

The separatory funnel is rinsed with 100 ml. of light petroleum ether, which is also passed through the sodium sulfate into the concentrator. After concentration in the usual manner to 1 or 2 ml., the concentrate is transferred to a 10-ml. volumetric flask with 5 ml. of light petroleum ether, then evaporated to dryness on a steam bath with the ground-glass stopper loosely in place to minimize entrainment losses. Next, 5 ml. of 95% ethyl alcohol is added and the mixture is evaporated on a hot plate until the volume is 1 to 2 ml. After the dilution of the concentrate to volume with 95% ethyl alcohol, the transmittancy is determined at 264  $m\mu$ , using a reference solution derived from a control sample which has been subjected to exactly the same cleanup and analytical procedures as the sample itself. A standard curve may be prepared from purified *p,p'*-dichlorobenzophenone (melting point 146.3° to 147.1° C.), but until interferences from different substrates have been evaluated, it is recommended that a standard curve be prepared from control extractives fortified with purified FW-293, using the exact procedure presented.

#### Comments on Procedures

The partition distribution of 4,4'-dichloro- $\alpha$ -(trichloromethyl)benzhydrol from petroleum ether (boiling point 60° to 80° C.) into acidified acetonitrile is shown in Table I.

The rates of hydrolysis of FW-293 in various concentrations of ammonia in 95% ethyl alcohol are listed in Table II.

The table shows that the ultraviolet procedure gives virtually a quantitative conversion into the benzophenone in the time specified.

With the split-blank procedure (2) the method is specific for FW-293, as only

**Table III. Typical Recoveries of 4,4'-Dichloro- $\alpha$ -(trichloromethyl)benzhydrol by the Split-Blank Method**

Added, $\gamma$	Recovered	
	$\gamma$	%
23	18	78.3
37	27	73.0
37	34	92.0
115	102	88.7
115	104	90.3
115	102	88.7
218	188	86.4
218	183	84.1

**Table IV. Typical Recoveries of 4,4'-Dichloro- $\alpha$ -(trichloromethyl)benzhydrol from Citrus Peel Extractives by the Total-Ketone Procedure**

Added		Recovered		
$\gamma$	P.p.m.	$\gamma$	P.p.m.	%
20	0.1	15	0.075	75
100	1.0	88	0.88	88
100	1.0	86	0.86	86
500	10.0	400	8.0	80
500	10.0	455	9.1	91

compounds which are very susceptible to hydrolysis with accompanying increase in absorptivity at 264  $m\mu$  will respond; also, minor interferences are automatically compensated. Only substances which absorb by themselves or which are very easily hydrolyzed to substances which strongly absorb ultraviolet energy will interfere.

The ultraviolet absorption characteristics of FW-293 and *p,p'*-dichlorobenzophenone are reproduced in Figure 1. A calibration curve for *p,p'*-dichlorobenzophenone prepared by the present procedure conforms to Beer's law from 10 to

280  $\gamma$  at 264  $m\mu$ . The split-blank procedure has an over-all efficiency of 73 to 92%, based upon recovery of *p,p'*-dichlorobenzophenone (Table III).

The total-ketone procedure will respond to most compounds having a



type structure, which

includes many of the probable metabolic and degradation products of 4,4'-dichloro- $\alpha$ -(trichloromethyl)benzhydrol. The total-ketone procedure has an over-all efficiency of 75 to 91%, based upon recovery of *p,p'*-dichlorobenzophenone from citrus peel extract (Table IV). Residues of FW-293 on and in citrus fruits determined by this procedure, as well as by the colorimetric (5) and total-chloride procedures, are presented in another paper (4).

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## INSECTICIDE SYNERGISM

### Mode of Action of Di-(*p*-Chlorophenyl)-(Trifluoromethyl)-Carbinol, as a Synergist to DDT Against DDT-Resistant Houseflies

The recently described di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol is a very active synergist to DDT for DDT-resistant houseflies and its mode of action has now been studied. The carbinol is absorbed rapidly through the fly cuticle and is fairly stable inside the fly body. The action of minute doses is felt over a period of many days. In vivo, the carbinol inhibits the dehydrochlorination of DDT by DDT-dehydrochlorinase but it also inhibits the penetration of DDT through the fly cuticle. As the compound counteracts its own synergistic activity, an optimum ratio of synergist to DDT exists for maximum effect.

