

SYNTHESIS OF CARBON-14 AND CARBON-13 LABELED CHLORINATED
POLYCYCLIC PESTICIDES

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SUMMARY

The preparations of the pesticides 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[cd]pentalene-U-C¹⁴ (mirex-U-C¹⁴), 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one-U-C¹⁴ (Kepone[®]-U-C¹⁴), ethyl 5-(1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-2-hydroxy-1,3,4-metheno-1H-cyclobuta[cd]pentalen-2-yl-1,1a,2,3,3a,4,5,5a,5b,6-C₁₀¹⁴)-levulinate (kelevan-C¹⁴), 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene-4,5,6,7,8-C₅¹⁴ (heptachlor-4,5,6,7,8-C₅¹⁴) and 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan-4,5,6,7,8-C₅¹⁴ (heptachlor epoxide-4,5,6,7,8-C₅¹⁴) from hexachlorocyclopentadiene-U-C¹⁴ are described. Specific activities were in the range of 1.8 to 3.2 mCi/mmol.

Further, the synthesis of mirex-C₂¹³ and Kepone[®]-C₂¹³ from isopropyl formate-C¹³ is reported. The determination of the carbon-13 enrichment of these compounds was accomplished by selected ion monitoring (SIM) of seven ions in the molecular clusters of mirex and Kepone[®], followed by calculation of the relative abundance of the various carbon-13 containing molecules from the areas of the SIM plots.

Key Words: Carbon-14, Chlorinated polycyclic pesticides, Carbon-13, Mass spectra, Selected ion monitoring

INTRODUCTION

The insecticidal compounds mirex, Kepone[®], kelevan and heptachlor have been, or continue to be, used on a widespread commercial basis. Their persistence in the environment, their toxicity to man and animals [1], and, in one case, their documented carcinogenicity [2] make knowledge of their metabolism, disposition, and environmental fate matters of considerable importance.

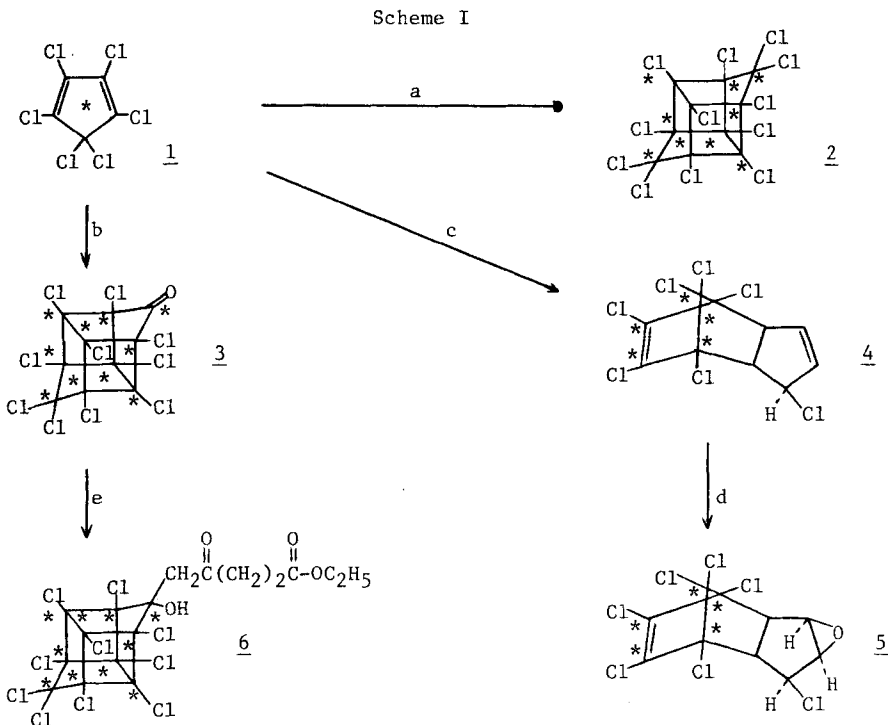
In order to facilitate metabolic and related studies of these compounds, we have synthesized mirex, Kepone[®], kelevan, heptachlor and heptachlor epoxide

labeled with carbon-14, as well as mirex and Kepone[®] enriched with carbon-13, and report the details of this work herein.

