Determination of Residues of GC-9160 in Cabbage, Lettuce, and Citrus Fruit

Annemarie Westlake, W. E. Westlake, and F. A. Gunther

A method for the determination of residues of GC-9160 [1,3,4-metheno-1H-cyclobuta(*cd*)pentalene-2-levulinic acid, 1,1*a*,3,3*a*,4,5,5,5*a*,5*b*,6-decachloro-octahydro-2-hydroxy, ethyl] is presented. GC-9160 was oxidized to Kepone, by refluxing with chromium trioxide in acetic acid, which was measured by gas chromatography. Electron-capture and the Coulson electrical-conductivity detectors were used. The conversion of GC-9160 to Kepone was 85 ± 3% efficient for quantities of 5 to 500 μg. Laboratory recoveries of GC-9160 from fortified lemon rind, using the electrical-conductivity detector ranged from 81 to 94% and for lemon pulp, 74 to 92%. Recoveries using the electron-capture detector were 52 to 86% for lemon rind and 60 to 90% for the pulp. Similar recoveries were found for orange rind, cabbage, and lettuce, using the electron-capture detector. The lower values, in all instances, were at the lowest level (0.07 p.p.m.).

The pesticide GC-9160 [1,3,4-metheno-1H-cyclobuta-(*cd*)pentalene-2-levulinic acid, 1,1*a*,3,3*a*,4,5,5,5*a*,5*b*,6-decachloro-octahydro-2-hydroxy, ethyl] (Allied Chemical) is one of the new Kepone derivatives that shows promise in controlling flea beetles, mites, and bud worms.

The compound cannot be determined at nanogram levels as such in gas chromatographic systems, as it does not chromatograph but can be converted to Kepone which is easily detectable by electron-capture gas chromatography according to Brewerton and Slade (1964). Gilbert *et al.* (1966) oxidized the corresponding alcohol to Kepone with chromic acid (chromium trioxide), but gave no procedural details. Oxidation of GC-9160 to Kepone by refluxing for 30 minutes with chromium trioxide in glacial acetic acid proved successful and also eliminated the need for subsequent cleanup for analysis by gas chromatography. Puma (1966) reported oxidation of GC-9160 by refluxing with butylamine but cleanup to separate the pesticide from other extractives in stripping solutions was required before refluxing, and still more cleanup after refluxing to remove substances that caused interference in the gas chromatographic analysis.

The procedure presented here was usable with methylene chloride stripping solutions of cabbage, lettuce, and citrus fruit after precleaning by adding activated charcoal, filtering, and evaporating the filtered solution to near dryness before oxidation. The charcoal treatment will remove most of the Kepone, if any is present from any source, but not GC-9160. The Kepone, then formed by the oxidation of GC-9160, was extracted into chloroform, the acetic acid was washed out, the chloroform extract was evaporated to dryness, and the residue was dissolved in acetone for determination.

MATERIALS AND METHODS

Apparatus. Gas chromatograph with electron-capture detector and a 2-foot × 1/8-inch stainless steel column packed with 10% DC-200 on Gas Chrom Q, 80–100 mesh.

Gas chromatograph with the Coulson electrical-conductivity detector and a 2-foot × 3-mm. i.d. borosilicate glass column packed with 10% DC-200 on Gas Chrom Q, 60–80 mesh.

Reagents. GC-9160, purified (100%) and Kepone, purified (88% Kepone, 12% water), Allied Chemical Corp.

Standard solutions of GC-9160 and Kepone in acetone in concentrations of 5, 10, 20, and 100 μg. per ml.

Department of Entomology, University of California Citrus Research Center and Agricultural Experiment Station, Riverside, Calif. 92502

Procedure. OXIDATION OF GC-9160 TO KEPONE. Aliquots of the GC-9160 standard solutions containing amounts of from 5 to 500 μg. of the insecticide, in 250-ml. Erlenmeyer flasks, were evaporated to dryness with a gentle jet of air at room temperature. Like quantities of Kepone were carried through the identical procedure, as reference standards.

