be determined in order to prove the presence of DDT or to detect its decomposition. All these chlorine determinations run into difficulty when the amount of DDT is less than about 1 mg., and they lack specificity. Furthermore, there is no method based on chlorine determinations by which the amounts of *p,p'*-DDT and *o,p'*-DDT present in mixtures can be estimated.

The terms used in this paper to designate DDT and related compounds are as follows: The generic term "DDT", originally abbreviated from dichlorodiphenyltrichloroethane, refers to the technical product, which ordinarily contains 70 to 77% of *p,p'*-DDT [1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and 15 to 25% of *o,p'*-DDT [1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenyl)-ethane]. One of the minor constituents is 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane which has been designated as *p,p'*-DDD (*22*). Gunther (*11*) has pointed out his error concerning the term "*p,p'*-DDD" made in a previous article (*10*). This compound has been named "1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane" in the present paper in conformity with the latest *Chemical Abstracts* nomenclature. The chemical composition of technical DDT is described by Gunther (*10*) and by Haller, Bartlett, Drake, Newman, and others (*13*). Dehydrochlorinated *p,p'*-DDT [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] is a decomposition product and bis(*p*-chlorophenyl)acetic acid (*8*, *27*), which has been called *p,p'*-DDA, is a metabolite of *p,p'*-DDT.

A search was made to find a suitable color reaction for DDT which could be made the basis of a colorimetric analytical method. Some of the exploratory work done in this direction is outlined below:

Tests for the trichloromethyl group using pyridine and alkali, resorcinol and alkali, or β-naphthol and alkali, as described by Snell and Snell (*23*), were all negative. Boiling ethanolic silver nitrate gave no precipitate of silver chloride. Nitration of DDT, reduction, and diazotization, followed by coupling with a suitable compound, give a color (orange with β-naphthol). Although this line of attack could probably be developed into a method for the analysis of DDT, it was not pursued further because it was

believed to be subject to interference from many aromatic compounds which could also be nitrated, reduced, diazotized, and coupled. Intensive nitration followed by reaction of an acetone solution of the nitrated derivative with alkali gives a red color. This test would also be subject to interference from many aromatic compounds (*3*). Intensely nitrated DDT gives a positive violet-red test when heated with the chemical-warfare reagent DB-3 (*28*), which might be useful for field tests. Rescently two new colorimetric methods for DDT have been described (*2*, *25*).

The method described in this paper depends on intensive nitration to polynitro derivatives and the production of intense colors upon addition of methanolic sodium methylate to a benzene solution of the nitration products (*22*). *p,p'*-DDT and *p,p'*-DDD give blue colors, and *o,p'*-DDT gives a violet-red color. Degradation products of DDT, such as dehydrochlorinated *p,p'*-DDT and *p,p'*-DDA (*3*, *27*), yield red colors. With the use of these reactions, a colorimetric analytical method was developed. Although all the factors and possible variations at each step of the analysis were not completely investigated, it is believed that the following description will satisfy the immediate need for a sensitive method of analysis for DDT by chemists, entomologists, and pharmacologists concerned with its wartime applications and public-health aspects.

APPARATUS

Glass Beads, 2 or 3 mm.

Test Tubes, 22 × 175 mm., with rims, to be used for nitrations.

Separatory Funnels, 125-ml. capacity. The glass stoppers should be ground to fit very well. By attaching them with Nichrome wire, they can be suspended loosely in the necks of the funnels while solutions are being drained. Stopcocks should be greased occasionally with a good grade of stopcock lubricant; vaseline is too thin. After each new greasing the excess grease should be removed by pouring ether or chloroform into the funnel and rotating the stopcock as the solvent drains. After each analysis the separatory funnels should be rinsed several times with warm water before being used again.

Glass Gooch-Crucible Holders. Body about 25 mm. in diameter and about 75 mm. long, stem about 30 mm. long.

SOLVENTS AND REAGENTS

Nitrating Acid. A mixture of c.p. fuming nitric acid (sp. gr. 1.49–1.50) and c.p. concentrated sulfuric acid (sp. gr. 1.84), 1 to 1 by volume.

Sodium Hydroxide Solution, 2%.

Sodium Chloride Solution. Distilled water saturated with c.p. sodium chloride. Technical salt is unsatisfactory because of dirt and colored impurities extractable by ether.

Cotton. Extracted with acetone in a Soxhlet extractor, dried for several hours at 105° to 110° C., and stored in a tightly stoppered bottle.

Ether. U.S.P. grade distilled before use. Ether that has been standing long enough to accumulate peroxides and aldehydes, or has been recovered after use in this method is unsatisfactory and should be purified before it is used again.

Benzene, c.p., dry. It is conveniently dried by distilling through a straight condenser until no more water distills over with the benzene, and then replacing the condenser with a dry one and continuing the distillation. Benzene that has been used in this method to dissolve the nitrated residues or to make dilutions thereof may be accumulated and recovered for reuse by distillation.