**D**IARYL-(TRIFLUOROMETHYL)-CARBINOLS, of the general formula (*p*-XC<sub>6</sub>H<sub>4</sub>)<sub>2</sub>.C(OH).CF<sub>3</sub> have recently been reported to be very active as DDT

synergists, when applied topically to resistant houseflies (1, 2, 15). The activity of the most potent member of the group, the di-(*p*-chlorophenyl)-(tri-

fluoromethyl)-carbinol, surpasses that of a related compound, 1,1-bis-(*p*-chlorophenyl)-ethanol (DMC), whose mode of action has been described in detail

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by Perry, Mattson, and Buckner (10). The structural similarity between the two compounds strongly suggests a similar mode of action—viz., inhibition of the DDT-dehydrochlorinase which forms part of the defense mechanism in DDT-resistant flies (6, 14). The behavior of the new compound has been studied in greater detail.

### Materials and Methods

All chemicals used were of analytical reagent grade.

A moderately resistant strain (T) and a highly resistant strain (R) classified according to Perry, Fay, and Buckner (8), of *Musca domestica vicina*, of sources reported previously (15), were used, characterized by an  $LD_{50}$  of 5 and 70  $\gamma$  of DDT per fly, respectively. A completely susceptible strain of the oriental housefly was not available. Unless otherwise stated, 3-to-4-day-old female flies were used in each test. The toxicants were applied in benzene solution to the ventral aspect of the thorax by a microloop that delivered 0.16  $\mu$ l. of benzene, as described in detail by Tahori (15). The flies were kept at 26° C. and 60 to 70% relative humidity. After each experiment the size of the microloop was rechecked with a standard dye solution.

For the microdetermination of di-(*p*-chlorophenyl) - (trifluoromethyl) - carbinol, the principle of the Schechter-Haller method (13) for the determination of DDT was employed. This method is based on the color reaction between the tetranitro derivative of DDT and sodium methoxide in methanol. Sodium methoxide was prepared freshly from sodium metal and methanol.

**Preparation of Tetranitro Derivatives of Diaryl-(trifluoromethyl)-carbinols.** The carbinol, 1 gram, was treated with 15 ml. of a 2 to 3 mixture of fuming nitric and concentrated sulfuric acids for 90 minutes on a boiling water bath. The reaction mixture was cooled, poured

into 50 ml. of ice water, and extracted with ether. The extract was evaporated and the residue recrystallized twice from 95% ethyl alcohol in the presence of charcoal. Table I summarizes the properties of the tetranitro derivatives of a number of carbinols and related compounds, and also gives an indication of the accuracy of the analytical method.

**Determination of Di-(*p*-chlorophenyl) - (trifluoromethyl) - carbinol in Flies.** The amount of unabsorbed carbinol (external) was determined by rinsing the flies for 30 seconds with three 10-ml. portions of chloroform. The combined rinsings were then evaporated on a water bath, and the residue was nitrated, as described above, with 3 ml. of a 2 to 3 mixture of fuming nitric and concentrated sulfuric acids. Ice-cold water, 25 ml., was added and the solution was extracted with two 10-ml. portions of ether. The combined ether extracts were washed successively with 20 ml. of 2% sodium hydroxide solution and two 20-ml. portions of a saturated solution of sodium chloride in water, and were filtered through a layer of anhydrous sodium sulfate into a dry 50-ml. flask. The sodium sulfate was washed with 5 ml. of ether and the washings were added to the extract. The ether solution was then evaporated to dryness and the residue was taken up with 1, 2, or 4 ml. of dry benzene, depending upon the expected quantity of carbinol; 0.5 ml. of the benzene solution was pipetted into a 5-ml. volumetric flask and was made up to the mark with a 10%  $\pm$  0.1 solution of sodium methoxide in methanol.

The absorbance of the resulting colored solution at 590  $m\mu$  was determined in a Beckman Model B spectrophotometer and the amount of the carbinol was calculated from a calibration curve. Blank values owing to other materials extracted from the fly were insignificant and no corrections had to be applied.

