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The Determination of Lindane Residues on Pickles

Critical Points in the Schechter-Hornstein Colorimetric Method

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TABLE OF CONTENTS

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The Determination of Lindane Residues on Pickles

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A semiquantitative colorimetric method for the determination of trace amounts of the insecticide benzene hexachloride (the mixed isomers of 1, 2, 3, 4, 5, 6-hexachloro-cyclohexane) or of lindane (99% + of the gamma isomer) has been developed by Schechter and Hornstein (1). These authors have applied the method in studies of the insecticidal residue on peanuts, mushrooms, and various food products (2, 3, 4, 5, 6). The Association of Official Agricultural Chemists has studied this method collaboratively during the past three years (7, 8, 9) with only partial success.

The Schechter-Hornstein method was selected for the determination of lindane residues on pickles. This investigation resulted from a cooperative project with the Departments of Entomology and Horticulture of the Missouri Agricultural Experiment Station. These departments have been conducting a series of experiments on the effect of trace amounts of BHC on the flavor of finished pickles and on the fermentation process used in making various types of finished pickles.

The major objectives of this investigation were to determine the critical points in the Schechter-Hornstein colorimetric method for lindane, and to determine the amount of lindane residues remaining on experimental pickles.

APPARATUS AND REAGENTS

Following is the list of apparatus and reagents used for the experiments:

Bausch and Lomb Spectronic "20" or other suitable colorimeter.
Constant temperature water or steam bath.
Digestion flasks, flat or round bottom, 50 to 500 ml.
Electric heating units, similar to Fisher No. 11-502-15, or of a design which allows the nitrating column to be kept away from the heat.
Erlenmeyer Flasks, 250 ml., glass stoppered.
Rings (iron) and support stands.
Schechter-Hornstein all-glass digestion and nitrating apparatus (1).
Separatory Funnels, 250 ml.

Shimer filter, body 25 mm. in diameter and 75 mm. long, stem about 30 mm. long.

Absorbent cotton —purify by immersion in ethyl ether, stir for five minutes, remove and squeeze out excess solvent. Repeat the treatment twice. Allow cotton to air-dry, then heat for two hours in oven at 110° C. and store in closed container.

Ether, anhydrous, reagent grade. If 100 ml., when evaporated, leaves a yellow or light brown residue, the ether should be redistilled before using.

Glacial acetic acid, A. R. grade—fractionate and discard the first and last portions.

Lindane. The reference sample used was lindane, 99% + of the gamma isomer submitted for collaborative study by A.O.A.C. in 1954.

Malonic acid, A. R. grade.

Methyl ethyl ketone, reagent grade.

Methylene chloride, reagent grade.

Mineral oil, refined nujol or its equivalent.

Nitrating acid, a mixture of C. P. fuming nitric acid (Specific gravity 1.49 to 1.50) and C.P. concentrated sulfuric acid (Specific gravity 1.84). 1 to 1 volume. Red fuming nitric acid (Specific gravity 1.59-1.60) may also be used.

Phosphoric acid, 85%, A.R. grade.

Potassium hydroxide, 40% W/V. Dissolve 470 g. of 85% reagent grade potassium hydroxide in distilled water, make to 1 liter.

Pyrex glass wool.

Sodium chloride solution, saturate double-distilled water with A.R. grade sodium chloride.

Sodium hydroxide solution, 2% W/V.

Trichloroethylene, technical grade.

Zinc metal, A.R. grade, 40 to 80 mesh.

ANALYTICAL PROCEDURE

The following procedure was set down for workers to follow:

(1) *Refluxing of Sample and Extraction of Meta-Dinitrobenzene*

Place the water-free sample containing residues of BHC, sample extract, or known amount of lindane in the reaction flask. Add 15 ml. of redistilled glacial acetic acid, 1.5 g. of 40 mesh or powdered zinc, and 2 g. of malonic acid. The addition of these reagents can be facilitated by using a large-mouth transfer funnel. Make sure that no particles adhere to the flask neck. Attach the flask securely to the reflux apparatus and carefully lubricate the ground glass joint and the stopcock with 85 percent phosphoric acid or some other lubricant free of aromatics. Add 5 ml. of the nitrating acid mixture to the nitrating column. Fill the outer jacket of the

reflux condenser to approximately the one-fourth mark with trichloroethylene, add a small amount of granulated zinc for smooth ebullition. Place the "cold fingers" in position and turn on the cold water. Heat gently at first, regulating the heat output of the heating mantles with variable transformers, finally reflux vigorously for 2.5 hours. The vapors of trichloroethylene should just reach the "cold finger" condenser. This requires about 30 minutes. After 2.5 to 3 hours remove the heat source and quickly separate the reaction flask from the rest of the apparatus, so that the acids are not forced out of the nitrating column. Wash the contents of the nitrating column into a 250 ml. separatory funnel as follows:

Place 10 ml. of ice-cold double-distilled water in the separatory funnel and drain the nitrating acid mixture into it. Rinse the nitrating column and inner tube three times with a total volume of 50 ml. of cold double-distilled water. Rinse similarly with 50 ml. of ether, then with another 50-ml. volume of cold distilled water. Shake the separatory funnel vigorously for one minute, allow the two layers to separate, then drain the lower acid portion into a second 250 ml. separatory funnel containing 30 ml. of ether. Shake the second separatory funnel vigorously for one minute, allow the ether to separate and discard the lower acid portion. Wash the ether in the first and second separatory funnels successively with 30 ml. of 2 percent sodium hydroxide solution; repeat with 30 ml. of a saturated solution of sodium chloride. Filter the ether extract in the first funnel through a 0.75-inch plug of absorbent cotton packed in a glass shimer filter funnel, and collect the ether solution in a 250 ml. glass-stoppered Erlenmeyer flask. Pour the ether extract from the second funnel into the first; use it as a rinse, and then filter through the cotton plug and collect in the same flask. Use three separate 15 ml. portions of ether as successive washes for the funnels and cotton.