Sodium Methylate Solution, 10.0 ± 0.1% (concentrations are expressed as weight per unit volume throughout this paper) of sodium methylate in dry c.p. methanol (10.0 grams per 100 ml. of solution). An excellent method (*18*) of drying the methanol is to reflux with magnesium turnings (5 to 10 grams per liter of methanol) and a small amount of iodine until the magnesium has completely dissolved and then to distill with the exclusion of moisture. The solution is prepared by dissolving the requisite amount of perfectly clean sodium or a good grade of powdered sodium methylate (available commercially) in the dried methanol with cooling, using a stirrer and a reflux condenser protected by a soda-lime tube. An aliquot of a clear portion of this solution should be diluted with water and titrated with standard hydrochloric acid, phenolphthalein being used as the indicator. The concentration of the solution should be adjusted to 10.0 ± 0.1% by the addition of sodium or sodium methylate or by dilution with dry methanol.

The sodium methylate solution that is added to the benzene to develop the color should be colorless and optically clear. If the sediment does not settle completely on standing, the solution should be filtered or centrifuged. Occasionally a turbidity or precipitate of crystalline material (probably sodium carbonate) will form when the centrifuged sodium methylate reagent is added to the benzene solutions. This difficulty can be obviated largely by cooling the standardized solution in a refrigerator for a day or two, centrifuging while cold, and decanting into another container.

Acetone, technical. Redistilled before using.

PROCEDURE

Preparation of Sample for Analysis. Unless the total sample has very little DDT (less than 100 micrograms), it is advantageous to use a portion of the sample which contains a reasonably large amount of DDT (0.5 mg. to several milligrams). It will then be possible to take an aliquot at the end of the procedure for the development of the color. Extract or strip the DDT from the sample with a suitable solvent and evaporate. Using acetone, transfer the residue or an aliquot thereof to a test tube for the nitration. In some cases, the aliquot may be taken directly from the extract before its evaporation. Care must be taken not to lose any of the sample mechanically during the evaporation of solvents prior to the nitration. The best procedure for evaporating organic solvents is to add a glass bead, immerse the test tube about one third of its length in a steam bath, and shake gently until the glass bead bounces and ebullition starts. When the solvent has been completely boiled out, remove the last traces by inserting a glass tube attached to a source of vacuum one third of the way into the test tube for at least half a minute, while it is still being heated. Unless the solvent is completely removed, it may react violently with the nitrating mixture in the next step of the procedure. If benzene or an aromatic solvent has been used, add 5 ml. of ethanol and evaporate to dryness in the same manner in order to remove the aromatic solvent by azeotropic distillation.

Nitration of Sample. Cool the test tube in a beaker of cold water and with a pipet add 2.0 or 5.0 ml. of the nitrating acid. Immerse the test tube one third to one half its length in a steam bath and heat for 1 hour. Since nitrations of even small quantities of materials may sometimes be violent, safety precautions should be observed. If there is much extraneous material, it is advisable to place the test tube in ice-cold water, add cooled nitrating acid, and warm the tube cautiously to prevent a sudden or violent nitration. When the initial reaction has subsided, the tube may be heated at 100° with safety. After the 1-hour nitration, cool the test tube in a beaker of cold water, add 25 ml. of ice-cold distilled water, and mix by gentle swirling. This stops the nitration, and the test tube may be left overnight if desired.

Extraction of Nitrated Product. Rinse the contents of the test tube quantitatively through a small funnel into a 125-ml. separatory funnel with about 25 ml. of water from a wash bottle and 50 ml. of ether. A small, irregularly shaped piece of glass placed in the funnel used for the transfer will prevent the glass bead from falling into the separatory funnel. Shake vigorously for at least 1 minute. After the layers have separated clearly, draw off and discard the lower layer. Wash the ether with 10-ml. portions of 2% aqueous sodium hydroxide until the washings are alkaline; one washing may be sufficient. Then wash the ether with two 10-ml. portions of salt solution. The final salt wash should be drawn off as completely as possible. Pack a 0.75-inch plug of cotton tightly in a glass Gooch-crucible holder, moisten it with ether, and allow the ether solution from the separatory funnel to filter slowly into a 125-ml. Erlenmeyer flask. Rinse the separatory funnel with 50 ml. of ether in four or five portions, passing this ether through the cotton in the Gooch funnel. If salt crystallizes in the neck of the separatory funnel, press the stopper of the funnel in place firmly with a rotating motion to prevent leakage of ether. Add a glass bead to the Erlenmeyer flask, warm the flask on a steam bath with a gentle swirling motion until the bead starts bouncing, and recover or evaporate the ether completely. While the flask is still being heated, insert a glass tube connected to a source of vacuum two thirds of the way into the flask for at least half a minute; then remove the flask and stopper it. The analysis may be interrupted at this point if desired.

The whole extraction procedure must be done carefully to avoid any loss, such as ether sprayed from the separatory funnel when the stopcock is opened to release pressure or when the glass stopper is removed. This type of loss can be minimized by allowing time for the ether to drain away from the stopcock or the stopper before performing these operations.

DEVELOPMENT OF COLOR. At this stage there is a choice of procedures, depending on the amount of DDT expected, the amount of solution necessary for use in making the photometric measurements, and whether it is desired to have some solution left to repeat the photometric measurements.

