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Received for review January 11, 1982. Accepted June 14, 1982. Paper No. 12,273, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

End of Symposium

ARTICLES

Fate of Kepone and Mirex in the Aquatic Environment

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The degradation of Kepone and mirex were examined in fish and hydrosoils. Fathead minnows were continuously exposed to three concentrations of [^{14}C]Kepone and [^{14}C]mirex in flow-through dilutor systems and then placed in fresh water for elimination phases. After 56 days [^{14}C]Kepone residues were concentrated 16 600 times by fathead minnows. However, only 1–5% or 0.1–0.23 μg/g of these residues was [^{14}C]Kepone. Several observations suggested that some [^{14}C]Kepone residues present in fathead minnows were chemically bound to biogenic compounds. Similar exposures of fathead minnows to [^{14}C]mirex resulted in bioconcentration factors as high as 51 400 times. The half-life of [^{14}C]mirex was greater than 28 days in fathead minnows, and no degradation products were detected in whole body samples. No evidence of [^{14}C]Kepone or [^{14}C]mirex degradation was observed in anaerobic and aerobic hydrosoil exposures.

Kepone ($C_{10}Cl_{10}O$) and mirex ($C_{10}Cl_{12}$) are closely related insecticides differing only by the presence of an oxygen atom substituted for two chlorine atoms. Although Kepone is used in about 55 commercial products, Harless et al. (1978) pointed out that most of these products are exported to other countries for use on bananas and potatoes. However, the compound has had limited use in the United States as an ant and roach bait. Production of Kepone by Life Sciences Products, Hopewell, VA, was discontinued in 1975 because of clinical evidence of Kepone intoxication in workers. Since then, interest in Kepone has continued because extensive residues were detected in the James River below Richmond, VA, extending to the mouth of the Chesapeake Bay. Stafford et al. (1978) found Kepone residues of 0.1–20 μg/g in fish and a corresponding bioconcentration factor of 20 000 times. Skalsky et al. (1979) reported that Kepone was bound to high-density lipoproteins in human plasma and suggested that lipoprotein binding may play a role in the toxicity of Kepone.

Large quantities of mirex have been used in the last decade for the control of fire ant infestation in nine southeastern states. Residues of mirex are widespread and were detected by Hawthorne et al. (1974) in fish and other aquatic organisms. A mirex committee (Van Middelem, 1972) reported that the amount of mirex sold from 1960 to 1970 as a flame retardant was over 4 times that sold for pesticide usage. Detection of mirex in fish from Lake Ontario (Kaiser, 1974) suggested that the compound had become a major environment contaminant. Jones and Hodges (1976) and Mehendale et al. (1972) reported that soil microorganisms and rats failed to degrade mirex in laboratory experiments. However, the discovery of Kepone as a degradation product of mirex in the environment (Carlson et al., 1976) has prompted considerable concern

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about the hazards associated with the widespread use of mirex.

We examined the fate and residue dynamics of Kepone and mirex in fathead minnows (*Pimephales promelas*). Lentic and lotic hydrosoils were exposed to Kepone and mirex and analyzed for degradation products. Several reaction products of Kepone and organic solvents were identified, and their chromatographic behavior during the cleanup of Kepone was characterized. Analytical methodology was developed for Kepone and mirex residues in fish, hydrosoils, and water.

MATERIALS AND METHODS

Reagents. All solvents used were nanograde (distilled in glass) from Burdick and Jackson, Muskegon, MI, or Mallinckrodt, St. Louis, MO. Sephadex LH-20 (25–100 μm) was obtained from Pharmacia Fine Chemicals, Piscataway, NJ, and Bio-Beads S-X3 (200–400 mesh) from Bio-Rad Laboratories, Richmond, CA. Tenax-GC column packing material was purchased from Applied Science Laboratories, Inc., State College, PA. Amoco carbon grade PX-21 was a gift from Amoco Research Corp., Chicago, IL. Polyurethane plugs (Gaymar Identi Plugs No. L800) were from Arthur H. Thomas Co., Philadelphia, PA. Silica gel for column chromatography (CC-7 Silicar, 70–230 mesh) was purchased from Mallinckrodt, St. Louis, MO, and stored at 130 °C during use. Silica gel thin-layer chromatography (TLC) plates (silica gel F254, 250 μm) were obtained from Brinkman Instruments, Westbury, NY.