(2) *Removal of Ether on Steam Bath*

Add a small glass bead and one drop of mineral oil to the ether solution and evaporate on an electrically heated water bath maintained at a temperature of 80 to 90° C. Constant rotation of the flask is essential to prevent loss of meta-dinitrobenzene when the volume of ether solution is about 5 to 10 ml. As the volume approaches 1 to 2 ml. (about 15 to 30 minutes), remove the flask from the heat source and bring to dryness by rotating the flask horizontally and pouring off the remaining ether vapors. Add exactly 10 ml. of methyl ethyl ketone and swirl to complete the solution of any residue. Add 1 ml. of 40 percent potassium hydroxide solution, shake vigorously for at least 1 minute, then place in the dark for 20 minutes for maximum color development. Decant part of the methyl ethyl ketone layer into an absorption cell or tube. Immediately read the percent transmittance or optical density at 565 mμ, as removal of the

colored substance from contact with the alkali causes a rapid fading of the violet color. Prepare a reagent blank with 10 ml. of methyl ethyl ketone that has been shaken vigorously with 1.0 ml. of 40 percent potassium hydroxide, and allow to stand in the dark for 20 minutes. Set the photometer at 100 percent transmittance or zero optical density with this reagent blank.

RESULTS AND DISCUSSION

The quantity of acetic acid used in the dechlorination reaction may vary, depending on the amount and type of sample. It has been recommended (1) that 1 g. of zinc be used with each 10 ml. of acetic acid, and 2 g. of malonic acid with the first 10 ml. of acetic acid and 0.5 g. more for each additional 10 ml. The acetic acid must be free of aromatics and later reports (10) indicate that water and alcohol must also be removed prior to usage.

The outer jacket of the nitrating apparatus contains trichloroethylene which maintains a constant temperature of 87° C. around the inner tube. This permits the benzene to be distilled over into the nitrating column while the acetic acid condenses and is returned to the reaction pot. Under normal operating conditions it takes about 30 minutes from the time heat is applied until the vapors of trichloroethylene reach the cold finger.

Ether Removal Studies

Once the benzene has been nitrated, extreme care must be taken to prevent its partial or total loss. As the determination is lengthy, it may be interrupted after the nitration reflux period or after the transfer of the nitrating acid mixture to the separatory funnel. No apparent loss occurred when known amounts of meta-dinitrobenzene were allowed to stand for 3 hours in the acid-ether solution. Thus, in this respect, the ether extraction and washing steps are not critical.

Mineral oil is added to the ether solution to help prevent volatilization of meta-dinitrobenzene during the ether evaporation step. It was found that if the ether was allowed to evaporate off at room temperature approximately 100 percent of the meta-dinitrobenzene was lost. It is best to evaporate the ether off rapidly on a steam water bath within 15 to 30 minutes, depending on the volume of ether at the start. The flask should be completely removed from the heat when approximately 1 to 2 ml. remain, and the remaining ether vapors poured off by rotating and holding the flask horizontally. Then the flask should be stoppered immediately, the methyl ethyl ketone and alkali added and the optical density read. As observed in Table 1, the color should be developed at this point rather than allowing the sample to stand, since even if the ether is rapidly removed, further standing open will cause large losses, as will a poorly stoppered flask. Overheating during the final

TABLE 1 -- ETHER REMOVAL STUDIES

Method Used	Meta-Dinitrobenzene Micrograms	Optical Density Determined	Optical Density Standard Avg.	Recovery %
Evaporated on standing at room temperature.	40	0.000	0.630	000.0
	40	0.120	0.630	19.0
	40	0.000	0.630	00.0
	40	0.208	0.630	33.0
	40	0.012	0.630	1.9
Evaporated rapidly, left open at room temperature overnight.	40	0.265	0.630	42.1
	40	0.246	0.630	39.0
	40	0.262	0.630	41.6
Evaporated rapidly, flask glass stoppered, color developed immediately.	40	0.610	0.630	96.8
	40	0.680	0.630	107.8
	40	0.610	0.630	96.8
	40	0.680	0.630	107.8
	40	0.665	0.630	105.6
	40	0.656	0.630	104.1
	40	0.650	0.630	103.2
	40	0.640	0.630	101.6
	40	0.665	0.630	105.6
	40	0.600	0.630	95.2
	40	0.560	0.630	88.9
	40	0.620	0.630	98.4
	40	0.650	0.630	103.2
	40	0.560	0.630	88.9
	40	0.630	0.630	100.0
	40	0.600	0.630	95.2

stages of evaporation also caused a loss. Thus, once the sample has been removed from the acid-ether solution in the separatory funnel, the remaining work must be carried on to completion with considerable care.

Effect of Time on Color Development

Data are presented in Tables 2 and 3 on the effect of time of standing in the dark on color development. The violet colored complex formed be-

TABLE 2 -- EFFECT OF TIME ON COLOR DEVELOPMENT

Time Minutes	Optical Density Determined[a] 1	2	3	4	Average
5	0.640	-----	0.640	0.620	0.633
10	0.660	0.645	0.640	0.620	0.641
15	0.660	0.640	0.630	0.618	0.637
20	0.660	0.630	0.640	0.620	0.638
25	0.640	0.622	0.630	0.615	0.627
30	0.640	0.625	0.635	0.610	0.628
35	0.640	0.620	0.628	0.600	0.622
40	0.640	0.618	0.630	0.600	0.622
45	0.640	0.620	0.615	0.605	0.620

a/ Optical density due to 40 micrograms of meta-dinitrobenzene in each case.

tween meta-dinitrobenzene and methyl ethyl ketone in the presence of alkali lacks stability and the time after color development at which it is read in the photometer is important. The optical density may be read anywhere between 5 and 60 minutes after the reagents are added, and it was observed that the maximum optical density occurred after 10 minutes. However, in analyzing a group of samples, one should for relative purposes always use the same time interval for color development. If the methyl ethyl ketone and alkali layers are separated, then the sample must be read immediately because the color fades very rapidly.

TABLE 3 -- EFFECT OF TIME ON COLOR DEVELOPMENT

Time	Optical Density Determined[a]		
Minutes	1	2	Average
20	0.620	0.620	0.620
30	0.610	0.610	0.610
40	0.615	0.605	0.610
50	0.612	0.600	0.606
60	0.605	0.605	0.605
70	0.595	0.590	0.593
80	0.590	0.585	0.588
90	0.580	0.580	0.580
120	0.580	0.580	0.580
150	0.555	0.550	0.553
180	0.535	0.530	0.533
210	0.530	0.515	0.523
420	0.400	0.400	0.400
420	0.400	0.380	0.390

[a/] Optical density due to 40 micrograms of meta-dinitrobenzene in each case.

