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Analytical Methodology for Kepone in Water and Sediment

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■ An analytical procedure for the determination of Kepone in water and sediment is described. The method adapts the regular, multiresidue procedure for the common chlorinated hydrocarbon pesticides and PCB's for the determination of Kepone. After elution of the common chlorinated hydrocarbon pesticides and PCB's from the Florisil column, Kepone is eluted using 200 mL of a solvent mixture consisting of 10% methanol and 5% benzene in hexane. This solvent mixture also produces consistent response of the electron capture detector to Kepone. The lower detection limit in water is 20 ng/L, and in sediment is 10 µg/kg. Average recovery of Kepone in sediments spiked with 4 µg/kg is 103%.

The chlorinated hydrocarbon pesticide Kepone, also known as chlordecone, is used for control of banana and potato bugs in foreign (non-USA) countries and was formerly used as part of a roach and ant poison sold in the U.S. (1). It was manufactured by Life Sciences Products until 1975, and the wastewater discharges from this firm have contaminated the James River from just below Richmond, Va., to the mouth of Chesapeake Bay.

This has caused the initiation of several studies on the occurrence and fate of Kepone in the environment. A study of the behavior of Kepone in association with dredging of the James River sediments revealed that the regular EC-GC multiresidue procedures for chlorinated hydrocarbon pesticides (2) were not adequate for analysis of Kepone in water and sediment samples. Two problems were encountered. The first was the increase of the electron capture response to standard Kepone solution as the percentage of methanol in the injection solvent increased. The second problem was the poor recovery of Kepone from the Florisil column normally used to separate organic pesticides from other coextracted organics. Both problems may result in erratic data on Kepone levels in environmental samples.

This paper describes an analytical methodology for the determination of Kepone in water and sediment. The approach taken in this procedure was to adapt the regular, multichlorinated hydrocarbon pesticide residue procedure for determination of Kepone, thus allowing the simultaneous study of a large number of chlorinated hydrocarbon pesticides and PCB's without repeating the extraction and cleanup procedures.

Kepone is the trade name of decachloro-octahydro-1,3,4-metheno-2H-cyclobuta[cd] = pentalen-2-one. Figure 1 shows the empirical formula and structure of Kepone and its hydrate. The pure compound is a white powder that decomposes

at 350 °C. It is reported to be slightly soluble in water and hydrocarbon solvents but soluble in alcohol, ketones, and acetic acid (3). A literature review indicated that very little information is available on the properties and behavior of Kepone in natural waters. The presence of the carbonyl group in the molecule would indicate the ease of formation of the hydrate in aqueous solutions. Oswald and Moseman (4) reported that Kepone exists in the diol form in aqueous and also in nonpolar organic solvents such as hexane and benzene. They also reported that in the presence of methanol, Kepone forms hemiacetal, the structure of which was confirmed by NMR, IR, and MS.

Different procedures have been developed for the analysis of Kepone in environmental samples (5-9). Most of these procedures recommend the addition of 1-2% methanol to the final sample extract or standard solution before injection into the GC, to provide optimum response to the EC detector. Several alternatives were used to overcome the low recovery of Kepone during the Florisil column cleanup procedure. In some procedures (5, 7, 8) a micro-Florisil column containing from 1.6 to 3 g of activated Florisil and from 1.6 to 2.5 g anhydrous sodium sulfate was used. Eluting solvents of varying composition were used with the micro-Florisil column to produce high recovery (more than 80%) of Kepone.

Base partitioning was another alternative used to separate Kepone from other chlorinated hydrocarbon pesticides (5). In another procedure (9) most coextracted organics were destroyed with fuming sulfuric acid. In one procedure (6) the use of Florisil column cleanup was completely avoided, and the

EMPIRICAL FORM



CHEMICAL NAME

Decachloro-octahydro-1,3,4-metheno-2H-cyclobuta[cd] = pentalen-2-one

STRUCTURE

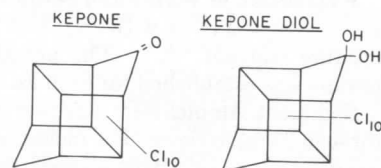


Figure 1. Empirical formula, chemical name, and structure of Kepone and its hydrate

EC detector temperature was increased to 337 °C. Most of the above-mentioned procedures focused primarily on the analysis of Kepone. For some studies, it may be required to investigate other chlorinated hydrocarbon pesticides and PCB's in addition to Kepone. In such situations the development of analytical methodology suitable for determination of all these compounds with only minor modifications is highly desirable. This paper reports on a modification of the regular chlorinated hydrocarbon analytical procedures for analysis of Kepone in natural waters and sediments.

