Both blotting and print papers are removed from the soaking bath and, together, squeezed between two dry pieces of blotting paper which must overlap the soaked papers by 0.75 inch on all sides. The pressure used should be approximately 550 pounds per square inch. The wider border of the dry papers will take up the excess solution squeezed out of the soaked papers.

The coated metal panel is then laid on the anode platen with the metal of the panel in contact with the metal of the anode and the imbibition papers placed emulsion side in contact with the area of which the desired porosity study is to be made. The soaked and squeezed blotting paper is laid congruently on the imbibition paper. The cathode platen is then placed over the blotting paper. This entire "sandwich" is centered in the press and necessary pressure applied. The current is switched on as soon as the pressure required is obtained. After 20 seconds current is switched off and pressure immediately released.

The imbibition paper is placed in a developing bath of 2.5 grams of potassium ferrocyanide and 2.5 grams of potassium ferricyanide per 100 ml. of distilled water. The prints are left in this bath for 0.5 hour, washed in warm water until the yellow color of the ferricyanide is removed, and then dried between blotting papers or on ferrotype tins.

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Infrared Spectroscopic Analysis of Five Isomers of 1,2,3,4,5,6-Hexachlorocyclohexane

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The methods of infrared spectroscopy have been applied to the analysis of the insecticide hexachlorocyclohexane. The spectra of five pure isomers of 1,2,3,4,5,6-hexachlorocyclohexane and of a mixture of heptachlorocyclohexane impurities have been determined in the 2- to 25-micron range. A relatively simple infrared method for the quantitative determination of each of the five isomers has been developed, which is satisfactory for the analysis of nearly pure samples and relatively free of interference from the usual type of impurities. The possible structures of the isomers are discussed.

RECENT advances in the use of the insecticide hexachlorocyclohexane and the inauguration of its commercial production forecast the widespread use of this material. It is, of course, important that both the insecticidal and the toxicological properties be thoroughly investigated and this has been hampered by the fact that the commercial insecticide, frequently designated as HCH (hexachlorocyclohexane) or BHC (benzene hexachloride), is not a pure compound but consists of a mixture of compounds, the concentration and identity of which are not easily determined. Thus, it is important that a method of assay be developed for use both in the evaluation of the insecticide and in the formulation of specifications for its purchase and application.

The industrial preparation of this compound by the chlorination of benzene to form 1,2,3,4,5,6-hexachlorocyclohexane results in a mixture of stereoisomers (alpha, beta, gamma, delta, and epsilon) as well as small quantities of hepta- and octachlorocyclohexane. These isomers differ only in the orientation of the chlorine atoms about the cyclohexane ring. This stereoisomerism induces a difference in insecticidal effectiveness, the gamma isomer, whatever its form, being the one especially endowed with insecticidal value. The toxicity of the isomers toward warm-blooded animals also varies, the gamma isomer being reported more toxic than DDT (*8*). In many cases—for example, where only the insecticidal effectiveness is concerned—it may be sufficient to know only the gamma isomer content of the product but in other cases, especially in the complete evaluation of the toxicological properties, it is important to know the complete composition.

It was the purpose of the present investigation to apply the methods of infrared spectroscopy to the analysis of the insecticide hexachlorocyclohexane. Since impurities such as hepta- and octachlorocyclohexanes exist in only very small quantities in the commercial insecticide, and the manufacturers believe that these impurities can be reduced to a negligible quantity (*7*), only the five stereoisomers are considered in the analysis. In addition to the development and evaluation of an analytical method it was desired to obtain any information relative to the structure of the isomers and its effect upon insecticidal or toxicological properties.

PREVIOUS WORK

Slade (*16*) and Jenkins (*8*) have prepared comprehensive reviews of the literature on hexachlorocyclohexane, and for the biological and toxicological aspects of this compound one is referred to these reviews. Although the compound was first prepared by Michael Faraday in 1825, it was not until 1912 that Van der Linden established the fact that the product was a mixture of at least four isomers (*16*). In 1943 the insecticidal value of one of these, the gamma isomer, was discovered by F. J. D. Thomas (*18*). For this reason concentrated efforts to investigate this compound have been recent and the results are probably unpublished. A new isomer has been reported by Kauer, DuVall, and Alquist (*9*), bringing the number of known isomers to five.

From Slade's article it appears that up to 1945 the methods of determining the composition of a crude preparation consisted of fractional solution and recrystallization of the crude with appropriate solvents. A more recent article by Ramsey and Patterson (*13*) on partition chromatography offers an alternate means of analysis. Kauer *et al.* mention that infrared spectroscopic techniques were used to determine the five isomers in hexachlorocyclohexane crudes. The spectra of Kauer *et al.*, which extend from 2 to 15 mu, are in substantial agreement with the present work.

SPECTROSCOPIC EQUIPMENT

All spectral measurements were made with a research-type prism spectrometer of high resolving power (*10*). Experimental records were made by recording, as a function of wave length, the energy transmitted by a blank and that transmitted by the sample as corresponding ink traces on the recorder chart. Slit widths were changed periodically by manual control.