Effect of Water in the Nitrating Column

The apparatus must be thoroughly dried between determinations. This can be accomplished with the aid of a good water aspirator. Remove all of the visible water, then rinse twice with acetone. Attach a clean, dry reaction flask to the ground joint and continue to apply suction until dry (approximately 15 minutes). Compressed air should not be used to dry the apparatus, as the air lines, compressor, etc., may contain oils and other contaminating substances. If the apparatus is not dry and water remains in the nitrating column, the effect will be similar to that shown in Table 4. Only a few drops of water present in the column will cause a loss of BHC. Therefore, wet samples or samples containing water can not be used, as the water distills over into the nitrating column and results in a loss in nitration of benzene and resultant meta- dinitrobenzene.

TABLE 4 -- EFFECT OF WATER IN NITRATING TUBE

BHC Added Micrograms	Water Added	Optical Density Determined[a]	Optical Density Standard[a]	Recovery %
40	1 ml.	0.249	0.283	88.0
40	1 ml.	0.231	0.283	81.6
40	5 ml.	0.000	0.283	00.0
40	5 ml.	0.000	0.283	00.0
40	10 ml.	0.000	0.283	00.0

a/ Optical density corrected for blank.

The Use of Dry, Ether Washed Cotton

It was considered possible that meta-dinitrobenzene may have been lost as a consequence of adsorption on cotton when the ether solution was dried by filtering it through a layer of cotton. Data are recorded in Table 5 on the optical density of ether solutions of meta-dinitrobenzene (40 μg.) which were passed through a 0.75 inch plug of sterile, absorbent, surgical cotton. This amount of meta-dinitrobenzene produced an average optical density of about 0.640. The absorbance values obtained for the six experimental trials were near the average. Thus, it was concluded that no losses occurred in this part of the method.

TABLE 5 -- EFFECT OF POURING AN ETHER SOLUTION OF META-DINITROBENZENE THROUGH DRY COTTON[a]

M-dinitrobenzene Added micrograms	Optical Density Determined
40	0.680
40	0.655
40	0.662
40	0.650
40	0.645
40	0.650

a/ Sterile absorbent, surgical cotton, twice washed in ether.

Standard Curve

Add 0, 1, 2, 3, 4, and 5 ml. of a solution of the gamma isomer (lindane) of BHC in glacial acetic acid to the reaction flask. Each ml. contains 0.01 mg. of BHC. Add 15 ml. of glacial acetic acid, 1.5 g. of zinc, and 2.0 g. of malonic acid and follow the procedure as presented in the section, "Analytical Procedure". After making a number of determinations, the averages of the resulting values should give a standard curve that obeys Beer's law. A great deal of difficulty was experienced in trying to obtain reproducible re-

sults, which, in part, is still unaccounted for. In all the work the best quality and purity of reagents were used. The acetic acid was fractionated, the first and last fractions being discarded. The lindane used was the gamma isomer supplied by the A.O.A.C. and used in collaborative study in 1954. Note the random absorbance values for known amounts of added lindane (Table 6). Compare the optical densities for 0, 10, 20γ, etc., added amounts of lindane. These data show the overlapping values obtained. The standard deviations range from about 12 to 25 percent of the average values obtained for a large number of independent values.

TABLE 6 -- ABSORBANCE VALUES OF LINDANE STANDARDS
PHOSPHORIC ACID AS LUBRICANT

BHC Added Micrograms	Optical Density Determined 1	2	3	4	5	Average	Standard Deviation
0	0.130	0.100	0.090	0.080	0.065		
	0.048	0.065	0.105	0.120	0.105		
	0.065	0.058	0.063	0.098	0.088		
	0.100	0.115	0.092	0.094	0.045		
	0.074	-----	-----	-----	-----	0.086	0.023
10	0.110	0.146	0.110	0.166	0.088		
	0.108	0.168	0.150	0.190	-----	0.137	0.034
20	0.250	0.210	0.184	0.190	0.167		
	0.133	0.250	0.195	0.220	-----	0.200	0.038
30	0.310	0.304	0.244	0.252	0.210		
	0.320	0.294	0.300	0.305	0.330	0.287	0.036
40	0.370	0.382	0.350	0.335	0.305		
	0.375	0.350	0.400	0.380	0.485		
	0.335	0.427	0.303	-----	-----	0.369	0.048
50	0.485	0.420	0.357	0.370	0.435		
	0.445	0.402	0.485	-----	-----	0.425	0.048
					Average Standard Deviation		±0.038

Phosphoric Acid as a Lubricant

As our investigations continued, it was found that one of the major reasons for the poor results was the use of phosphoric acid as a lubricant on the ground glass seals of the reaction flask and the nitrating apparatus. Although phosphoric acid forms what appears to be a good seal, the presence of even a small amount of it in the reaction flask caused a loss of BHC. Refer to Tables 7 and 8, and Figure 1. Regardless of how small an amount of phosphoric acid is used to make a seal, the refluxing acetic acid will work up around the ground glass joint and cause some of the phosphoric acid to come back down into the reaction pot with a consequent loss of BHC. Figure 1 very clearly shows the effect of phosphoric acid in the reaction flask on the recovery of lindane. One drop, or about 40 mg. of phosphoric acid,

caused more than a 50 percent loss. In view of these results, the problem of finding a suitable lubricant became a necessity.