Absorbed carbinol (internal) was determined by grinding the flies with 5 grams of anhydrous sodium sulfate and 1 gram

of glass sand, and extracting the material with chloroform in a Soxhlet apparatus for 2 hours. The same procedure as above was then followed. In this case, blank experiments were carried out with untreated flies, for each series of tests, and their values subtracted from the observed absorbance.

**Determination of DDT in Flies.** External and internal DDT, either alone or in the presence of di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol, was determined by the method of Schechter and coworkers (13). The fuming sulfuric acid, which is used in this procedure to remove interfering fats, also removes the carbinol effectively.

### Results

**Penetration of Di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol Through Cuticle of Fly.** Groups of 100 female flies of strain T, which had received 4  $\gamma$  of the carbinol per fly, were sacrificed after 1, 2, 4, 8, and 24 hours. The amount of external and internal carbinol was determined. The results, representing the average of three parallel experiments, are summarized in Table II. In all of the experiments, the loss of toxicants to the containers was insignificant.

**Persistence of Di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol in Flies.** Table II shows that 4  $\gamma$  of di-(*p*-chlorophenyl) - (trifluoromethyl) - carbinol remain almost unchanged in the fly body for at least 24 hours. However, this quantity exerts a toxic effect after 4 to 5 days. In order to investigate the fate of the carbinol over longer periods, it became necessary to apply a much smaller dosage. As these quantities are not accessible to the above chemical method of analysis, the following bioassay was derived:

About 1000 2-day-old female flies of strain T were divided into three equal groups, A, B, and C. Group A received 0.04  $\gamma$  of the carbinol per fly; group B, 0.16  $\gamma$  per fly; while group C served as control and received the solvent only. The flies were kept in glass containers and supplied with sugar and water. Immediately after treatment, and each day for a period of 7 days, 50 flies were withdrawn from each container and treated topically with 0.4  $\gamma$  of DDT per fly. Mortality was counted after 24 hours and the data, representing the average of two repetitions, are shown in Table III.

**Effect of Di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol on Penetration and Detoxification of DDT.** DDT and mixtures of DDT and the carbinol were applied topically to groups of 50 female flies of strain R. After administration of the toxicant, they were kept in glass containers with free access to food and water. Determinations of external and internal DDT and DDE were made

**Table I. Tetranitro Derivatives of Diaryl-(trifluoromethyl)-carbinols and Related Compounds**

Parent Compound	Melting Point of Tetranitro Derivative	Nitrogen, %		Maximum of Color Obtained with 10% $CH_3ONa$ , $m\mu$	Log <sub>max.</sub>	% Average Recovery for 50-500 $\gamma$
		Calcd.	Found			
( <i>p</i> -ClC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .C(OH).CF <sub>3</sub>	205	11.1	11.0	590	4.086	90
( <i>p</i> -BrC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .C(OH).CF <sub>3</sub>	225	9.5	9.4	590	4.193	90
( <i>p</i> -FC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .C(OH).CF <sub>3</sub>	Not isolated <sup>a</sup>	..	..	590	...	80
( <i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .C(OH).CF <sub>3</sub>	166 <sup>b</sup>	11.3	11.1	590	4.234	80
( <i>p</i> -CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .C(OH).CF <sub>3</sub>	175	12.1	11.8	No color	...	..
( <i>p</i> -ClC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .CH.CF <sub>3</sub>	210	11.5	11.3	600	4.076	80
( <i>p</i> -FC <sub>6</sub> H <sub>4</sub> ) <sub>2</sub> .CH.CF <sub>3</sub>	186 <sup>c</sup>	12.3	12.2	600	4.026	60

<sup>a</sup> Carbinol probably yields a mixture of isomers that could not be fractionated by simple crystallization.

<sup>b</sup> Nitration was carried out with fuming nitric acid alone, heating for 30 minutes only on a boiling water bath.

<sup>c</sup> Preparation has been reported by Mechoulam, Cohen, and Kaluszyn (5).