RESULTS AND DISCUSSION

All carbon-14 labeled compounds were prepared from the common intermediate hexachlorocyclopentadiene-U-C¹⁴, (1), (hereafter referred to as "hex") which was synthesized according to the method of McKinney and Pearce [3]. Yields obtained by us were similar to those previously reported. The overall radiochemical yield of 1 was 18%, based on BaC¹⁴O₃.

Samples of "hex"-U-C¹⁴ were subsequently converted to chlorinated polycyclic pesticides as shown in Scheme I. Thus, mirex-U-C¹⁴ (2) [4] and Kepone[®]-U-C¹⁴ (3)

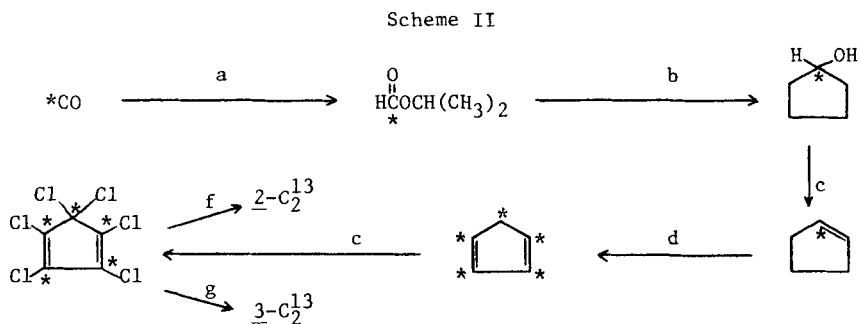


a AlCl₃, Δ; b SO₃, hydrolysis; c cyclopentadiene, Δ; SeO₂, HOAc; ZnCl₂, HCl;

d CrO₃, H₂SO₄; e CH₃C(=O)(CH₂)₂C(=O)OC₂H₅, xylenes, reflux

[4] were prepared according to the methods of Newcomer and McBee [4] and of Gilbert and Giolito [5], respectively. Heptachlor-4,5,6,7,8-C¹⁴₅, (4), and heptachlor epoxide-4,5,6,7,8-C¹⁴₅, (5), were synthesized utilizing adaptations of reported literature methods [6], and kelevan-C¹⁴, (6), was prepared in 57% yield by treating a xylene solution of 3 with an excess of ethyl levulinate at reflux for 24 hr [7].

The syntheses of the carbon-13 labeled compounds were designed to minimize C¹³-C¹³ coupling in the NMR, and consequently the synthesis pathway to "hex"-1,2,3-C¹³_{1/3} was altered to provide singly labeled (above natural abundance) material; see Scheme II. Thus, cyclopentadiene-C¹³ was prepared according to the



^a iPrOH, Na, 150 psi; ^b BrMg(CH₂)₄MgBr, THF; ^c H₃PO₄, K₂S₂O₇; ^d Br₂, CCl₄; quinoline, HMPA; ^e 5% NaOCl, H₂NSO₃Na; ^f AlCl₃, Δ; ^g SO₃, hydrolysis.

procedure of Larson, Vergamini and Whaley [8] and subsequently converted to "hex"-1,2,3-C¹³_{1/3} by chlorination using a method based on that of Kleinman [9], but utilizing 5% sodium hypochlorite and a catalytic amount of sodium sulfamate [10]. The resulting "hex"-1,2,3-C¹³_{1/3} was converted to mirex-C¹³₂ and Kepone[®]-C¹³₂ as described above for the C¹⁴-labeled compounds.

The mass spectral fragmentation patterns of the C¹³-labeled mirex and Kepone[®] are consonant with those reported earlier [11], but the presence of

excess C^{13} alters the isotopic distribution patterns for the ions in the spectrum. The predominant mode of fragmentation for both molecules is cleavage of the pentacyclic nucleus in half. In the case of mirex, both halves are identical, and the $(C_5Cl_6)^+$ ion (m/e 270) displays sequential loss of at least four chlorine atoms. The same losses of multiple chlorine atoms are observed in the molecular ion series for Kepone[®] although the Kepone[®] spectrum is complicated by the reduced symmetry of the molecule, and the secondary fragmentation pattern is more complicated.