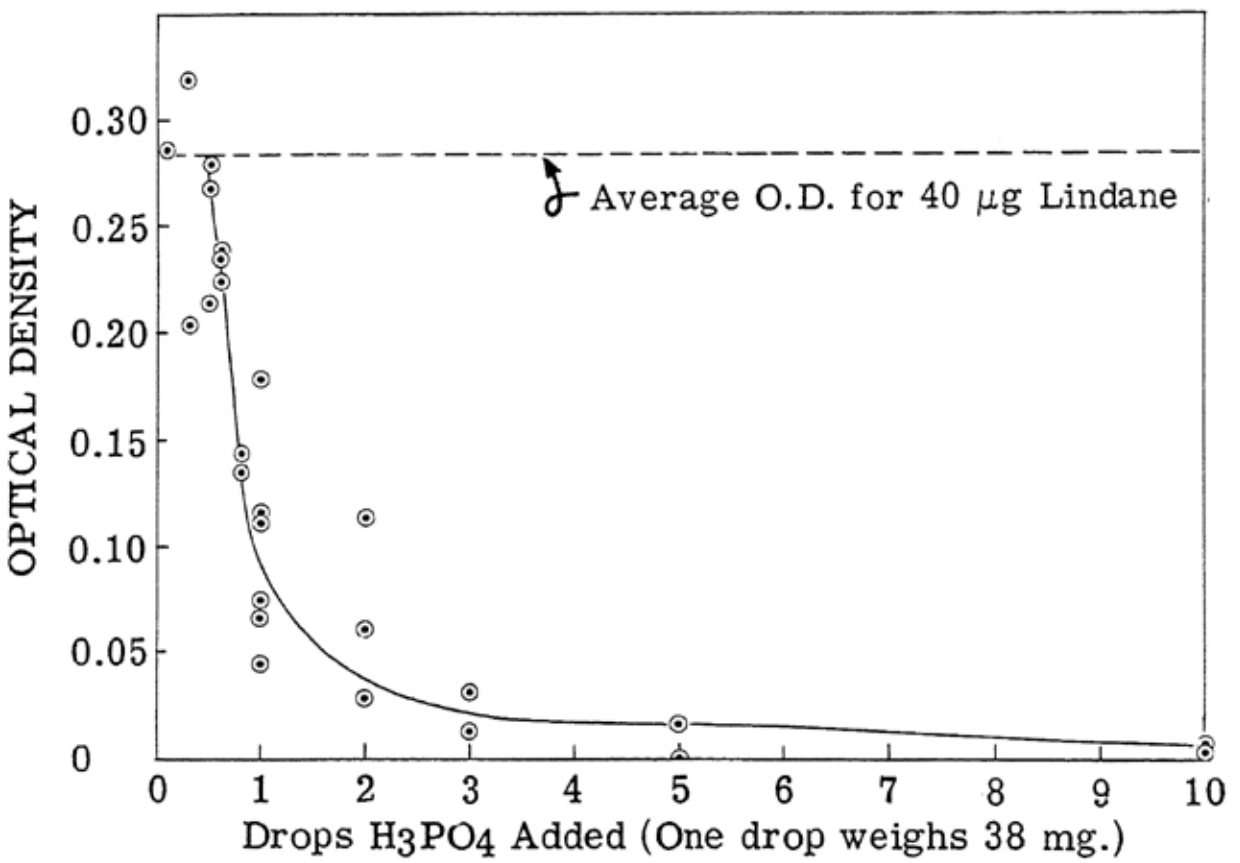

FIGURE 1

TABLE 7 -- EFFECT OF PHOSPHORIC ACID IN THE REACTION FLASK ON RECOVERY OF LINDANE

BHC Added Micrograms	Phosphoric Acid Added Drops[a]	Optical Density Determined[b]	Optical Density Standard[b]	Recovery %
20	1	0.031	0.114	27.2
20	2	0.029	0.114	25.4
20	2	0.000	0.114	00.0
20	3	0.021	0.114	18.4
20	10	0.000	0.114	00.0

a/ One drop of 85% phosphoric acid weighs 38 mg. (45 drops/ml.).

b/ Optical density corrected for blank.

TABLE 8 -- EFFECT OF PHOSPHORIC ACID IN THE REACTION FLASK ON RECOVERY OF LINDANE

BHC Added Micrograms	Phosphoric Acid Added Drops[a]	Optical Density Determined 1	2	3	Avg.	Avg.[b]	Standard[b]	Recovery %
40	0.1	0.372	-----	-----	0.372	0.286	0.283	101.0
40	0.3	0.405	0.450	0.290	0.381	0.295	0.283	104.2
40	0.5	0.353	0.365	0.300	0.339	0.253	0.283	89.4
40	0.6	0.325	0.320	0.310	0.318	0.232	0.283	82.0
40	0.8	0.220	0.230	-----	0.225	0.139	0.283	49.1
40	1	0.130	0.198	0.152				
		0.200	0.265	0.160	0.184	0.098	0.283	34.6
40	2	0.115	0.200	0.148				
		0.200	-----	-----	0.166	0.080	0.283	28.3
40	3	0.099	0.118	-----	0.109	0.023	0.283	8.1
40	5	0.082	0.102	-----	0.092	0.006	0.283	2.1
40	10	0.091	0.092	-----	0.092	0.006	0.283	2.1

a/One drop of 85% phosphoric acid weighs 38 mg. (45 drops per ml.).
b/Optical density corrected for blank.

Studies on Various Lubricants

A number of lubricants were tried, some of which were mineral oil, sulfuric acid, glycerol, dichloroacetic acid, and acetic acid. A lubricant was needed that would give a good seal, yet not cause a loss of BHC. After conducting a number of determinations, it was concluded that acetic acid and dichloroacetic acid appeared to be the best lubricants. The data are presented in Tables 9, 10, and 11. The addition of two drops of sulfuric acid in the

TABLE 9 -- EFFECT OF VARIOUS LUBRICANTS ON RECOVERY OF LINDANE

Lubricant	Micrograms BHC Added + Lubricant[a]	Optical Density Determined 1	2	Average[b]	Standard[b]	Recovery %
Mineral Oil	40	0.360	0.350	0.269	0.283	95.1
Mineral Oil	40 + 2 drops	0.300	0.201	0.165	0.283	58.3
H_2SO_4	40	0.290	0.332	0.225	0.283	79.5
H_2SO_4	40 + 2 drops	0.068	0.066	0.000	0.283	00.0
Glycerol	40	0.325	0.330	0.242	0.283	85.5
Glycerol	40 + 2 drops	0.270	0.235	0.167	0.283	59.0

a/ Two drops of lubricant placed in reaction flask where indicated.
b/ Optical density corrected for blank.

TABLE 10 -- ABSORBANCE VALUES OF LINDANE STANDARDS DICHLOROACETIC ACID AS LUBRICANT

BHC Added[a] Micrograms + Lubricant	Optical Density Determined
40	0.355
40	0.315
40	0.325
40	0.340
40	0.325
40	0.310
40	0.305
40	0.320
40	0.285
40	0.305
40 + 2 drops	0.305
40 + 2 drops	0.315
40 + 2 drops	0.335
	ave. 0.318
	Standard Deviation ±0.017

a/ Two drops of lubricant placed in reaction flask when indicated.

TABLE 11 -- ABSORBANCE VALUES OF LINDANE STANDARDS ACETIC ACID AS LUBRICANT

BHC Added Micrograms	Optical Density Determined 1	2	Average	Standard Deviation	Standard Deviation %
Blank	0.045	0.074			
	0.050	0.058			
	0.054	0.045	0.054	0.011	20.4
10	0.115	0.110	0.113	0.003	2.7
20	0.165	0.180	0.173	0.011	6.4
30	0.250	0.262	0.256	0.008	3.1
40	0.318	0.310			
	0.330	0.326			
	0.337	0.345			
	0.320	0.332			
	0.305	0.315			
	0.280	0.325			
	0.308	0.290			
	0.305	0.320	0.317	0.016	5.0
50	0.400	0.405	0.403	0.003	0.7
			Average Standard Deviation	±0.0086	

reaction flask caused a complete loss of 40 μg. of added BHC. Acetic acid gave a good seal and, of course, could not hinder the reaction in the flask as it was the solvent used.