The wave-length measurements of the transmission curves are accurate to ±0.01 micron or better and the per cent transmittance

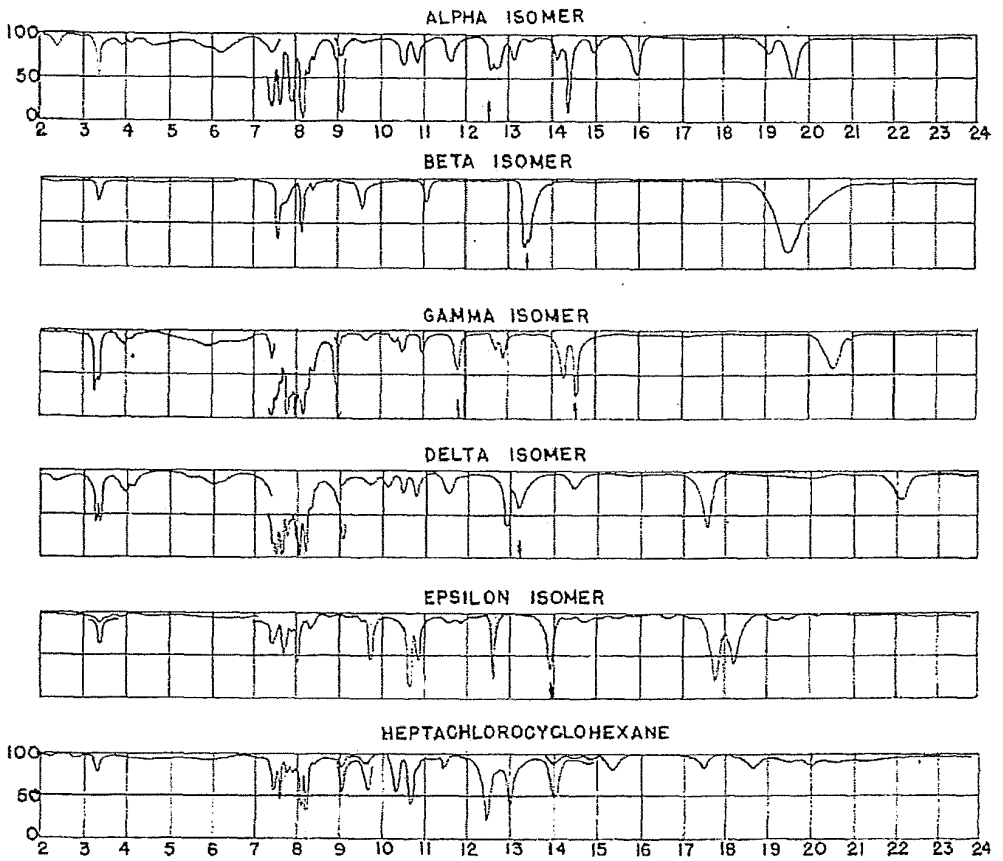

Figure 1. Spectra of Hexachlorocyclohexane Isomers and Heptachlorocyclohexane

measurements are accurate to about ±1 to 2% over the range 20 to 80% transmittance. Quantitative spot measurements were made by setting on a particular wave length instead of by scanning through a band. By taking the average of several deflections the spot measurements can be made accurate to ±0.5% transmittance.

Samples studied in solution were placed in amalgam-sealed absorption cells constructed and calibrated by the method of Smith and Miller (*17*). Samples studied in the solid state were prepared by grinding with mortar and pestle and mixing with petrolatum to form a paste which was placed between potassium bromide plates with an appropriate spacer. The blank for the solid samples was made in a similar manner using a potassium bromide and petrolatum paste.

BASIS OF ANALYTICAL METHOD

Infrared spectroscopy can be used both for analytical purposes and for investigation of molecular structure. The bands in the infrared spectrum are produced by preferential absorption of various wave lengths of infrared energy, the energies absorbed corresponding to the energies of certain molecular vibrations. Thus, each compound possesses a unique spectrum characterized by its atomic composition and arrangement. The spectra of geometrical isomers, although they may have several bands which coincide, will, in general, be sufficiently different at other wave lengths to be distinguishable.

The spectra of the five known isomers of hexachlorocyclohexane are shown in Figure 1. Discontinuities are due to different concentrations. Each isomer has at least one absorption band in a spectral region where the others are relatively transparent. These differences in spectral absorption constitute a basis for spectroscopic identification of an isomer, either alone or in a mixture with other isomers. The presence of the various isomers in a mixture can be recognized by absorption maxima at the following wave lengths:

Isomer	Wave Length, mu
Alpha	12.55
Beta	13.46
Delta	13.22
Gamma	11.51 and 14.53
Epsilon	13.96

If the region beyond 15 mu is overlooked, for reasons discussed later, a consideration of the intensities of the absorptions shows that the magnitudes of the spectral differences at these wave lengths are greater than at any other set of wave lengths which might be chosen.

