Longer lags also allow more time for some of the compounds to decompose.

TABLE OF IGNITION TEMPERATURES

Table III gives the ignition temperatures employed by the Bureau of Mines for a number of compounds. Many have been determined by the bureau; the remainder were selected from the results of other investigators. A variety of methods was, of course, used in the different determinations.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Standards, Pt. III, pp. 90–1 (A.S.T.M. D286-30), 1942.
(2) Baron, J., and Laffitte, P., *Compt. rend.*, **206**, 1386 (1938).
(3) Bunte, K., and Block, A., *Gas- und Wasserfach*, **78**, 325 (1935).
(4) Coward, H. F., *J. Chem. Soc.*, **1934**, 1382.
(5) Coward, H. F., Jones, G. W., Dunkle, C. G., and Hess, B. E., Mining Met. Invest., U. S. Bur. Mines, Carnegie Inst. Technol., Mining Met. Advisory Boards, *Bull.* 30 (1926).
(6) Dixon, H. B., *Rec. trav. chim.*, **44**, 305 (1925).
(7) Dixon, H. B., and Coward, H. F., *J. Chem. Soc.*, **95**, 514 (1909).
(8) Egerton, A., and Gates, S. F., *J. Inst. Petroleum Tech.*, **13**, 244 (1927).
(9) Factory Mutuals, *Ind. Eng. Chem.*, **32**, 880 (1940).
(10) Freitag, *Current Met. Abstracts, Metals & Alloys*, **3**, 85 (March 1932).
(11) Goldman, F., *Z. physik. Chem.*, **5**, 316 (1929).
(12) Grebel, A., *Mem. et compt. rend. trav. soc. ing. civils France*, **83**, 35 (1930).
(13) Holm, H., *Z. angew. Chem.*, **26**, 273 (1913).
(14) Jones, G. W., and Beattie, B. B., *Ind. Eng. Chem.*, **26**, 557 (1934).
(15) Jones, G. W., Kennedy, R. E., and Miller, W. E., U. S. Bur. Mines, *Rept. Investigations* **3648** (1942).
(16) Jones, G. W., Kennedy, R. E., and Scott, G. S., *Ibid.*, **3597** (1941).
(17) Jones, G. W., *et al.*, U. S. Bur. Mines, unpublished data.
(18) Jones, G. W., and Miller, W. E., U. S. Bur. Mines, *Rept. Investigations* **3567** (1941).
(19) Jones, G. W., Miller, W. E., and Seaman, H., *Ind. Eng. Chem.*, **25**, 694 (1933).
(20) Jones, G. W., and Scott, G. S., U. S. Bur. Mines, *Rept. Investigations* **3881** (1946).
(21) Jones, G. W., Scott, G. S., and Miller, W. E., *Ibid.*, **3640** (1942).
(22) Jones, G. W., Seaman, H., and Kennedy, R. E., *Ind. Eng. Chem.*, **25**, 1283 (1933).
(23) Jones, G. W., Yant, W. P., Miller, W. E., and Kennedy, R. E., U. S. Bur. Mines, *Rept. Investigations* **3284** (1935).
(24) Laffitte, P., and Baret, G., *Bull. soc. chim.*, (*4*) **51**, 281 (1932).
(25) Lewis, J. S., *J. Chem. Soc.*, **1930**, 2241.
(26) Mason, W., and Wheeler, R. V., *Ibid.*, **121**, 2079 (1922).
(27) Masson, H. J., and Hamilton, W. F., *Ind. Eng. Chem.*, **20**, 813 (1928).
(28) Moore, H., *J. Inst. Petroleum Tech.*, **6**, 186 (1919).
(29) Moore, H., *J. Soc. Chem. Ind.*, **36**, 109 (1917).
(30) Naylor, C. A., and Wheeler, R. V., *J. Chem. Soc.*, **1931**, 2456.
(31) Nuckolls, A. H., "Underwriters' Laboratories Method for Classification of Hazards of Liquids" (March 1929).
(32) Nuckolls, A. H., Underwriters' Laboratories, *Report* **S-1528** (1919).
(33) *Ibid.*, **2375** (1933).
(34) Ormandy, W. R., and Craven, E. C., *J. Inst. Petroleum Tech.*, **12**, 650 (1926).
(35) Sortman, C. W., Beatty, H. A., and Heron, S. D., *Ind. Eng. Chem.*, **33**, 357 (1941).
(36) Tanaka, Y., and Nagai, Y., *Proc. Imp. Acad.* (*Tokyo*), **2**, 219 (1926).
(37) Thompson, N. J., *Ind. Eng. Chem.*, **21**, 134 (1929).
(38) White, A. G., and Price, T. W., *J. Chem. Soc.*, **115**, 1462 (1919).
(39) Wollers, G., and Emcke, V., *Krupp. Monatsh.*, **2**, 1 (1921).
(40) Zerbe, C., and Eckert, F., *Angew. Chem.*, **45**, 593 (1932).