Technical mirex (908 g) used for flow-through exposures was purchased from Mississippi Authority for the Control of Fire Ants, Jackson, MS. Technical Kepone (200 g) was a gift of Allied Chemical, Morristown, NJ. Duplicate electron capture gas chromatographic (EC-GC) determinations indicated a purity of 99.1% for technical mirex and 94.1% for Kepone.

Radiolabeled Chemicals. Both [^{14}C]Kepone and [^{14}C]mirex (cage structures of Kepone and mirex molecules each contain eight ^{14}C-labeled atoms) were obtained from Pathfinder Laboratories, Inc., St. Louis, MO. The specific activity of [^{14}C]Kepone was 15.5 mCi/mM (based on a molecular weight of 490.68) and of [^{14}C]mirex was 7.25 mCi/mM. Stock solutions of [^{14}C]Kepone and [^{14}C]mirex were analyzed by EC-GC and compared with liquid scintillation determinations of the same [^{14}C]Kepone and [^{14}C]mirex stocks for confirmation of specific activities.

All gravimetric and EC-GC determinations of [^{14}C]-Kepone were corrected for the presence of four waters of hydration resulting from environmental exposure. The purity of [^{14}C]Kepone was confirmed by TLC, electron impact gas chromatography–mass spectrometry (EI-GC–MS), and EC-GC. These analyses suggested that the radiolabeled Kepone standard was >99% pure. Chlorinated impurities representing >0.5% of the radioactivity present in the ^{14}C-labeled standard or >0.5% of the EC-GC response to the ^{14}C-labeled standard were discernible above background. A small amount of the possible 9-Cl homologue of [^{14}C]Kepone was detected, which represented <1% of the EC-GC response to [^{14}C]Kepone.

The purity of [^{14}C]mirex was examined by EC-GC and TLC. We detected no radiolabeled impurities, suggesting that >99% of the radioactivity was associated with [^{14}C]mirex.

Apparatus. All GC columns used in the study were glass (2 mm i.d. × 1.8 m) and packed with one of the following liquid phases on 80–100-mesh Chromosorb W HP (w/w): 3% OV-101, 3% OV-7, 3% SE-30, or 3% OV-17. Special care was required to deactivate glass surfaces and other adsorption sites for adequate GC response to Kepone. Glass surfaces of GC columns were deactivated with a 10% solution of dichlorodimethylsilane in toluene.

A Varian Model 3700 GC equipped with small-volume ^{63}Ni EC detectors was used for the analysis of most Kepone and mirex sample extracts. Selected samples were analyzed on two Micro Tek 220 GC's equipped with Tracor ^{63}Ni EC detectors in both linearized (free-standing Tracor linearizers) and nonlinearized modes. Column temperatures generally ranged between 170 and 220 °C. Carrier gases for linearized and nonlinearized modes were argon–methane (90:10 v/v) and nitrogen, respectively. The column flow rates were set at 28–32 mL/min. All GC's were equipped with on-column injectors, which were maintained at 10–20 °C above the column temperature. EC detectors were set at 350 °C. The Varian EC-GC was fitted with a volatiles trap for ^{14}C analyses. All components contacting volatiles were glass or glass-lined stainless steel to the EC cell and the GC effluent was directed through Tenax columns (0.4 cm i.d. × 10 cm) via a silicone septum to determine which peaks were radioactive. The trapping efficiency of injected [^{14}C]Kepone standards was 40–50% at GC column temperatures of 180–230 °C, suggesting losses due to adsorption, condensation, or aerosol formation.

A Finnigan Model 4023 automated GC–mass spectrometer (MS) system, equipped with a quadrapole mass filter, was used to identify Kepone derivatives. The GC–MS was interfaced with a Nova 3, INCOS 3.1 data system. Source and GC–MS interface temperatures were set at 250 °C. The filament was maintained at 70 eV during both chemical ionization (CI) and EI modes. Methane was used as a reagent gas for CI-MS analysis. Several samples were introduced into the source via a liquid chromatograph belt-drive interface.