The C^{13} enrichments of the C^{13} -labeled mirex and Kepone[®] were determined from the relative intensities of peaks in their molecular ion (M^+) clusters. (See Figures 1 and 2.) The relative intensity data, determined by SIM, were used to calculate the fractional contributions of molecules with zero to three C^{13} per molecule (see Tables I and II). From these contributions, the C^{13} enrichments were determined to be approximately 80%.

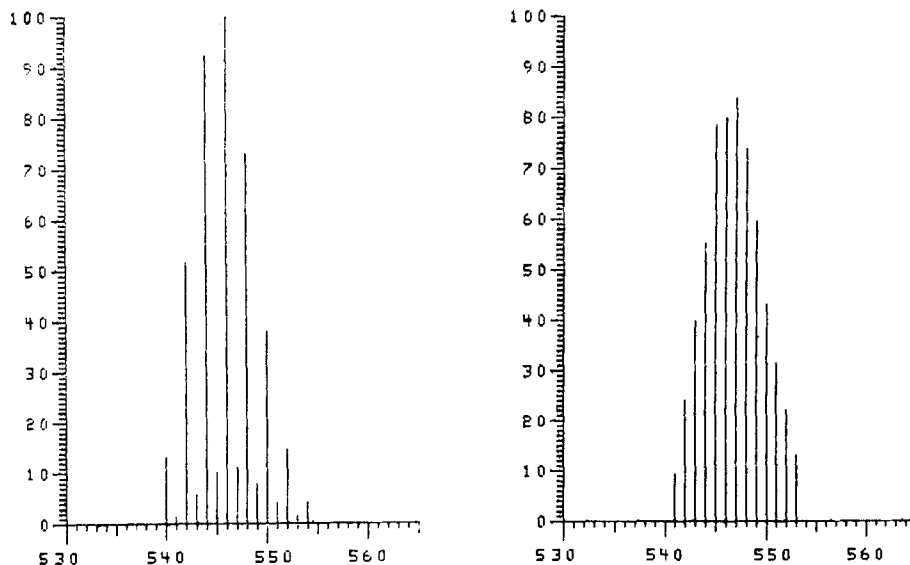


Figure 1 - Molecular ion series of natural abundance (left) and C^{13} -enriched (right) mirex

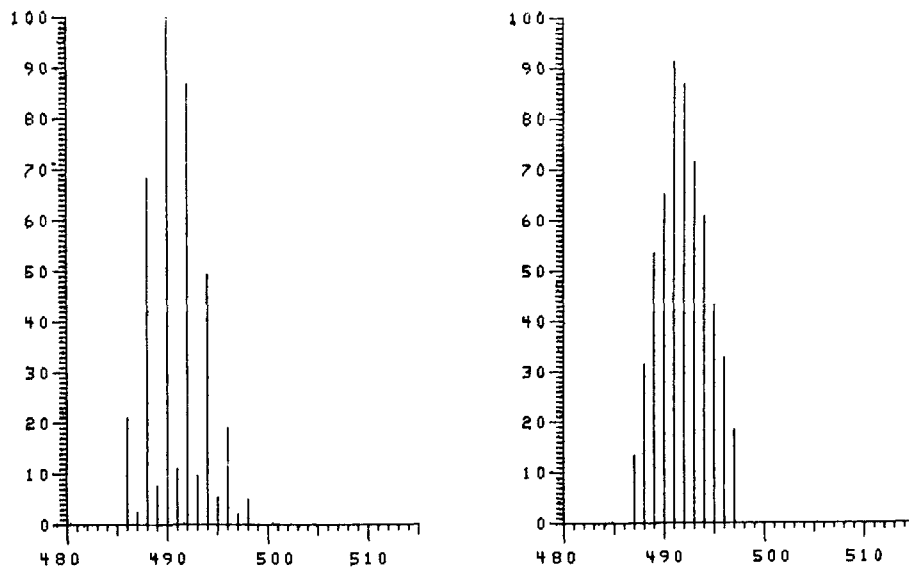


Figure 2 - Molecular ion series of natural abundance (left) and C^{13} -enriched (right) Kepone[®]