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Determination of the Gamma Isomer of Hexachlorocyclohexane

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A method is described for determination of the gamma isomer of hexachlorocyclohexane in the technical product and dust mixtures. The method is based on the dehydrochlorination of two 0.100-gram samples, dissolved in 50.0 ml. of 95% ethyl alcohol, one for 15 minutes and the other for 50 minutes, at 0° C. with 10.0 ml. of 1 *N* ethanolic potassium hydroxide. The per cent chloride difference multiplied by a factor yields the percentage of gamma isomer.

THE insecticidal value of hexachlorocyclohexane is believed to be due to the presence of the gamma isomer of hexachlorocyclohexane (*7*), and in the enforcement of the Agricultural Code of the State of California, pertaining to the labeling and sale of economic poisons, it is necessary to determine the gamma isomer in commercial dust mixtures offered for sale as insecticides.

A search of the literature and written inquiry disclosed that the only workable methods developed at that time were based on infrared absorption (*8*) or bioassay using insects. The infrared absorption method, though accurate under certain conditions, demands the use of the relatively expensive infrared spectrophotometer, and the bioassay method requires the skill of a highly trained entomologist.

EXPERIMENTAL

Hexachlorocyclohexane is dehydrochlorinated by alcoholic sodium or potassium hydroxide, losing three atoms each of hydrogen and chlorine and forming trichlorobenzene (*2*, *9*). The individual isomers have different rates of reaction to dehydrochlorination (*2*), depending on time, temperature, and concentration of reactants. The reaction goes rapidly to completion at elevated temperatures.

Samples of highly refined alpha, beta, gamma, delta, and epsilon isomers of hexachlorocyclohexane were obtained and their melting points determined as shown in Table I. The reaction rates of the individual isomers were determined at various temperatures and concentrations. Reaction rate curves under the best conditions found were plotted (Figure 1). From these data it was deduced that the alpha and delta isomers should be almost completely dehydrochlorinated before the gamma isomer was appreciably dehydrochlorinated, then using a longer period of time the alpha, delta, and gamma isomers should all be dehydrochlorinated. Since the beta isomer is inert under these conditions, and the epsilon isomer content in the technical product is low enough not to interfere seriously, the difference in chloride produced in a definite short and long period of dehydrochlorination should be a function of the gamma isomer content.

Table I. Melting Points of Purified Isomers

Isomer	Melting Point Range, ° C.
Alpha	157.5–158.0
Beta	309 (sublimes)
Gamma	112.0–112.8
Delta	138.0–138.4
Epsilon	218.5–219.3

Duplicate samples containing known percentages of the five isomers were prepared. Portions of the samples weighing 0.100 gram were dissolved in 50.0 ml. of 95% ethyl alcohol, and dehydrochlorinated at 0° C. (as described below) with 10.0 ml. of 1 *N* ethanolic potassium hydroxide for 5, 10, 15, and 20 minutes for the short periods for dehydrochlorination of the alpha and delta isomers, and 30, 40, 50, and 60 minutes as the periods for dehydrochlorination of alpha, delta, and gamma isomers. The reactions were stopped at the required time by the addition of 10 ml. of 1 to 3 nitric acid, and the chloride was determined by the Volhard method. The percentage of chloride formed during the shorter intervals of time was subtracted from the chloride produced during the longer intervals. These chloride values were then plotted against the percentage of gamma isomer in the samples.