Two Autoprep automated gel permeation chromatographs (GPC) from Analytical Biochemistry, Columbia, MO, were used for sample cleanup and separation of Kepone derivatives. Kepone and mirex GPC separations were achieved by using 2.5 cm i.d. columns packed with 70 g of Sephadex LH-20 (ethyl acetate/toluene/methanol, 80/10/10 v/v/v) or 50 g of Bio-Beads S-X3 (methylene chloride/cyclohexane, 15/85 v/v), respectively. Both columns were set at a flow rate of 4.8–5.0 mL/min.

A Berthold LB 276 TLC scanner (7547 Wildbad, West Germany), supplied with an argon/methane (90/10 v/v) counting gas, provided chromatograms of radioactive spots or bands. The integrator was set at 2 K and the time constant was maintained at 0.5 s unless stated otherwise. Radioactive residues ($^{14}CO_2$) in several samples (hydrosoils and fish tissues) were liberated with a Biological Material Oxidizer from R. J. Harvey Instrument Corp., Hillsdale, NJ. The recovery of $^{14}CO_2$ evolved from combustion of samples was 80–85%. All radiometric determinations during these studies were done with a Beckman liquid scintillation system (LS-3133T) from Beckman Instruments Inc., Fullerton, CA. A quench curve was established to correct for counting efficiency. The scintillation fluid used contained Beckman fluoralloy dry mixture dissolved in toluene and Triton X-100.

Partial Chronic Exposures. Fathead minnows were exposed to [^{14}C]Kepone and [^{14}C]mirex in flow-through dilutor systems as described by Hawthorne et al. (1974). Fish were exposed to 4, 41, and 380 ng/L of [^{14}C]Kepone for 56 days and then placed in untreated flowing water for 28 days. Samples were taken at 28 and 56 days of exposure and 7, 14, and 28 days after cessation of the exposure. The sampling regime of the flow-through exposure of fathead

minnows to mirex was similar to that for Kepone, and the water concentrations were 0.37, 3.8, and 33 μg/L.

Hydrosoil Exposures. Aerobic and anaerobic hydrosoils from a small man-made reservoir (Little Dixie, Columbia, MO) and from a tributary of the James River in Virginia (Bailey Creek) contaminated with Kepone were exposed to a single application of [^{14}C]Kepone and [^{14}C]mirex. Details of hydrosoil collection, preparation, and exposure to Kepone and mirex were described by Skaar et al. (1981). Sample extracts were purified by using carbon/foam column chromatography, as described by Huckins et al. (1978), prior to TLC and EC-GC analysis. Environmental residues of Kepone present in the anaerobic and aerobic Bailey Creek hydrosoil samples were determined by EC-GC and averaged 0.38 and 0.54 μg/g, respectively (based on dry weight).

Tissue Analysis. Fathead minnow samples from laboratory exposures were stored at −25 °C to minimize reactions occurring during sample storage. Radioactive residues in samples were monitored throughout extraction and cleanup procedures by LS counting. For detection of the presence of nonextractable ^{14}C-labeled residues, subsamples or samples of <0.5 g were analyzed by a biological material oxidizer. A few samples were counted directly by grinding with a tissue homogenizer in the presence of Triton X-100 and fluoralloy cocktail.

Fish samples (whole body ≤5 g) from mirex and Kepone exposures were ground with 4 times their weight of anhydrous sodium sulfate (activated at 550 °C for 2 h) and placed in columns designed by Hesselberg and Johnson (1972). These columns were 1 cm i.d. glass with 150-mL solvent reservoirs and fitted Teflon stopcocks. Mirex residues were eluted with 150 mL of 5% diethyl ether in petroleum ether at <3 mL/min, followed by 50 mL of acetone–ethyl acetate (50:50 v/v) to ensure complete recovery of possible degradation products. Column extracts were slowly concentrated to an ≃2-mL volume in porcelain casseroles on an explosion-proof hot plate set at 55 °C. Residues in the 5% diethyl ether in petroleum ether extracts were diluted to a 10-mL volume with 15% methylene chloride in cyclohexane and 5 mL was chromatographed on GPC (S-X3, 15% methylene chloride in cyclohexane). The 0–105-mL fraction containing lipids was discarded after counting 1-mL aliquots, and mirex residues were collected in the 105–200-mL fraction. Mirex residues in GPC fractions were concentrated under filtered N_2 streams at 55 °C to volumes appropriate for GC analysis.

