Colorimetric Microdetermination of the Acaricide 4,4′-Dichloro-alpha-(trichloromethyl)benzhydrol (FW-293)

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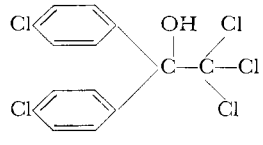
A microanalytical method for the determination of the new acaricide 4,4′-dichloro-α-(trichloromethyl)benzhydrol, or FW-293, is presented. Under mildly alkaline conditions, FW-293 affords stoichiometric proportions of chloroform and p,p′-dichlorobenzophenone. In the present residue method, the liberated chloroform is swept from extraneous materials in the above reaction mixture in a special apparatus and converted quantitatively to the Fujiwara-type red dye (λ_{max} 530 mμ, molar absorbance index 14,150 in aqueous pyridine) with a pyridine-water–sodium hydroxide mixture. Under optimum conditions, the method responds to 10 γ of FW-293 with 99% efficiency, in the presence of various fruit and nut extractives.

THE COMPOUND 4,4′-dichloro-α-(trichloromethyl)benzhydrol [1,1-bis (*p*-chlorophenyl)-2,2,2-trichloroethanol, also known as compound FW-293 and registered under the trade-mark Kelthane] has been described as possessing high acaricidal activity and is showing promise for the control of several plant-feeding mites. This white crystalline compound possesses the following properties:

Structural formula,



Molecular weight, 370.5
Per cent chlorine, 47.85
Melting point, 78.5–79.5° C.
Boiling point, 180° C./<0.1 mm.

At present, magnitudes of residues existing on and in treated edible products are important and necessary in the establishment of tolerances for the ultimate residues of new insecticidal and acaricidal materials. Several total-chlorine methods exist (*7*) for the establishment of the maximum amount of parent 4,4′-dichloro-α-(trichloromethyl)-benzhydrol that could exist in a treated substrate, regardless of alterations from metabolism or degradation. Parallel residue determinations by more specific methods would be most valuable to provide information as to the possible extent of degradation of the parent compound. This and the following paper (*8*) present details of two such definitive methods while a third paper (*9*) presents typical field residue data accrued by combinations of colorimetric, ultraviolet, and total chlorine techniques. The final paper (*11*) in this series is concerned with the field effectiveness of FW-293 against citrus mites in California.

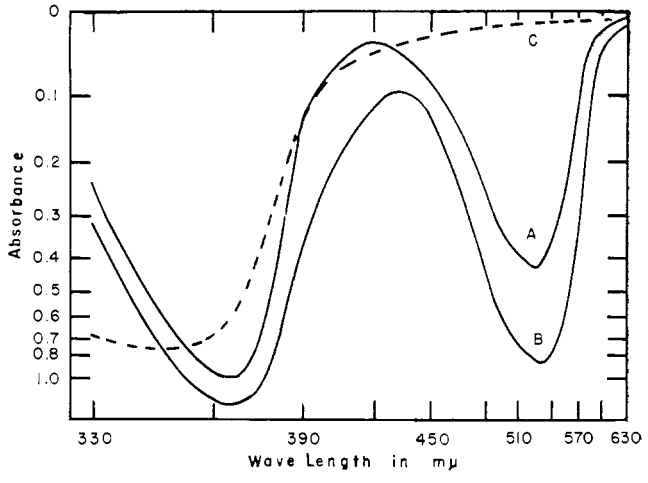
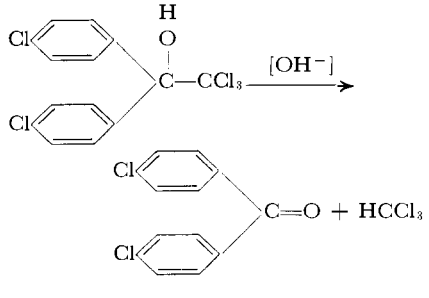


Figure 1. Absorption characteristics of the Fujiwara reaction product from chloroform

A. 38γ of chloroform (equivalent to 124γ of FW-293) in pyridine
B. Volatile component from 248γ of FW-293 reaction with base
C. Reagent blank

Under mildly alkaline conditions, FW-293 will undergo a haloform type reaction to yield 4,4′-dichlorobenzophenone and chloroform:



Chloroform was substantiated as one of the products of this reaction by a gas chromatographic analysis where the peak obtained from the above volatile product was identical with the peak obtained from a sample of purified chloroform. Infrared examination of several milligrams of the above volatile material confirmed that the volatile product was indeed chloroform.