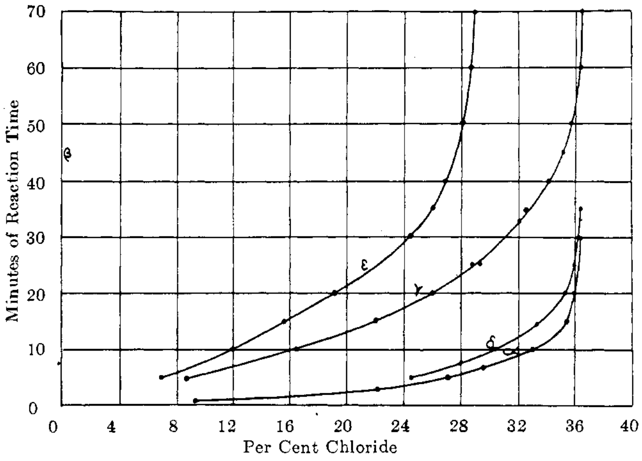

Figure 1. Effect of Time on Dehydrochlorination of Hexachlorocyclohexane Isomers

0.100 gram of isomer in 50.0 ml. of ethyl alcohol at 0° C. with 10.0 ml. of 1 *N* ethanolic potassium hydroxide

The 10-minute period was not long enough to dehydrochlorinate completely mixtures containing over 70% alpha plus delta isomers, while the 40-minute period was too short for a gamma content in excess of 40%. The periods of 15 and 50 minutes seemed to fulfill the requirements, yielding a straight line expressable by the equation:

$$8(\% \text{ chloride in 50 minutes} - \% \text{ chloride in 15 minutes}) - 8.20 = \% \text{ gamma isomer}$$

Two dust mixtures containing known percentages of pure alpha, beta, gamma, and delta isomers of hexachlorocyclohexane were prepared in triplicate with talc as a diluent, only enough mixture being made for each test to eliminate the error of nonuniformity of one large batch. One mixture was extracted with acetone and another with anhydrous ether in a Soxhlet extraction apparatus. After the solvent had been removed, the residues were analyzed for total hexachlorocyclohexane content by dehydrochlorination, by refluxing 0.100 gram of the residues with 10 ml. of 1 *N* ethanolic potassium hydroxide for 20 minutes and determining chloride by the Volhard method, using nitrobenzene to coagulate the silver chloride (*1*). Hexachlorocyclohexane loses 3 molecules of hydrogen chloride when refluxed with ethanolic potassium hydroxide. A weight of the residues equivalent to 0.100 gram of hexachlorocyclohexane was dissolved in 50.0 ml. of 95% alcohol and dehydrochlorinated at 0° C. with 10.0 ml. of 1 *N* ethanolic potassium hydroxide, one sample for 15 minutes and a second sample for 50 minutes. Other portions of the dust mixtures were analyzed for total hexachlorocyclohexane content by dehydrochlorination and the unextracted dusts equivalent to 0.100 gram of hexachlorocyclohexane were refluxed for 20 minutes with 50.0 ml. of 95% alcohol to dissolve the hexachlorocyclohexane. The cooled solutions were then dehydrochlorinated for 15 and 50 minutes at 0° C. Results are shown in Table II.

Table II. Comparison of Methods for Analysis of Dust Mixtures

Sample No.	Gamma Isomer, %	Gamma Isomer Added	Gamma Isomer as Determined, %: Acetone extracted residue	Ether extracted residue	Dust sample without extraction
1[a]	In total HCH	12.00	14.52	11.64	11.64
	In prepared sample	0.60	0.73	0.58	0.58
2[b]	In total HCH	20.00	25.56	20.20	21.30
	In prepared sample	0.20	0.26	0.20	0.21
3[c]	In total HCH	10.00	...	...	10.53
	In prepared sample	1.00	...	...	1.05

[a] Dust mixture containing 5% total hexachlorocyclohexane, composed of alpha 70.0%, beta 5.0%, gamma 12.0%, and delta 13.0%, diluted with talc. Finished dust contained 0.60% gamma isomer.

[b] Dust mixture containing 1% total hexachlorocyclohexane, composed of alpha 55.0%, beta 2.0%, gamma 20.0%, and delta 23.0%, diluted with talc. Finished dust contained 0.20% gamma isomer.

[c] Dust mixture containing 10% total hexachlorocyclohexane, composed of alpha 70.0%, beta 3.0%, gamma 10.0%, delta 15.0%, and epsilon 2.0%, diluted with talc containing 0.5% sulfur. Finished dust contained 1.00% gamma isomer.

REAGENTS

Ethyl alcohol, 190-proof, 95% by volume.

Ethanolic potassium hydroxide, 1.000 *N*. Dissolve 57 to 58 grams of C.P. potassium hydroxide pellets in about 950 ml. of 95% ethyl alcohol with the aid of heat, cool, filter into a 1-liter volumetric flask, mix, and standardize against 0.5 *N* hydrochloric acid, using methyl orange indicator. Adjust to 1.000 *N* by the addition of alcohol or alkali.