This basis for qualitative analysis of a mixture is sufficient for quantitative analysis if the intensity of absorption at each wave length is measured under controlled conditions.

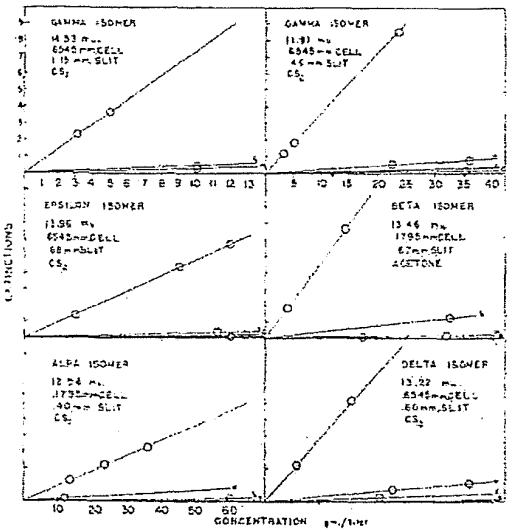

Figure 2. Working Curves

In the absence of molecular interaction, the law of Beer and Lambert states that for a solution of an absorbing material in a transparent solvent $E = \log I_0/I = Kcl$, where E = extinction, I_0 = light transmitted by a blank (pure solvent), I = light transmitted by the solution, K = extinction coefficient of absorbing material, c = concentration in grams per liter, and l = length of solution path in centimeters. If the absorbing material is a pure compound its extinction coefficient, K, for a given wave length can be determined by measuring the extinction for a solution of concentration c in a cell of length l. For a material containing several components, this equation may be written:

$$E = K_1c_1l + K_2C_2l + K_3c_3l + \ldots\ldots\ldots \quad (1)$$

$$K_1c_1l = E - K_2c_2l - K_3c_3l - \ldots\ldots\ldots \quad (2)$$

where the subscripts refer to components 1, 2, 3, etc. The K's can be determined from measurements on the pure components and if the above equation for a mixture of n components is applied to a sample at n wave lengths the n simultaneous equations obtained determine the concentrations of the n components in the mixture. These equations can be solved exactly by any of several methods—for example, by the use of special computing machines, or by a graphical method of successive approximations described below.

In the application of this method to mixtures of the five isomers of hexachlorocyclohexane Equation 1 is applied at each of the selected analytical wave lengths. At each wave length only one component absorbs strongly and therefore all but one of the right-hand terms of equations will be small. By neglecting these small correction terms it is possible to obtain an approximate value of the concentration of the main absorbing components from the extinctions of the sample measured at the various wave lengths. These approximate concentrations can then be used to calculate the magnitude of the correction terms and thus to determine more accurate values of the concentrations.

To obtain the experimental measurements required in the application of the method to hexachlorocyclohexane a solvent which is transparent at each of the analytical wave lengths is required. Carbon disulfide appeared to be the only sufficiently transparent solvent and an investigation of solubilities has shown that satisfactory concentrations of all the isomers except beta can be obtained in this solvent (Table I).

Since the beta isomer is nearly insoluble, the use of carbon disulfide effects a simplification in the analysis by limiting the number of variable components to four. The beta isomer, then, may be determined by total insolubles (assuming no foreign matter in the sample) or by difference, using the measured values of the other four isomers. A superior approach, however, is to determine the beta isomer directly by a separate spectroscopic analysis, using an acetone solution for measurement of the extinction at the beta wave length.

ANALYTICAL PROCEDURE

Calibration of the method is based upon absorption measurements on solutions of the pure isomers at the various analytical wave lengths. The absorption measurements (calculated as per cent transmittance) are converted to extinctions by Beer's law and plotted against concentration to give the working curves of Figure 2. The curves appear to be linear and straight lines have been drawn. The exact analytical wave-length setting of the spectrograph is best determined by slowly scanning the band and plotting the record obtained. This is an especially necessary procedure at the 11.81 mu gamma band which is on the side of a carbon disulfide (solvent) band. Since it can be shown that the optimum accuracy for a measurement is obtained for a sample that transmits 37.5% of the light, a procedure of analysis specifying certain cell thicknesses and sample concentrations will yield most accurate results for only a certain small range of sample composition. The procedure presented here is devised for samples containing approximately 70% alpha, 5% beta, 15% gamma, 10% delta, and less than 5% epsilon. This corresponds closely with the composition of the commercial products at the present time; for samples having appreciably different composition the results will be somewhat less accurate unless cell thicknesses and sample concentrations are properly adjusted. However, reasonable accuracy is obtained for transmittance measurements between 20 and 80%, so that even high gamma preparations such as that reported by Gunther (3) could be analyzed with only small changes of the analytical procedures.