Experimental

The instrument was a Varian Model 2100 gas chromatograph equipped with an Sc³H electron capture detector. Columns were glass U shaped of 4 mm i.d. × 6 ft L. One column was packed with 80–100 mesh Chromasorb WHP coated with 4% SE-30/6% OV-210. Another column was packed with 80–100 mesh Chromasorb WHP coated with 1.5% OV-17/1.95% QF-1.

The GC operating conditions were: carrier gas, purified nitrogen; flow rate, 60–70 cc/min; temperatures—detector 275 °C, injector 225 °C, column 200 °C; input range, 10^{–10} × 10 or 10^{–10} × 8 A; linearity range of standard Kepone, 10 000 to 50 pg.

Apparatus and Reagents. For extraction an empty 1-gal solvent bottle with Teflon-lined cap was used. A 2-L separatory funnel with a Teflon stopcock was used. The Soxhlet extractor was an all-glass unit consisting of a condenser, 250-mL extractor flask, and large-size extractor glass thimbles with extra coarse fritted disc. The Chromaflex column was 19 mm i.d. × 300 mm L with a 250-mL reservoir and stopcock. A Kuderna Danish concentrating apparatus with a 500-mL capacity and 3-ball Snyder column and graduated concentrating tubes of 10 and 4 mL was used. Solvents were benzene, hexane, methanol, ethyl-ether and acetone, all pesticide quality. Anhydrous, granular sodium sulfate was extracted for 20 h with hexane in an all-glass Soxhlet extractor, air dried, and stored in an oven at 130 °C. Silanized glass wool was extracted and stored the same as the sodium sulfate.

Florisil, activated at 1200 °C, 60–80 mesh, should be purchased in one lot. One pound was placed in a glass jar and stored in an oven at 130 °C for immediate use. Elution patterns for each lot were evaluated for the proper strength of the eluting solvent. Standard Kepone was purified solid of 87.3% purity. A stock solution of 20 ng/μL was prepared in benzene. Diluted standards were prepared as required using hexane, methanol, and benzene. Standards of other chlorinated hydrocarbon pesticides were high purity; concentrated stock solutions of 20 ng/μL were dissolved in 2,2,4-trimethyl pentane or benzene and stored in the refrigerator. Fresh standards were prepared as required by dilution of the stock solution.

Procedure. All glassware was washed with detergent and hot water, chromic acid, tap water, distilled water and acetone in that order. When using an ultrasonic cleaner and detergent, there was no need to use the chromic acid. The cleaned glassware was put in an oven at 130 °C for at least 2 h; then the ends of each piece were wrapped in aluminum foil and stored. Just prior to use, all glassware used for pesticide residue concentrations was rinsed with a few milliliters of hexane.

Extraction of Water and Sediment. Three liters of water were extracted twice with 75 mL of 35% ethyl-ether hexane mixture (solvent "A"). (The percentage of ethyl-ether in hexane was established for each lot of Florisil.)

Sediment samples were air dried and homogenized to pass through 25 mesh sieves. The moisture content in the sediment was determined by drying a known weight of the sediment at 103 °C to a constant weight. For pesticide residue analysis, two aliquots of 10 g dry sediment were placed in a glass thimble

padded with a layer of glass wool. A 100-ng heptachlor epoxide in 1 mL hexane was added as an internal standard to one of the aliquots. Each sample was extracted with 75 mL of solvent "A" in the Soxhlet extractor for 4 h. The cycling rate was adjusted to 30 cycles/h.