Nitric acid, 1 to 3, 1 volume of C.P. concentrated nitric acid with 3 volumes of distilled water.

Silver nitrate, 0.1 *N*, containing 16.989 grams of silver nitrate per liter of solution. Standardize by precipitating with a slight excess of dilute hydrochloric acid and weighing as silver chloride.

Ammonium thiocyanate, 0.1 *N*, containing 7.611 grams of ammonium thiocyanate per liter of solution. Standardize against the 0.1 *N* silver nitrate.

Ferric alum indicator, 10%. Dissolve 10 grams of C.P. ferric ammonium sulfate in about 50 ml. of distilled water, filter, decolorize with concentrated nitric acid, and dilute to 100 ml.

Nitrobenzene, purified grade mononitrobenzene.

Anhydrous ethyl ether, reagent grade.

PROCEDURE

The original calibration graph was based on the use of 0.100 gram of 100% pure hexachlorocyclohexane isomer mixtures. The technical product and diluted dust mixtures must first be analyzed to determine their hexachlorocyclohexane content. If the dust mixture is extracted with ether, the thoroughly mixed residue must be analyzed, so that 0.100 gram of total hexachlorocyclohexane is dehydrochlorinated for 15 and 50 minutes at 0° C.

Determination of Total Hexachlorocyclohexane. This may be calculated from total combined chlorine. The lime ignition method of Kimball and Tufts (*4*) is suitable for determination of total chlorine in samples of technical hexachlorocyclohexane.

Those authors have modified the method (*5*) for the analysis of hexachlorocyclohexane by weighing a sample equivalent to approximately 0.15 gram of hexachlorocyclohexane in a 2.5-inch (6.25-cm.) length of ordinary soda straw, crimped at one end. The lower 3 inches of the ignition tube containing the sample in the soda straw are filled with a mixture of hydrated lime containing 15% anhydrous calcium nitrate. The remainder of the tube is filled with lime alone. A blank determination including a 2.5-inch piece of empty soda straw must be run and the chloride found subtracted from subsequent determinations.

The Parr peroxide bomb (*6*) can be used to determine total chlorine in samples of hexachlorocyclohexane and its mixtures, but it sometimes gives low results. A sample of the technical product in excess of 0.1 gram is difficult to oxidize completely, and low results are obtained. A sample of a dust mixture corresponding to 0.1 gram of total hexachlorocyclohexane can usually be completely oxidized. The total chlorine is determined in the fusion mixture by the Volhard method.

Total hexachlorocyclohexane can be calculated from the total chlorine determination by the formula:

$$\frac{\text{Ml. of 0.1 } N \text{ AgNO}_3 \times 0.4848}{\text{sample weight}} = \%\text{ total hexachlorocyclohexane}$$

Total hexachlorocyclohexane can also be determined quantitatively by dehydrochlorination. When hexachlorocyclohexane is refluxed with ethanolic sodium or potassium hydroxide, 3 molecules of hydrogen chloride are removed, producing trichlorobenzene (*2*).

A 0.1- to 0.2-gram sample of technical hexachlorocyclohexane or an equivalent weight of a dust mixture is refluxed 20 minutes in a 125-ml. Ŧ Erlenmeyer flask with approximately 15 ml. of 1 N ethanolic potassium hydroxide. If a large sample weight of a dilute dust is used, sufficient alcohol may be added to cover the sample. After refluxing, the flask is cooled and the inside walls of the flask are washed down with distilled water. The contents are made slightly acid with 1 to 3 nitric acid, using a drop of phenolphthalein solution as an indicator. An excess of 0.1 N silver nitrate along with 3 ml. of 10% ferric alum solution and 5 ml. of nitrobenzene is added to the flask. The flask is stoppered and shaken to coagulate the silver chloride (*1*), and the excess silver nitrate is then titrated with 0.1 N ammonium thiocyanate. An indicator blank must be applied.

$$\frac{\text{Ml. of 0.1 } N \text{ AgNO}_3 \times 0.9696}{\text{sample weight}} = \%\text{ total hexachlorocyclohexane}$$

Data obtained by the three methods, using purified alpha isomer, are given in Table III. The other four isomers gave similar results.