Procedure 1. Add accurately measured amount of benzene—for example, 5.00 ml.—to the residue in the Erlenmeyer flask and swirl gently until it is dissolved. Use a volume of benzene at least equal to one third the volume necessary for use in the absorption cell or tube of the photometer. With a pipet add 2 volumes (10.00 ml. for 5.00 ml. of the benzene solution) of the sodium methylate reagent to 1 volume of benzene solution. Swirl gently until the solution is homogeneous, pour into the absorption cell or tube of the photometer, and prepare to make the most important measurements 15 minutes after the sodium methylate reagent has been mixed with the benzene. This procedure should be used only when it is known that the amount of DDT is very low and in the range where the color developed will be suitable for direct measurement in the photometer. If there is a possibility that the color developed will be too dark for direct measurement, it is preferable to use procedure 2 rather than add more benzene and sodium methylate to the colored solution to dilute it.

Procedure 2. Add a measured amount of benzene—for example, 25.00 ml.—to the Erlenmeyer flask, and swirl gently until the residue is dissolved. To an aliquot—for example, 5.00 ml.—add twice its volume of sodium methylate reagent, mix thoroughly by gentle swirling, and pour into the absorption cell or tube. In some cases it is possible to mix the solutions directly in the absorption cell or tube. If the color is too deep, a photometric measurement may be made to obtain a rough estimate. Dilute part or all of the remaining benzene solution to a more suitable volume before removing a new aliquot for development of the color. If the color is too light for good photometric measurement, rinse the pipet used for the first transfer with benzene into the Erlenmeyer flask, evaporate all the solvent on the steam bath, swirling the flask gently to start the bead bouncing, and, when all the benzene is evaporated, remove the last traces by inserting a glass tube attached to a source of vacuum. This residue in the Erlenmeyer flask should now be treated as in procedure 1.

PHOTOMETRIC MEASUREMENTS. Spectrophotometric or photometric measurements should be made at the most important wave lengths or with the most important filters as close as possible to 15 minutes after the sodium methylate solution has been mixed with the benzene. Measurements at other wave lengths or with other filters can be made just before or after the most significant readings have been taken.

Absorption cells or tubes should be stoppered tightly. Absorption cells usually have glass covers or stoppers, but if test tubes are used, as in many routine photometric measurements, rubber stoppers washed free of sulfur are preferable to cork stoppers, contact with which will turn the solution yellow. Since the solutions on which optical measurements are made are strongly alkaline, absorption cells constructed with alkali-resistant cement should be used. The solutions should be left in the cells no longer than is necessary to make photometric measurements, after which the cells should be cleaned immediately. Although it might be expected that the alkaline solutions would attack and etch glass cells, no such difficulty has been experienced during several months of use.

In any application of the method it is important to run a blank analysis on a sample of the same type of material being analyzed which has not been treated with DDT. The results, in terms of DDT or extinction values (never in terms of per cent transmission), should be applied as corrections to the values obtained at each wave length or filter used in the analysis of the DDT-treated samples. If appropriate blanks are not run, the results of the analysis may be high. Blank analyses should be made by diluting the blank runs in the same manner as the DDT-treated samples, or else the corrections should be calculated to the same weight of untreated material as used in the analysis of the treated material.

DISCUSSION OF THE METHOD

PREPARATION OF SAMPLE. DDT decomposes with evolution of hydrogen chloride when heated at a high temperature, and it may decompose at 100° or lower in the presence of traces of certain catalysts, such as ferric chloride or iron (*6*). To minimize the possibility of decomposition, solvents may be removed from samples at room temperature by means of a draft of air. Tests should be made to demonstrate that there is no decomposition under the particular conditions of preparation of the sample and nitration employed by the analyst. It is advisable to get rid of as much extraneous material as possible before analyzing samples. In this connection solvents which extract less extraneous material than others may be used.

NITRATION OF SAMPLE. A number of nitrating mixtures other than the recommended 1 to 1 fuming nitric acid–concentrated sulfuric acid were tried, such as 1 to 1 red fuming nitric acid–concentrated sulfuric acid, 1 to 1 fuming nitric acid–25% fuming sulfuric acid, 1 to 1 red fuming nitric acid–25% fuming sulfuric acid, and 1 to 1 concentrated nitric acid–concentrated sulfuric acid. The last mixture gave colors that were too light, and none of them seemed to have any particular advantage over the recommended mixture.

Although the nitration seems to be completed in less than 1 hour, it was considered that in many applications of the method a 1-hour period of heating with the nitrating mixture would give more complete destruction of extraneous material. The nitrating mixture destroys to a large extent many plant extracts, oils, etc., by oxidation and conversion to alkali-soluble products, which are removed when the ether solution is washed with aqueous sodium hydroxide. However, some interfering substances are not destroyed completely.

The amount of nitrating acid used is not critical. Where small samples are used and the amount of extraneous material is not large, 2 ml. can be employed; where larger amounts of extraneous material are regularly encountered, 5 ml. are preferable. Since the quantity of acid used will make a slight difference in the calibration curves on known amounts, these curves should be prepared on the basis of whatever amount of acid is to be used for analysis of samples.