TABLE I

C^{13} CONTENTS OF NATURAL ABUNDANCE AND C^{13} -ENRICHED MIREX

Species	Fractional Contribution	
	Natural Abundance	C^{13} -Enriched
$C_0^{13}C_{10}^{12}Cl_{12}$	$1.000 \pm 0.023^\dagger$	$0.202 \pm 0.004^\dagger$
$C_1^{13}C_9^{12}Cl_{12}$	$0.010 \pm 0.001^\dagger$	$0.509 \pm 0.002^\dagger$
$C_2^{13}C_8^{12}Cl_{12}$	$-0.001 \pm 0.024^\dagger$	$0.313 \pm 0.004^\dagger$
$C_3^{13}C_7^{12}Cl_{12}$	$0.000 \pm 0.02^\dagger$	$-0.024 \pm 0.002^\dagger$

† Average deviation for three determinations.

TABLE II

C¹³ CONTENTS OF NATURAL ABUNDANCE AND C¹³-ENRICHED KEPONE[®]

Species	Fractional Contribution	
	Natural Abundance	C ¹³ -Enriched
C ₀ ¹³ C ₁₀ ¹² C ₁₀ ⁰	1.020 ± 0.009 [†]	0.196 ± 0.007 [†]
C ₁ ¹³ C ₉ ¹² C ₁₀ ⁰	0.013 ± 0.001 [†]	0.482 ± 0.006 [†]
C ₂ ¹³ C ₈ ¹² C ₁₀ ⁰	-0.030 ± 0.009 [†]	0.315 ± 0.008 [†]
C ₃ ¹³ C ₇ ¹² C ₁₀ ⁰	-0.003 ± 0.002 [†]	0.008 ± 0.012 [†]

† Average deviation for three determinations.

EXPERIMENTAL

Isopropyl formate-C¹³ was purchased from Prochem Isotopes, Summit, New Jersey, or synthesized utilizing the literature procedure [8]. IR spectra were determined with a Beckman Acculab I, either neat or in Nujol. Radioactivity was determined in a Packard Model 3003 liquid scintillation counter using Econofluor[®] (New England Nuclear) as the counting medium. Radiochemical purity was determined in a Packard Model 7201 radiochromatogram scanner. All gas chromatographic analyses were performed using a Bendix Model 2500 GC equipped with dual FID detection. Three glass columns were used: (a) 1.8 m x 2 mm ID, 1.5% OV-17/1.95% OV-210 on 80 to 100 mesh Gas Chrom Q; (b) 1.8 m x 2 mm ID, 5% OV-210 on 80 to 100 mesh Gas Chrom Q; and (c) 1.0 m x 2 mm ID, 10% DC-200 on 80 to 100 mesh Supelcoport. A 50 ml/min carrier gas (nitrogen) flow was used for all three columns. The injection port and detector were held at 250° and oven temperatures were appropriately adjusted for the compound being analyzed.

Carbon-13 enrichments of labeled and natural abundance mirex and Kepone[®] were determined by mass spectrometry from the isotopic abundance patterns of their molecular ions. Spectra were obtained with a Varian/MAT 311-A interfaced

with a Varian 620/i MS data system. Samples were introduced into the ion source via a direct insertion probe. A fairly constant sample flow rate into the source was maintained without heating the probe. The intensities of seven ions of the molecular clusters were measured for 5 to 8 min for each compound by selected ion monitoring (SIM). The C^{13} contents of each compound were calculated from the normalized areas under the SIM curves. A series of four linear equations describing the contribution of molecules with from zero to three C^{13} 's was constructed based on the method of Kiser [12] and was solved using the ion intensity data from three sets of four ions in the molecular cluster. The contributions were averaged and average deviations determined. The C^{13} enrichments were then determined by comparing the fraction of molecules with one or more C^{13} 's to the C_0^{13} fraction.

Hexachlorocyclopentadiene-U- C^{14} (1)

This compound was prepared from $BaC^{14}O_3$ according to the procedures previously described in the literature [3]. Analysis by GC, columns (a) and (b), and radiochromatography indicated the material to be $\geq 98\%$ chemically and radiochemically pure. The IR was identical to an authentic sample of unlabeled material. The specific activity was 3.2 mCi/mmol.