Determination of Gamma Isomer. Two samples of the technical product, the ether-extracted residue, or the original dust mixture, equivalent to 0.100 gram of total hexachlorocyclohexane are washed into separate 125-ml. Ŧ Erlenmeyer flasks with 50.0 ml. of 95% ethyl alcohol. The flasks are heated under reflux for about 10 minutes to dissolve the hexachlorocyclohexane. When cool, the flasks are stoppered and packed in crushed ice, which covers the lower half of the flask. The flasks, still packed in ice, are placed in a refrigerator for at least 1.5 hours until the contents are cooled to 0° C. This may take 2 hours, as shown by the data in Table IV. A tightly stoppered 250-ml. Erlenmeyer flask containing about 100 ml. of N ethanolic potassium hydroxide is packed in crushed ice and also placed in the refrigerator. A 10-ml. volumetric pipet should be placed in the refrigerator to cool at least 30 minutes before it is ready to use.

Table III. Analysis for Total Hexachlorocyclohexane Using Purified Alpha Isomer as Chlorine Standard

Method	Chlorine Found, %	Theory	Hexachlorocyclohexane Calculated from Chlorine, %
Lime ignition	73.19	73.16	100.04
Parr bomb	73.14	73.16	99.97
Dehydrochlorination at reflux temperature	36.52	36.58	99.84

Table IV. Time Necessary to Cool 50 Ml. of Alcohol Packed in Crushed Ice[a]

Time, Minutes	Alcohol Temperature, ° C.
0	27.0
5	9.2
35	0.7
45	0.5
60	0.4
90	0.2
120	0.0

[a] Alcohol contained in a 125-ml. Ŧ Erlenmeyer flask with cork stopper.

Using the cold 10-ml. volumetric pipet, exactly 10.00-ml. portions of the 1 N ethanolic potassium hydroxide are withdrawn from the 0° C. temperature storage flask and transferred to each of the cold flasks containing the hexachlorocyclohexane solution. The time of addition to each flask is carefully noted. The contents of the flasks are mixed by rotating the flasks. In exactly 15 minutes 10 ml. of 1 to 3 nitric acid are added to one of the flasks and the flask is rotated to mix the contents. In exactly 50 minutes the reaction in the second flask is stopped by the addition of 10 ml. of 1 to 3 nitric acid. The flasks are removed from the ice bath and an excess of 0.1 N silver nitrate (10.00 ml. to the 15-minute flask and 15.00 ml. to the 50-minute flask), 2 ml. of 10% ferric alum solution, and 5 ml. of nitrobenzene are added to the flasks and the flasks are stoppered and shaken. The excess silver nitrate is titrated with 0.1 N ammonium thiocyanate.

It is imperative that the end-point color be the same in both the 15-minute and 50-minute reaction flasks. The volume of 0.1 N silver nitrate used during the 15-minute reaction period is subtracted from the volume used during the 50-minute period; no indicator blank correction is necessary. This difference in volumes multiplied by 0.3546 and divided by 0.1-gram sample weight gives the per cent chloride difference between the two reaction periods. The per cent chloride difference, on multiplying by 8 and subtracting 8.20 from the product, gives the percentage of gamma isomer in total hexachlorocyclohexane. This percentage of gamma isomer multiplied by the percentage of total hexachlorocyclohexane in the sample yields the percentage of gamma isomer in the original sample.

Extraction of Dust Mixtures. Most dust samples, containing as low as 0.5% gamma isomer content, can be dehydrochlorinated at 15 and 50 minutes without extracting the hexachlorocyclohexane with ether. Some commercial samples are rather highly colored, owing to a yellow or brown diluent. The color makes the end point so uncertain that an extraction with anhydrous ether in a Soxhlet extraction apparatus is necessary. The proper size of sample to be extracted depends on the percentage of total hexachlorocyclohexane. A residue weight of 0.5 to 1.0 gram will give enough sample for duplicate analyses. An extraction period of 16 hours has been found sufficient for most samples, but the extraction thimble should be checked after this period of extraction for the presence of hexachlorocyclohexane by allowing the thimble to dry; if the odor of hexachlorocyclohexane is not apparent the extraction is considered completed. Complete mixing of the dry ether extract is necessary because the alpha and beta isomers crystallize out before the ether is completely evaporated.

The anhydrous ether must be removed from extracted hexachlorocyclohexane in the Soxhlet extraction flask at a low temperature; otherwise some of the isomers of hexachlorocyclohexane will be removed by sublimation. The method found best was to place the extraction flask on a steam bath and run a stream of air into the flask. This facilitates the removal of the ether and keeps the temperature down. As the temperature should not be allowed to go above the boiling point of ether, the last of the ether should be removed with the flask off the steam bath.