Table I. Solubility of Hexachlorocyclohexane in Carbon Disulfide[a]

	G./cc.
Gamma	0.1995
Alpha	0.0500
Delta	0.0930
Beta	0.0007
Epsilon	0.0125

[a] Solubilities determined by weighing residue upon evaporation of solvent from 1 cc. of saturated solution at room temperature (approximately 25° C.).

There are several additional considerations in the choice of cell thickness, concentrations, and slit widths. If the solvent absorption is high, as at 11.81 mu, a larger slit and/or a thinner cell is necessary to obtain full-scale deflections, and if the absorption band is very strong, as in the 13.46 mu beta band, the thinner cell enables an appreciable concentration range to be covered.

An unknown mixture should be sampled by some adequate procedure and about 0.3 gram weighed into each of two 10-ml. volumetric flasks and made up to volume with carbon disulfide and acetone, respectively. The appropriate solution is used at the various analytical wave lengths under the same conditions used to determine the working curves. The transmittance measurements are converted to extinctions and the approximate concentrations are then determined by the working curves. The approximate concentrations for alpha, delta, gamma, and epsilon (all too high) now enable the extinctions at each wave length to be corrected for the absorption of the interfering components at that wave length. These corrections are also determined graphically from the correction curves and are evaluations of the small negative terms of Equation 2. The new values are overcorrected, but a second evaluation using the new values will usually give results that are within the experimental error. The

beta isomer is then determined in the acetone solution and corrections are applied using the now known percentages of alpha, gamma, delta, and epsilon.

EXPERIMENTAL RESULTS

The necessary calibration curves, shown in Figure 2, were constructed from measurements on samples of the pure isomers. Absence of impurity absorption in the spectra (Figure 1) and narrow melting point ranges, as determined by the usual capillary method, indicated that the samples were of suitable purity for this work:

Isomer	Melting Point, ° C.
Gamma	111.8–112.2
Delta	136.0–136.2
Alpha	155.3–155.8
Epsilon	202–227 (sublimed
Beta	199–210 (sublimed)

The calibration curves were then used in the analysis of several synthetic solutions of pure isomers (Table II). For one solution detailed results which indicate the method of successive approximations are presented. Separate solutions were used for the beta isomer determinations, since this required a different solvent. Most of the data in Table II were obtained before the new epsilon isomer was reported, but a quantity of this isomer sufficient for making one, five-component mixture was later obtained and the results are included as the last analysis.

Table III contains the results of several analyses of two crude samples from the commercial production of the insecticide. The presence of epsilon isomer was neglected in these analyses, and consequently the results for all other isomers are uncorrected for the epsilon isomer content. This does not affect the usefulness of the data, since the corrections introduced by the small amount of epsilon in these crude samples are of negligible size in all but the most accurate analytical attempts. At a later date the epsilon content of these samples was determined by a separate analysis and found to be 2 and 1% for products 1 and 2, respectively. It has been determined that the presence of 2% of epsilon found for product 1 would cause errors of only 0.30, 0.02, and 0.06%, respectively, in the original experimentally determined concentrations of alpha, beta, delta, and gamma. These combined errors are well within the experimental error of the analysis.

The totaled percentages, including epsilon, are 104.2 and 101.2% for products 1 and 2, respectively. The high values obtained for the total concentrations in these analyses are discussed below after the inherent accuracy of the method itself has been considered. The spectra of products 1 and 2, as well as a third product, No. 3, are presented in Figure 3. Although the complete analysis for the latter product is not included, it was found to have substantially more epsilon isomer (4%) than the other products.

DISCUSSION

Since this method, like any method employing working curves, is empirical, its inherent errors are negligible, the ultimate accuracy being limited chiefly by the accuracy of the calibration data (working curves). If the analyses are performed under the same conditions used for the calibration, the latter need not be absolute but only experimentally reproducible. Thus, the accuracy of the method is determined almost entirely by the experimental reproducibility realized in the laboratory.

If care is used in sampling, making standard solutions, handling sample, etc., the most important errors affecting reproducibility are probably those in the percentage transmittance measurements. However, for any given reproducibility a high degree of accuracy may be obtained by averaging a sufficiently large number of measurements. Hence it may be assumed that the calibration data (working curves) can be determined as accurately as desired

Table II. Analysis of Known Solutions

(Alpha, gamma, delta determinations. Known concentrations: gamma = 7.15 grams per liter, delta = 8.11 grams per liter, alpha = 5.09 grams per liter. Total sample = 21.25 grams per liter)

Wave Length	Substance	% Transmittance	Extinction, Total	Extinction, Sample	Experimental Concentration, G./Liter
14.53	Gamma	21.50	0.0611	0.3621	7.53
	CS_2	79.50	0.0900		
13.22	Delta	38.82	0.4109	0.3650	9.10
	CS_2	59.90	0.0459		
12.58	Alpha	81.30	0.0852	0.0541	7.00
	CS_2	94.55	0.0241		
11.81	Gamma	51.25	....	0.2900	7.90
	CS_2	...	.....		