As a result of this study of the alkaline decomposition of FW-293, the analytical methods for the chloroform moiety and for the 4,4′-dichlorobenzophenone moiety were developed. The latter method is the subject of another paper (*8*). The present method for the colorimetric determination of chloroform is based upon the Fujiwara reaction (*5*), in which chloroform in a pyridine-water–sodium hydroxide mixture is heated to 100° C. to produce a red dye (absorption maxima, 530 mμ and 364 mμ, as shown in Figure 1; molar absorbance index at 530 mμ, 14,150 in aqueous pyridine).

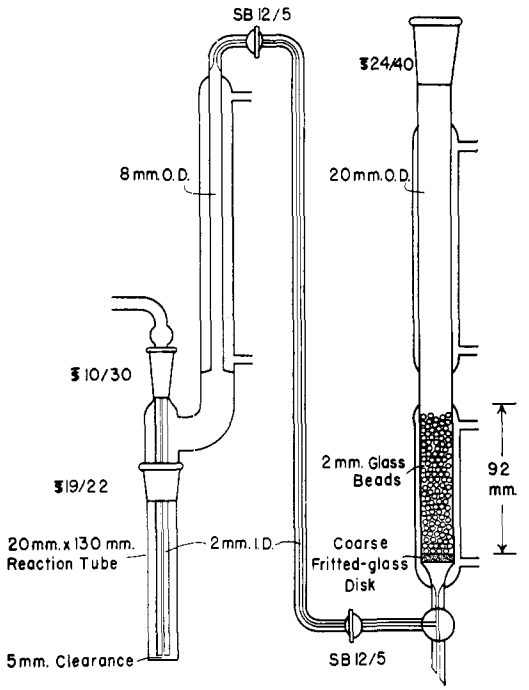


Figure 2. Chloroform evolution apparatus

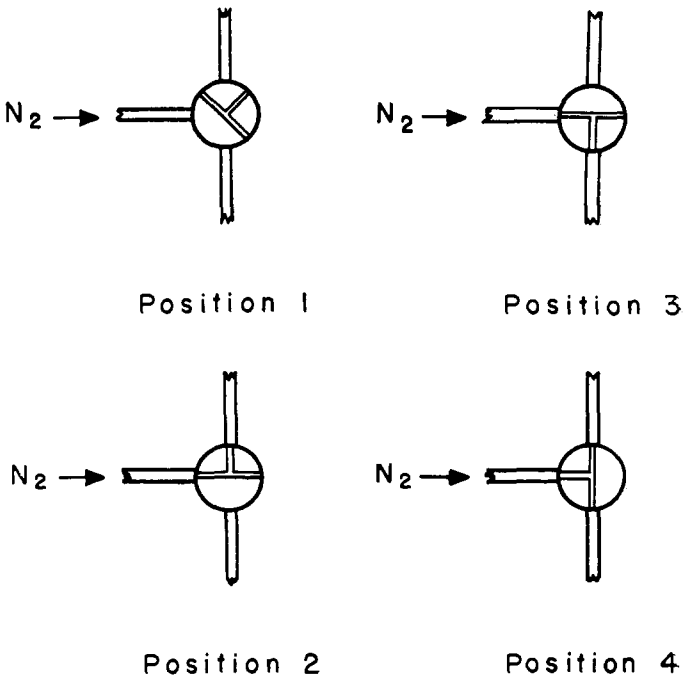


Figure 3. Positions of stopcocks during analysis

Other workers (*1–4, 6, 10, 12–15, 17*) have investigated the Fujiwara reaction as applied to the colorimetric determination of chloroform, but their conditions for the color-developing step are so many and varied that a complete study was reconducted of major variables including time of heating, temperature, concentration of reagents, and stability of color produced.

Procedure

Reagents All chemicals are analytical reagent grade, except where specifically indicated.

Sodium hydroxide, 50% aqueous solution.

Pyridine. Store in an amber bottle over sodium hydroxide pellets.

Mineral or paraffin oil.

Acetone.

Nitrogen. The commercial compressed grade is satisfactory.

n-Hexane. A commercial grade is satisfactory.

Special Apparatus Spectrophotometer or colorimeter. Any type that responds satisfactorily at 530 mμ.

Kuderna-Danish evaporative concentrators (*7*).

Chloroform evolution apparatus (see Figure 2). The reaction tube is from a Kuderna-Danish evaporative concentrator.

Flowmeter. Any flowmeter registering 80 cc. of nitrogen per minute.