EXAMPLE. A commercial dust was analyzed for total hexachlorocyclohexane by refluxing 1.000 gram of the material with approximately 15 ml. of 1 N ethanolic potassium hydroxide. After the inside of the flask had been cooled and washed down with water and acidified with nitric acid, 10.00 ml. of 0.1 N silver nitrate were added. The excess silver nitrate required 5.90 ml. of 0.1 N ammonium thiocyanate for the back-titration. An indicator blank of 0.10 ml. of 0.1 N ammonium thiocyanate (Table V), for this volume of solution was subtracted from the volume of 0.1 N ammonium thiocyanate solution, giving a final volume of 5.80 ml. of 0.1 N ammonium thiocyanate solution.

$$10.00 - 5.80 = 4.20 \text{ ml. of 0.1 } N \text{ silver nitrate solution used}$$

$$\frac{4.20 \times 0.9696}{1.0} = 4.07\% \text{ total hexachlorocyclohexane in material}$$

$$\frac{100 \times 0.1}{4.07} = 2.457 \text{ grams of original material, equivalent to 0.1 gram of total hexachlorocyclohexane}$$

Two 2.457-gram portions of the material were dissolved in 50 ml. of 95% ethyl alcohol and cooled to 0° C. One portion was dehydrochlorinated at 0° C. for 15 minutes with 10.00 ml. of 1 N ethanolic potassium hydroxide, the other for 50 minutes. The 15-minute period required $10.00 - 2.42 = 7.58$ ml. of 0.1 N silver nitrate. The 50-minute period portion required $15.00 - 6.18 = 8.82$ ml. of 0.1 N silver nitrate.

$8.82 - 7.58 = 1.24$ ml. of 0.1 N silver nitrate difference

$$\frac{1.24 \times 0.3546}{0.1} = 4.40\% \text{ chloride difference}$$

$(4.40 \times 8) - 8.20 = 27.00\%$ gamma isomer in hexachlorocyclohexane

$4.07 \times 0.27 = 1.10\%$ gamma isomer in original sample

$4.07 - 1.10 = 2.97\%$ other isomers of hexachlorocyclohexane in original sample

DISCUSSION

The accurate determination of total hexachlorocyclohexane from the total chlorine content of technical hexachlorocyclohexane and its mixtures is a difficult task because a very small error in determining the total chlorine content will cause a rather large error when calculated to hexachlorocyclohexane.

Table V. Typical Indicator Blank Data

Solution Volume, Ml.	Excess 0.1 N NH_4SCN, Ml.
50	0.00
100	0.10
200	0.15
300	0.22

A common source of error in the Volhard method for chlorine is the amount of standardized ammonium thiocyanate solution necessary to impart a visible color to the solution being titrated after all excess silver has been precipitated. This volume of solution constitutes an indicator blank, and varies with the volume of solution being titrated and the shade of color desired by the chemist for the end point. The Research Analytical Laboratory of the Hooker Electrochemical Company (*5*) has developed a procedure for use in control work which increases the accuracy of the Volhard method by applying an indicator blank. That laboratory determines the indicator blank by titrating 1.00 ml. of 0.1 N silver nitrate with 0.1 N ammonium thiocyanate in the same volume as used in the chloride titration. The indicator blank is not directly proportional to the volume and should be determined for different volumes of solution, as shown in Table V. The indicator blank may be added to the volume of silver nitrate or subtracted from the volume of thiocyanate solution. Failure to apply an indicator blank can cause a 2 to 3% error in the determination of total hexachlorocyclohexane in the technical product.

This investigation was almost completed before the existence of a fifth isomer, epsilon, was announced (*3*). The effect of the epsilon isomer on the method was demonstrated by analyzing mixtures containing known percentages of all five of the obtainable isomers of hexachlorocyclohexane (Table VI).

The presence of free chloride in hexachlorocyclohexane and its products should be checked in the following manner:

A 0.1- to 1.0-gram sample is refluxed with 25 ml. of 95% alcohol for 10 minutes, cooled, and acidified with a few milliliters of 1 to 3 nitric acid. An excess of 0.1 N silver nitrate, 5.00 ml., is added along with ferric alum solution and nitrobenzene. After shaking, the excess silver nitrate is titrated with 0.1 N ammonium thiocyanate. Any silver nitrate used must be subtracted from the volume used when determining total hexachlorocyclohexane, keeping the proper volume to weight ratio.

