determination. In preliminary experiments, even a 50% excess of iron produced a loss of magnesium within the limits of error which could be tolerated in analytical control work. If an insufficient amount of iron is added to precipitate all the phosphate present, considerable difficulty in end point determination in the titration step is encountered and reliable results cannot be obtained.

Likewise, in the acid-soluble procedure the total phosphorus pentoxide figure should theoretically be used in calculating the amount of iron to be added. It can be assumed that the total phosphorus pentoxide in any given grade will not in most cases exceed the guaranteed available phosphorus pentoxide value by more than 2%. (A 10% sample will not normally have a total phosphorus pentoxide content of more than 12%.) So here again the figure can serve as a guide. When samples have been analyzed for total and water-soluble phosphorus pentoxide before magnesium analysis is started, the iron excess can be held to an absolute minimum by using these figures rather than estimating from the expected available phosphorus pentoxide as suggested here.

A pH meter is used in the neutralization step. The 0.5N sodium hydroxide is normally used in the water-soluble procedure to make pH adjustments before and after the addition of the iron solution. Because of the initial high degree of acidity in the acid-soluble procedure, the pH adjustment to pH 4.0, prior to the addition of the iron solution, is made with the stronger 10% sodium hydroxide solution. If an indicator were used instead of a pH meter, the indicator color would mask the end point in the final titration with murexide and Eriochrome Black T. Buffering the solution to pH 5 is not feasible because the final titration has to be made in a medium buffered to pH 10 for the titration with Eriochrome Black T, and in an alkaline solution of pH 12 for the calcium titration with murexide. As the amount of sodium hydroxide necessary for neutralization depends on the amount of iron solution used and the original acidity of the fertilizer solution, a pH meter is most convenient for this step.

The final titration procedure is nearly identical with that described by Cheng, Kurtz, and Bray (*2*) for limestone analysis.

Interference

During more than a year of actual use of this method in the laboratory under routine conditions the following interferences were observed:

Manganese. Manganese present in fertilizer in amounts greater than a few hundredths of 1% influences the indicator color of both murexide and Eriochrome Black T, making the end points very indefinite and unreliable.

Organic matter. As a completely clear uncolored solution is desirable in the final titration, the lightly colored solutions, resulting from organic fertilizers, might interfere with a proper end point determination. However, in this laboratory such samples have not been encountered. A moderate amount of organic matter does not cause a coloring severe enough to interfere.

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PESTICIDE DETERMINATION

Critical Points in the Schechter-Hornstein Colorimetric Method for Lindane

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The Schechter-Hornstein colorimetric procedure for lindane was studied to determine its critical points and to improve its precision and accuracy. Careful attention in the analytical method must be given to cleanliness, contamination, moisture, removal of ether solvent, and timing. Low and erratic results were obtained when small amounts (20 mg.) of phosphoric acid lubricant were placed in the reaction pot. The precision of the Schechter-Hornstein method was improved significantly with acetic and dichloroacetic acid lubricants. Average deviation for phosphoric was 16.1%, acetic was 3.6%, and dichloroacetic was 5.3%. The method was applied to determine the residual concentration of lindane on finished pickles.

Chemical residues determinations on plant and animal tissues have become a necessity because of the increased use of the newer insecticides. In many cases the toxic level to man and animals has been undetermined. The Schechter-Hornstein colorimetric method (*1*, *8*) has been collaboratively studied (*3*, *5*, *6*) and used for the estimation of the residual amount of lindane on plant tissues and in foods (*4*). This method is probably the best in use today, but it is lengthy and has a number of critical points at which losses of lindane or interference may occur. The objective of this article is to present findings on extensive studies on the reproducibility and accuracy of the method and its application to the determination of lindane residues on pickles.

The sample of lindane used in all the work was a referee sample obtained from the Association of Official Agricultural Chemists in 1953 and contained 99%+ of the gamma isomer. All other chemical reagents used were of the highest purity—reagent quality.