1,1a,2,2,3,3a,4,5,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[cd]-pentalene-U- C^{14} , (Mirex-U- C^{14}) (2)

A mixture of 1.36 g (5.0 mmol) of "hex"-U- C^{14} (1.5 mCi/mmol) and 1.4 g (12.0 mmol) of anhydrous aluminum chloride was stirred for 1 hr at 90° (nitrogen blanket). To the resulting dark brown mixture was added 2 ml of CCl_4 and the mixture stirred an additional 3 hr at 75° . After cooling, the mixture was poured over ice (10 g) and 20 ml of CCl_4 was added. The resulting organic layer was separated, washed with 2 x 10 ml of water, dried ($MgSO_4$), filtered and the CCl_4 evaporated (in vacuo), yielding a dark viscous oil. Crystallization twice from

benzene afforded 756.5 mg (1.38 mmol, 56%) of mirex-U-C¹⁴ at a specific activity of 2.97 mCi/mmol (4.1 mCi total); TLC, R_f 0.60 silica gel/heptane:acetone (49:1); and R_f 0.65 silica gel/hexane:acetone (4:7). Radiochromatography and gas chromatography, columns (a) and (b), indicated a chemical and radiochemical purity of ≥ 98%.

1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one-U-C¹⁴, (Kepone[Ⓜ]-U-C¹⁴) (3)

To a 10-ml flask equipped with a Teflon-coated rubber septum and glass-coated magnetic stirring bar was added 2.97 g (10.9 mmol) of "hex"-U-C¹⁴ (1.5 mCi/mmol). The sulfur trioxide, 1.31 g (16.3 mmol) was then added slowly via syringe while cooling the reaction vessel in an ice bath. An immediate reaction occurred as evidenced by the formation of a deep purple color. The resulting mixture was allowed to warm to room temperature and was stirred for an additional 12 hr. The contents were then poured slowly into 80 ml of 6% NaOH solution and the resulting suspension stirred for 1.5 hr. Upon neutralization of the mixture with concentrated H₂SO₄, a white precipitate formed which was collected by filtration. The Kepone[Ⓜ]-U-C¹⁴ was rendered anhydrous by azeotropic distillation of water using toluene and subsequent recrystallization from xylene. Sublimation of the resulting white solid at 150 to 160° ca. 1 mm, afforded a total of 1.60 g (3.27 mmol, 60%) of Kepone[Ⓜ]-U-C¹⁴ at a specific activity of 3.2 mCi/mmol; gas chromatographic analysis, using column (c), indicated a purity level of ≥ 98%.

1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene-4,5,6,7,8-U-C¹⁴, (Heptachlor-4,5,6,7,8-U-C¹⁴) (4)

A mixture of 546 mg (2.0 mmol, 3.2 mCi/mmol) of "hex"-U-C¹⁴ and 200 mg (3.0 mmol) of cyclopentadiene was stirred at room temperature for 15 min (nitrogen blanket). The reaction vessel was slowly heated to a temperature of 70° and maintained at this temperature for 0.5 hr. The temperature was then raised as

rapidly as possible to 160° (5 min) and the heat removed immediately. After cooling to room temperature, the excess cyclopentadiene was evaporated (*in vacuo*) and the resulting chlordene-C¹⁴ crystallized on standing, TLC R_f 0.40 silica gel/heptane. The chlordene-C¹⁴ was transferred to a 10-ml flask and 1 ml of acetic acid and 10 drops of water were added. Selenium dioxide, 127 mg (1.14 mmol) was then added and hydrogen chloride gas passed through the solution for 6 hr. The reaction mixture was subsequently treated with 10 ml of water and extracted with 5 x 20 ml of ether. The ether solution was backwashed with 3 x 10 ml of water and the organic layer collected, dried (Na₂SO₄) and evaporated (*in vacuo*), yielding a light yellow oil. The oil was dissolved in 20 ml of hot methanol and the resulting solution treated with 10 mg of charcoal, filtered, and evaporated to a volume of ~ 5 ml, at which time the product began to crystallize. After cooling the solution in an ice bath for 15 min, the resulting crystals were collected and dried (*in vacuo*), yielding 708 mg (63%) of heptachlor-C¹⁴ at a specific activity of 3.2 mCi/mmol. An additional run was made and a 70% yield was obtained. TLC, R_f 0.30 silica gel/heptane. Radiochromatography indicated a radiochemical purity of ≥ 98%, and no impurities could be detected by gas chromatography using columns (a) and (b).