Mirex residues were determined by EC-GC and concentrations were calculated by comparing peak areas (width at half-height times height) to a standard curve. Recoveries of mirex residues from fish tissue spiked with 0.6–1 μg/g [^{14}C]mirex ranged from 60 to 98%. The average recoveries based on GC and radiometric determinations were 84.0 and 85.8%, respectively. Solvent blanks, control fish, and triplicate spikes of [^{14}C]mirex or [^{14}C]Kepone in untreated fish were analyzed along with each sample set (10–20 samples). All residue values reported in these studies were corrected for recovery.

Kepone residues were recovered from tissue/sodium sulfate mixtures with 150 mL of ethyl acetate/acetone (50/50 v/v). The columns were subsequently washed with 50 mL of methanol to monitor possible losses due to adsorption. The ethyl acetate–acetone column eluates were purified by using Sephadex LH-20 GPC (ethyl acetate/toluene/methanol, 80/10/10 v/v/v). Kepone residues were eluted from the GPC in the 85–230-mL fraction and concentrated as described for mirex to a 1-mL volume. Further purification of Kepone residues was accomplished by using 1 cm i.d. glass columns packed with 4 g of silica gel. Interfering PCBs were eluted in the first 50-mL wash of the column with 0.5% benzene in hexane (v/v). Kepone residues were recovered with 50 mL of 5% methanol in ethyl acetate. Samples were then concentrated to appropriate volumes for EC-GC analysis (≃0.1 μg/mL).

Measurement and quality control procedures for Kepone residues were similar to those described for the analysis of mirex in fish. However, tissue samples spiked with Kepone were carefully examined throughout the cleanup procedures for Kepone–solvent artifacts. We did not detect the aldol condensation product of Kepone and acetone in any spiked samples. Radiometric recoveries of Kepone residues from control fish tissues spiked with 0.1–10 μg/g [^{14}C]Kepone ranged from 53 to 98%. The average recovery values of these spiked samples based on radiometric determinations was was 84%, whereas GC recovery values of the samples averaged only 53% (see Conclusions for a possible explanation of this discrepancy).

Selected fractions from GPC separations of Kepone and mirex samples were examined on TLC. The solvent system used for TLC of [^{14}C]Kepone residues was 33% acetone in benzene (v/v) and the solvent system used for TLC of [^{14}C]mirex residues was 5% diethyl ether in hexane (v/v). Several [^{14}C]Kepone samples were developed twice to increases R_f values of radioactive spots or bands.

Hydrosoil Analysis. [^{14}C]Kepone and [^{14}C]mirex hydrosoils (30 g of hydrosoil: 10 g of soil/20 g of water) were placed in 50-mL centrifuge tubes, and the pH was adjusted to 1 by adding small amounts of concentrated HCL. Ethyl acetate (15 mL) and 1 g of shiny copper powder were added to each tube, and the samples were thoroughly agitated by using a Vortex-genie mixer. The aqueous and organic phases were separated by centrifugation, and the organic layer was transferred to a porcelain casserole. The extraction step was repeated twice, and the ethyl acetate fractions from each sample were combined and concentrated to a volume of 1–2 mL. Columns of carbon/foam (1 cm i.d. × 10 cm) were prepared as described by Huckins et al. (1978) and used for the cleanup of hydrosoil extracts. To prevent column overloading and channeling, we purified only half of each sample extract and maintained the flow rate of 2 mL/min. [^{14}C]Mirex was collected in the first 60-mL petroleum ether column wash, and the column was rinsed with 50 mL of ethyl acetate. Extracts from [^{14}C]Kepone hydrosoil samples were also chromatographed on carbon/foam columns. The first 100 mL of petroleum ether was discarded after counting, and 100 mL of 5% methanol in ethyl acetate was used to recover [^{14}C]Kepone. Both [^{14}C]mirex and [^{14}C]Kepone columns were washed with 49 mL of methanol to ensure removal of all radioactive residues. All radioactive fractions from the column cleanup of [^{14}C]mirex and [^{14}C]Kepone hydrosoil samples were concentrated in calibrated centrifuge tubes for EC-GC and TLC analysis, as described for the characterization of ^{14}C-labeled residues in fish.