Application of Method to Lindane Residues on Pickles

This investigation resulted from a cooperative project with the Departments of Entomology and Horticulture of the Agricultural Experiment Station, in which a series of experiments was

conducted on the effect of trace amounts of lindane on the flavor of pickles and on the fermentation processes of making various types of finished pickles. About 500 independent determinations were made for residual lindane on control, salt stock, sour, sweet, and dill pickles. Also, a series of recovery studies was conducted on dry methylene chloride extracts of pickles containing added lindane at various known levels. The recoveries were not considered satisfactory because of interfering substances and erratic results in obtaining the standard curve. The absorbance values obtained for lindane residues on pickles could be expressed only as an apparent lindane content. Lindane cannot be positively identified unless the final solution is a violet-cherry color, and even then there may be questions concerning it. The yellow, orange, or green colors which were obtained may have been produced by methylene chloride–extracted substances that were carried through the analytical procedure. The off-colors may also have been due to a combination of lindane or *m*-dinitrobenzene and other interfering materials carried through the procedure which did not allow full development of the violet color. The sulfonation technique employed by Hornstein (*2*), or the acetic acid boiling-off technique employed by Lichtenstein (*7*), on the removal of interfering substances, may be an answer in part to the difficulties encountered.

Critical Studies on Method Involving Standards

Because of the erratic results with standards and the experimental pickle samples, further studies were made in an attempt to improve the method. To determine if some reagent or extractant might have been responsible for the poor results, experiments were conducted on the effects of sodium sulfate, ethyl acetate, methyl ethyl ketone, propionaldehyde, ethyl alcohol, and chlorophyll when placed directly in the reaction flask on the absorbance of standards. No effect was observed on the recovery of lindane as a result of these added reagents.

After the dechlorination reaction and nitration of the benzene, extreme care must be exercised in order to prevent a partial or total loss of the resultant *m*-dinitrobenzene. If necessary, the determination may be interrupted or stopped after the nitration reflux period, or after the transfer of the nitrating mixture into cold water in the separatory funnel. Repeated experiments showed that no loss in *m*-dinitrobenzene occurred when the nitrating acids were allowed to stand overnight in the nitration column, or when known amounts of *m*-dinitrobenzene were allowed to stand for 3 hours in the acid-water-ether solution in the separatory funnel. Thus in this respect, the ether extraction and washing steps are not critical.

Mineral oil is added to the ether solution in order to prevent volatilization of *m*-dinitrobenzene during the ether evaporation step. If the ether were allowed to evaporate off slowly, or if the flasks were left unstoppered, approximately 100% of the *m*-dinitrobenzene was lost. Also, it was found best to evaporate the ether off rapidly on a steam water bath at 80° C. within a period of 15 to 30 minutes, depending on the original sample volume. Constant rotation of the flask was essential to prevent loss of *m*-dinitrobenzene when the volume of the ether solution approached 5 to 10 ml. The flask should be completely removed from the heat when approximately 1 to 2 ml. remains and the ether vapors poured off by rotating and holding the flask horizontally; then the flask should be immediately stoppered, the color developed, and the determination completed. When stoppered flasks were allowed to stand, *m*-dinitrobenzene was lost as a result of poor seals. Overheating of the ether solution during the final stages of evaporation also caused a loss of *m*-dinitrobenzene. Once the *m*-dinitrobenzene has been removed from the acid-water-ether solution in the separatory funnel, the remaining work must be carried on to completion without interruption and with considerable care.