1,4,5,6,7,8,8-Heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan-4,5,6,7,8-C¹⁴₅, (Heptachlor epoxide-4,5,6,7,8-C¹⁴₅) (5)

To 556 mg of heptachlor-C¹⁴ (1.5 mmol, 1.8 mCi/mmol) was added 28 ml of oxidizing agent [13] and 1.5 ml of concentrated sulfuric acid. The resulting mixture was heated at ~ 100° for 7 min with rapid stirring. After cooling, the solution was diluted with ~ 200 ml of water and extracted with 5 x 50 ml of Skelly F. The combined extracts were backwashed with 2 x 20 ml of water and the organic layer collected, dried (Na₂SO₄) and evaporated (*in vacuo*). The resulting viscous oil was eluted through a 4 mm x 70 mm column of silica gel using 1% acetone in

heptane as the eluant. Evaporation of the solvents and recrystallization of the resulting residue from 95% ethanol afforded 220 mg (0.56 mmol, 37%) of heptachlor epoxide-C₅¹⁴ as a white crystalline solid, specific activity 1.8 mCi/mmol; TLC, R_f 0.24 silica gel/heptane:acetone (99:1). Radiochromatography and gas chromatography established the chemical and radiochemical purity at ≥ 98%.

Ethyl 5-(1,1a,3,3a,4,5,5a,5b,6-decachlorooctahydro-2-hydroxy-1,3,4-metheno-1H-cyclobuta[cd]pentalen-2-yl-1,1a,2,3,3a,4,5,5a,6-C₁₀¹⁴)-levulinate, (Kelevan-C¹⁴)

(6)

To 844 mg (1.72 mmol, 3.2 mCi/mmol) of anhydrous Kepone[®]-U-C¹⁴ in 15 ml of xylenes was added 450 μl of ethyl levulinate. A nitrogen atmosphere was established and the mixture slowly heated to reflux and maintained at this temperature for 24 hr. The resulting mixture was cooled and the xylenes evaporated (in vacuo). The residue was dissolved in hexanes (~ 10 ml) and ethanol was added until crystallization occurred. Recrystallization from hexanes:ethanol (99:1) afforded 622 mg (57%) of kelevan-C¹⁴ at a specific activity of 3.2 mCi/mmol. TLC, R_f 0.30, silica gel/hexanes:acetone (4:1). Radiochromatography indicated a radiochemical purity level of ≥ 98%. Gas chromatographic analysis, columns (a) and (b), indicated no chemical impurities.

Hexachlorocyclopentadiene-1,2,3-C_{1/3}¹³ (1-C¹³)

Cyclopentadiene-C¹³ was prepared according to the literature procedure [8] and converted to "hex"-1,2,3-C_{1/3}¹³ using the method of Kleinman [9]. The yields obtained were comparable to those reported in the literature, except for the chlorination step, which gave consistent yields in pilot reactions but a lower yield when cyclopentadiene-C¹³ was used [10]. Analysis by GC, columns (a) and (b), indicated a purity level of ≥ 98%.

1,1a,2,2,3,3a,4,5,5a,5b,6-Dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta[cd]-pentalene-C₂¹³, (Mirex-C₂¹³) (2-C¹³)

To 4.12 g of anhydrous aluminum chloride, under N₂, was added 4.0 g (14.7 mmol) of "hex"-1,2,3-C_{1/3}¹³ and the pasty mixture warmed to 90° for 1 hr. Carbon tetrachloride, 6 ml, was then added and the bath temperature reduced to 80°. After three additional hours the mixture was washed onto 30 g of ice using 60 ml of CCl₄. The dark organic layer was separated, washed three times with water, dried (MgSO₄) and evaporated (*in vacuo*). The dark oily product was taken up in hexane and purified on a column of 50 g SiO₂ using hexane as the eluant. The eluate was reduced in volume to ca. 15 ml, whereupon mirex-C₂¹³ separated as white crystals. The solids were filtered, dried, and recrystallized from hexane to give 2.48 g (62% yield) of mirex-C₂¹³. Analysis by gas chromatography, column (c), indicated a purity level of ≥ 98%.