Water Analysis. Methods for the analysis of Kepone and mirex residues in water were similar. Water samples of 1 or 2 L (≤50 ng/L) were collected from each test aquarium and placed in separatory funnels equipped with Teflon stopcocks. Each water sample was extracted 3 times with 50-mL aliquots of methylene chloride. The organic phases were drained into 250-mL Erlenmeyer flasks (containing ≃10 g of anhydrous Na_2SO_4) and shaken for several minutes. We then transferred sample extracts to a 250-mL porcelain casserole, using 5-mL washes of ethyl acetate (Kepone) or petroleum ether (mirex). Methylene chloride was removed from sample casseroles

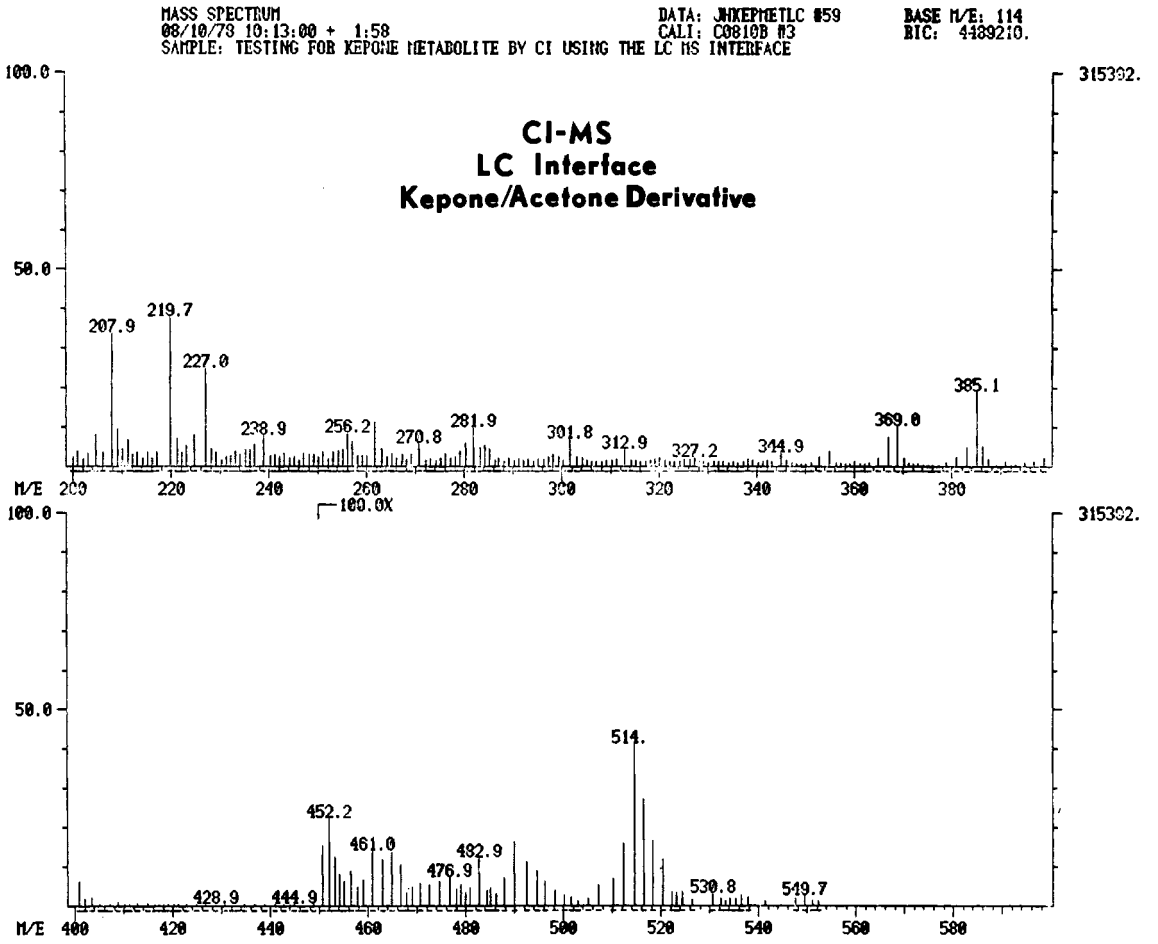

Figure 1. CI (methane)-MS of the aldol condensation product of acetone and Kepone. The sample was introduced directly into the source via a liquid chromatograph belt interface. See Materials and Methods for instrumental conditions.

by using an explosion-proof hot plate set at 45 °C. After the removal of methylene chloride, sample volumes were adjusted with ethyl acetate (Kepone) or petroleum ether (mirex) to provide acceptable EC-GC response. By use of these methods the recovery of Kepone residues in water samples averaged 90 ± 6.1% ($\bar{x}$ ± SD). The recovery of mirex residues in water was 90 ± 5.7% ($\bar{x}$ ± SD). The minimum detection limit for Kepone and mirex in water samples was 0.02 μg/L.

RESULTS AND DISCUSSION

Kepone/Solvent Interactions. Analysis of Kepone in fish, water, and hydrosoil samples was complicated by the reactive nature of the parent Kepone molecule. Kepone exists in the hydrated form (*gem*-diol) with one or four water molecules and, according to Harless et al. (1978), has not been isolated in the free carbonyl form. Several organic solvents commonly used in pesticide residue analysis react with Kepone. Methanol reacts with Kepone to form the hemiketal, and Gilbert et al. (1966) found that under reflux conditions for 18 h, acetone reacts with Kepone to form the aldol condensation product. We characterized the analytical behavior of several possible Kepone–solvent derivatives to prevent misinterpreting these compounds as biotransformation products.