Florisil Column Cleanup. The column was prepared by placing 20 g of activated Florisil over a layer of glass wool and 0.5 in. Na₂SO₄. The column was pre-eluted with 50 mL hexane, the pre-eluate was discarded, and just prior to exposure of the sulfate layer, the extract was quantitatively transferred to the column. The first eluate was received in a 500-mL Kuderna Danish (KD) concentrator. The elution rate was adjusted to 5 mL/min, and an extra 50 mL of solvent "A" was added to complete the elution of the chlorinated hydrocarbon pesticides and PCB's. In the case of sediment extract (total volume of 75 mL), an additional 125 mL of solvent "A" was used. A final 25 mL of hexane was added, and before exposure of the sulfate layer, the stopcock was closed and another KD unit was placed. Kepone was then eluted with 200 mL of solvent consisting of 10% methanol and 5% benzene in hexane (solvent "B") at the rate of 5 mL/min.

Both eluates were concentrated down to 10 mL or less, using a water bath at 90 °C. Care was taken to avoid complete drying of the extract or mechanical loss of the solvent during the concentration step, especially for concentration to less than 10 mL. The first eluate I from solvent "A" contained the common chlorinated hydrocarbon pesticides and PCB's that could be analyzed according to the EPA (2) procedures. The second eluate II contained Kepone and its decomposition products that could be analyzed by EC-GC under the regular operating conditions. The final volume of the second eluate II was always adjusted to contain 10% methanol and 5% benzene in hexane before injection into the GC. The LDL in water was 20 ng/L and in sediment 10 μg/kg. These values were based on extraction of 3 L of water or 10 g of sediment and concentrating the extract to 1 mL.

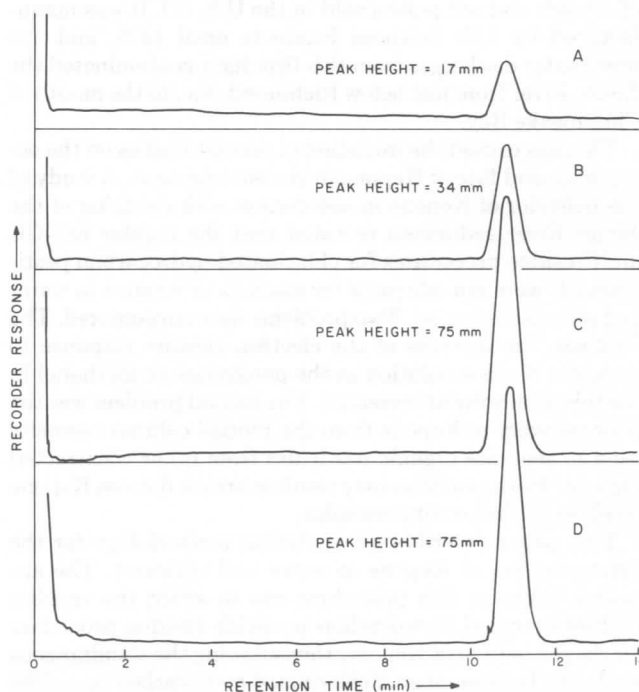


Figure 2. Increase of EC response to standard Kepone solution in presence of increasing percentage of methanol

A) EC chromatograms of 5 μL of standard Kepone (400 pg/μL) in hexane on GC column 4% SE-30/6% OV-210. B) 5 μL of the same standard in 1% CH₃OH–5% C₆H₆ in hexane. C) 5 μL of the same standard in 5% CH₃OH–5% C₆H₆ in hexane. D) 5 μL of the same standard in 10% CH₃OH–5% C₆H₆ in hexane

Table I. Recoveries of Kepone^a from Florisil Column

% Recovery	Replicate			
	1	2	3	4
First aliquot of solvent "B" ^b (100 mL)	60.7	64.7	57.1	85.1
Second aliquot of solvent "B" ^b (100 mL)	48.6	35.6	42.1	14.9
Total % recovery	109.3	100.3	99.2	100

^a 100 μ g of Kepone in 5 mL of benzene. ^b Solvent "B" consists of 10% CH₃OH-5% C₆H₆ in hexane.