Twenty milliliters of glacial acetic acid, 2 grams of chromium trioxide, and a boiling chip were added to each residue, and the resulting solutions were refluxed for 30 minutes. Eighty milliliters of distilled water was then added to each assembly through the condenser, and the flasks were cooled to room temperature.

Each solution was transferred to a 250-ml. separatory funnel with 80 ml. of chloroform and shaken for 30 seconds. After phase separation, the chloroform was drained into a 500-ml. separatory funnel; the reaction solution was extracted twice more with 80-ml. portions of chloroform, with final combination of the three extracts in the 500-ml. separatory funnel. This extract was washed three times with 40-ml. portions of distilled water to remove the acetic acid, then filtered through a 2-inch layer of anhydrous sodium sulfate in a 36-mm. filter tube into a 500-ml. Kuderna-Danish evaporator. The sodium sulfate was washed twice with 25-ml. portions of chloroform and the solution was reduced almost to dryness on a steam bath, then carefully blown to complete dryness with a gentle stream of air. The chloroform must be *completely* removed as it will give a strong response in any of the detection systems used. The dry residue was then dissolved in sufficient acetone to provide a Kepone concentration of about 10 μg. per ml.

EXTRACTION OF LETTUCE, CABBAGE, AND CITRUS FRUIT. A representative sample of vegetable or of citrus rind was chopped in a Hobart food cutter to about 1/8-inch size and a 500-gram subsample was tumbled at 60 r.p.m. for 1 hour with 1000 ml. (2 ml. per gram of sample) of methylene chloride. The phases were allowed to separate (about 30 minutes) and the methylene chloride solution was decanted through a fluted Sharkskin filter paper containing about 10 grams of anhydrous sodium sulfate. Lemon and orange pulp samples were blended, using a modified drill press (Gunther and Blinn, 1955) with methylene chloride (1 ml. of solvent per gram of sample) for 2 minutes and stored in sealed containers at 4° C. for 2 days for separation of the phases; the methylene chloride solution was then filtered as above and stored at 4° C. in screw-cap bottles until analysis. Extractives were stored for 1 or 2 days at most, after fortification. No storage stability study was made.

CLEANUP. One hundred-fifty milliliters of a methylene

chloride stripping solution, equal to 75 grams of sample (80–85 ml. for citrus pulp samples, equal to 80–85 grams of sample) was the standard analytical aliquot. Stripping solutions from control samples were fortified with known amounts of GC-9160 and Kepone to obtain laboratory-efficiency recovery data.

Two grams of activated charcoal were added to each analytical aliquot. After standing with occasional shaking for 30 minutes, this mixture was filtered through Whatman No. 2 paper. Exactly 120 ml. of filtrate (60.0 ml. for pulp samples), equal to 60.0 grams of sample was evaporated almost to dryness in a 500-ml. Erlenmeyer flask (do not allow to go completely to dryness) on a steam bath and the remaining solvent was removed with a gentle jet of air. The initial evaporation might preferably be done in a Kuderna-Danish concentration apparatus. The residues were then oxidized *in toto* as described under "Oxidation of GC-9160 to Kepone."

GAS CHROMATOGRAPHIC DETERMINATION. The operating conditions for the instruments equipped with the two types of detectors are given in Table I.

Electron-capture detector. A standard curve was prepared by injecting 1, 2, 3, 4, 5, and 6 μl. of a standard solution containing 1 ng. of Kepone per μl. One to 5 μl. of sample solutions were injected for analysis after adjusting to appropriate volumes to keep the Kepone content in the 1–6 ng. range.

Coulson electrical-conductivity detector. Kepone standard solution containing 10 ng. per μl. was injected in volumes of 0.5, 1.0, 2.0, 3.0, and 3.5 μl. (5–35 ng. of Kepone) to obtain a linear standard curve. Samples were injected in volumes of 1 to 5 μl.