Corrections (First Approximation)

Wave Length	Substance	Extinction	Corrections: Alpha	Gamma	Delta	Total	Corrected Extinction	Corrected Concentration
14.53	Gamma	0.3621	0.023	....	0.038	0.061	0.3011	6.50
13.22	Delta	0.3650	0.023	0.0075	.....	0.0305	0.3345	8.30
12.58	Alpha	0.0541	.....	0.0025	0.0040	0.0065	0.0576	6.00
11.81	Gamma	0.2900	0.014	....	0.004	0.018	0.2720	7.40

Second Approximation

14.53	Gamma	0.3621	0.029	....	0.027	0.047	0.3151	6.93
13.22	Delta	0.3650	0.020	0.0075	.....	0.0275	0.3375	8.40
12.58	Alpha	0.0541	.....	0.0025	0.004	0.0065	0.0576	6.00
11.81	Gamma	0.2900	0.013	....	0.004	0.017	0.2730	7.50
								21.35

Other Solutions

Wave Length	Isomer	Known Concentration	Experimental Concentration
14.53	Gamma	6.07	5.80
13.22	Delta	3.87	3.94
12.58	Alpha	32.41	32.40
(11.81)	(Gamma)	(53.07)	(53.10)
		42.35	42.14
14.53	Gamma	6.48	5.11
13.22	Delta	4.56	4.70
12.58	Alpha	35.59	35.81
(11.81)	(Gamma)	(53.48)	(53.90)
		95.61	95.62

Beta Isomer Determinations

13.46	Beta	4.41	4.40
13.46	Beta	6.21	6.20
13.46	Beta	5.71	5.50
13.46	Beta	7.89	7.85

Five-Component Determination

14.53	Gamma	4.37	4.20
13.90	Epsilon	2.05	1.95
13.22	Delta	7.77	7.55
12.58	Alpha	10.08	11.50
	(Beta)	(2.61)	
(11.81)	(Gamma)	(14.37)	(14.35)
		25.17	25.20

Table III. Analysis of Crude Hexachlorocyclohexane Industrial Products

	Gamma, 14.53 mu	Gamma, 11.81 mu	Alpha, 12.53 mu	Delta, 13.22 mu	Beta, 13.46 mu
Product 1[a]	14.29 ± 0.83	15.15 ± 0.70	70.29 ± 2.5	8.53 ± 0.39	10.55 ± 0.36
Product 2	12.14	13.08	73.55	6.70	6.77

[a] Average of 10 determinations.

The accuracy of an analysis, then, will be limited chiefly by the reproducibility of the transmittance measurements on the unknown sample and can be calculated for any given reproducibility of transmittance measurement.

Assuming, for example, that the measurements are made at the optimum value of 37.5% transmission, an experimental uncertainty of 0.5% transmittance corresponds to ±0.0055 in optical density or to ±1.3% in the extinction determination. Neglecting, for the moment, the absorption of interfering components, this uncertainty in the optical density determination at each analytical wave length leads to the following uncertainties in the concentrations of the various isomers:

Wave Length	Isomer	Uncertainty[a] Concentration, Grams/Liter	Uncertainty[a] Percentage (on Total Sample)[b]
14.53	Gamma	±0.074	±0.25
11.81	Gamma	±0.151	±0.50
12.58	Alpha	±0.583	±1.95
13.22	Delta	±1.35	±0.45
13.96	Epsilon	±0.114	±0.38
13.46	Beta	±0.120	±0.40

[a] Analytical uncertainty resulting from an experimental uncertainty of 0.5% in transmittance measurement.
[b] Total sample concentration = 30 grams per liter.

The figures in the right-hand column represent the analytical precision which can be obtained under the conditions given. Greater precision in transmittance measurement and the use of average values would lead to correspondingly less uncertainty in the analytical results.

It was assumed in the previous paragraph that the correction for the absorption of interfering isomers at each analytical wave length could be determined precisely and that introduction of this correction, therefore, would not contribute to the uncertainty of the analytical results. The validity of this assumption may be shown in the following manner. By computing (from Figure 2) the quantities of the various isomers which have equal absorption at each of the analytical wave lengths as given in Table IV, it is seen that the absorption at each analytical wave length is relatively insensitive to the presence of other components, and therefore errors resulting from inaccuracies in the corrections for interfering absorptions are negligible.