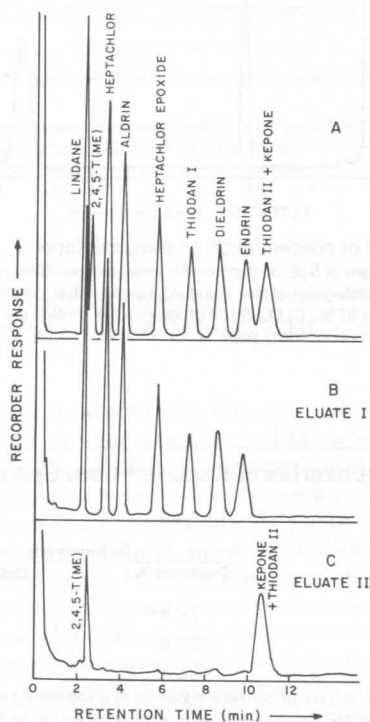


Figure 3. Separation of Kepone from nine chlorinated pesticides
A) EC chromatograms of 10 μ L of mixture of nine chlorinated pesticides and Kepone in solvent "B", total volume 10 mL, GC column 4% SE-30/6% OV-210. B) EC chromatogram of 10 μ L eluate I of total volume 10 mL in solvent "A". C) EC chromatogram of 10 μ L eluate II of total volume 10 mL in solvent "B"

Results

The above-mentioned procedure was tested for its reliability and precision. The approach used to solve the problems associated with the Kepone analysis was to produce a stable signal for Kepone on the EC detector and to recover Kepone from the regular Florisil column. The EC response to standard Kepone in solvents such as benzene or hexane gradually increased as the percentage of methanol in the solvent increased up to 5%. Above this percentage the EC signal was constant. Figure 2 shows the increase of the EC response to 5 μ L standard Kepone solution (400 pg/ μ L) with an increase of the percentage of methanol in the solvent. The 5% benzene was added to effect the complete miscibility of methanol in hexane.

To test the efficiency of Kepone elution from the Florisil column using solvent "B", two experiments were run. In the first experiment four Florisil columns were prepared. To each column 100 μ g of Kepone in 5 mL of benzene was added and eluted with two aliquots of 100 mL of solvent "B". Each aliquot was separately collected in a 100-mL volumetric flask. Table I shows the recoveries obtained from each aliquot. The first aliquot of solvent "B" eluted from 57.1 to 85.1% of the

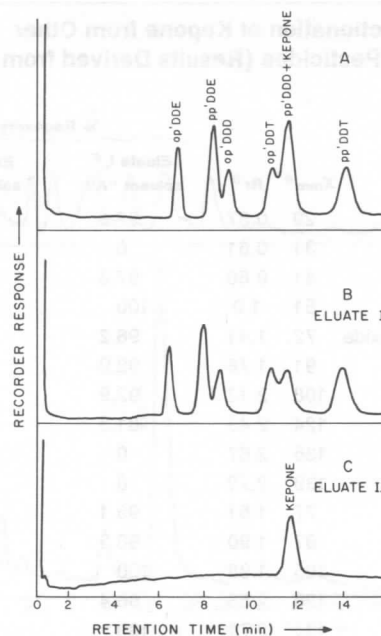


Figure 4. Separation of Kepone from DDT and its analogs

A) EC chromatogram of 10 μ L of mixture of DDT and its analogs and Kepone in solvent "B", total volume 10 mL, GC column 4% SE-30/6% OV-210. B) EC chromatogram of 10 μ L eluate I of total volume 10 mL in solvent "A". C) EC chromatogram of 10 μ L eluate II of total volume 10 mL in solvent "B"

Kepone. Total recoveries using 200 mL of solvent "B" were 109.3, 100.3, 99.9, and 100.0%, respectively.

In the second experiment, Kepone was mixed with some of the common chlorinated pesticides. In one test 1 μ g Kepone was mixed with 0.5 μ g of each of the following chlorinated pesticides: lindane, 2,4,5-T (ME), heptachlor, aldrin, heptachlor epoxide, thiodan I, dieldrin, endrin, and thiodan II, using solvent "B". The total volume of the mixture was 10 mL. Thus, the concentration of each of the chlorinated pesticides was 50 pg/ μ L, and that of Kepone was 100 pg/ μ L. Figure 3A shows the EC chromatograms of 10 μ L of the standard mixture on the GC column packed with 4% SE-30/6% OV-210. The figure shows that the retention time of Kepone (136 mm) overlaps with that of thiodan II (139 mm). This standard mixture was subjected to the Florisil column fractionation using solvents "A" and "B" as described in the procedure. Figure 3B shows the EC chromatogram of 10 μ L eluate I, and Figure 3C the EC chromatogram of 10 μ L eluate II. Apparently, solvent "A" eluted all the chlorinated pesticides except 2,4,5-T (ME), thiodan II, and Kepone. The last three compounds were effectively eluted with solvent "B".