The nitration of an organic compound rarely gives a 100% yield of a single product. Usually a number of isomeric nitrated products are formed, and products of lower and higher nitration are sometimes present. Ordinarily a certain amount of material

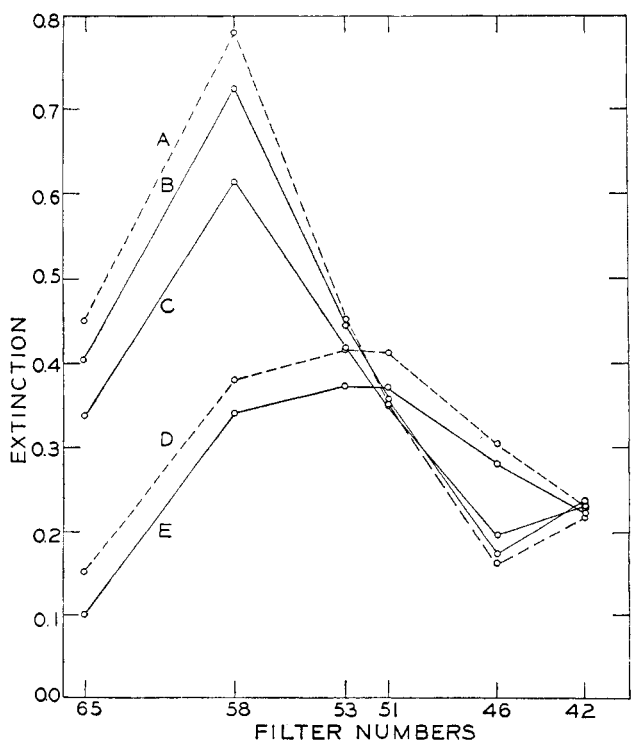


Figure 1. Filter Photometer Color Curves

A. Pure tetranitro-*p,p'*-DDT
B. 0.1 mg. of *p,p'*-DDT carried through method
C. 0.1 mg. of technical DDT carried through method
D. Pure tetranitro-*o,p'*-DDT
E. 0.1 mg. of *o,p'*-DDT carried through method

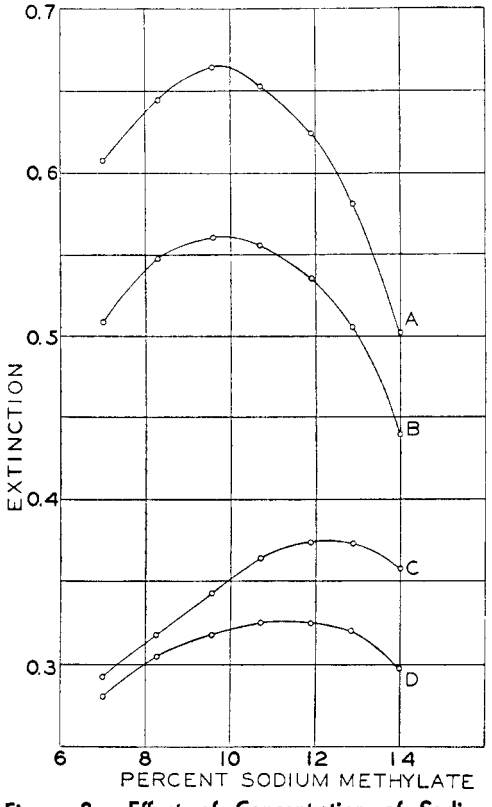


Figure 2. Effect of Concentration of Sodium Methylate on Intensity of Color

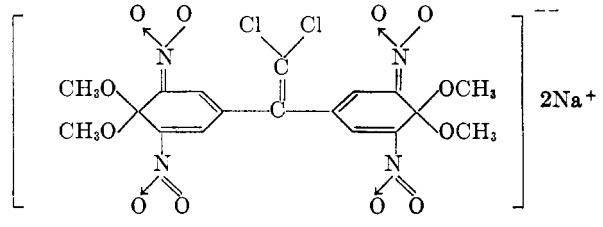
A. *p,p'*-DDT, No. 58 filter *C.* *o,p'*-DDT, No. 51 filter
B. Technical DDT, No. 58 filter *D.* *o,p'*-DDT, No. 58 filter

is oxidized to degradation products, or even completely oxidized When *p,p'*-DDT and *o,p'*-DDT are carried through the method, the main products are the tetranitro compounds described by Schechter and Haller (*22*), as borne out by a comparison of photometric measurements given in Figure 1.

Extraction of Nitrated Product. Benzene would be advantageous for the extraction, in that the color could be developed in the extract directly. However, for routine use ether is preferable because it gives more rapidly a clear separation of the layers. If the shaking is vigorous and the layers are permitted to separate clearly, a single extraction with ether is as good as a double extraction, within the precision of the method. To prevent possible decomposition of the chromogenic compounds, contact with the aqueous alkali should be no longer than necessary for thorough extraction and separation of the layers. Shaking the ether with saturated salt solution washes out the alkali and also partially dries the ether. Filtration through oven-dried cotton prevents any salt droplets from coming through and further dries the ether.

