PESTICIDES AND FLAVOR

Palatability and Chemical Studies on Peanuts Grown In Rotation with Cotton Dusted with Insecticides Containing Benzene Hexachloride

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Palatability and chemical studies were made to determine whether benzene hexachloride dust used to control cotton insects may result in sufficient soil accumulation of the insecticide to affect adversely the quality of peanuts grown in rotation with cotton. Initial studies, carried out for selecting and training palatability panel members, showed considerable variation among individuals with respect to sensitivity to off-flavors in peanut butter and illustrate some problems involved in evaluating palatability judgments when the flavor is at or near the judge's threshold for its detection. When quantities of benzene hexachloride dust considerably in excess of recommendations were applied to cotton, trained judges generally detected off-flavors in peanut butter made from peanuts that followed the cotton. Chemical analyses showed that benzene hexachloride content of the peanuts correlated generally with palatability scores and with the quantity of insecticide applied. In other palatability tests responses of judges were affected by the form in which the insecticide was present. There was no detection of off-flavor when technical benzene hexachloride was incorporated directly in peanut butter at a concentration several times higher than in peanuts that followed treated cotton and yielded definitely off-flavored peanut butter.

Palatability panel judgments have greater validity when panel members have been selected for their qualifications for the specific job. Because sensitivity to tastes differs among individuals, it is highly desirable in studies of the effect of pesticides on food flavor to select as panel members persons who are sensitive to the flavor being studied. Precision and reliability of panel judgments are further increased by training of the selected panel members on the specific food and flavor being evaluated. The ideal panel would consist, therefore, of individuals selected for their sensitivity to the flavor in question and trained in its evaluation in the food product being studied.

In actual practice this ideal must sometimes be compromised to meet the exigencies of time, available funds, and available panel candidates. Palatability panel evaluations are, at best, time-consuming and expensive. The cost of a panel evaluation is increased when time must be spent in training judges.

In 1947 and 1948 a number of commercial processors reported the occurrence of undesirable off-flavors in peanut butter and candies made from certain lots of peanuts. The Bureau of Entomology and Plant Quarantine had recommended that neither technical benzene hexachloride nor lindane be applied directly to the soil in which peanuts are to be planted in the season of application or in which peanuts are already growing (*2*). However, left unanswered was the question as to whether soils previously planted with cotton that had been dusted or sprayed with benzene hexachloride might contain sufficient residues of the insecticide to affect adversely the flavor of peanuts grown in rotation with the cotton.

To provide information on this question 82 samples of peanuts were collected in 1950 by the Bureau of Entomology and Plant Quarantine from fields previously planted to cotton in four of the principal cotton-producing areas. To ensure uniform sampling of the peanuts for palatability tests, a portion of each sample was processed into peanut butter in a laboratory of the Bureau of Plant Industry, Soils, and Agricultural Engineering. The resulting peanut butter samples were scored for the presence or absence of off-flavor by a trained panel.

The results of that experiment provided no definite evidence that applications by commercial growers of benzene hexachloride-containing insecticides to cotton caused significant off-flavors in peanuts that were grown subsequently in the same fields. However, the nature of the samples was such that flavor of the peanuts may have been affected by several variables that were not subject to control. For this reason the results were not considered conclusive and investigations were continued with additional lots of peanuts produced with more rigid control of experimental variables.

The new material consisted of nine lots of Virginia Jumbo peanuts grown at the Tidewater Field Station, Holland, Va. Eight were from plots where cotton treated with standard 3-5-40 benzene hexachloride–DDT–sulfur dust (3% gamma isomer of benzene hexachloride

Table I. Mean Flavor Scores by 14 Individuals for Peanut Butter Containing Different Amounts of BHC