The apparatus must be thoroughly dried between determinations. When the apparatus was not dried and water remained in the nitrating tube, or if water distilled into the nitrating column from a wet sample, losses occurred. As little as 1 ml. of water present in the nitrating column caused a 16% loss and 5 ml. or more caused a total loss. Therefore, wet samples or samples containing water cannot be directly used, as the water may distill over into the nitrating column and result in poor nitration of the benzene to *m*-dinitrobenzene. It is perhaps best to extract the residues from wet samples with an organic solvent such as methylene chloride. This may also cause trouble, as interfering substances may be extracted which will produce apparent lindane values. Hornstein (*2*) presented a sulfonation technique by which extracted interfering substances were removed. Drying samples in a forced-draft or vacuum oven at 65° C. will cause a loss of lindane.

m-Dinitrobenzene may possibly have been lost as a consequence of adsorption on dry cotton when the ether–*m*-dinitrobenzene solution was dried by passing it through a 0.75-inch layer of cotton. However, data obtained in six experimental trials showed that no losses occurred in this part of the method.

The effect of time on color development of the complex formed between reagent grade *m*-dinitrobenzene and methyl ethyl ketone was studied. Four independent determinations were conducted at 5-minute intervals to 45 minutes, then at 10- or 30-minute intervals to 420 minutes. The violet colored complex lacks stability and the time after color development at which it is read in the photometer is important. The absorbance may be read anywhere between 5 and 60 minutes after the reagents are added. Repeated work showed that a maximum absorbance occurred after 10 minutes. However, for relative purposes, the same time interval for color development should always be used.

A great deal of difficulty was encountered in obtaining a workable standard curve. The results were erratic and lacked precision, partly because of the use of phosphoric acid as a lubricant on the ground-glass seals of the reaction flasks and the nitrating apparatus. Although phosphoric acid appeared to form a good seal, the presence of even small amounts of it in the reaction flask caused a serious loss of lindane. One drop, or about 38 mg. of phosphoric acid in the reaction flask, caused a loss of 65% of the lindane added, and 2 to 3 drops caused almost a total loss. This is undoubtedly more than will work down into the reaction flask, but only 0.6 drop (23 mg.) caused an 18% loss, and 0.8 drop (30 mg.) caused a 51% loss of lindane. Thus, just a few milligrams of phosphoric acid present in the reaction flask will cause considerable loss of lindane.

Following this observation on the effect of phosphoric acid on the recovery of lindane, a series of recovery experiments was conducted using various types of lubricants—e.g., glycerol, acetic acid, dichloroacetic acid, sulfuric acid, mineral oil, and several prepared lubricants. Acetic and dichloroacetic acids were considered good usable lubricants. The others have the same disadvantages as phosphoric acid. If phosphoric acid is used as a lubricant, extreme care must be used so that none of it gets into the reaction flask, for even when a small amount is used to make a seal, there is still the disadvantage that the refluxing acetic acid works up around the ground-glass joint and causes some of the phosphoric acid to drain into the reaction flask with a consequent loss of lindane.

No losses of lindane occurred when acetic and dichloroacetic acids were placed in the reaction flask. The following average standard deviations for all absorbance values of standards in the range of 0 to 50 γ were obtained: For phosphoric acid, ± 0.0380 absorbance unit on 70 determinations (16.1% average deviation); acetic acid, ± 0.0086 absorbance unit on 30 determinations (3.6% average deviation); and dichloroacetic acid, ± 0.0170 absorbance unit on 17 determinations (5.3% average

deviation). As a result of these studies, acetic and dichloroacetic acids are recommended as lubricants for the nitration apparatus. Good seals of the joints were obtained, and the reproducibility of absorbance values of standards, when these lubricants were used, was good—the percentage deviation being about one third to one fifth of the deviation found when phosphoric acid was used as the lubricant.

These findings emphasize the limitations and some of the critical points of the present colorimetric method for lindane. As a result of further research, especially on the removal of interfering materials, the Schechter-Hornstein method can probably be modified to include a wider variety of plant and animal products.

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INSECTICIDE RESIDUES

Colorimetric Determination of Toxaphene

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A method has been developed for determining as little as 0.5 mg. of toxaphene or 0.25 mg. of Strobane in 5 ml. of solution, and has been applied to alfalfa and butterfat extract. In tests with other chlorinated insecticides, interferences were encountered only with chlordan and heptachlor.