Acetic acid was used by other workers as the solvent (1) because it promoted the dechlorination reaction, had a convenient boiling point, did not interfere in the nitration reaction, and was not readily decomposed by the nitrating acid mixture.

As a result of these studies, acetic and dichloroacetic acids are recommended as lubricants. Good seals of the joints and good reproducibility of the absorbance values for standards were obtained when these lubricants were used. No losses of BHC were evident when these reagents were placed in the reaction pot. The following average standard deviations for all absorbance values of standards in the range of 0 to 50 μg. were obtained. For phosphoric acid, ± 0.0380 O. D. units on 70 determinations (16.1% avg. dev.); acetic acid, ± 0.0086 O. D. units on 30 determinations (3.6% avg. dev.); and dichloroacetic acid, ± 0.0170 O. D. units on 17 determinations (5.3% avg. dev.).

A comparison of the average absorbance values obtained with phosphoric and acetic acids as lubricants for lindane standards over the range of

0 to 50 μg. is given in Table 12. The average values for both are in fair agreement, although slightly better recovery values were obtained when acetic

TABLE 12 -- A COMPARISON OF ABSORBANCE VALUES FOR LINDANE STANDARDS PHOSPHORIC AND ACETIC ACIDS AS LUBRICANTS

BHC Added Micrograms	Average Optical Density Determined[b]				Difference
	Phosphoric	Acetic	Phosphoric[a]	Acetic[a]	%
0	0.086	0.054	-----	-----	----
10	0.137	0.113	0.051	0.059	13.6
20	0.200	0.173	0.115	0.119	4.2
30	0.287	0.256	0.201	0.202	0.5
40	0.369	0.316	0.283	0.262	7.4
50	0.425	0.403	0.339	0.349	2.9

a/ Optical density corrected for blank

b/ Each absorbance value is an average of a number of independent runs as shown in Tables 6 and 11.

acid was used as the lubricant. These data are presented graphically in Figure 2. The values obtained when using each acid as a lubricant are close enough

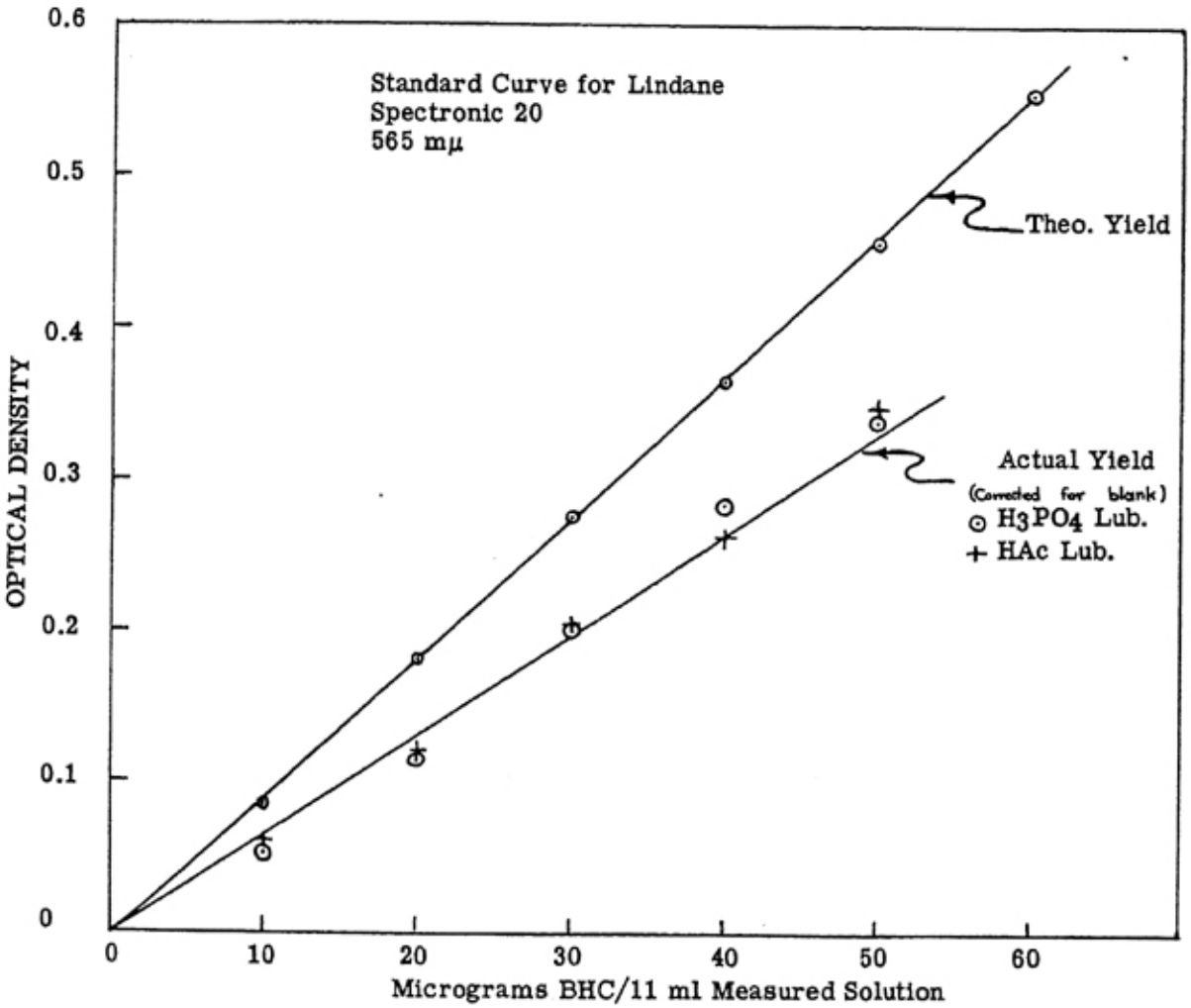

FIGURE 2

together so that a single straight line curve may be drawn. The experimental curve, as discussed in the original article (1), does not approach the theoretical curve because a part of the benzene is nitrated to ortho and para dinitrobenzene which does not form a colored complex with methyl ethyl ketone and alkali. The experimental recovery was found to be approximately 71 percent of the theoretical.