BHC Content of Sample, P.P.M.	Mean Flavor Scores[a] for Successive Sessions							Mean of All Sessions
	1	2	3	4	5	6	7	
0	10	9.9	9.9	9.1	...	9.5	8.7	9.5
7	1.4	...	...	...	...	...	...	1.7
	1.4	1.9	...	...	...	...	...	
3.5	...	...	3.5	...	...	...	...	2.9
	...	2.7	2.4	...	...	...	...	
1.8	...	...	...	5.3	4.6	...	...	5.0
0.9	...	...	...	7.9	8.2	8.4	...	8.2
0.4	...	...	...	...	9.5	9.2	8.8	9.2
0.2	...	...	...	...	...	...	8.6	8.6

[a] A score of 10 indicates no off-flavor; 8, barely perceptible off-flavor; 6, perceptible off-flavor; 4, slightly strong off-flavor; 2, strong off-flavor; and 1, very strong off-flavor.

present as approximately 12% gamma technical benzene hexachloride, 5% DDT, 40% sulfur) had been grown the previous year, and the ninth was from a plot with a history of no prior exposure to benzene hexachloride. These peanuts were also processed into peanut butter. Previous studies on peanut butter prepared in the laboratory of the Bureau of Plant Industry, Soils, and Agricultural Engineering had shown that it was not possible with the available equipment to control the roasting process precisely. These uncontrolled variations in roast affected both color and flavor of the peanut butter and thus added to the difficulty of evaluating the characteristic of immediate interest. To reduce the effect of this variable, the peanuts used for control samples were roasted to light, medium, and dark shades to permit closer matching with the experimental samples during the palatability tests.

Training of Judges

Prior to undertaking the panel evaluation of these samples, further opportunity was found for testing and training of judges. For this purpose there was available peanut butter that had been prepared earlier from peanuts grown in soil treated with technical benzene hexachloride at a dosage rate to give 1 pound of gamma isomer per acre. Chemical analysis of this sample, using a modification (*1*) of the Schechter-Hornstein method (*3*), showed a technical benzene hexachloride content of approximately 7 p.p.m. Aliquots of this peanut butter were mixed with a natural-flavored peanut butter to provide test samples that were made up of $^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$, and $^1/_{32}$ of the benzene hexachloride–contaminated material. On the basis of the determined benzene hexachloride content of the undiluted sample, the estimated benzene hexachloride contents of these dilutions were 3.5, 1.8, 0.9, 0.4, and 0.2 p.p.m., respectively. Portions of these samples were served, three at each panel session, at 3-minute intervals to 14 individuals in a series of tests providing for decreasing concentrations of the contaminated peanut butter as the threshold test progressed. Scoring was on a 10-point scale; a score of 10 indicated no off-flavor; 8, barely perceptible off-flavor; 6, perceptible off-flavor; 4, slightly strong off-flavor; 2, strong off-flavor; and 1, very strong off-flavor. The progression of these threshold tests is illustrated in Table I, which summarizes averaged scores of the 14 individuals tested and the mean of all judgments on each sample.

During the first session a portion of the reference sample was served with duplicate samples of the undiluted peanut butter containing 7 p.p.m. of benzene hexachloride. At the second session portions of the reference sample, the undiluted benzene hexachloride sample, and the one-half dilution sample were scored. As shown by the table, seven series of three samples each were rated, finishing with the reference sample served during the seventh session in combination with samples containing 0.4 and 0.2 p.p.m. of benzene hexachloride. In addition to illustrating the sequence of dilution tests, this table indicates the extent of flavor defect in the sample made from peanuts grown in benzene hexachloride–treated soil. Even though about half of the individuals on this panel of 14 had not had previous experience or training in recognizing flavor defects caused by benzene hexachloride contamination of peanut butter, the over-all score given the undiluted sample was 1.7, indicating that it was consistently rated as so strongly off-flavored as to be inedible. The panel detected the off-flavor definitely in the peanut butters containing 3.5 and 1.8 p.p.m. of benzene hexachloride, possibly in the sample containing 0.9 p.p.m., but not in those containing 0.4 or 0.2 p.p.m.