1,1a,3,3a,4,5,5,5a,5b,6-Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene-2-one-C₂¹³, (Kepone[⊖]-C₂¹³) (3-C¹³)

A 4.5 g (16.3 mmol) portion of "hex"-1,2,3-C_{1/3}¹³, in a 6-ml vial equipped with Teflon septum, stirring bar, and cooled in an ice bath, was treated with 1.55 ml (39 mmol) of sulfur trioxide. The resulting purple mixture was allowed to warm slowly to room temperature and stirred for 14 hr. The reaction mixture was poured slowly into 140 ml of 6% aqueous NaOH and stirred 1.5 hr. The mixture was neutralized with concentrated H₂SO₄ and extracted with several portions of ether. The combined ether solutions were washed, dried (Na₂SO₄) and evaporated (*in vacuo*) to give a yellow-white solid which was triturated with three small portions of hexane. The resulting white solid was collected by filtration, dried (*in vacuo*), and sublimed (150 to 160°, ca. 1 mm) to give 2.30 g of Kepone[⊖]-C₂¹³ as white crystals (55% yield). Analysis by gas chromatography, column (c), indicated a purity level of ≥ 99%.

ACKNOWLEDGMENT

We gratefully acknowledge the support of this work by the Environmental Protection Agency, Contract No. 68-02-2236.

REFERENCES

1. "Registry of Toxic Effects of Chemical Substances," U.S. Department of Health, Education and Welfare, Rockville, Maryland (1975).
2. International Agency for Research on Cancer, Monographs on the Evaluation of Carcinogenic Risk of Compounds to Man (Lyon), 5, 209 (1974).
3. McKinney R.M. and Pearce G.W. - J. Agri. Food Chem., 8: 457 (1960).
4. Due to the presence of only one C¹³-enriched position in "hex," the compound's dimerization to mirex or Kepone[®] would provide material with two enriched positions, one in each half of the polycyclic molecule.
5. Newcomer J.S. and McBee E.T. - J. Amer. Chem. Soc., 71: 952 (1949).
6. Gilbert E.E. and Giolito S.L. - U.S. Patents 2,616,825 and 2,616,928 (1952); Chem. Abstr., 47: 2424e (1953).
7. (a) Kleinman J. and Goldman A. - U.S. Patent 2,672,486 (1953); Chem. Abstr., 48: 6642a (1954). (b) Singh J. - Bull. Environ. Contam. Toxicol., 4: 77 (1969).
8. Gilbert E.E., Lombardo P., Rumanowski E.J. and Walker G.L. - J. Agri. Food Chem., 14: 111 (1966).
9. Larson S.D., Vergamini P.J. and Whaley T.W. - J. Label. Compounds, 11: 325 (1975).
10. Kleinman M. - U.S. Patent 2,658,085 (1953); Chem. Abstr., 48: 12798 (1954).
11. Significant amounts of incompletely chlorinated by-products inevitably formed in this reaction and were separated by fractional distillation. The yield of $\geq 98\%$ pure "hex"-U-C¹³ obtained was 20%; however, in several pilot runs of a similar size the yield was consistently 40 to 45%.
12. (a) Dilling W.L. and Dilling M.L. - Tetrahedron, 23: 1225 (1967); (b) Alley E.G., Layton B.L. and Minyard J.P., Jr. - J. Agri. Food Chem., 22: 442 (1974).
13. (a) Kiser R.W. - Introduction to Mass Spectrometry and Its Applications, Prentice Hall, Inc., Englewoods Cliff, New Jersey, 1965, pp. 216-233; (b) Complete details concerning these determinations have been presented elsewhere: American Society for Mass Spectrometry, 26th Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, Missouri, May 29, 1978.
14. The oxidizing agent was prepared by dissolving 5 g of CrO₃ in a mixture of 5 ml of water and 50 ml of acetic acid.