Determination of Kepone Dechlorination Products in Finfish, Oysters, and Crustaceans

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Samples of edible portions of crab, fish, and oysters were fortified with Kepone, monohydro-Kepone, and dihydro-Kepone at two levels and were analyzed for Kepone by the gas–liquid chromatographic method of Carver et al. Recoveries of monohydro- and dihydro-Kepone closely approximated those of Kepone. Fifteen samples of fish and crab known to be contaminated with high residue levels of Kepone contained an average of 6.8 ± 1.4% of the total residue as monohydro-Kepone. Trace levels of dihydro-Kepone (<1 ppb) were detected in these samples. This method permits environmental monitoring of these residues along with Kepone residues.

Environmental contamination by Kepone in the vicinity of Hopewell, Virginia, and the nearby James River has been monitored by Federal and State agencies since its discovery in 1975. Methods have been developed for the determination of Kepone in many sample types such as finfish, shellfish, and crustaceans (Moseman et al., 1977; Carver et al., 1978), air sediment, soil, and water (Moseman et al., 1977; EPA, 1975, 1976; Commonwealth of Virginia, 1976) and human blood, urine, stools, and bile (Blanke et al., 1977; Adir et al., 1978; EPA, 1975, 1976). The presence of Kepone dechlorination products (monohydro- and dihydro-Kepones) in fish and soil (Harless et al., 1978; Borsetti and Roach, 1978), avian tissue and eggs (Stafford et al., 1978), and other environmental samples (Harless et al., 1978, and references cited therein) has previously been demonstrated.

Depending on the rate of dechlorination of Kepone in the environment, the possibility exists for an increase in the number and concentrations of its alteration products. Accordingly, methodology capable of determining the two known Kepone dechlorination products is needed for monitoring purposes. Any method used for the gas–liquid chromatographic (GLC) determination of very low concentrations of these compounds in environmental samples must provide a chromatographic background which is free of interferences. The clean-up procedure in the method used in this work furnishes such a chromatographic background. We studied the behavior of these compounds, when added to edible portions of lake trout, crab, and oyster samples, in a method known to be adequate for Kepone determination (Carver et al., 1978). Concentrations of Kepone and its dechlorination products in 13 finfish and two crab samples obtained from the James River are also reported.

EXPERIMENTAL SECTION

Materials. Approximately 10 mg each of monohydro-Kepone (≥95% purity) and dihydro-Kepone (≥98% purity) were obtained from Dr. R. D. Zehr, Health Effects Research Laboratory, EPA, Research Triangle Park, NC. These compounds were prepared by photolysis of Kepone (Harless et al., 1978). Wilson and Zehr (1979) determined the exact structures of these compounds to be 1a,3,3a,-4,5,5,5a,5b,6-nonachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one (monohydro-Kepone) and 1a,3,4,5,5,5a,5b,6-octachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one (dihydro-Kepone). Standard solutions were prepared both before and immediately after desiccation of the solid standards over P_2O_5 at 138 °C for 6 h at atmospheric pressure. The GLC results of sample analyses reported in this work are based upon a comparison with standard solutions prepared from the desiccated solid standards of monohydro- and dihydro-Kepone.

Solid Kepone standard was obtained from the Pesticides Reference Standards Section, Chemistry Branch, Registration Division, EPA, Washington, DC. The standard was weighed as the tetrahydrate (88.5% Kepone, 11.5% water), and the results are reported as anhydrous Kepone after the correction factor has been applied (Carver et al., 1978).

Edible portions of lake trout, oyster, and crab samples used for recovery analyses were composited, analyzed, and found to be free of Kepone (detection limit <0.005 ppm). The finfish and crab samples containing high concentrations of environmentally accumulated Kepone residues were all collected in 1976 and 1977.

Procedure. The method of Carver et al. (1978) was used for the analyses. The samples are extracted with isopropyl alcohol and benzene, and the extract is concentrated and redissolved in hexane. The hexane solution is shaken with oleum and then extracted with aqueous alkali. The aqueous alkali extract is acidified and extracted with 1:1 ethyl ether/petroleum ether. Benzene is added to the ether extract and the solution is reduced in volume. Methanol is added before the GLC determination so that the final solution contains 2% methanol.

Analysis. A Barber-Colman Model 5360 gas chromatograph equipped with a ^3H pin-cup electron-capture detector (ECD) and a Hewlett-Packard Model 5710 gas chromatograph equipped with a ^{63}Ni linearized, constant current ECD were used.