Sample Preparation The sample of plant part to be examined for residues of FW-293 is equilibrated with *n*-hexane in a 1 to 2 weight-to-volume ratio, and an aliquot of the stripping solution is evaporatively concentrated in a Kuderna-Danish evaporative concentrator (*7*), to approximately 3 ml., on a steam bath. The remaining solvent is removed at room temperature with the aid of a gentle stream of dry air, then the tube containing the residue is used as a reaction

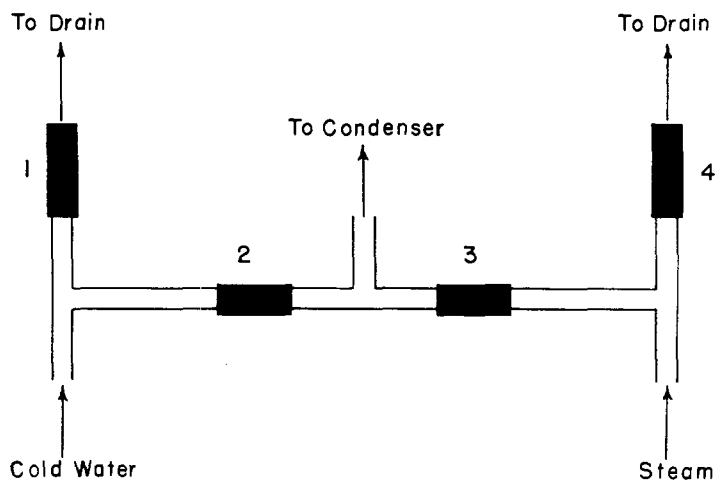


Figure 4. Method of operation of cooling and heating system

1. To circulate cold water, pinch rubber tubing at 1 and 3
2. To circulate steam, open 1, pinch 2, then open 3 and pinch 4
3. To recirculate cold water, reverse second procedure

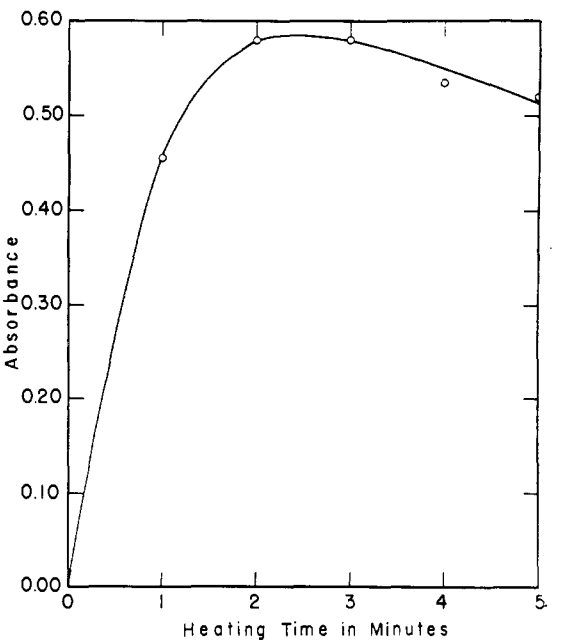


Figure 5. Absorbance–heating time curve at 100° C.

chamber in the chloroform evolution apparatus (Figure 2).

Apparatus Preparation In Figure 3 are shown the sequential operations of the stopcocks and in Figure 4 are shown the sequential operations of the water and steam systems of the chloroform evolution apparatus. The nitrogen flow rate is adjusted to 80 cc. per minute through a 3-cm. piece of themometer capillary tubing inserted into the nitrogen line just ahead of the reaction tube bubbler. Tap water is circulated through all the condensers.

To fill the receiving chamber, the stopcock in Figure 3 must be in position 1. Exactly 9 ml. of pyridine, 0.6 ml. of water, and 0.4 ml. of 50% aqueous sodium hydroxide solution are added to this chamber.

Reaction and Sweeping. After the addition of 3 ml. of paraffin oil to the receiver tube containing the evaporated sample, it is warmed slightly to solubilize the residual plant waxes and oils, then placed, without seating, under the nitrogen inlet (Figure 2). Two milliliters of 50% sodium hydroxide solution are added with a hypodermic syringe and, immediately, the tube is seated against the joint. The nitrogen inlet tube must be well below the surface of the sodium hydroxide layer. The stopcock is now turned to position 2 (Figure 3). A vigorously boiling water bath is placed around the reaction tube and the nitrogen is allowed to flow for 25 minutes. The stopcock is then turned to position 3 to vent the nitrogen to the atmosphere and thus prevent the escape of the chloroform from the receiving chamber during the heating cycle.