Development of Color. Benzene is a better solvent than methanol for the nitrated residue and is miscible with 2 volumes of the sodium methylate–methanol reagent. The curves shown in Figure 2 illustrate the effect of the concentration of sodium methylate on the intensity of the color as measured with an Aminco type F filter photometer (see Results for a description of the filters). *p,p'*-DDT, *o,p'*-DDT, and technical DDT were carried through the procedure, and the color was developed on aliquots of the final benzene solutions (0.10 mg. per 5.00 ml. of benzene) with the use of different concentrations of methanolic sodium methylate. The results indicate that, for *p,p'*-DDT and technical DDT, the most intense color was developed close to 10.0% of sodium methylate in methanol as measured with the No. 58 filter, and this concentration was adopted in the authors' work. The maximum intensity for the *o,p'*-DDT was developed near 11% of sodium methylate, as measured with the No. 58 filter and near 13% as measured with the No. 51 filter. The absorption curve of a benzene solution of tetranitro-*o,p'*-DDT plus methanolic sodium methylate (5.0 grams of sodium per 100 ml. of solution) exhibits two absorption peaks (*22*), one at 590 and the other at 511 millimicrons. It is interesting to note from Figure 2 that each peak is affected in a different manner by different concentrations of sodium methylate. Unless there is a special interest in determining *o,p'*-DDT, or the relative amounts of *p,p'*- and *o,p'*-DDT in mixtures, there is no advantage in using higher than the recommended 10.0% sodium methylate reagent, since the sensitivity with regard to *p,p'*-DDT is thereby decreased.

The color may be due to the following type of reaction product, one of the structures of the resonance hybrid of the complex from tetranitro-*p,p'*-DDT being shown:



The reaction of polynitro compounds with sodium alcoholates has been investigated by Jackson and Earle (*16*) and by Meisenheimer (*19*).

Photometric Measurements. Filter photometers are subject to a number of difficulties and sometimes give deviations from Beer's law because of broadness of the bands passed by their filters, stray light effects, etc. Their limitations must therefore be kept in mind. The difficulties and sources of error in filter photometry are adequately discussed by States and Anderson (*24*) and by Hamilton (*14*). Hogness *et al.* (*15*) and Brode (*4*) give good discussions of absorption spectrophotometry. In general, better results can be obtained with a spectrophotometer than with a filter photometer.

The absorption peak when *p,p'*-DDT is carried through the method using 10% sodium methylate reagent is at 596 millimicrons, and the two peaks given by *o,p'*-DDT are at 590 and 506 millimicrons (unpublished data). To determine *p,p'*-DDT or technical DDT, measurements should be made at the wave length or filter that gives the maximum absorption for *p,p'*-DDT (ca. 596 millimicrons) obtained on the instrument used by the analyst. Calibration curves should be made with known amounts of the type of material to be determined, whether it is *p,p'*-DDT or some batch of technical DDT.

Schechter and Haller (*22*) indicated that it would be feasible to determine the relative amounts of *p,p'*- and *o,p'*-DDT in mixtures of the two. Although technical DDT has as its major constituents *p,p'*-DDT (about 70 to 77%) and *o,p'*-DDT (about 15 to 25%), it does contain small amounts of other compounds and unidentified material [Haller *et al.* (*13*)]. The composition may vary with the method of manufacture, and even from one batch to the next. While calculations of the amounts of *p,p'*- and *o,p'*-DDT, assuming that these are the only two compounds present, give results of the right order of magnitude, the effect of the minor constituents, such as *p,p'*-DDD, should not be ignored. These calculations can be made if analytical calibration curves are prepared for known amounts of each of the two isomers at two suitable wave lengths or filters (in the range of 595 to 600 millimicrons and in the range of 500 to 510 millimicrons). Such computations from photometric data on mixtures are adequately discussed by Miller (*20*), Knudsen *et al.* (*17*), and others. It should be emphasized that these calculations will hold only over the regions of the calibration curves which follow Beer's law, so that a spectrophotometer or a photometer having filters of narrow wave length range should be used.

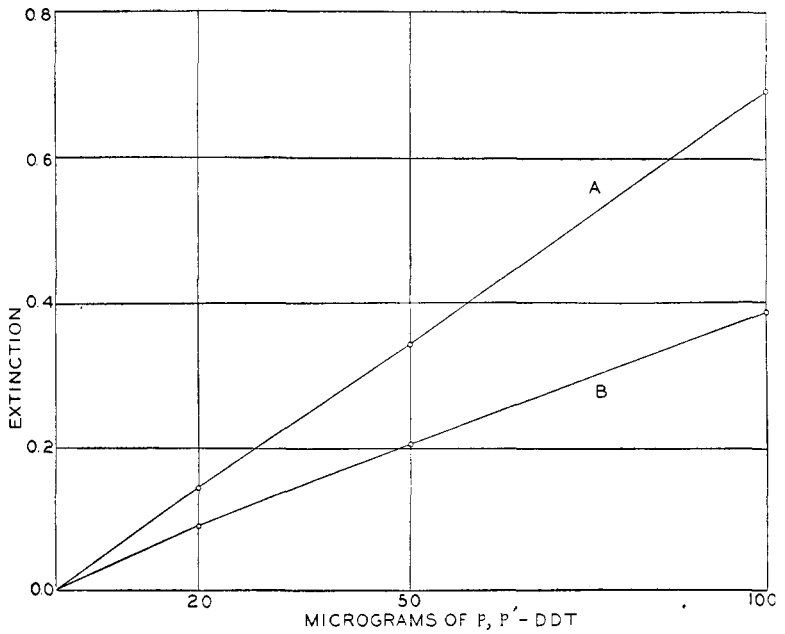


Figure 3. Analytical Calibration Curves for *p,p'*-DDT

A. With No. 58 filter
B. With No. 65 filter

For more accurate determination of the percentage of *p,p'*-DDT in technical DDT, the crystallization procedure of Cristol *et al.* (*5*) probably is more suitable.