Individual Reactions of Judges

In Table II are presented flavor scores given the peanut butter samples by 3 of the 14 individuals whose scores were included in the averages of Table I. The three sets of scores were selected to illustrate diverse types of flavor response ob-

Table II. Scores of Three Individuals for Flavor of Peanut Butter Containing Different Amounts of BHC

Individual Tested	BHC Content of Sample, P.P.M.	Flavor Scores for Successive Sessions						
		1	2	3	4	5	6	7
1	0	10	10	10	6	...	8	5
	7	1	...	...	...	...	...	...
		1	2	...	...	...	...	...
	3.5	...	...	4	...	...	...	...
		...	2	1	...	...	...	...
	1.8	...	...	...	10	5	...	...
	0.9	...	...	...	8	5	4	...
	0.4	...	...	...	...	10	9	3
	0.2	...	...	...	...	...	...	5
2	0	10	10	10	10	...	10	10
	7	2	...	...	...	...	...	...
		1	1	...	...	...	...	...
	3.5	...	...	1	...	...	...	...
		...	1	1	...	...	...	...
	1.8	...	...	...	1	1	...	...
	0.9	...	...	...	8	10	10	...
	0.4	...	...	...	...	10	10	10
	0.2	...	...	...	...	...	...	10
3	0	10	10	10	10	...	10	8
	7	1	...	...	...	...	...	...
		1	1	...	...	...	...	...
	3.5	...	...	4	...	...	...	...
		...	4	4	...	...	...	...
	1.8	...	...	...	5	6	...	...
	0.9	...	...	...	8	9	9	...
	0.4	...	...	...	...	10	9	10
	0.2	...	...	...	...	...	...	9

Table III. Mean Scores of a Panel of Six Selected Judges for Flavor of Peanut Butter Containing Different Amounts of BHC

BHC Content of Sample, P.P.M.	Mean Flavor Scores for Successive Sessions							Mean of All Sessions
	1	2	3	4	5	6	7	
0	10	10	10	10	...	9.8	9.7	9.9
7	1.3	...	...	...	...	...	...	
	1.7	1.5	...	...	...	...	...	1.5
3.5	...	...	2.8	...	...	...	...	
	...	3.0	2.5	...	...	...	...	2.8
1.8	...	...	...	4.0	3.3	...	...	3.7
0.9	...	...	...	8.0	8.3	8.7	...	8.3
0.4	...	...	...	...	9.8	9.2	9.7	9.6
0.2	...	...	...	...	...	...	9.5	9.5

served among the individuals tested. The ratings by the first individual are characteristic of scorings by persons who exhibited the least sensitivity to the flavor in question. Detection was definite in the undiluted and in the first dilution of the off-flavored sample—7.0 and 3.5 p.p.m. of benzene hexachloride, respectively. Beyond that level there is no evidence of distinction between the reference sample (0 p.p.m. of benzene hexachloride), and the samples containing concentrations of 0.2 through 1.8 p.p.m. This individual's scores also illustrate a tendency observed frequently during these tests—i.e., to score the reference sample erratically when it was rated along with dilutions of the contaminated peanut butter in which the amount of off-flavor was near or beyond the judge's threshold level.

Scoring by the second individual illustrates another type of reaction. These scores indicate a threshold level for the off-flavor that is somewhat higher than that shown by the first series of scores, and is, within the limits of the dilutions tested, sharply defined. They also show that the second individual was not discriminating as to intensity of off-flavor at levels in which it was detected.

The third series of scores (Table II) indicates approximately the same threshold level as shown by the second individual. Furthermore, scoring by the third individual exhibits ability to evaluate differences in intensity of off-flavor within the range in which it is detected. Detection on the basis of scores appeared to be definite in the peanut butter containing 1.8 p.p.m. of benzene hexachloride, questionable in the next dilution, 0.9 p.p.m.