Table VI. Analysis of Mixtures Containing Known Percentages of Pure Isomers of Hexachlorocyclohexane for Gamma Isomer

Per Cent of Pure Isomers in Mixture					Gamma Isomer Found, %	Recovery, %
Alpha	Beta	Delta	Epsilon	Gamma		
75.0	5.0	15.0	...	5.0	5.40	108.0
80.0	5.0	10.0	...	5.0	5.96	119.2
72.0	10.0	10.0	3.0	5.0	6.20	124.0
70.0	5.0	15.0	...	10.0	9.84	98.4
75.0	5.0	10.0	...	10.0	9.40	94.0
70.0	3.0	15.0	2.0	10.0	11.64	116.4
65.0	5.0	15.0	...	15.0	15.88	105.9
55.0	5.0	20.0	...	20.0	20.20	101.0
55.0	3.5	20.0	1.5	20.0	23.00	115.0
55.0	3.5	20.0	1.5	20.0	22.60	113.0
50.0	15.0	5.0	...	30.0	31.48	105.0
50.0	14.0	5.0	1.0	30.0	29.56	98.5
50.0	5.0	10.0	...	35.0	34.36	98.2
20.0	5.0	35.0	...	40.0	40.04	100.1
15.0	10.0	30.0	...	45.0	44.60	99.1
5.0	5.0	40.0	...	50.0	49.96	99.9
5.0	4.5	40.0	0.5	50.0	49.64	99.3
2.2	2.8	25.0	...	70.0	68.27	97.5
1.5	2.0	16.5	...	80.0	85.40	106.8
..	..	..	...	100.0	92.96	93.0
					Av.	104.6

Most samples contain no free chloride, but one sample of commercial hexachlorocyclohexane was found to contain 1.06% free chloride.

No chloride blank or indicator blank need be applied to the time differential determinations of the gamma isomer, as the sample weights are the same and their effect is canceled.

It is imperative that exactly the same sample weight be taken for the 15-minute and 50-minute dehydrochlorination reactions.

Anhydrous ether is the best solvent for extracting dust mixtures. Ordinary solvent ether can be used, but the last traces of alcohol are difficult to remove. Hexachlorocyclohexane is very soluble in acetone, but changes apparently take place during the evaporation of the solvent, giving high results for the gamma isomer, as shown in Table II.

The accuracy of the method falls down on samples containing more than 70% gamma isomer.

The method as written is not applicable to mixtures containing DDT, DDD, chlorinated camphene, chlordan, or other insecti-

Table VII. Determination of Gamma Isomer

	Per Cent Gamma Isomer Found		
Sample No.	By infrared analysis[a]		By dehydrochlorination
1	12.8		13.32 13.08 12.76
		Av.	13.05
2	13.7		13.88 14.52 14.52
		Av.	14.31

[a] Reported by Kimball and Tufts.

Table VIII. Analysis of Commercial Hexachlorocyclohexane Dust Samples

	Guaranteed on Label		Found by Analysis	
	Gamma isomer, %	Other isomers, %	Gamma isomer, %	Other isomers, %
1	6.0	..	6.83	40.20
2	4.0	..	4.29	24.32
3	5.7	..	6.42	43.69
4	5.0	45.0	5.35	45.50
5	1.0	..	1.13	5.98
6	0.5	..	0.52	3.06
7	1.0	..	1.33	3.84
8	6.0	21.3	6.85	22.24
9	1.0	3.5	1.06	3.53
10	1.0	7.3	1.08	6.38
11	1.0	2.5	1.10	2.97
12[a]	1.0	7.0	1.34	7.09

[a] Sample contained small amount of sulfur, possibly contamination from unclean mixer.

cides containing labile chlorine. Controlled dehydrochlorination might possibly be used in developing methods for the analysis of these complex insecticides in the presence of one another. Work is being continued along this line of investigation.

The presence of sulfur, whether part of a formula or contamination, in a hexachlorocyclohexane dust causes some difficulty in the analysis for the gamma isomer. The sulfur is converted to sulfide during the dehydrochlorination reaction at 0° C., and when silver nitrate is added to the acidified solution a precipitate of silver sulfide is formed.

This was overcome by adding a large enough excess of 0.1 *N* silver nitrate solution to the 15- and 50-minute reaction flasks, following dehydrochlorination at 0° C., and acidification with nitric acid, to precipitate completely both sulfide and chloride. The flasks were then placed on a hot plate and most of the alcohol was boiled off, 2 ml. of concentrated nitric acid were added to each flask, and the silver sulfide was decomposed by continued boiling. After cooling, the excess silver nitrate was titrated in the regular way and the gamma isomer per cent calculated as described. Sample 3, Table II, shows the results obtained on a dust containing sulfur.