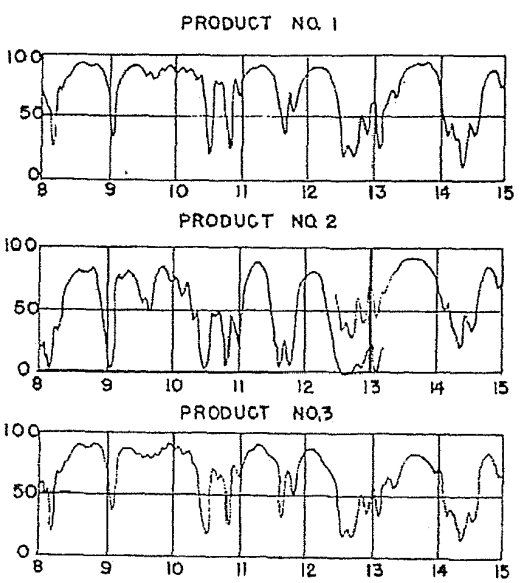

Figure 3. Spectra of Hexachlorocyclohexane (Commercial Crudes)

It is concluded, therefore, that if the per cent transmittance is measured to within ±0.5, it is possible by a careful application of the method to obtain analytical results which are correct to within ±0.5% for the beta, gamma, delta, and epsilon isomers and to within ±2% for the alpha isomer, the values based on total sample. This theoretical accuracy (or reproducibility) agrees well with the experimental reproducibility obtained in Table III.

Table IV. Parts of Interfering Isomer Equivalent to One Part of Absorbing Isomer

Microns	Gamma	Alpha	Delta	Epsilon	Beta
11.81	1	17	578	35	.
14.53	1	23	16	400	
12.58	42	1	25	0.7	
13.22	35	12.4	1	125	
13.96	23	73	443	1	
13.46	..	91	11	135	1

In addition to these factors the over-all accuracy (as distinguished from precision or reproducibility) of the analyses for the crude samples in Table III will also be determined by the accuracy of the working curves and by the possible presence of interfering impurities, such as hepta- and octachlorocyclohexanes. The high values of total concentration are very probably due to errors in the working curves, since it has been observed that in general all analyses made in this investigation are consistently high, and in the particular case of the gamma determination the values obtained in the "check" determination at 11.81 mu are consistently higher than those determined at 14.53 mu. It is clear that these small calibration errors could be eliminated in routine work and should not be considered in evaluating the method. The presence of isomeric hepta- and octachlorocyclohexane impurities may cause errors in the results. However, comparison of the spectra of the crudes (Figure 3) with the spectra shown in Figure 1 substantiates the manufacturer's claim that very little impurity is present. Hence, it is believed that the results have been unimpaired and that future products will likewise have negligible corrections from such sources of error. In addition, the error caused by loss of solvent in filling the absorption cells would tend to give high results. This error could be avoided by using special absorption cell and procedures, the devising of which would be feasible in routine applications.

It has been indicated that both 14.53 and 11.81 mu seem to be suitable for gamma isomer analysis and since a two-band check for the active component would be appropriate, analyses at both bands have been included in the present scheme. Nevertheless, the recommendation of the longer wave length must be stressed. The set of working curves at 14.53 mu is more sensitive to the presence of gamma isomer and provides a more accurate determination. In addition, the 11.81 mu band has the disadvantage of being on the side of a strong carbon disulfide absorption band, making it difficult to locate or check the position of the center of this band. Greater accuracy in the epsilon determination may be had by using an absorption cell of greater thickness than was used in this investigation.

In the analytical work described here no correction was made for the small amount of beta isomer which will dissolve in the carbon disulfide solutions. Some finite interference in the analysis for the other isomers will exist, although under the conditions of the analysis this interference was too small to be measured. However, if the method is to be applied to sample solutions containing at least 2% of beta (the quantity required to saturate the sample solution), then any interference, however small, arising from the presence of beta in the sample may be completely eliminated by using in place of the "solvent" blank one which is saturated with pure beta isomer.

In the application of the method to routine analysis it might be desirable to measure directly the transmittance of the sample-

filled cell relative to the solvent-filled cell instead of measuring separately the transmittance of each cell relative to a blank rock-salt or potassium bromide plate. This can be done by employing cells of known and approximately equal thicknesses, one filled with solvent (or beta-saturated solvent) and the other with sample solution.

There are other combinations of bands that could be used in this analysis but those chosen appear to be the best available from 2 to 15 mu. Beyond 15 mu where the vibrations become more characteristic of the molecule as a whole one would expect the bands for different isomers to be more isolated. Such was found to be the case. They are strong and there is no interference between isomers if beta is first eliminated by using carbon disulfide solutions. This is necessary because beta has one band at 10.55 mu which overlaps the alpha band at 10.64 mu (see Figure 1). The equations then would be simplified to one term and any isomer could be determined without knowing the concentration of the other isomers, experimentally or otherwise. However, since many laboratories are not equipped to investigate the region beyond 15 mu, advantage has not been taken of these great spectral differences. Instead, analysis has been confined to the region below 15 mu in order that the analytical method might be used in any infrared laboratory.

It appears possible with the set of chosen wave lengths to use only one solution in acetone to determine all five isomers. One would then have to obtain correction curves for all isomers (including beta), for they are all soluble in acetone. This solvent has strong absorption at about 14.35 and 12.75 mu but is relatively transparent at all the analytical wave lengths. Since it is a polar solvent there might be some nonlinearity in working curves caused by molecular interaction. However, in using this solvent for beta determination no such interaction was noticed.