On the basis of this series of tests, six individuals, plus two alternates, were selected to serve on the judging panel. These judges all demonstrated ability to identify the samples of peanut butter containing 1.8 p.p.m. of benzene hexachloride and generally showed ability to detect at the next dilution of 0.9 p.p.m. Data presented in Table III giving the average panel scores of these six selected judges on the dilution samples show that the reference sample (0 p.p.m. of benzene hexachloride) was never seriously underscored. The mean score of 9.9 shows that the total possible score of 360 points from 36 individual evaluations of the control sample was missed by only 3 points throughout the tests. Scores given the one to four dilution of the off-flavored peanut butter (1.8 p.p.m. of benzene hexachloride) demonstrate that the panel unquestionably identified the off-flavor at this level. While the score difference between the average of 8.3 given the next higher dilution (0.9 p.p.m. of benzene hexachloride) and that of 9.9 for the reference sample may be of doubtful significance, it is possibly indicative of borderline sensitivity at this level of off-flavor.

Such an assumption finds further support in the results presented in Table IV. Here are enumerated the number of times that reported off-flavors described were typical of those for benzene hexachloride - contaminated samples — i.e., musty, earthy, or benzene hexachloride. Because descriptions of flavors aid in the evaluation of panel scores, judges are asked to describe the character of observed flavors. Flavors recognized as typical of benzene hexachloride were not at any time ascribed to the reference sample, but were generally noted in the samples containing 7.0, 3.5, and 1.8 p.p.m. of benzene hexachloride. At the next higher dilution (0.9 p.p.m. of benzene hexachloride) notations of typical benzene hexachloride flavors accompanied 5 of the 18 judgments made on this sample. These reports of typical off-flavors, in conjunction with the average panel score of 8.3 given this sample, may be taken as further evidence that the flavor associated with a peanut butter containing approximately 0.9 p.p.m. of benzene hexachloride was recognized, but that this level probably approaches the threshold for its detection by this panel.

This selected panel was used to rate the peanut butter samples prepared from the peanuts grown at the Tidewater Field Station on plots used the previous year for cotton treated with technical benzene hexachloride at two different levels. The design used for this palatability study was a twice replicated, completely balanced lattice with a randomized distribution of samples for testing by six judges on four days. The reference sample was presented each day as a known sample and was included as a coded control at least once in the samples given to each judge. Each judge rated the flavor of three samples at each judging session using the 10-point scale. A variance analysis of the scores was made.

The results, including average panel scores for these samples, are presented in Table V. Two of the samples from peanuts that followed cotton receiving 3.8 pounds of gamma benzene hexachloride per acre, and all four from peanuts that followed the more heavily treated cotton (5.14 pounds of gamma benzene hexachloride per acre) were rated significantly lower on off-flavor than the control. These differences were statistically significant at the 5% level. While there is some overlapping of scores between treatments, the general mean scores of 8.1 and 7.1 for the samples following the low and high benzene hexachloride cotton treatments are both significantly lower at the 5% level than the score of 9.8 given the control sample, and also differ significantly from each other. The results appear to provide rather satisfactory evidence that the peanut butter made from peanuts that followed benzene hexachloride–treated cotton was characterized by definite off-flavors properly attribut-

Table IV. Number of Times Six Judges Noted Off-Flavors Typical of BHC in Peanut Butter Containing Different Amounts of BHC

BHC Content of Sample, P.P.M.	Number of Observations of BHC Flavors in Successive Sessions						
	1	2	3	4	5	6	7
0	0	0	0	0	...	0	0
7	6	...	...	...	...	...	...
	6	5	...	...	...	...	...
3.5	...	...	5	...	...	...	...
	...	4	5	...	...	...	...
1.8	...	...	...	4	4	...	...
0.9	...	...	...	1	2	2	...
0.4	...	...	...	...	1	1	0
0.2	...	...	...	...	...	...	0

able to prior exposure to benzene hexachloride of the soils in which the peanuts were produced. However, a look inside the hat from which this statistical rabbit has been extracted may lessen confidence in such a categorical conclusion.