In applications of the colorimetric method it is advisable to prepare calibration curves and also to make readings at a number of wave lengths or filters. There is usually less interference from extraneous materials at the higher wave lengths, since many interferences, such as those from plant extracts, exhibit an absorption curve which shows gradually increasing absorption with decreasing wave length.

While a calibration curve at a wave length of 640 millimicrons, or with a filter at 650 millimicrons, will have a lower sensitivity for determining *p,p'*-DDT, the results read from such a curve will have least interference from extraneous materials and practically none from possible decomposition or degradation products of *p,p'*-DDT.

FADING OF THE COLORS. The use of impure solvents and reagents can give rise to serious difficulties. Technical benzene sometimes contains impurities which contaminate the distillate with hydrogen sulfide, and this causes rapid fading of the developed color. Contamination of the benzene or of the sodium methylate reagent with sulfur, such as that from rubber stoppers, will increase the rate of fading. Sulfur can be removed from stoppers by boiling them in strong sodium hydroxide solution, washing, and drying.

The presence of water in the benzene used to dissolve the nitrated residue, or of water or sodium hydroxide in the sodium methylate solution, will also cause rapid fading. With good reagents and solvents the authors have found the fading to be 2 to 3%, in terms of either *p,p'*-DDT or technical DDT, 1 hour after the first reading. Six per cent during the first hour should be regarded as the maximum permissible amount of fading. While methanolic sodium hydroxide will produce the blue color, the rate of fading is so fast that satisfactory photometric measurements cannot be made.

The colors given by *p,p'*-DDT, *o,p'*-DDT, and technical DDT develop fully in 2 to 3 minutes and then fade very slowly. The red colors given by some of the possible decomposition or degradation products of DDT require about 10 minutes to develop fully and are relatively stable.

RESULTS

An Aminco type F photometer was used with filters 65, 58, 53, 51, 46, and 42, having wave lengths of maximum transmission at 650, 580, 530, 514, 460, and 424 millimicrons, respectively, and test tubes 2 cm. in diameter. All photometric readings were made on 5.00 ml. of the benzene solution plus 10.00 ml. of sodium methylate reagent and were converted to extinction values, or $\log\left(\frac{100}{\%\text{ transmission}}\right)$. Figures 1, 4, and 5 show photometric measurements made with the various filters in the photometer; they do not represent spectrophotometric curves. Figure 1 shows the readings obtained when 0.10 mg. of *p,p'*-DDT, *o,p'*-DDT, and technical DDT were carried through the method and the colors developed according to procedure 1. For comparison, pure tetranitro-*p,p'*-DDT and tetranitro-*o,p'*-DDT were dissolved in benzene, 5.00-ml. aliquots containing 0.15 mg. (equivalent to 0.10 mg. of *p,p'*-DDT and *o,p'*-DDT, respectively) were mixed with 10.00 ml. of sodium methylate reagent, and the results plotted (dotted lines) in the same figure.

Figure 3 illustrates the type of analytical calibration curve obtained with small amounts of *p,p'*-DDT (20, 50, and 100 micrograms) when 2.0 ml. of nitrating acid were used and the color was developed according to procedure 1. At each concentration the average deviation with the No. 58 filter in a number of runs by three workers was about 2 micrograms and the maximum deviation about 4 micrograms. A Beckman DU spectrophotometer gave straight-line calibration curves for the same amounts. The deviation from Beer's law when the No. 65 filter was used is due to the absorption characteristics of this filter. This "stray light"