Experimental Pickle Samples

The Department of Horticulture is conducting a study on the effects of residual amounts of BHC on the fermentation of pickles and the organoleptic properties of the finished fermented product. All of the experimental pickle samples were obtained from these investigations.

The fermentation method and procedures for making sour, dill, and sweet pickles were similar to the processes used by most of the commercial pickle manufacturers in the United States. The cucumbers were placed in brine (40° salometer or about 10 percent by weight) where natural fermentation took place. Additional salt was added once per week until the salometer reading was 60 degrees.

The bacterial fermentation produces about 0.6 to 0.7 percent of lactic acid which is needed to cure and preserve the "salt stock". As the concentration of acid reaches this value, the pickles change from the chalky cucumber to a translucent texture. This change usually requires 6 months or longer at room temperature. The pickles will contain about 12 to 15 percent salt by weight, and are called "salt stock" pickles.

To convert the "salt stock" pickles to desirable ones, they were leached with water which reduced the salt content to 1 to 2 percent. This took approximately 24 to 48 hours, depending on the method used, and usually required three volumes of water (first two were cold washes and the last 62° C). Alum and turmeric were added to give firmness and uniform color.

The sour pickles were prepared by adding a 6 percent solution of acetic acid. They were allowed to stand for 24 hours. Then the solution was removed and a fresh 6 percent solution of acetic acid was added. The jars were sealed mechanically.

The dill pickles were prepared by adding the dill solution to the jars.

The sweet pickles were prepared by treating with 6 percent acetic acid solution for 24 hours, removing the acid, adding a fresh solution, and then gradually adding sugar (other spices, etc.) until the sweet pickles were produced which contained 40 percent sugar. As with the other pickles, a mechanical seal was used.

Preparation of Pickle Samples for BHC Analysis

All samples were prepared for chemical analysis in accordance with standard A.O.A.C. procedures. The raw cucumber samples were ground in

a Waring Blendor for 5 minutes and then stored in polyethylene containers at -17.7° C., until analyzed. The salt stock, sour, sweet, and dill pickles were stored at room temperature until prepared for analysis. One gallon jars of pickles were then dumped on 8-mesh screens 12 inches in diameter and drained for 2 minutes. After draining, the pickles were placed in a Waring Blendor and blended for five minutes. The blended samples were then stored in polyethylene containers at 4.4° C. in the dark, until analyzed.

A method was adapted for the extraction of lindane from the blended samples, as it was found in preliminary work that the wet pickle samples cannot be placed directly in the dechlorination reaction flask. The extraction method used was as follows:

> Add 100 ml. of methylene chloride to an 80 g. sample in a glass stoppered Erlenmeyer flask and shake vigorously for 5 to 10 minutes. Allow the mixture to stand and separate for a few minutes, then draw off 65 to 75 ml. of the lower methylene chloride solution and place in a glass stoppered Erlenmeyer flask containing 10 to 15 g. of anhydrous sodium sulfate. Shake the flask vigorously for 5 minutes to ensure removal of water from the extract solution.
>
> Take a 25 ml. aliquot of the extract and place in a reaction flask. This aliquot represents a 20 g. sample. Place the reaction flask on a steam water bath at 80° C. and carefully drive off the methylene chloride until one ml. remains, and finally take to dryness by pouring off the remaining vapors.
>
> The substances left in the flask will be lindane (if present) and any other methylene chloride soluble materials. Add 15 ml. of acetic acid, 2.0 g. malonic acid, and 1.5 g. of zinc metal or dust to the flask. After all of the reagents have been added, the flask is attached to the digestion and nitrating apparatus. The remainder of the method is similar to that given under "Analytical Procedure".

Recovery of Lindane from Dry Methylene Chloride Extracts

To determine whether the methylene chloride extraction method gave good recovery, studies were made on pickles to which known, varying amounts of lindane were added. From the Percent Recovery column in Table 13 something appears wrong. The erratic results do not set a pattern nor do they prove whether or not the extraction was complete. The recoveries ranged from 39.8 to 127 percent. The non-reproducible results may have been due, in part, to the use of phosphoric acid as a lubricant.

Some recovery studies (see Table 14) were attempted using wet pickles, before the effects of water in the nitrating column were known, with similar erratic results. Most of the percent recoveries were higher for some unknown reason, but off colors were developed in the methyl ethyl ketone which may

TABLE 13 -- RECOVERY OF LINDANE FROM DRY METHYLENE CHLORIDE EXTRACT OF PICKLES
PHOSPHORIC ACID AS LUBRICANT

Sample	BHC Added Micrograms	Optical Density: Pickles + Blank + BHC	Optical Density: Pickles + Blank	Optical Density: Due to Added BHC	Standard[a]	Recovery %
1. Salt Stock	30	0.230	0.150	0.080	0.201	39.8
1. Salt Stock	30	0.235	0.150	0.085	0.201	42.3
1. Salt Stock	40	0.380	0.150	0.230	0.283	81.3
1. Salt Stock	40	0.310	0.150	0.160	0.283	56.5
2. Sour	30	0.300	0.139	0.161	0.201	80.1
2. Sour	30	0.310	0.139	0.171	0.201	85.1
2. Sour	40	0.436	0.139	0.297	0.283	104.9
2. Sour	40	0.360	0.139	0.221	0.283	78.1
2. Sour	40	0.410	0.139	0.271	0.283	95.8
2. Sour	40	0.490	0.139	0.351	0.283	124.0
3. Sweet	20	0.268	0.135	0.133	0.114	116.6
3. Sweet	20	0.266	0.135	0.131	0.114	114.9
3. Sweet	30	0.340	0.135	0.205	0.201	102.0
3. Sweet	30	0.390	0.135	0.255	0.201	127.0
3. Sweet	30	0.285	0.135	0.150	0.201	74.6
3. Sweet	30	0.295	0.135	0.160	0.201	79.6
3. Sweet	40	0.355	0.135	0.220	0.283	77.7
3. Sweet	40	0.332	0.135	0.197	0.283	69.6
3. Sweet	40	0.358	0.135	0.223	0.283	78.8
3. Sweet	40	0.330	0.135	0.195	0.283	68.9
3. Sweet	40	0.435	0.135	0.300	0.283	106.0
3. Sweet	40	0.480	0.135	0.345	0.283	121.9
4. Dill	40	0.360	0.147	0.213	0.283	75.3
4. Dill	40	0.305	0.147	0.158	0.283	55.8
4. Dill	40	0.330	0.147	0.183	0.283	64.7
17. Salt Stock	30	0.310	0.100	0.210	0.201	104.5
17. Salt Stock	30	0.315	0.100	0.215	0.201	107.0

[a] Optical density corrected for blank.