The unit equipped with the ^3H ECD was operated under conditions outlined by Carver et al. (1978). The following operating conditions were used for the unit equipped with the ^{63}Ni ECD: carrier gas, 5% methane in argon; flow rate, 60 mL/min; detector temperature, 300 °C; injector temperature, 250 °C; column, glass, 6 ft × 4 mm i.d.; column packing, 5% OV-101 on 80/100 mesh Chromosorb W(HP); column temperature, 200 °C; linear ECD control output attenuation ×4 to ×128. Under these conditions, the retention of Kepone and *p,p'*-DDT relative to aldrin were identical with those observed with the unit equipped with the ^3H ECD.

RESULTS AND DISCUSSION

It was assumed that monohydro- and dihydro-Kepone might exist in a hydrated form, as is normally observed

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0021-8561/79/1427-1035$01.00/0

Table I. Recoveries of Kepone and Its Dechlorination Products after High and Low Level Fortification of Samples[a]

sample	Kepone fortification, ppm	Kepone mean recov., %	monohydro-Kepone fortification, ppm	monohydro-Kepone mean recov., %	dihydro-Kepone fortification, ppm	dihydro-Kepone mean recov., %
high level fortification						
lake trout	0.0903	87.9	0.0516	81.7	0.0224	74.7
crab	0.0903	87.3	0.0516	93.6	0.0224	90.3
oysters	0.0974	83.6	0.0632	80.5	0.0262	79.0
overall mean ± SD		86.4 ± 8.9		85.2 ± 9.3		81.3 ± 8.9
low level fortification						
lake trout	0.0487	83.3	0.0316	83.8	0.0131	77.9
crab	0.0487	70.1	0.0316	69.4	0.0131	67.8
oysters	0.0487	86.3	0.0316	85.8	0.0131	76.9
overall mean ± SD		79.9 ± 10.2		79.4 ± 9.1		74.2 ± 5.9

[a] Values are means of triplicate analyses for each sample type; overall means include all nine samples.

Table II. Levels of Kepone, Monohydro-Kepone, and Dihydro-Kepone Present in Finfish Samples from the James River and Chesapeake Bay[a]

sample	Kepone, ppm[b]	monohydro-Kepone, ppm[c]	dihydro-Kepone ppm[c,d]	monohydro-Kepone as % of total residue present
bluefish	0.590	0.045	Tr (0.0006)	7.1
crab	1.36	0.094	Tr (0.0005)	6.5
crab	0.634	0.048	Tr (0.0003)	7.0
croaker	0.726	0.041	Tr (0.0006)	5.4
croaker	0.580	0.044	Tr (0.0006)	7.0
croaker	0.383	0.018	Tr (0.0002)	4.5
croaker	0.126	0.008	Tr (0.0001)	6.2
croaker	0.066	0.005	Tr (0.0003)	6.8
croaker	0.706	0.057	Tr (0.0008)[e]	7.5
mullet	0.100	0.010	Tr (0.0002)	8.9
mullet	0.534[f]	0.035[f]	Tr (0.0006)	6.2
spot	0.609	0.040	Tr (0.0006)	6.2
spot	0.670	0.034	Tr (0.0006)[e]	4.8
spot	0.235	0.020	Tr (0.0007)	7.8
trout[g]	0.178	0.019	Tr (0.0007)	9.6
av ± SD				6.8 ± 1.4

[a] Values are uncorrected for recovery. [b] Anhydrous. [c] Standard was dried at 138 °C for 6 h before preparation of standard solutions. [d] Values in parentheses are estimates (see text). [e] Observed on two different GLC columns (see Carver et al., 1978). [f] The presence of Kepone and monohydro-Kepone in this sample was confirmed by GC/MS. [g] Average of data obtained from analysis of two subsamples.

with Kepone. A GLC comparison after the compounds were dried indicated that monohydro-Kepone and dihydro-Kepone lost 8.4 and 5.8%, respectively, of their weights. Because of the limited quantities of standards available, no attempts were made to dry the compounds further or to determine the amount of residual water present.

Monohydro- and dihydro-Kepone gave linear responses (peak heights) on the ^{3}H ECD when amounts from 2 to 5 and 0.8 to 2 ng, respectively, were injected. The amounts of these compounds and of Kepone necessary to cause a 50% full-scale recorder deflection under the conditions employed were as follows: Kepone 6.3 ng, monohydro-Kepone 3.4 ng, and dihydro-Kepone 1.5 ng. With the ^{63}Ni ECD, a linear response for all quantities injected was obtained for both monohydro-Kepone (0.05–5 ng injected) and dihydro-Kepone (0.03–2.8 ng injected).