**Table II. Effect of Time on Penetration of Diaryl-(trifluoromethyl)-carbinols (4  $\gamma$  per T-Strain Fly)**

Compound	$\gamma/100$ Flies					
	Hours					
	1	2	4	8	24	
Di-( <i>p</i> -chlorophenyl)-(trifluoromethyl)-carbinol	External	216	164	82	38	36
	Internal	161	200	294	348	354
	Total	377	364	376	386	390
Di-( <i>p</i> -chlorophenyl)-(trifluoromethyl)-carbinyl acetate	External	319	270	240	112	77
	Internal	54	113	161	293	317
	Total	373	383	401	405	394
Di-( <i>p</i> -bromophenyl)-(trifluoromethyl)-carbinol	External	259	183	66	50	16
	Internal	129	193	330	340	326
	Total	388	376	396	390	342
Di-( <i>p</i> -methoxyphenyl)-(trifluoromethyl)-carbinol	External	280	266	171	91	33
	Internal <sup>a</sup>	51	63	51	..	..
	Total	331	329	222	91	33
DDT	External	280	249	238	209	91
	Internal <sup>b</sup>	44	46	72	109	202
	Total	324	295	310	318	293

<sup>a</sup> No internal carbinol could be detected in 8- and 24-hour tests.

<sup>b</sup> DDT + its metabolite DDE, calculated as DDT.

24 hours later, for living and moribund flies only. The results shown in Table IV are the average of two parallel experiments, which show a pronounced effect of the carbinol on the penetration of DDT through the fly cuticle.

The effects of other compounds were compared with that of the carbinol. For this purpose, the relatively inactive di-(*p*-tolyl)-(trifluoromethyl)-carbinol and the structurally different DDT synergist, *N*-methyl-4-bromobenzene-sulfo-4'-chloro-anilide (7), were chosen. Topical applications of the toxicants in benzene solutions were carried out to strain R flies as described previously. Only the external DDT was determined. The results shown in Table V are the average of two determinations. These two compounds also affect the penetration of DDT through the fly cuticle, but to a lesser degree than di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol.

The effect was further investigated by exposing resistant flies to deposits of DDT and di-(*p*-chlorophenyl)-trifluoromethyl-carbinol, thus eliminating any interference that might have been caused by the solvent. Groups of 50 female flies of strain R were kept in contact with deposits of DDT, the carbinol, and mixtures of the two compounds on filter paper. Two hours later, knock-down counts were made and the flies removed for the determination of DDT and DDE. Carbinol determinations in the presence of DDT are difficult to perform and were not carried out. The results, the average of two parallel experiments, are shown in Table VI. The observed toxicity of the deposits is

due to the fumigation effect of the carbinol. That such a fumigation effect actually exists was confirmed by keeping flies on pieces of muslin, which was stretched about 1 cm. high above either deposits of the carbinol or a saturated solution of the carbinol in water. Within 12 hours, complete knock-down of the flies was observed.

Combinations of DDT and di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol probably have a lower vapor pressure than the carbinol alone. Thus the observation becomes understandable that such a combination is less toxic than the carbinol alone.

**Inhibition of DDT-Dehydrochlorinase by Di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol.** Results in Tables IV and VI suggest that DDT-dehydrochlorinase is inhibited by the carbinol. This was confirmed by experiments with the enzyme in vitro, using a modification of the method of Sternburg, Kearns,

and Moorefield (74). Fly homogenates were prepared by grinding about 20 grams of flies of strain R with 40 ml. of cold 0.137M phosphate buffer of pH 7.4, for 1 minute in a refrigerated Waring Blendor. The brei was strained through cheesecloth and the liquid centrifuged at 5000 r.p.m. for 20 minutes in a refrigerated centrifuge. The supernatant was decanted and used immediately. One milligram of DDT crystallized on 600 mg. of glass beads (15 to 60 microns in diameter) was treated with 2 ml. of the enzyme solution and 0.5 ml. of 0.018M glutathione at 37° C. for 3 hours. When the reaction was carried out in presence of the carbinol, the latter was added together with the DDT, using an acetone solution of the two compounds for the deposition on the glass beads. The extraction, the correction for the presence of the carbinol, and the determination of DDT and DDE in the reaction mixture were carried out according to Moorefield and Kearns (6). The results are shown in Table VII. Experiments have also been carried out with a substrate-synergist ratio of 1 to 1—the results, however, were not conclusive, as reasonable recoveries of the substrate (DDT + DDE) and of the carbinol could not be effected by extraction.