TABLE 14 -- RECOVERY OF LINDANE FROM WET PICKLES
PHOSPHORIC ACID AS LUBRICANT

Sample Number	BHC Added Micrograms	Optical Density: Pickles + Blank + BHC	Optical Density: Pickles + Blank	Optical Density: Due to Added BHC	Standard[a]	Recovery %
13. Salt Stock	20	0.265	0.168	0.097	0.114	85.1
13. Salt Stock	30	0.432	0.168	0.264	0.201	131.3
13. Salt Stock	50	0.620	0.168	0.452	0.339	133.3
14. Sour	20	0.335	0.198	0.137	0.114	120.2
14. Sour	30	0.370	0.198	0.172	0.201	85.6
14. Sour	50	0.770	0.198	0.572	0.339	168.7
18. Sour	30	0.360	0.067	0.293	0.201	145.8
18. Sour	40	0.438	0.067	0.371	0.283	131.1

[a] Optical density corrected for blank.

account for this. This was also true of some of the analyses on wet pickle samples that gave varying results. Not only did water distill over into the nitrating column, but certain organic substances must have been steam distilled over and produced color reactions that were not due to lindane. This also may have been true for the methylene chloride extracts, in which the solvent may have extracted some organic materials that contributed to the color development and appeared to be lindane.

Data are presented in Tables 15 and 16 giving the lindane content on a large number of different types of pickle samples. The values reported can be expressed only as "apparent lindane content". Colored solutions were obtained which yielded an optical density that gave a calculated p.p.m. of lindane in the sample. This did not mean that lindane was present, unless

TABLE 15 -- LINDANE RESIDUES ON PICKLES (1952-1953)
PHOSPHORIC ACID AS LUBRICANT

Sample Number	Type	Color Developed	P.P.M.[a]
1A Salt Stock	Control	Orange	0.75
2A Salt Stock	Control	Green	1.65
3A Salt Stock	Spray	Yellow	0.05
4A Salt Stock	Spray	Yellow	0.50
5A Sweet	Control	Dirty violet	0.65
6A Dill	Control	Orange	0.37
7A Sour	Control	Yellow	0.20
8A Sweet	Control	Orange	0.45
9A Dill	Control	Orange	0.24
10A Sour	Control	Violet	1.19
11A Sweet	Spray	Yellow	0.31
12A Dill	Spray	Pale violet	0.48
13A Sour	Spray	Orange	0.49
14A Sweet	Spray	Yellow and violet	1.12
15A Dill	Spray	Yellow-green	0.88
16A Sour	Spray	Yellow-green	0.85
17A Raw	Spray	Violet	11.72
18A Raw	Control	Dirty violet	1.70
19A Salt Stock	Spray	Violet	10.00
20A Salt Stock	Control	Yellow	0.21
21A Sour	Control	Yellow	0.40
22A Sour	Spray	Violet	12.00
23A Dill	Control	Pale violet	0.72
24A Dill	Spray	Violet	4.00
25A Sweet	Spray	Violet	2.75
26A Sweet	Control	Violet	2.15
27A Raw	Spray	Violet	10.40
28A Raw	Control	Violet	3.08

a/ Each value is an average of a number of independent analyses. (From 2 to 16).

the solution was a violet-cherry color, and even then there was a question concerning it. The yellow, orange, or green color, etc., may have been produced by some methylene chloride extracted substances which were carried through the analytical procedure. The off-colors also could have been due to a combination of meta-dinitrobenzene and some other interfering material which did not allow full development of the violet colored complex. This is especially true of the 1952-1953 samples and in a few cases for the 1954 samples.

All of the pickle samples in the 1954 series had received six applications of lindane in the field by spraying. The samples appeared to be free of lindane after the fermentation and pickling processes. Inositol and lindane were added to the fermentation vats of eight of the samples at a level of 10 p.p.m. on a total volume basis. These substances were not added for recovery purposes as such, but to determine how much of them would carry through the pickling processes, and if fermentation would be retarded by their presence. The data are presented in Table 16. The apparent lindane content of these samples was considerably higher than that of samples which did not have added lindane and/or inositol in the vats. The values ranged from about 4 to 8 p.p.m.

An extremely high value for the optical density was occasionally obtained for a sample that on repeated analyses yielded lower values. The explanation for this could well be from contamination due to benzene or some ring compound or from compounds extracted from the pickle samples by methylene chloride. Extreme care was always taken to prevent contamination. To determine if some reagent or extractant might be responsible for the erratic results, an experiment was set up to determine the effect of sodium sulfate, ethyl acetate, methyl ethyl ketone, propionaldehyde, ethyl alcohol, and chlorophyll placed directly in the reaction flask on the optical density of standards (Table 17). Ethyl acetate was the only substance of this group that showed any effect. The effect of adding ethyl acetate was much the same as the random infinite optical density value obtained for one of these unexplained higher values for a sample, although the color and optical density evidently are not due to ethyl acetate, but from some aromatic impurity in it. The ethyl acetate was of analytical grade and an ultraviolet absorption spectrum of it made on the Model 11 Cary Spectrophotometer revealed no benzene. However, the presence of some undetermined substance which absorbed at 260 mμ. was noted. It was believed to be aromatic in nature and responsible for the color.

TABLE 16 -- LINDANE RESIDUES ON PICKLES (1954)
PHOSPHORIC ACID AS LUBRICANT

Sample Number	Substance Added[a]	Color Developed	P.P.M.[b]
1. Salt Stock		Orange	0.54
2. Sour		Orange	0.41
3. Sweet		Orange	0.42
4. Dill		Orange	0.20
5. Salt Stock		Orange	0.00
6. Sour		Orange	0.00
7. Sweet		Orange	0.05
8. Dill		Orange	0.08
9. Salt Stock		Orange	0.00
10. Sour		Orange	0.00
11. Sweet		Orange	0.40
12. Dill		Orange	0.07
13. Salt Stock		Orange	0.06
14. Sour		Orange	0.00
15. Sweet		Orange	0.00
16. Dill		Orange	0.00
17. Salt Stock		Orange	0.12
18. Sour		Orange	0.00
19. Sweet		Orange	0.00
20. Dill		Orange	0.06
21. Salt Stock	10 ppm Lindane + 10 ppm Inositol	Violet	4.25
22. Sour	10 ppm Lindane + 10 ppm Inositol	Violet	3.73
23. Sweet	10 ppm Lindane + 10 ppm Inositol	Violet	4.40
24. Dill	10 ppm Lindane + 10 ppm Inositol	Violet	5.50
25. Salt Stock	10 ppm Lindane	Violet	7.30
26. Sour	10 ppm Lindane	Violet	4.68
27. Sweet	10 ppm Lindane	Violet	8.10
28. Dill	10 ppm Lindane	Violet	7.00

a/ Substance added to fermentation vat with brine on a total volume basis.
b/ Each value is an average of a number of independent analyses (From 2 to 16).