Finally, since the analytical bands are relatively broad, the quality of the spectrographic equipment used for this analysis is well above the minimum requirement. This instrument has a resolution of approximately 0.7 cm.$^{-1}$ at 10 mu when an estimated 3 or 4 cm.$^{-1}$ would be satisfactory. The resolutions claimed for most commercial instruments by their manufacturers are usually within this range.

STRUCTURE OF THE ISOMERS

The alicyclic compounds have not been too thoroughly investigated and their stereochemistry is not well understood. Sachse (*15*) in his theory of strainless rings postulated that a ring such as cyclohexane would be able to take two configurations now commonly designated the C or "boat" form and the Z or "chair" form. Such an idea (for cyclohexane) is supported by the work of Hassel (*4*) who investigated monochlorinated cyclohexane and came to the conclusion that within one of these forms (the Z form) the chlorine in a monochloro derivative was able to assume two positions in the molecule, making two forms that were interconvertible. The two positions of the chlorine are on valence bonds parallel and perpendicular to the axis of the molecule, there being six equivalent bonds of each type in the Z form of cyclohexane. In an earlier article by Hassel and Ottar (*5*) it is stated that the possibility of other less symmetrical forms is not excluded. These other less symmetrical forms would be the C form and the planar form. The word "planar" is used here in its strictest sense—that is, all the carbon atoms are contained in one plane, the plane of the ring. It is apparent from the literature that this term has been loosely used. For example, Parodi (*11*) states that cyclohexane is a planar molecule. Likewise, the hexahydroxy derivative, inositol, is discussed by R. L. Shriner and R. Adams (*2*) as a planar ring. The connotation of "planar" meaning a molecule whose atoms determine several planes which are parallel to one plane simplifies the discussion, but oversimplifies when one is discussing the number of likely isomers in a derivative. Thus, inositol is stated to have eight possible isomers and one would assume that 1,2,3,4,5,6-hexachlorocyclohexane would have the same number. However, if one builds Fischer models of 1,2,3,4,5,6-hexachlorocyclohexane in the Z and C forms the following facts become apparent.

For the Z or Chair Form. 1. There are sixteen possible configurations if one assumes only one chair form is possible.
2. Of these sixteen, five are relatively strainless and one of the five is the mirror image of another.
3. There are eight different possible configurations assuming all chairs are interconvertible and strained bonds such as in the eleven strained configurations of statement 2 are stable.

For the C or Boat Form. 1. There are eight possible isomers if the two chlorines on the bow and stern of the boat are directed to the outside of the boat (it is too strained in the other position).
2. Of these eight only two are relatively strainless and they are mirror images.

Patterson and White (*12*) discuss the Z form in much the same manner. If the logical assumption is made that those forms which are strainless are the ones which will probably form in the preparation of an isomeric mixture, it is evident that there should be seven isomers of 1,2,3,4,5,6-hexachlorocyclohexane, of which two pairs are mirror images (see Figure 4). This leaves five isomers whose infrared spectra would be unique (the mirror images being spectroscopically identical).

It should be relatively easy to identify the optically active pairs by their activity but none of the pure isomers investigated appeared to be optically active. A saturated solution of the crude insecticide in a 20-cm. cell showed no activity, which would seem to substantiate the fact that the optically active isomers, if present, are racemates.

Actual structural determinations have been confined to the beta isomer (*1*, *6*). X-ray analysis easily characterizes it to be in the cubic system. It therefore has a centrosymmetric structure and has been assigned the 1,3,5, configuration in the chair form. The other structures must await further investigation of their x-ray diagrams which is being attempted at the present time. X-ray data for the new epsilon isomer are said to be included in the report of its separation (*9*).

The external crystal structure of these isomers may be informative on their internal molecular arrangement and may be helpful in distinguishing between isomeric crystals (Table V).

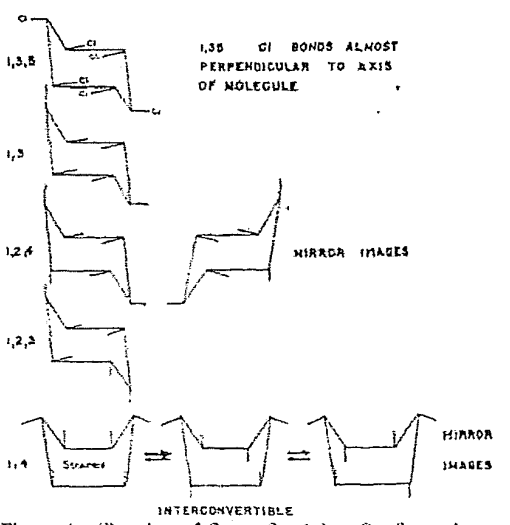

Figure 4. Sketches of Seven Strainless Configurations of 1,2,3,4,5,6-Hexachlorocyclohexane

Table V. Crystallographic Data

Isomer	Type	Sign	Approximate 2V	Birefringence	Range of Indices
Beta				None	1.630
Alpha	Biaxial	+	20°	Medium	1.60–1.626
Gamma	Biaxial	+	63°	Medium	1.60–1.635
Delta	Biaxial	+		Very high	1.576–1.674
Epsilon	Biaxial	+	73°	Medium	1.60–1.635