**Discussion**

Table II shows that the penetration of di-(chlorophenyl)-(trifluoromethyl)-

**Table III. Persistence of Synergistic Effect of Di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol for DDT**

(0.4  $\gamma$  of DDT per T-strain fly)

Time after Carbinol Administration, Days	% Mortality after 24 Hours		
	Group A (0.04 $\gamma$ carbinol/ fly)	Group B (0.16 $\gamma$ carbinol/ fly)	Group C (control)
0	93	96	0
1	76	93	0
2	72	90	0
3	45	86	0
4	26	89	0
5	20	68	2
6	11	41	2

**Table IV. Effect of Di-(*p*-chlorophenyl)-trifluoromethyl-carbinol on Penetration and Dehydrochlorination of DDT in Resistant R-Strain Houseflies**

$\gamma$ Toxicant/Fly		DDT, External, %	DDT, Internal, %	DDE <sup>a</sup> , Internal, %	Dehydrochlorination, % <sup>b</sup>	DDT Unrecovered, %	Mortality, %
DDT	Carbinol						
11	0	21	20	29	59	30	4
11	0.5	28	35	15	30	22	44
11	1.1	31	37	11	23	21	58
11	3.3	50	24	6	20	20	38
11	11	65	10	4	29	21	30
0	11	..	..	..	..	..	8

<sup>a</sup> Given as equivalent amount of DDT.

<sup>b</sup> Calculated:  $\frac{\text{internal DDE}}{\text{internal DDT} + \text{DDE}} \times 100$ .

**Table V. Effect of N-Methyl-4-bromobenzenesulfo-4'-chloroanilide and Di-(p-tolyl)-(trifluoromethyl)-carbinol on Penetration of DDT Through Cuticle of the R-Strain Fly**

$\gamma$ Toxicant/Fly		$(p\text{-BrC}_6\text{H}_4)\text{SO}_2\text{N}(\text{CH}_3)$ $(p\text{-Cl}_3\text{CH}_2)$		$(p\text{-CH}_3\text{C}_6\text{H}_4)_2\text{C}(\text{OH})\text{CF}_3$	
DDT	Synergist	External DDT, %	Mortality, %	External DDT, %	Mortality, %
11	0	22	0	22	0
11	0.64	21	33	36	15
11	2.2	32	55	35	18
11	3.3	33	52	38	23
11	11	46	58	46	38
0	11	..	..	..	25

**Table VI. Effect of Di-(p-chlorophenyl)-(trifluoromethyl)-carbinol on Pickup, Penetration, and Dehydrochlorination of DDT in Resistant R-Strain Flies Kept in Contact with Deposits of Toxicants for 2 Hours**

( $\gamma$  per 100 flies)

Composition of Deposit, G./Sq.M.		DDT, External	Carbinol, External	DDT, Internal	DDE, Internal	Carbinol, Internal	Mortality, %
DDT	Carbinol						
0	2	...	41	...	...	25	28
2	0	12	...	2.7	6.3	...	0
2	2	50	...	15	3	...	0
0	13	...	490	...	...	372	100
13	0	126	...	41	40	...	0
13	13	113	...	.. <sup>a</sup>	.. <sup>a</sup>	...	80

<sup>a</sup> Internal DDT and DDE were not found in measurable quantities.

carbinol through the fly cuticle is very rapid. Acetylation of the hydroxyl group, or replacement of the *p*-substituted chlorine atoms by bromine atoms or methoxy groups, lessens the rate of penetration. DDT itself, whose affinity to chitin and solubility in the cuticular material are well known features of its insecticidal properties (3, 12), penetrates far less. The greater penetration of the carbinol may be because its molecule contains both lipophilic and hydrophilic groups (hydroxyl); the latter enable the compound to penetrate the inner aqueous phase of the endocuticle (16, 17).