Color Development. With the stopcock in position 3, the water line is closed and the steam line is opened (Figure 4). Steam is introduced vigorously for 2.5 minutes after the residual water in the jacket has started to boil, then it is closed off and the cold water is recirculated for 4 minutes.

The final colored solution in the receiving chamber is drained with position 4 of stopcock (Figure 3) into a 10-ml. volumetric flask, previously purged with nitrogen. The receiving chamber is rinsed through the top of the condenser with 1 ml. of pyridine. In the event the dyed solution and the rinsings do not drain from the chamber, a standard-taper glass stopper gently inserted and withdrawn from the top of the condenser will force the solution through the fritted-glass plate. The solution in the flask is then diluted to the mark with pyridine and the absorbance is read at 530 mμ against a pyridine blank. Standard calibration and recovery curves prepared from purified FW-293 by the above procedure followed Beer's law from 10 to 120 γ of the acaricide.

Discussion

The original reaction proposed by Fujiwara (*5*) in 1917 involved heating chloroform, pyridine, alkali, and water at 100° C. to afford a red color. A number of investigators have modified conditions for this reaction for individual problems. As no comprehensive evaluation of their many conditions has been published, it was necessary to standardize them for the present purpose.

The absorption characteristics of the colored solution obtained in the Fujiwara reaction with chloroform and with the volatile product from the action of sodium hydroxide upon FW-293 afforded identical transmittance–wave length curves in the visible region, with maxima at 530 and 364 mμ (Figure 1).

Color Development. The colored solution obtained with extremely small concentrations of base rapidly faded on exposure to the atmosphere, as acid gases such as carbon dioxide will rapidly destroy the red color (*13*). The use of carbonate-free sodium hydroxide eliminated this fading of color. With concentrations of sodium hydroxide less than 50%, the colored solutions were not optically clear. Also, the optimum color development varied with the amount of water present. Effects of varying amounts of water and of sodium hydroxide are shown in Table I. The optimum concentrations of critical reagents for 10 ml. of solution are therefore 0.4 ml. of 50% sodium hydroxide solution and 0.6 ml. of water. Under these conditions, the optimum heating time for color development is 2.5 minutes as shown in Figure 5. The colored solution developed under these conditions is stable for 3 hours. Ethyl alcohol added to the final colored solution will destroy the color.

Reproducibility of the color developing step is shown in Table II, with data representing four levels of chloroform concentration in pyridine.

Recovery of Chloroform. Known amounts of chloroform were introduced directly into the receiving chamber with the other reagents, and the color was developed in the usual manner for comparison with the color developed from the parent acaricide. These results, summarized in Table III and in Figure 5,

Table I. Effect of Varying Amounts of Water and 50% Sodium Hydroxide Solution on Color Development

Water, Ml.	50% NaOH, Ml.	Absorbance
0.0	0.2	0.105
0.6	0.2	0.560
0.6	0.4	0.630
0.6	0.6	Cloudy
0.8	0.6	Cloudy
0.8	0.4	0.580
0.8	0.2	0.530

Table II. Reproducibility of Color Developing Step at Four Levels of Chloroform Concentration

Chloroform, γ	Absorbance
4.8	0.063 ± 0.002
9.6	0.112 ± 0.008
14.3	0.171 ± 0.001
19.1	0.217 ± 0.004