The average accuracy obtained on commercial samples should be in the neighborhood of 5%, though greater accuracy has been obtained. The method tends to give high results for the gamma isomer, as indicated in Tables VI and VII.

Some typical results obtained in analysis of commercial dust mixtures are presented in Table VIII.

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LITERATURE CITED

(1) Caldwell, J. R., and Moyer, H. V., IND. ENG. CHEM., ANAL. ED., **7**, 38 (1935).
(2) Cristol, S. J., *J. Am. Chem. Soc.*, **69**, 338 (1947).
(3) Kauer, K. C., Du Vall, R. O., and Alquist, F. N., *Ind. Eng. Chem.*, **39**, 1335 (1947).
(4) Kimball, R. H., and Tufts, L. E., IND. ENG. CHEM., ANAL. ED., **10**, 530 (1938).
(5) Kimball, R. H., and Tufts, L. E., private communication.
(6) Parr Instrument Co., Moline, Ill., *Direction Booklet* **116**.
(7) Slade, R. E., *Chemistry & Industry*, 40, 314 (1945).
(8) Tufts, L. E., and Kimball, R. H., Physical Chem. Lab., Hooker Electrochemical Co., Niagara Falls, N. Y., *Rept.* **4706** (March 17, 1947).
(9) Van der Linder, L., *Ber.*, **45**, 236 (1912).

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ANTHOCYANIN PIGMENTS

Colorimetric Determination in Strawberries and Strawberry Products

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The amount of red anthocyanin pigment in strawberry products can be quantitatively determined by measuring the light absorption in extracts made therefrom at 500 mμ and at pH 3.40 and 2.00. The measurement consists of subtracting the optical density at 500 mμ of an anthocyanin solution at pH 3.4 from its optical density at pH 2.0, at known concentration. The increase in color intensity under such conditions is proportional to the concentration of anthocyanin in the solution. A solution of Congo red is suggested as a standard of color intensity. Examples illustrate applicability of the method, which may be used for objective comparison of the red color in different samples of strawberries and in strawberry products of different origin, and to follow color deterioration in the product after manufacture.

IN CONNECTION with work in this laboratory on the changes of strawberry preserves during storage, a quantitative method for the determination of the red anthocyanin pigments of strawberries was desired.

During the storage of strawberry products at temperatures above 0° C., two main types of nonenzymatic color reactions occur: the red anthocyanin color is lost and secondary brown pigments develop. None of the existing methods has been found satisfactory for the quantitative determination of the actual loss of red pigment. Usually data are obtained with the Lovibond-type colorimeter (*1*, *9*), which give an indication of the degree of color deterioration in the juice when the ratio of red to yellow light absorption is calculated. Yet these measurements are not strictly quantitative or even directly related to the loss of anthocyanin. Attempts to separate the anthocyanin quantitatively from other colored materials present in the juice by chromatographic adsorption and various solvents were unsuccessful.

The pH of the medium strongly affects the light absorption of anthocyanin (*3*, *8*). For instance, when the pH of fresh strawberry juice is lowered from 3.5 to 2.0 the absorption at 500 mμ, expressed as optical density, is more than doubled. Figure 1 shows the effect of decreasing pH on the absorption at 500 mμ of pure anthocyanin pigment at constant concentration. (The anthocyanin used in these experiments was isolated in crystalline form from Dresden strawberries. Work is now in progress on the characterization of this pigment.) Further experimentation showed that this absorption change can be used for the quantitative determination of anthocyanin in strawberries and strawberry products.

Anthocyanins are amphoteric substances which form oxonium salts with mineral and organic acids. It is highly probable that in neutral or nearly neutral solutions the pigment exists in the free state (*5*) and upon acidification the equilibrium between the color base and the oxonium salt is shifted, resulting in a molecule of higher resonance and therefore exhibiting greater light absorption.

METHOD OF MEASUREMENT IN STRAWBERRY PRODUCTS

When the pH of a solution containing anthocyanins is lowered, the light absorption in the visible range increases. Under the experimental conditions used, this increase is proportional to the concentration of anthocyanin. The readings are taken at 500 mμ, since this is the highest peak of absorption in the visible range and therefore maximum sensitivity is obtained at this wave length. Furthermore, the absorption peaks of ex-