Recent federal legislation has made it necessary to establish tolerances for pesticide residues. The establishment of a tolerance implies that analytical methods are or will be available for accurately estimating residues. Analytical data obtained by nonspecific tests such as bioassay techniques, total chlorine determination, or cholinesterase inhibition are considerably strengthened if a reasonably specific chemical method is also available for determining the pesticide in question.

A tolerance of 7 p.p.m. has been established for toxaphene on many agricultural crops. Although bioassay and total-chlorine methods can be used in obtaining residue data, no specific method for toxaphene is at present available. Kenyon (*2*) described an infrared procedure of qualitative value, and Johnson (*1*) described a test for the detection of toxaphene in formulations in which pyridine and methanolic potassium hydroxide are used to give a Fujiwara-type test.

In general, toxaphene and Strobane respond to the same reagents in similar fashion, as they are closely related materials. Toxaphene is a mixture of chlorinated camphenes, Strobane is a mixture of chlorinated terpenes, both approximately 68% chlorine.

In this paper, a method is described for determining a minimum of 0.5 mg. of toxaphene or 0.25 mg. of Strobane in a final volume of 5 ml. of solution. Although this is by no means as high a sensitivity as might be desired, it should prove useful in view of the 7 p.p.m. tolerance. For example, a 500-gram sample containing 4 p.p.m. of toxaphene would yield 2 mg. of the insecticide, an amount readily determined by this procedure.

The method is based on the reaction of the several related compounds in toxaphene or Strobane with thiourea in the presence of alkali to give a yellow color that may be measured photometrically. Although thiourea reacts with acyl, alkyl, and heterocyclic halides in inert solvents to give pseudothioureas, this reaction does not take place when toxaphene or Strobane is refluxed with thiourea in isopropyl alcohol or isopropyl alcohol plus water. However, in the presence of a small amount of strong alkali the development of a yellow color starts almost immediately and is enhanced by heating. The reaction appears to be catalyzed by a strong base and does not take place in a medium that is only slightly alkaline.

Experimental

Reagents

Isopropyl alcohol, 99%.

Thiourea, recrystallized from methanol, 2% w./v. solution in 2% w./v. aqueous potassium hydroxide.

Magnesium silicate (chromatographic grade Florisil), obtainable from Floridin Co., Tallahassee, Fla.

n-Hexane, redistilled.

Methylene chloride, redistilled.

Sulfuric acid, concentrated.

Preparation of Standard Curve. An isopropyl alcohol solution of toxaphene containing 1 mg. of toxaphene per ml. is prepared. The solution, 1, 2, 3, and 4 ml., respectively, is pipetted into borosilicate glass test tubes, which can be glass-stoppered and which are calibrated to the 5-ml. mark. Each solution is made up to 4 ml. with isopropyl alcohol, and 1 ml. of the thiourea–potassium hydroxide solution is pipetted into each test tube. The test tubes are stoppered and heated for 1 hour in a constant-temperature bath at 70° C. after which, they are removed from the bath, and the solution is allowed to come to room temperature. If necessary, the volume is adjusted to 5 ml. with isopropyl alcohol. The yellow color is stable for several hours.

The absorbance is read at 400 mμ, using a Beckman Model DU spectrophotometer and 1-cm. absorption cells. To set the instrument at zero absorbance, isopropyl alcohol is used. The standard curve is prepared by plotting absorbance against milligrams of toxaphene. A standard curve for Strobane can be prepared in a similar manner.

Preparation and Analysis of Samples. The absorption of the thiourea reaction products is measured at a wave length where considerable interference from naturally occurring products may be expected. Extraneous yellow colors are often encountered and, to apply this method successfully, one must use adequate cleanup procedures, particularly as large samples must be processed.