As flies do not seem to be able to metabolize the carbinol, at least not rapidly, the effect of small doses is felt over many days. That the susceptibility of treated flies decreases progressively with time might be due to the excretion of the carbinol which is soluble in water to the extent of 20 p.p.m. In this respect, it differs widely from a related compound, 1,1-di-(*p*-chlorophenyl)-ethanol (DMC), which has been assumed to be rapidly metabolized to di-(*p*-chlorophenyl)-acetic acid via 1,1-di-(*p*-chlorophenyl)-ethylene, both of which are devoid of synergistic activity (10). On the other hand, di-(*p*-methoxyphenyl)-(trifluoromethyl)-carbinol could not be recovered in appreciable quantities from treated flies and is probably metabolized into an inactive compound, whose presence cannot be demonstrated by the analytical method used. This explanation is supported by the observation (11) that resistant flies can survive a dosage of 200  $\gamma$  of this carbinol.

The *in vivo* inhibition of the dehydrochlorination of DDT to DDE, by the

carbinol, is appreciable but not complete even at a dosage at which the toxicity of the carbinol begins to make itself felt. Perry, Mattson, and Buckner (10) have shown that, even when using DMC and DDT at a 10 to 1 ratio, causing 100% kill, the inhibition of the dehydrochlorination amounted only to 84.3%. Regarding the penetration of DDT, which is adversely affected by the carbinol, there exists a quantitative relationship between the amount of synergist applied and the amount of unabsorbed (external) DDT. Maximum mortality has been obtained with 11  $\gamma$  of DDT and 1.10  $\gamma$  of carbinol. This ratio appears to represent the compromise between two opposed properties of the carbinol, its synergistic effect and its adverse effect on the penetration of DDT. The effect on the penetration of DDT is more pronounced when the toxicants come in contact with the flies without a solvent (Table VI). In the residues of DDT alone, the pickup of DDT is 12  $\gamma$  per 100 flies, the sum of

**Table VII. Inhibition of DDT-Dehydrochlorinase by Di-(p-chlorophenyl)-(trifluoromethyl)-carbinol**

(Substrate: 1 mg. of DDT crystallized on 600 mg. of glass beads. Source of enzyme: R strain)

Molar Ratio Carbinol:DDT	$\gamma$ DDE Formed <sup>a</sup>	Inhibition, % <sup>b</sup>
No carbinol	155	0
1-10	20	87
1-100	80	49

<sup>a</sup> Given as equivalent amount of DDT.  
<sup>b</sup> Calculated according to Moorefield and Kearns (6).

internal DDT and DDE is about 10  $\gamma$ . In the presence of the carbinol, the pickup is 50  $\gamma$  but internal DDT and DDE amount only to 18  $\gamma$ . By increasing greatly the amount of the carbinol in the deposit without changing the ratio of DDT to carbinol in the mixture, the penetration of DDT is completely inhibited. As the carbinol enters the fly cuticle much more quickly than DDT, it is evidently preferentially adsorbed on the chitin micelles, thus blocking, at least partly, the entry of DDT (12). Two other polar compounds, a related carbinol, and a sulfonamide (Table V) exhibit the same property, but to a lesser degree. However, these two compounds are solids and, therefore, may act by retaining mechanically the DDT crystals, while the di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol is an oily liquid which may even serve as a solvent for DDT. The synergist, piperonyl cyclonene, applied at a high ratio to DDT, has been shown to inhibit the penetration of the DDT into flies (9).

In preliminary experiments, the rearing of strain R larvae in the presence of DDT-di-(*p*-chlorophenyl)-(trifluoromethyl)-carbinol combinations for four generations did not raise the resistance level of the strain. This is probably due to the larvicidal properties of the carbinol (7), causing selection with regard to the carbinol, and not to the combination. On the other hand, March, Metcalf, and Lewallen (4) reported a 100-fold rise in the resistance of the fifth generation when larvae were exposed to the influence of DDT and 1,1-di-(*p*-chlorophenyl)-ethane. It was also demonstrated that selection with regard to combinations of DDT and various synergists caused an increase in the DDT-dehydrochlorinase content of flies of a previously resistant strain (6).