This point is illustrated by the data presented in Table VI. Here are shown individual scores on the control and on the treated samples. The experimental design provided for scoring of each sample once by each judge and twice by two judges to provide a total of eight scores per sample. Panel mean scores, given in this table as direct arithmetic means, differ slightly from those of Table V which were adjusted by the analysis of variance, for block differences attributable to scoring on different days.

The individual scores for sample a, which provided one of the significantly low mean scores, range from a low of 3 to the maximum of 10. Neither judge D nor judge F succeeded in scoring this sample twice at comparable levels. Scoring on sample c, also from peanuts that followed cotton treated with 3.8 pounds per acre of gamma benzene hexachloride, was more uniform. Here, the maximum score range was two points and agreement between scores on the two repeats was satisfactory. Individual scores for the samples shown from peanuts that followed the cotton treated with 5.14 pounds per acre of gamma benzene hexachloride also range from a low of 3 for sample e, and 2 for h, to the maximum of 10 in both instances. Variations of this type, as noted in the previously described dilution tests, are typical of those to be expected when the flavor being judged is present at about the threshold level of detection.

In spite of the apparent inconsistencies shown by these scores, the fact that the control sample was generally scored 10, for a total score of 77 out of a possible maximum of 80 points, adds some confidence to an assumption of real detection of off-flavors in the samples from peanuts that followed benzene hexachloride–treated cotton. This assumption finds further support in judges' descriptions of observed flavors. Among the 32 judgments on the samples from peanuts that followed cotton given the 3.8-pound-per-acre treatment, there were 11 notations (35% of judgments) of off-flavors typical of benzene hexachloride. Among the 32 judgments on samples from peanuts that followed the more heavily treated cotton, 18 (56% of the scores), were accompanied by such descriptions. No typical benzene hexachloride flavors were ascribed to the control samples.

Referring again to Table V, correlation of benzene hexachloride content with cotton treatment is generally comparable with that shown by flavor scores. Benzene hexachloride content was generally higher in peanuts that followed the most heavily treated cotton. However, two of the samples (b, d) that followed the more lightly treated cotton showed approximately the same benzene hexachloride content as one (f) of those that followed the heavier treatment. Lowest flavor scores were not assigned to the samples that showed the highest chemically determined benzene hexachloride levels. The previously described dilution tests indicated that the panel's threshold level for the benzene hexachloride off-flavor in peanut butter was reached when the benzene hexachloride content was reduced to approximately 0.9 p.p.m. However, three samples that contained approximately one half or less than that amount of chemically determinable benzene hexachloride were scored significantly low on off-flavor.

These findings may be summarized briefly as follows: Six of the eight samples of peanut butter made from peanuts that followed benzene hexachloride–treated cotton were scored significantly lower than the control sample and the mean scores of 7.1 and 8.1 correlated with the respective cotton treatments of 5.14 and 3.8 pounds of gamma benzene hexachloride per acre. Forty-five per cent of all judgments on the samples from peanuts that followed treated cotton were accompanied by descriptions of typical benzene hexachloride flavors and the number of such notations correlated with the rate of benzene hexachloride application. No comparable flavors were ascribed to the control sample. Chemical analyses showed no detectable amounts of benzene hexachloride in the control sample but benzene hexachloride was found in all eight of the samples of peanuts that followed benzene hexachloride–treated cotton and in quantities that correlated generally with the cotton treatments, approaching 1 p.p.m. in three of the samples that followed the most heavily treated cotton. In total, therefore, these results add up to rather convincing evidence that benzene hexachloride insecticides applied as dust to cotton can result in sufficient soil accumulation and carry-over of the insecticide to affect adversely the flavor of peanuts grown subsequently in the same soils.