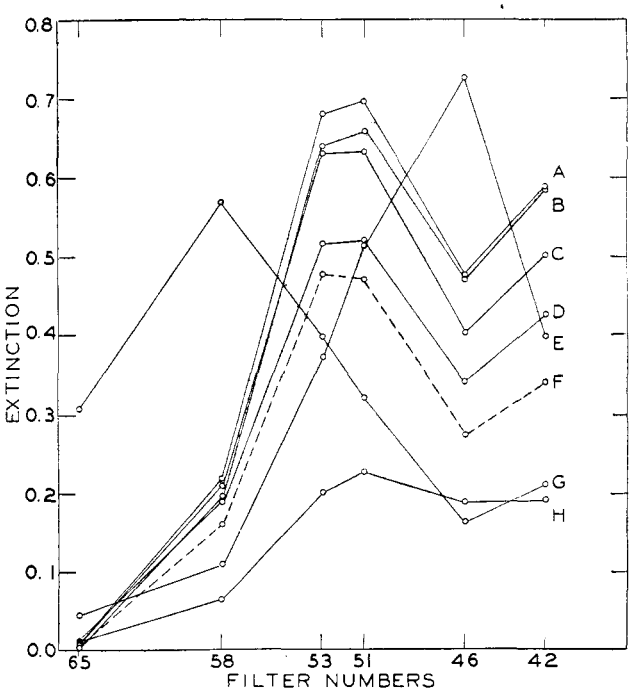


Figure 4. Filter Photometer Color Curves

A. 0.1 mg. of dehydrochlorinated *p,p'*-DDT
B. 0.1 mg. of 4,4'-dichlorobenzophenone. *C.* 0.1 mg. of *p,p'*-DDA
D. 0.1 mg. of 4,4'-dichlorobenzohydrol. *E.* 0.6 mg. of *o*-dichlorobenzene
F. 0.068 mg. of pure 4,4-dichloro-3,3 5,5-tetranitrobenzophenone
G. 0.1 mg. of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane, or *p,p'*-DDD
H. 0.1 mg. of bis(*p*-chlorophenyl)methane
Each compound was carried through method except *F*

effect is described by States and Anderson (*24*). When 10 mg. of DDT were used, and the color was developed after proper dilution into a readable photometric range according to procedure 2, a calculated result of 9.9 mg. was obtained.

Figure 4 shows the results obtained by applying the color test to a number of compounds related to DDT, using procedure 2. *p,p'*-DDD gives a blue color practically identical to that of *p,p'*-DDT but slightly less intense.

Some of the possible breakdown products of *p,p'*-DDT—such as dehydrochlorinated *p,p'*-DDT, 4,4'-dichlorobenzophenone (*10*), 4,4'-dichlorobenzohydrol, bis(*p*-chlorophenyl)methane (*27*), and *p,p'*-DDA (*8, 27*)—give red colors with negligible absorption when the No. 65 filter is used. Consequently there could be little or no interference from these breakdown products on the analysis for *p,p'*-DDT as read from its calibration curve at this filter. In an experiment with a mixture containing 0.050 mg. of *p,p'*-DDT and 0.025 mg. of dehydrochlorinated *p,p'*-DDT, the result when read from the analytical calibration curve at the No. 65 filter indicated the presence of 0.050 mg. of *p,p'*-DDT with no interference from the dehydrochlorinated *p,p'*-DDT. However, in the case of technical DDT, if there is considerable decomposition, it would be difficult to calculate or interpret the results because of the complexity of the system, at least four components (*p,p'*- and *o,p'*-DDT and their dehydrochlorinated derivatives) being present.

If the results for DDT read from calibration curves at several filters agree after being corrected for the blank analysis at each filter, it is evident that the DDT has not decomposed to any appreciable extent. The red colors are due to the formation of a considerable amount of 4,4'-dichloro-3,3',5,5'-tetranitrobenzophenone (probably accompanied by isomers and other nitro derivatives) during the nitration of these degradation products of DDT. The extinction values on the color given by synthetic 4,4'-dichloro-3,3',5,5'-tetranitrobenzophenone in benzene solution plus sodium methylate are also given (dotted line) in Figure 4 for comparison.

4-Chloro-3,5-dinitrobenzoic acid has been detected as another product of the nitration of dehydrochlorinated *p,p'*-DDT, 4,4'-dichlorobenzophenone, and *p,p'*-DDA. In the analysis of these compounds the 2% alkali washes of the ether solutions were acidified and extracted with ether, the ether was washed with salt solution and evaporated to dryness, and the residues were dissolved in benzene. On addition of sodium methylate reagent a violet-red color was developed in each case. The same color was developed when *p*-chlorobenzoic acid was carried through the method with the omission of the alkali wash. A comparison of photometric readings (Figure 5) obtained on these solutions with readings (dotted line) made on a sample of synthetic 4-chloro-3,5-dinitrobenzoic acid in benzene plus sodium methylate reagent indicated that each of the solutions contained this compound.

The fact that *p,p'*-DDA yields 4,4'-dichloro-3,3',5,5'-tetranitrobenzophenone and 4-chloro-3,5-dinitrobenzoic acid on intensive nitration is rather remarkable, since it involves decarboxylation of the DDA in addition to nitration and oxidation reactions. The dichlorotetranitrobenzophenone formed is neutral and remains in the ether in the analytical method, while the chlorodinitrobenzoic acid goes into the alkali wash. *p,p'*-DDA has been shown in pharmacological studies (*27*) to be a metabolite of *p,p'*-DDT, and a method for its detection and estimation has considerable importance in these investigations. Use can be made of its acidic properties to separate DDA from DDT and any neutral degradation products prior to the application of this colorimetric test. It does not seem to be possible at present to differentiate some of the other breakdown products of DDT, such as dehydrochlorinated *p,p'*-DDT and 4,4'-dichlorobenzophenone, on the basis of this color test alone. In such cases supplemental chlorine determinations on the samples would be of value.