In another test the same amount of Kepone (1 μ g) was mixed with 0.5 μ g of the following DDT compounds: *op*'DDE, *pp*'DDE, *op*'DDD, *op*'DDT, *pp*'DDD, and *pp*'DDT. The rest of the test was similar to the previous one. Figure 4A shows the EC chromatogram of 10 μ L of standard mixture. Note that here the retention time of Kepone (136 mm) overlaps with that of *pp*'DDD (138 mm). Figure 4B shows the EC chromatograms of 10 μ L eluate I, which includes all the DDT compounds. Figure 4C shows the EC chromatogram of eluate II, which includes Kepone. Table II shows the relative retention time (Rr) and the percent recovery of each compound from either the first or second eluate. Solvent "A" effected more than 90% recovery of all the compounds investigated except endrin, for which the recovery was 81.3%. No endrin was recovered in the second eluate. Also, solvent "B" effected complete recovery (100%) of Kepone, thiodan II, and 2,4,5-T (ME). The results of these two experiments indicated that both solvents "A" and "B" could be used for the complete separation of Kepone from mixtures containing 16 organic

Table II. Fractionation of Kepone from Other Chlorinated Pesticides (Results Derived from Figures 3 and 4)

Compound	X_{mm}^a	Rr^b	% Recovery	
			Eluate I, ^c solvent "A"	Eluate II, ^d solvent "B"
Lindane	29	0.57	97.3	0
2,4,5-T	31	0.61	0	100
Heptachlor	41	0.80	97.3	0
Aldrin	51	1.0	100	0
Heptachlor epoxide	72	1.41	96.2	0
Thiodan I	91	1.78	92.9	3.6
Dieldrin	108	2.12	92.9	8.8
Endrin	124	2.43	81.3	0
Kepone	136	2.67	0	100
Thiodan II	139	2.72	0	100
<i>op'</i> DDE	77	1.51	95.1	0
<i>pp'</i> DDE	97	1.90	96.3	0
<i>op'</i> DDD	105	1.98	100	0
<i>op'</i> DDT	129	2.53	96.4	0
<i>pp'</i> DDD	138	2.71	100	0
<i>pp'</i> DDT	171	3.35	93.1	0

^a X_{mm} = distance between injection and midpeak in mm. ^b Rr = relative retention time with respect to aldrin, column 4% SE-30/6% OV-210. ^c Solvent "A" = 35% ethyl-ether in hexane. ^d Solvent "B" = 10% CH₃OH-5% C₆H₆ in hexane.

Table III. Relative Retention Time (Rr) of Kepone and Its Decomposition Product on Two Chromatographic Columns

EC-GC Operating Conditions—See Text

Column	Rr^a	
	Kepone	Kepone decomposition product
4% SE-30/6% OV-210	2.63	0.98
1.5% OV-17/1.95% QF-1	2.87	0.82

^a Rr = Relative retention time with respect to aldrin.

pesticides. The herbicide 2,4,5-T(ME) and thiodan II were two of the compounds eluted with Kepone using solvent "B".

The effect of the KD concentration on Kepone was investigated. In triplicate tests 10 μ g of Kepone in 10 mL of solvent "B" was diluted to 200 mL and concentrated to 8 mL using the KD concentrator. The final volume was adjusted to 10 mL maintaining the 10% methanol and 5% benzene in hexane. Percent recoveries of Kepone were 89, 97, and 77.6, respectively. The 77.6% recovery was associated with the appearance of a secondary peak of $Rr = 0.98$ on the SE 30 column. Figure 5A shows the EC chromatograms of 5 μ L of standard Kepone (1 ng/ μ L). Figure 5B shows the EC chromatogram of 5 μ L of one of the replicates after dilution and concentration, recovering 97%. Figure 5C shows the EC chromatogram of 5 μ L of another replicate recovering 77.6% of Kepone and showing the secondary decomposition peak at $Rr = 0.98$. Table III shows the Rr values of standard Kepone and the secondary peak noted upon concentration on the two chromatographic columns used.