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## FLAVOR CHANGES

# Effects of Chlorinated Hydrocarbon Insecticides on Flavors of Vegetables

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Aldrin, dieldrin, endrin, chlordan, heptachlor, lindane, and toxaphene were tested for their possible effects on the flavor of 12 vegetable crops. The treated vegetables were evaluated in the form or forms in which they are most commonly consumed. The triangular taste tests indicated that lindane treatments caused considerable damage to the flavor of most of the crops studied. Other insecticides were less pronounced in their effects on flavor. The results of flavor evaluations after prolonged storage of insecticide-treated raw and canned vegetables were similar to results obtained with fresh samples. Changes in flavor were more pronounced in canned samples than in raw or cooked samples.

THE EFFECTS OF INSECTICIDES on the flavor of fruits and vegetables have been investigated, since 1947, by many research workers in the fields of entomology and food technology. Extensive studies have been conducted on the effects of benzene hexachloride on the flavor of food crops to which it has been applied. These studies have been concerned with the effects of this chemical on flavor when applied to soils in which the crops were subsequently grown, and when applied to the foliage of fruits and vegetables. A review of the effects of benzene hexachloride and lindane has been compiled by Hinreiner and Simone (7). Other synthetic organic insecticides have been investigated for their possible effects on the flavor of fruits and vegetables, but the available information is limited.

Aldrin and dieldrin have been investigated extensively and the results, most of which are listed in the "Agricultural Handbook for Aldrin and Dieldrin" (73), appear favorable for both chemicals. Gilpin and coworkers (3) have investigated the effects, on the flavor of peanuts, of aldrin and dieldrin dust applications to the soil and foliage of peanuts and found that the flavor was not affected. These chemicals did not affect the flavor of lima beans and carrots when applied to the soil (9). Soil and foliage applications of aldrin had no

effect on the flavor of potatoes (4, 7, 8, 10, 15), tomatoes (4, 6, 15), carrots (6, 7), sweet potatoes (7), and beets (6). Hening, Davis, and Robinson (6) found off-flavors in pureed squash when aldrin had been applied to the soil at the rate of 4 pounds per acre. They did not detect a change in flavor, however, when the squash was grown in soils containing 8 pounds of aldrin per acre. Applications of dieldrin to soils have not had much effect on the flavor of crops subsequently grown in the treated areas. Potatoes (7, 8, 10), carrots (6, 7, 9), beets, tomatoes, and squash (6), and sweet potatoes (7) have been reported to be free from flavor changes due to soil treatments with dieldrin.

A relatively new compound, endrin, is being studied for its possible effects on the flavor of vegetable crops. Hinreiner and Simone (7) have reported no off-flavors due to applications of endrin to the soils in which potatoes, carrots, and sweet potatoes were grown.

The effects of chlordan treatments on the flavor of a number of vegetable crops have been reported. Stone, Foley, and Bixby (15) found that applications of 20 pounds of chlordan to the soil caused off-flavor in potatoes. Boswell reported off-flavors in potatoes when chlordan was applied to the soil at 8 and 15 pounds per acre, but not when applied at the rate of 75 pounds per acre (2). Potatoes, however, generally were not

off-flavor when lighter rates of application of chlordan were employed (4, 5, 7, 8, 10). No significant differences in flavor due to chlordan treatments were noted with carrots (6, 7, 9, 14), snap beans (9, 17, 14), sweet potatoes (7), peas (14), tomatoes (4, 6, 15), cucumbers (14), or beets (6). Gould and associates (4) reported off-flavors in tomatoes, carrots, potatoes, and lima beans when chlordan sprays were employed under certain conditions. The effects of chlordan treatments are subject to variations due to climatic conditions and soil type (2).

Heptachlor treatments have not had any measurable effect on the flavor of the vegetables studied. Lindgren, Anderson, and Frost (9) reported no changes in flavor of lima beans and carrots grown in soils treated with 5 pounds per acre of heptachlor. Kirkpatrick and coworkers (8) found heptachlor treatments to have no effect on the flavor of potatoes in two out of three years. Hinreiner and Simone (7) tested sweet potatoes, carrots, and potatoes in soils treated with heptachlor and did not detect changes in flavor.

A study was made of the effects of seven of these chlorinated hydrocarbon insecticides on the flavor of 12 vegetables. All chemicals were supplied by their basic manufacturers and included aldrin, dieldrin, endrin, chlordan, heptachlor, lindane, and toxaphene. These compounds were tested on snap beans, beets,

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