Source of Off-Flavor

Some of the findings of this study emphasize one question that has arisen frequently in this work: Is the observed off-flavor due to benzene hexachloride per se, to decomposition products of benzene hexachloride, or possibly to changes in the food product itself caused by physiological response of the plant to the chemical? The off-flavor in the peanut butter made from peanuts grown in benzene chloride–treated soil and shown by chemical analysis to contain approximately 7 p.p.m. of benzene hexachloride was regularly described as characteristic of benzene hexachloride. If the dilution test did provide a true

Table V. Mean Panel Scores for Flavor and Benzene Hexachloride Content of Peanut Butter from Peanuts Grown Following BHC-Treated Cotton

Cotton Treatment Gamma BHC per Acre, Lb.	Sample	Panel Flavor Scores: Sample mean	Panel Flavor Scores: Treatment mean	BHC Content, P.P.M.
0	Control	9.8	9.8	0.00
3.8	a	6.6[a]		0.20
	b	9.2		0.40
	c	9.4		0.20
	d	7.2[a]	8.1[a]	0.50
5.14	e	7.1[a]		0.90
	f	6.1[a]		0.45
	g	7.4[a]		0.70
	h	7.7[a]	7.1[a]	0.80

[a] Significantly lower than score for control at 5% level.

Table VI. Flavor Scores by Individual Judges for Peanut Butter from Samples of Peanuts that Followed BHC Treated Cotton

Cotton Treatment Gamma BHC per Acre, Lb.	Sample	Flavor Scores by Judges: A	B	C	D	E	F	Panel Mean Score
0	Control	10, 10	10	10, 10	8	9	10	9.6
3.8	a	5	10	10	3, 6	4	4, 10	6.5
	b	10	10, 10	10	9	6, 10	6	8.9
	c	10	9, 8	10	9	9	10, 8	9.1
	d	8, 9	9	8	9	8	4, 4	7.4
5.14	e	8	9	10, 10	5, 9	3	4	7.3
	f	8, 9	9	7	7	7, 8	3	7.2
	g	6	4	8	5, 9	10, 7	4	6.6
	h	10	9, 9	5, 10	8	10	2	7.9

measure of the panel's threshold to benzene hexachloride flavor in peanut butter at a concentration of approximately 0.9 p.p.m., then the results shown in Table V would lead to the conclusion that the chemical determination of benzene hexachloride is much more sensitive, at least for this product, and could, therefore, replace palatability tests. However, three of the samples from peanuts that followed benzene hexachloride-treated cotton and were scored significantly lower on flavor than the control showed chemically determined benzene hexachloride levels considerably lower than that associated with the indicated threshold level of the palatability panel. In so far as these scores are meaningful, therefore, they may indicate that the off-flavor is not entirely benzene hexachloride. That the flavor of benzene hexachloride in foods may be affected by the condition in which it is present is illustrated by limited experiments carried out on peanut butter.

A solution containing 300 p.p.m. of benzene hexachloride in refined peanut oil was prepared and an aliquot of this solution was incorporated in a sample of peanut butter to provide a benzene hexachloride concentration of 10 p.p.m. An equal quantity of benzene hexachloride–free peanut oil was incorporated in a second sample of the same peanut butter. When portions of these samples were submitted to the panel, no difference in flavor was observed, but both were scored lower than a control sample of the same peanut butter with no added oil. To avoid the use of additional oil, which apparently lowered flavor scores, a portion of oil was removed by suction from another sample of the peanut butter. Enough technical benzene hexachloride was dissolved in this oil to provide for approximately 15 p.p.m. of technical benzene hexachloride in the peanut butter when the oil solution and peanut butter were remixed. Again, there was no definite differentiation between this sample and controls containing no benzene hexachloride. Similar results were obtained on panel evaluation of a peanut butter sample containing approximately 15 p.p.m. of 1,2,4-trichlorobenzene, a major degradation product that might be expected from benzene hexachloride. Since even inexperienced judges readily detected off-flavors in peanut butter made from peanuts grown in benzene hexachloride–treated soils and containing as little as 1.8 p.p.m. of benzene hexachloride, these results show definitely that the condition in which the chemical is present has a pronounced effect on flavor response.