The recovery of Kepone from spiked sediments was investigated. Three types of sediments were used. One type was a mixture of silt and sand, the other was mostly clay, and the third was almost all sand. All three sediments were initially extracted with 50% ethyl-ether in hexane, in the Soxhlet extractor for 8 h to remove organic residues. Each type of sedi-

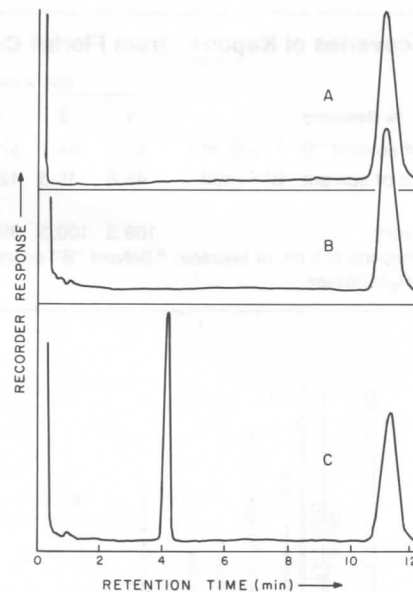


Figure 5. Effect of concentration on standard Kepone

A) EC chromatogram of 5 μ L of standard Kepone solution (1000 pg/ μ L) in solvent "B". B) EC chromatogram of the standard Kepone after dilution and concentration, recovering 97%. C) EC chromatogram after dilution and concentration, recovering 77.6%, secondary peak apparent at $Rr = 0.98$

Table IV. Recoveries of Kepone^a from Spiked Sediments

Type of sediment	% Recovery	
	Duplicate A	Duplicate B
Silt and sand	77.4 ^b	96.8
Clay	101.6	97.5
Sand	106.4	114.5

^a 40 μ g Kepone in 2 mL of benzene added to 10 g sediment, i.e., concentration of Kepone is 4 mg/kg. ^b Secondary peak of $Rr = 0.98$ on SE-30 column was observed.

ment was run in triplicate. Three aliquots, each weighing 10 g, were added to a glass thimble padded with a layer of glass wool. Two of the aliquots were spiked with 40 μ g of Kepone in 2 mL of solvent "B". Caution was taken to introduce the standard through the sediment particles. The spiked sediments were left for 2 h to equilibrate. The third aliquot was used as a sediment blank to check the background residue left in the sediment.

All the sediments were then subjected to the outlined analytical procedures. Table IV presents the recovery data. In the clay and sandy sediments, average recoveries were 99.6 and 110.5%, respectively. In the silty and sandy sediment, percent recovery for one of the duplicates was 96.8 and for the other, 77.4%. The latter low recovery was associated with the appearance of the secondary peak of $Rr = 0.98$ on the SE 30 column.

In association with the dredging operations of the James River, several water and sediment samples were collected from the area in mid-July 1976. All samples were analyzed for 15 chlorinated hydrocarbon pesticides, total PCB's, and Kepone. The results of these studies are presented by Saleh and Lee (10). Kepone was analyzed using the above-mentioned procedure. Figure 6 shows EC chromatograms of eluates I and II for a water sample. Figure 7 shows the EC chromatogram of both eluates in a sediment sample collected from Bailey Creek, which is close to the Hopewell sewage plant. Duplicate anal-

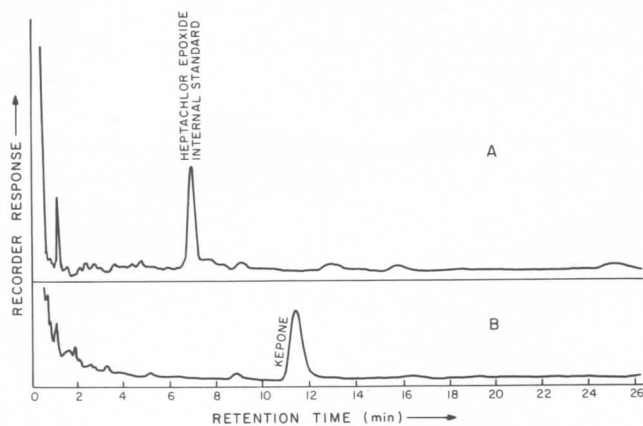


Figure 6. EC chromatograms of water sample collected from the James River

A) 5 μ L eluate I containing chlorinated hydrocarbon pesticides and PCB's, total volume 1 mL \equiv 3 L sample, GC column 4% SE-30/6% OV-210. B) 5 μ L eluate II containing Kepone, total volume 1 mL \equiv 3 L sample

yses were run on selected water and sediment samples. Table V shows the results and the standard deviation.