RESULTS AND DISCUSSION

The influence of reaction time on the oxidation of GC-9160 to form Kepone was evaluated; 30 minutes proved optimum (see Table II) in refluxing glacial acetic acid. The oxidation of from 5 to 500 ng. of GC-9160 gave an average yield of 85 ± 3% (Table III), as based upon the recovery of Kepone subjected to the same procedure; there was no dependency of recovery upon amount of GC-9160. There are minor losses of Kepone, or oxidized GC-9160 in the total procedure, probably during the extraction and washing steps. Chloroform, methylene chloride, benzene, and hexane were tested as extraction solvents for Kepone; chloroform consistently afforded the highest recoveries and also extracted less of the acetic acid.

Cleanup of methylene chloride stripping solutions of citrus rind, cabbage, and lettuce prior to oxidation, by shaking with activated charcoal removed the bulk of the extractable plant material and the small amounts of any remaining interfering compounds were destroyed during the oxidation step. Determination of GC-9160 in the aforementioned substrates is possible without charcoal cleanup but the background interference is greater, and the lower limit of detectability is raised correspondingly. Laboratory recoveries of the GC-9160 in orange rind, cabbage, and lettuce stripping solutions are listed in Table IV.

Table I. Conditions Used for Two Types of Gas Chromatographic Detectors

	Electron Capture	Coulson Electrical Conductivity
Column, °C.	190	175
Injection block, °C.	200	200
Detector temperature, °C.	190	...
Furnace, °C.	...	800
Carrier gas	nitrogen	helium
Flow rate, ml./min.	80	...
Retention time, min.	3.2–3.8	2.8

Table II. Dependency of Rate of Oxidation on Reaction Time in Refluxing Glacial Acetic Acid

(1 mg. of GC-9160 and 1 mg. of Kepone (12% H_2O) as reference standard)

Min. Reflux	% Recovery (Electron-Capture) Kepone reference	Oxidized GC-9160
10	97	69
20	89	76
30	97	81
30	95	83
30	...	81
40	...	78
60	...	78

Table III. Recoveries after 30-Minute Oxidations of Different Amounts of GC-9160

Amount, μg.	% Recovery (Electron-Capture)[a] Kepone reference	Oxidized GC-9160
500	...	75
200	93	84
150	...	75
100	92	83
50	...	83
20	94	81
10	97	76
5	...	82
	Average 94 ± 2	80 ± 3[b]

[a] Based on Kepone standard curve.
[b] 85 ± 3% corrected for Kepone recovery (Table II).

Table IV. Laboratory Recoveries of GC-9160 from Fortified Orange Rind, Lettuce, and Cabbage Stripping Solution (Electron- Capture)

Added p.p.m.	Added μg.	Orange Rind Found, μg.	Orange Rind Recovery, %	Lettuce Found, μg.	Lettuce Recovery, %	Cabbage Found, μg.	Cabbage Recovery, %
2.67	200	160	80	147	74	152	76
2.00	150	111	74	115	77	117	78
1.33	100	82	82	67	67	78	78
1.33	100	79	79	68	68	75	75
0.67	50	40	80	34	70	39	79
0.27	20	15	73	13	67	15	73
0.13	10	7	73	6	59	7	69
0.07	5	3	63	3	58	3	62
Control	0	ND[a]	...	ND[a]	...	ND[a]	...
Average recovery of GC-9160 at levels above 0.1 p.p.m.			78		68		75

[a] ND: None determinable.