Acknowledgment

The authors are indebted to Fred W. Poos and Lorin C. Fife, Bureau of Entomology and Plant Quarantine, for supplying the peanut samples; to Victor R. Boswell and staff, Bureau of Plant Industry, Soils, and Agricultural Engineering, for preparing the peanut butters; and to the following members of the Bureau of Human Nutrition and Home Economics staff: Elsie H. Dawson for advice on planning the palatability studies, Eleanor L. Geissenhainer and Ruth A. Redstrom for assistance in carrying out the palatability studies, and Albert B. Parks and Elsie F. Dochterman for statistical analysis of the data.

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Received for review May 21, 1953. Accepted August 20, 1953. Presented during Symposium on Influence of the Use of Pesticides on the Flavor of Fresh and Processed Fruits and Vegetables, sponsored by AMERICAN CHEMICAL SOCIETY *and American Association of Economic Entomologists, Philadelphia, December 16, 1952. Parts of a two-year study to determine if the flavor of peanuts may be affected by growing them in soils previously used for the production of cotton that was treated with benzene hexachloride (BHC) insecticides. Some of the palatability and chemical studies constituting one segment of a cooperative project carried out jointly by the Bureaus of Entomology and Plant Quarantine; Plant Industry, Soils, and Agricultural Engineering; Human Nutrition and Home Economics; and the Production and Marketing Administration of the U. S. Department of Agriculture.*

TRACER STUDIES—INSECTICIDES

Preparation of Carbon-14 Labeled DDT

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RADIOACTIVE DDT LABELED WITH CARBON-14 has been used in studies of the disposition of the compound in insects (*2, 4, 5*). The writers have been advised that the labeled material used contained carbon-14 in the benzene ring. Presumably the rings were labeled through the procedure described by Fields *et al.* (*3*). DDT labeled by this method is necessarily costly, owing to the low over-all yield inherent in any procedure involving a mutiple of synthesis steps. In the case of studies involving insects, only milligram quantities of the labeled compound are needed as a rule. For toxicological studies with higher animals, relatively much greater quantities are required. As labeled DDT was desired principally for the latter purpose, study of a more efficient synthesis was undertaken.

Methods have been developed for preparing $CH_3C^{14}H_2OH$ in good yield (*1*). Chlorination of ethyl alcohol to chloral, and subsequent condensation with chlorobenzene to form DDT, appeared to be most direct, promising relatively high over-all yield. It was apparent from the literature and from preliminary trial runs that the chlorination of the alcohol was highly complex and would be the limiting reaction as far as yield of DDT is concerned. Consequently, considerable time was devoted to developing a satisfactory chlorination procedure.

An outline of the synthesis steps employed and the yield obtained has been published (*7*).

Ethyl Acetate ($CH_3C^{14}OOC_2H_5$) Barium carbonate (1.20 grams) containing 20 millicuries of activity was added to 8.65 grams of inactive barium carbonate (total 50 millimoles) and the mixture was charged along with some glass beads to the carbon dioxide generating flask, *A*, of the modified Sakami (*9*) apparatus shown in Figure 1. The apparatus was evacuated and checked for leaks. When tight, flask *B* was immersed in liquid nitrogen and slow addition of concentrated sulfuric acid to flask *A* from reservoir *C* was begun (a glass wool plug inserted in the outlet of *A* prevented mechanical carry-over of sulfuric acid into *B*). Generation of carbon dioxide was completed in about 5 hours, flask *A* being finally filled with acid to displace all gas. Then 170 ml. of 0.38 *M* methyl magnesium iodide solution was gradually added from reservoir *D* to the solid $C^{14}O_2$ in *B*. *B* was intermittently removed from the bath and the entire