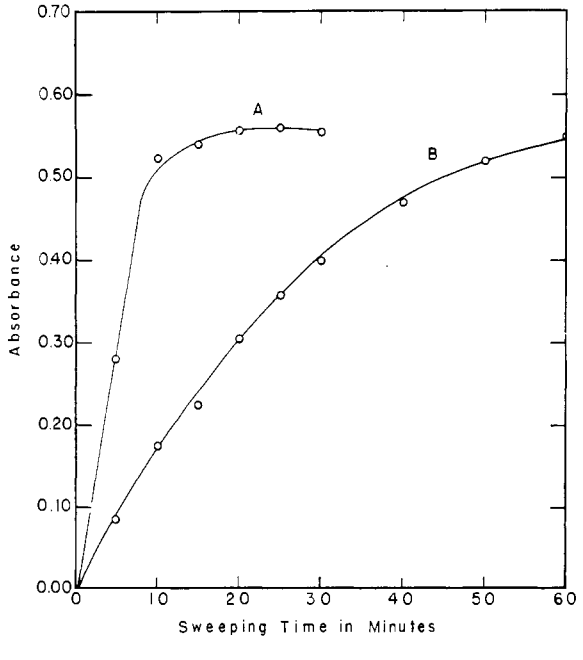


Figure 6. Time of sweeping required

A. Absorbance–sweeping time at 100° C.
B. Absorbance–sweeping time at 30° C.

Table III. Recovery of Chloroform from FW-293

Fw-293, γ	Chloroform equiv., γ	Absorbance FW-293	Absorbance Chloroform	% Recovery
10	3.2	0.035	0.036	97.2
20	6.3	0.068	0.072	94.4
40	12.6	0.131	0.144	91.0
80	25.2	0.255	0.288	88.5

Table IV. Reliability of Over-all Method

FW-293, γ	Absorbance
10	0.039 ± 0.001
20	0.073 ± 0.003
40	0.133 ± 0.002
80	0.257 ± 0.003

Table V. Apparent Recovery of FW-293 in Extractives from Valencia Orange Peel

Orange Peel, G.	FW-293, P.p.m. Added	FW-293, P.p.m. Recovered	% Recovery
5	10.0	12.5	125
5	10.0	10.2	102
25	9.0	8.9	99
25	9.0	8.8	98
25	4.5	4.3	96
25	4.5	4.2	93
50	1.0	0.8	80
50	1.0	1.0	100
50	0.1	0.1	100
50	0.1	0.1	100

show that the combination of the stoichiometry of the reaction with alkali and the efficiency of the collection of chloroform afford an average recovery of 93%.

The reliability of the over-all method, shown in Table IV, is equivalent to that for the color development step alone (Table II).

Recovery data obtained at 10, 1.0, and 0.1 p.p.m. of FW-293 added to the total petroleum ether extractives from Valencia orange peel are shown in Table V and indicate little interference from the orange extractives.

The time required to sweep the liberated chloroform completely from the reaction mixture was established as 20 minutes at 100° C., as shown in Figure 6; sweeping at a room temperature of 30° C. required at least 60 minutes.

Interferences. The following materials could contribute colored solutions under the conditions of this procedure (*16*): methyl chloride, methylene chloride, chloroform, carbon tetrachloride, methyl chloroform, tetrachloroethane, bromoform, or any large molecule capable of liberating these compounds under present conditions. There are currently no known commercial insecticides that will interfere with the present method, including *p,p'*-DDT, *p,p'*-TDE, BHC, and Perthane (ethyl analog of TDE). Petroleum ether extractives from the following crops have been found not to interfere: almonds, apples, grapes, lemons, oranges, peaches, pears, and tomatoes.

Acknowledgment

The authors are indebted to C. F. Gordon, A. H. Joseph, and F. W. Wilson, Rohm & Haas Co., and to J. H. Barkley, Citrus Experiment Station, for assistance and suggestions.

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Received for review October 25, 1956. Accepted March 22, 1957. Presented at the First Delaware Valley Regional Meeting, ACS, February 16, 1956.

PESTICIDE DETERMINATION

Ultraviolet Spectrophotometric Microdetermination of the Acaricide 4,4'-Dichloro-alpha-(trichloromethyl)-benzhydrol (FW-293)

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The *p,p'*-dichlorobenzophenone resulting from the alkaline hydrolysis of the new acaricide, 4,4'-dichloro-α-(trichloromethyl)benzhydrol, or FW-293, may be determined, quantitatively, directly by its absorption at 264 mμ (molar absorbance index 21,540 in ethyl alcohol) or indirectly by the absorption of its 2,4-dinitrophenylhydrazone in alcoholic alkali at 510 mμ. In residue applications, chromic anhydride oxidation of extraneous extractives is recommended. Under optimum conditions the method responds to 10 γ of FW-293 with 84% efficiency in the presence of citrus peel extractives.

AN ANALYTICAL METHOD, based upon the absorption of ultraviolet energy at 264 mμ by the *p,p'*-dichlorobenzophenone moiety of the acaricide, 4,4'-dichloro-α-(trichloromethyl)benzhydrol, is presented. A colorimetric method for the microdetermination of this acaricide has been discussed (*5*).

The purified crystalline acaricide (melting point 78.5° to 79.5° C.) exhibits the ultraviolet spectrum, *A*, shown in Figure 1; the ultraviolet absorption characteristics, *B*, of *p,p'*-dichlorobenzophenone are also shown. Molar absorbance index values for the parent acaricide