Bis(*p*-chlorophenyl)sulfone, a minor constituent of technical DDT, and *p*-dichlorobenzene were found to give no color when carried through the analytical procedure. *o*-Dichlorobenzene gave an orange color (Figure 4). Care should be taken in interpreting results of this method when aromatic halogen compounds that might interfere are known to be present; however, there are not likely to be any such materials in spray residues.

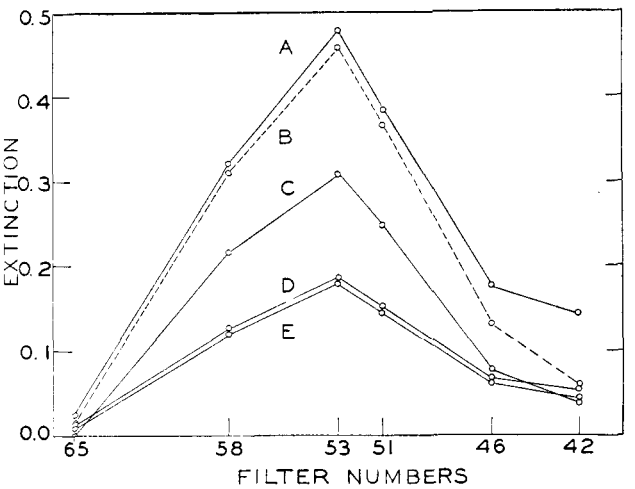


Figure 5. Detection of 4-Chloro-3,5-dinitrobenzoic Acid

A. From alkali wash in analysis of 0.8 mg. of *p,p'*-DDA
B. 0.1 mg. of pure 4-chloro-3,5-dinitrobenzoic acid
C. 0.05 mg. of *p*-chlorobenzoic acid carried through method, alkali wash omitted
D. From alkali wash in analysis of 0.5 mg. of 4,4'-dichlorobenzophenone
E. From alkali wash in analysis of 0.5 mg. of dehydrochlorinated *p,p'*-DDT

This method is being applied to the analysis of spray residues, water samples, etc. The chemistry involved in the nitration of DDT and its breakdown products and the reactions of these and related nitro derivatives together with pertinent spectrophotometric data are also being investigated.

LITERATURE CITED

(1) Annand, *J. Econ. Entomol.*, 37, 125 (1944).
(2) Bailes and Payne, IND. ENG. CHEM., ANAL. ED., 17, 438 (1945).
(3) Bost and Nicholson, *Ibid.*, 7, 190 (1935).
(4) Brode, "Chemical Spectroscopy", 2nd ed., New York, John Wiley & Sons, 1943.
(5) Cristol, Hayes, and Haller, IND. ENG. CHEM., ANAL. ED., 17, 470 (1945).
(6) Fleck and Haller, *J. Am. Chem. Soc.*, 66, 2095 (1944).
(7) Froelicher, *Soap & Sanit. Chem.*, 20 (7), 115 (1944).
(8) Grummitt, Buck, and Stearns, *J. Am. Chem. Soc.*, 67, 156 (1945).
(9) Gunther, IND. ENG. CHEM., ANAL. ED., 17, 149 (1945).
(10) Gunther, *J. Chem. Education*, 22, 238 (1945).
(11) *Ibid.*, 22, 279 (1945).
(12) Hall, Schechter, and Fleck, *U. S. Bur. Entomol. Plant Quarantine* ET-211 (1944).
(13) Haller, Bartlett, Drake, Newman, and others, *J. Am. Chem. Soc.*, 67, 1591 (1945).
(14) Hamilton, IND. ENG. CHEM., ANAL. ED., 16, 123 (1944).
(15) Hogness, Zscheile, and Sidwell, *J. Phys. Chem.*, 41, 379 (1937).
(16) Jackson and Earle, *Am. Chem. J.*, 29, 89 (1903).
(17) Knudsen, Meloche, and Juday, IND. ENG. CHEM., ANAL. ED., 12, 715 (1940).
(18) Lund and Bjerrum, *Ber.*, 64, 210 (1931).
(19) Meisenheimer, *Ann.*, 323, 205 (1902).
(20) Miller, "Quantitative Biological Spectroscopy", Minneapolis, Burgess Publishing Co., 1939.
(21) Neal, von Oettingen, and others, U. S. Pub. Health Service, *Pub. Health Repts., Supplement* 177, 2 (1944).
(22) Schechter and Haller, *J. Am. Chem. Soc.*, 66, 2129 (1944).
(23) Snell and Snell, "Colorimetric Methods of Analysis", Vol. 2, New York, D. Van Nostrand Co., 1937.
(24) States and Anderson, *J. Optical Soc. Am.*, 32, 659 (1942).
(25) Stiff and Castillo, *Science*, 101, 440 (1945).
(26) Umhoefer, IND. ENG. CHEM., ANAL. ED., 15, 383 (1943).
(27) White and Sweeney, U. S. Pub. Health Service, *Pub. Health Repts.*, 60, 66 (1945).
(28) Zais, *J. Chem. Education*, 21, 489 (1944).

PART of this work was done under a transfer of funds, recommended by the Committee on Medical Research, from the Office of Scientific Research and Development to the Bureau of Entomology and Plant Quarantine.