TABLE 17 -- EFFECT OF VARIOUS SUBSTANCES IN REACTION FLASK
ACETIC ACID AS LUBRICANT

BHC Added Micrograms	Substance Added	Optical Density Determined 1	2	3	Average	Standard
40	50 mg. Na_2SO_4	0.500	0.405	0.300	0.402	0.317
40	2 drops Ethyl Acetate	1.200	1.160	0.920	1.093	0.317
40	2 drops Methyl Ethyl Ketone	0.327	0.410	0.305	0.347	0.317
40	2 drops Propionaldehyde	0.370	0.480	0.355	0.402	0.317
40	2 drops Ethyl Alcohol	0.270	0.425	0.313	0.336	0.317
40	50 mg. Chlorophyll	0.348	0.345	0.278	0.324	0.317

SUMMARY AND CONCLUSIONS

The objectives of this investigation were to determine the critical points in the Schechter-Hornstein colorimetric method for lindane, and to determine the amount of lindane residues on pickles.

Findings and Recommendations:

Extreme cleanliness and the best analytical techniques must be practiced.

The nitrating column and reaction flask must be dry. The apparatus may be dried with the aid of vacuum using a water aspirator. Compressed air should not be used to dry the apparatus, as it may contain aromatics.

After the meta-dinitrobenzene is extracted from the acid solution with ether, the determination should be carried to completion without interruptions.

No apparent loss occurred when known amounts of meta-dinitrobenzene were allowed to stand for 3 hours in the acid-ether solution.

In removing the ether on the steam bath, when the volume approaches 5 ml., constant rotation of the flask is necessary to prevent loss of meta-dinitrobenzene. When the volume is 1 to 2 ml., the flask should be removed from the heat and rotated in a horizontal position to remove the remaining ether vapors, then stoppered and the color immediately developed. The flasks must not be left open or allowed to stand stoppered for any period of time. The ether should be evaporated as rapidly as possible (15 to 30 minutes).

Maximum optical density of the colored complex forms within 10 minutes. The color may be read after 5 minutes, and up to 60 minutes. However, for comparative purposes, the same time interval should be used for all readings. If the MEK solution is decanted from the alkali the optical density must be read at once.

No losses of meta-dinitrobenzene were evident upon passing the ether solution through dry, ether washed, absorbent cotton.

If phosphoric acid is used as a lubricant *extreme care* must be used so that none of it gets into the reaction flask; otherwise low and erratic results will be obtained. This finding does not agree with those of other workers.

Mineral oil and glycerol are usable as lubricants but have the same disadvantages as phosphoric acid. Sulfuric acid has many disadvantages.

Acetic acid and dichloroacetic acid are recommended as lubricants. Good seals of the joints were obtained. Good reproducibility was obtained for absorbance values of standards when these lubricants were used. Acetic acid appeared to be the best. No losses of BHC occurred when these lubricants were placed in the reaction flask. The average standard deviations for all absorbance values of standards in the range of 0 to 50 μg were:

Phosphoric acid as lubricant on 70 determinations $\pm$0.0380 Absorbance units. (16.1% Average deviation.)

Acetic acid as lubricant on 30 determinations ± 0.0086 Absorbance units. (3.6% Average deviation.)

Dichloroacetic acid as lubricant on 13 determinations ± 0.0170 Absorbance units. (5.3% Average deviation.)

Ethyl alcohol, chlorophyll, propionaldehyde, sodium sulfate, and methyl ethyl ketone, when added to the reaction flask, had no apparent effect on recovery of lindane.

The recovery of added BHC from pickles by a methylene chloride extraction procedure was not considered satisfactory. In many cases the final color developed was orange, red, green, dirty yellow, etc. Certain organic molecules were extracted by the solvent and interfered in the final color development. It cannot be said that lindane is present unless a violet-red colored solution is obtained and then its presence is not definitely proven.

The recovery of BHC added to wet pickles, in most cases, was positive. Certain organic molecules, in trace amounts, must have been steam distilled into the reaction column. Wet samples cannot be placed in the reaction pot.

It appears from this investigation on the reproducibility and various points in the colorimetric method for lindane that additional research on the method should be conducted. Perhaps the sulfonation technique employed by Hornstein (5) on the removal of interfering substances from mushrooms is an answer to some of the difficulties encountered.

LITERATURE CITED

1. Schechter, M. S., and Hornstein, I., Colorimetric Determination of Benzene Hexachloride, Anal. Chem. *24* 544-8 (1952).
2. Hornstein, Irwin, Determination of Technical Benzene Hexachloride in Peanuts and Soils, Anal. Chem. *24* 1036-7 (1952).
3. Reynolds, H., Gilpin, G. L., and Hornstein, I., Palatability and Chemical Studies on Peanuts Grown in Rotation with Cotton Dusted with Insecticides Containing Benzene Hexachloride, J. Agr. Food Chem., *1* 772-6 (1953).
4. Hornstein, I., Reynolds, H., and Gilpin, G. L., Benzene Hexachloride Content and Flavor of Peanuts Grown in Rotation with Cotton Dusted with This Insecticide, J. Agr. Food Chem., *2* 776-8 (1954).
5. Hornstein, Irwin, Determination of Lindane in Mushrooms, J. Agr. Food Chem., *3* 848-9 (1955).
6. Hornstein, Irwin, McGregor, W. S., and Sullivan, W. N., Lowering the Volatility of Lindane Cattle Sprays by Addition of Film-forming Material, J. Agr. Food Chem., *4* 148-9 (1956).
7. Hornstein, Irwin, Report on Benzene Hexachloride, J. Assoc. Official Agr. Chemists, *35* 266-8 (1952).
8. Klein, A. K., Report on Benzene Hexachloride in Foods, J. Assoc. Official Agr. Chemists, *36* 589-94 (1953).
9. Klein, A. K., Report on Benzene Hexachloride in Foods, J. Assoc. Official Agr. Chemists, *37* 576-8 (1954).
10. Klein, A. K., Private Communication, June, 1955.