The beta isomer was found to be isometric, which checks the cubic classification by x-ray analysis. Its index of refraction, by the immersion method and Becke line, was determined to be 1.630. The solubility of the compounds in the usual organic immersion oils necessitated the use of potassium mercuric iodide-water solutions which were found to change index rapidly. As a universal stage was not used, the orientation of the small crystals was unknown. The data are therefore offered as a preliminary set of measurements. However, the other four isomers are known to belong to the orthorhombic or monoclinic crystal classes. The very high birefringence of the delta isomer and the low 2V angle for alpha distinguish these crystals from the rest. To distinguish gamma from epsilon would require more exact information on their three refractive indices, alpha, beta, and gamma. These data were obtained in conjunction with O. S. Tuttle of the Crystal Section at the Naval Research Laboratory, to whom the author is indebted.

The similarity of gamma and delta goes further than crystal structure. Their infrared absorption spectra have some peculiar correspondences. The doublet structure of these two isomers at the C—H vibration frequencies of 3.34 and 3.39 mu is contrasted with the singlet structure of the other three isomers. In the region from 7.5 to 8.5 mu there is a definite similarity of shape and intensity between bands for gamma and delta, as well as alpha. At larger wave lengths where the total molecular configuration is more important, the similarity disappears.

Beta and epsilon isomers are also spectrally similar in the shorter wave-length regions (2 to 10 mu). The most striking feature of these two spectra is their simplicity when compared with those of the other isomers. Simplicity or symmetry of structure is the usual cause for simplified absorption spectra and therefore one might look for a more or less symmetrical molecule for the epsilon isomer. Of the remaining four configurations (1,3,5 = beta) the 1,2,3, chair form and the 1,4 boat form are the most symmetrical (see Figure 4). The former has a plane of symmetry and a center of symmetry and the latter has two planes of symmetry. Since the epsilon concentration is the lowest in the preparation of the insecticide, the structure of higher energy content would probably be epsilon. This corresponds to the 1,4 boat form (4). Validity of this designation rests on rather slender evidence and it is offered as a suggested structure to assume when matching future data with possible structures.

Another spectral peculiarity, which must also be dealt with in general terms at the present time, is the apparent doubling of the beta bands at 7.6 and 8.12 mu in the alpha, delta, and gamma isomers. The single beta band at 7.60 mu splits into two at 7.42 to 7.62 mu for alpha, 7.52 to 7.67 mu for delta, and 7.45 to 7.82 mu for gamma. Similarly, with the beta band at 8.12 mu the two bands for alpha are at 7.92 to 8.18 mu at 8.09 to 8.24 mu for delta, and at 8.05 to 8.21 mu for gamma. According to Rasmussen (*14*), who investigated cyclohexane by infrared methods, the bands in the range 700 to 1350 cm.$^{-1}$ (7.5 to 14 mu) are due to CH_2 rocking and twisting vibrations or C—C stretching vibrations. The apparent splitting of the beta bands could be due to the two kinds of —CHCl— groups, depending upon which bond of each carbon the chlorine had added. The two types of bonds would correspond to those mentioned by Hassel in his monochloro derivatives. The chlorine atoms in the beta structure are attached by only one of the types of bonds (perpendicular to the axis of the molecule). This might explain the doublet C—H vibrations mentioned previously but the singlet C—H vibration for the alpha isomer contradicts the hypothesis. Further work along these lines will be reported at a later date.

SUMMARY AND CONCLUSIONS

The spectra of five pure isomers of the insecticide 1,2,3,4,5,6-hexachlorocyclohexane and of a mixture of heptachlorocyclohexane impurities have been determined in the 2- to 25-micron range. The differences in the spectral absorption of the isomers are sufficiently great to permit the qualitative and quantitative analysis of mixtures by infrared methods.

An infrared method for the quantitative determination of each of the five isomers in the insecticide has been developed and a recommended procedure is described in detail. The method is relatively simple for a five-component analysis and could be applied either to control or to research determinations. It has been successfully applied to the analysis of a few synthetic mixtures of pure isomers and to crude samples from the commercial production of the insecticide. For all but one of the isomers the precision of the method is numerically equal to the precision of the per cent transmittance measurements, and the over-all accuracy of the method is limited only by its precision.

It is concluded that the method is entirely satisfactory for the analysis of nearly pure samples of the insecticide and is relatively free of interference from the usual type of impurities encountered. It is estimated that ±0.5% accuracy in analytical results can readily be obtained in most infrared laboratories by a careful application of the method.

The possible structures of the isomers are discussed; seven are relatively strainless forms and of these two pairs are optical isomers. Preliminary crystallographic data for the five known isomers are given which distinguish all but two by optical properties. The accepted cubic classification for the structure of the beta isomer is checked by x-ray and crystallographic data.

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