Discussion

The problems associated with Kepone analysis can be attributed to its molecular structure and the presence of the carbonyl group. Though the exact explanation of the increase of the EC response to Kepone in the presence of methanol is unknown, it is possible to attribute the phenomenon to the increase of electron affinity upon elutriation of Kepone through the EC detector. It is recognized that whether Kepone is present as a hydrate in nonpolar solvents or as a hemiacetal in the presence of methanol, it is decomposed to the anhydrous form upon injection on the GC with the injector port at 225 $^{\circ}$ C. The low recovery of Kepone from the regular Florisil column can be attributed to the strong binding forces that hold Kepone molecules on the Florisil particles. Apparently, the polarity of the ethyl-ether hexane is not enough to counteract these binding forces. Initial experiments performed using an increasing percentage of ethyl-ether in hexane up to 80% did not yield satisfactory recovery results. Probably, the formation of Kepone hemiacetal in the presence of methanol plays an important role in releasing Kepone from the Florisil particles.

The stability of Kepone is another area that needs to be investigated. The occasional decomposition of Kepone upon concentration or during the Soxhlet extraction procedure was also noted upon storing the standard Kepone solution for a period of one month.

The outlined procedure allows for the analysis of Kepone in addition to several other chlorinated hydrocarbon pesti-

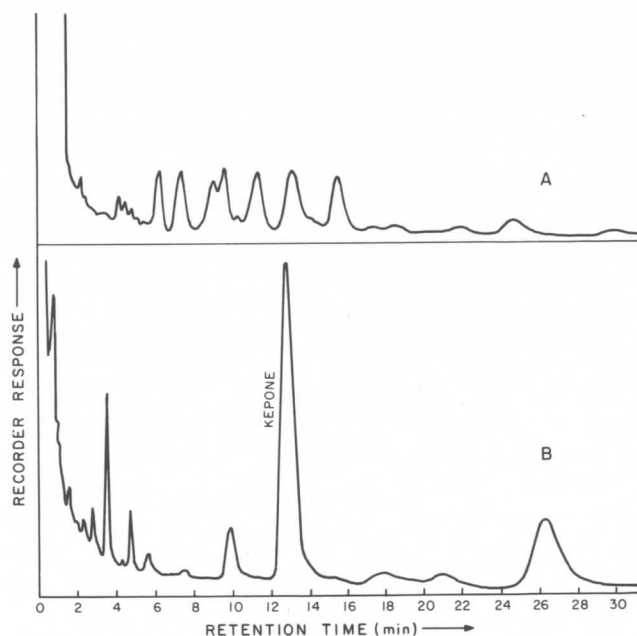


Figure 7. EC chromatograms of sediment sample collected from Bailey Creek

A) 5 μ L eluate I containing chlorinated hydrocarbon pesticides and PCB's, total volume 10 mL \equiv 10 g sediment, GC column 4% SE-30/6% OV-210. B) 5 μ L eluate II containing Kepone total volume 100 mL \equiv 10 g sediment

cides and PCB's using a single extraction and single Florisil column. Also, the procedure allows the separation of Kepone from other chlorinated hydrocarbon pesticides that have retention times close to that of Kepone, such as *pp'*DDD on the SE-30 column and endrin on the OV-17 column.

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Table V. Evaluation of Precision of Kepone Procedure Results of Duplicate Analyses of Water and Sediment Collected from the James River and Bailey Creek

Kepone concn	Duplicate A	Duplicate B	Av	SD ^a
Water (in ng/L)	44.4	46.6	45.5	1.6
Sediment (in mg/kg) ^b	1.45	1.34	1.40	0.08

^a SD = standard deviation. ^b Results are based on dry weight.