Because of the structural similarities of the monohydro- and dihydro-Kepone photoproducts to Kepone, the method would be expected to recover these compounds efficiently. Table I gives the average recoveries for triplicate analyses of three sample types at two different levels of fortification with these compounds. The fortification levels of monohydro- and dihydro-Kepone were chosen to provide GLC responses (peak height) approximately equivalent to the response for Kepone at each level. Analysis of the data by the Student's t test indicated no statistically significant differences in recovery of these compounds at either level of fortification.

Fifteen samples of edible portions of fish and crab with relatively high concentrations of environmentally accumulated Kepone residues were obtained from the James River. Results of the analyses of these samples for Kepone, monohydro-Kepone, and dihydro-Kepone are reported in Table II; values were not corrected for the method losses reported in Table I. The level of monohydro-Kepone, as a percentage of the total residue present, fell within a relatively narrow range and did not correlate with the date of sample collection. The concentrations of dihydro-Kepone were so low (S/N ≤4) that background interferences may have contributed significantly to the response, although the chromatograms appear not to have significant noise (see Figure 1). Therefore, the amounts of dihydro-Kepone are reported as trace levels in Table II.

The presence of monohydro-Kepone in one of these samples was confirmed by gas chromatography–mass spectrometry (Borsetti and Roach, 1978), but similar attempts to confirm dihydro-Kepone failed because of the low level present. Two samples were subjected to GLC analysis on two different columns, and peaks were observed on each column at the retention time of dihydro-Kepone (see Table II). The presence of dihydro-Kepone in avian tissue and eggs (Stafford et al., 1978) and in other environmental samples (Harless et al., 1978) from the James River area strongly suggests that residues of this compound

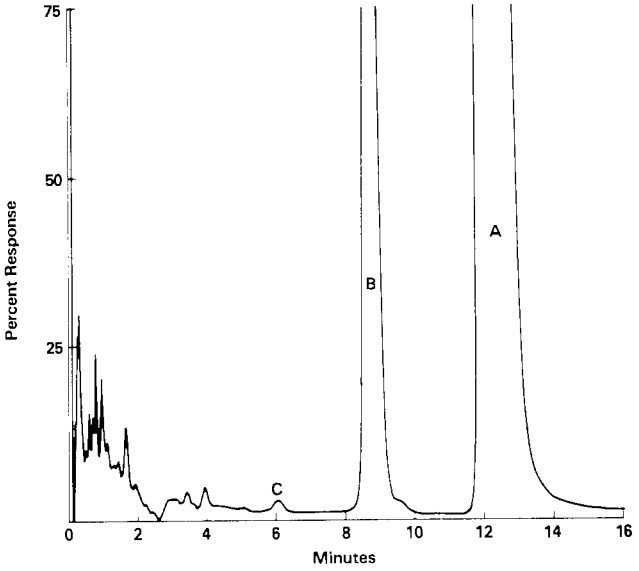

Figure 1. Gas chromatogram of spot sample (235 mg equiv sample injected) with biologically incorporated Kepone (A, 0.706 ppm), monohydro-Kepone (B, 0.057 ppm), and dihydro-Kepone (C, ca. 0.0008 ppm). GLC conditions were those reported by Carver et al. (1978).

would be present in fish collected from the same area.

The complex and incompletely understood residue pattern associated with Kepone presents some special and difficult toxicological considerations. Considering the large amount of Kepone in and around Hopewell and the James River area (EPA, 1975), the levels of monohydro- and dihydro-Kepone in the environment can be expected to increase in the future. Photolysis (Alley et al., 1974) and/or metabolism [e.g., microbial metabolism similar to that observed with mirex by Alley et al. (1974) and Carlson et al. (1976)] may be pathways for the conversion of Kepone to these compounds in the environment. The environment should be monitored for Kepone contamination by using methods that allow detection and quantitation of monohydro- and dihydro-Kepone residues, since these compounds will also be present.

Acute and chronic toxicity studies for Kepone are available in the literature (e.g., Schimmel and Wilson, 1977; Hansen et al., 1977; Walsh et al., 1977). The acute toxicity of monohydro-Kepone is approximately equal to that of Kepone in mysid shrimp, while dihydro-Kepone appears to be less acutely toxic by at least an order of magnitude. Because of limitations on the availability of these compounds, no chronic or acute toxicity tests were performed on other organisms (Nimmo, 1978). However, the presence of these compounds in environmental samples should not be ignored, especially since this method permits their determination simultaneously with Kepone.

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Received for review December 1, 1978. Accepted April 12, 1979.