Table V. Recovery of Kepone from Fortified Extractives from Orange Rind, Lemon Rind and Pulp, Lettuce, and Cabbage (Electron-Capture)

	Added		Found	
	p.p.m.	μg.	μg.	Recovery, %
Orange rind	1.33	100	15	15
	0.67	50	4	8
Lemon rind	1.33	100	12	12
	0.67	50	3	7
Lemon pulp	1.18	100	6	6
	0.59	50	2	4
Lettuce	1.33	100	10	10
	0.67	50	4	7
Cabbage	1.33	100	12	12
	0.67	50	3	6

Table VI. Recoveries of GC-9160 from Fortified Extractives of Lemon Rind and Pulp

Added		Electron-Capture		Electrical-conductivity	
p.p.m.	μg.	Found (μg.)	Recovery (%)	Found (μg.)	Recovery (%)
		Rind			
2.67	200	172	86	...	...
2.00	150	101	67	142	94
1.33	100	74	74	81	81
1.33	100	84	84	82	82
0.67	50	36	71	42	84
0.27	20	15	77	18	89
0.13	10	5	52	9	89
0.07	5	3	52	...	...
Control	0	ND[a]	...	ND[a]	...
		Pulp			
2.50	200	148	74	148	74
1.88	150	109	73	116	77
1.18	100	75	75	80	80
1.18	100	90	90	92	92
0.59	50	39	77	44	89
0.24	20	15	75	15	74
0.12	10	8	77	8	83
0.06	5	3	60	...	...
Control	0	ND[a]	...	ND[a]	...

[a] ND None determinable.

If Kepone is present, from 85 to 96% will be retained by the charcoal (Table V) as used in the present procedure. For complete separation of Kepone, a charcoal column may be used. Kepone is soluble in sodium hydroxide solution of unspecified concentration (Gilbert *et al.*, 1966) and, if a simultaneous determination of this compound is desired, an aliquot of the stripping solution may be partitioned from benzene into sodium hydroxide solution with further cleanup by acidifying and extracting into chloroform with transfer to acetone followed by gas chromatography (Puma, 1966).

Table VI shows the recoveries of GC-9160 from lemon rind extractives fortified at several levels, and analyzed with both electron-conductivity and electron-capture detectors.

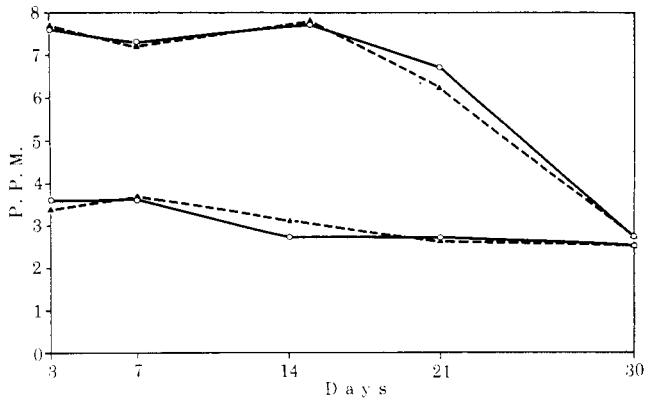

Figure 1. Residues of GC-9160 on and in unwashed orange rind

Upper lines = 1.0 lb./100 gal. of spray; lower lines = 0.5 lb./100 gal. O—O = Electroncapture detector; ▲---▲ = Coulson electrical-conductivity detector

The electrical-conductivity detector consistently gave slightly higher recoveries than the electron-capture detector, perhaps because of lack of background interference due to crop extractives for the former, or because of the stainless steel column used with the electron-capture detector. The minimum levels of detectability are essentially the same (Table VI).

The rind extractives from field-treated oranges were analyzed with the two gas chromatographic detectors and the resulting degradation curves for two different dosages of the same formulation are shown in Figure 1. It should be emphasized that only a few field samples were analyzed for the primary purpose of confirming the method. The data give an indication of the rate of degradation but a properly designed and longer lasting field experiment will be required to validate these preliminary results. The agreement between the two detectors employed is excellent and it appears that either is acceptable. The authors prefer the electrical-conductivity detector, however, because of its specificity for the chlorine-containing compound and corresponding lack of response to other extractives in the injected solutions.

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