We found that Kepone reacted with acetone after 5 weeks at room temperature (22 °C), forming the aldol condensation product. The reaction occurred rapidly once it began since the aldol product was not detected in 4-week samples of the Kepone solutions. CI-MS analysis revealed an empirical formula of $C_{13}H_4Cl_{10}O$ (Figure 1), which is consistent with the loss of one water molecule from the Kepone–acetone aldol condensation product. Weekly or more frequent monitoring of dilutor stocks (acetone solutions) with EC-GC was required to ensure the integrity of the Kepone used for flow-through exposures to fish. The Kepone–acetone condensation reaction occurred very slowly in refrigerated (5 °C) stocks, and after 8 months less than 1% of the radioactivity in these [^{14}C]Kepone stocks appeared to be associated with the aldol condensation product.

The formation of the dimethyl ketal derivative of Kepone and methanol ($C_{12}H_6Cl_{10}O_2$) during residue analysis is possible under acidic conditions. The acidic nature of Kepone hydrate permitted rapid methylation with diazomethane at 22 °C to form the dimethyl ketal. We examined the dimethyl ketal derivative on CI-MS (Figure 2) and EC-GC. The retention time of the ketal was 2.62 times greater than that of Kepone on GE-SE-30. The GPC elution profiles of the methyl hemiketal and dimethyl ketal differed significantly from that of Kepone hydrate. Figure 3 shows the LH-20 GPC elution profiles of these Kepone derivatives and [^{14}C]Kepone hydrate.

Moseman et al. (1977) reported that the addition of methanol to hexane solutions of Kepone increased the sensitivity of Kepone to EC-GC analysis. Methanol increases the solubility of Kepone in hexane. However, we observed a significant decrease (up to 25%) in the EC-GC response to ethyl acetate solutions of Kepone containing increasing amounts of methanol. No corresponding decrease in EC-GC response was observed for standards of mirex and *p,p'*-DDE in similar methanolic solutions, suggesting an interaction specific for Kepone. When equal amounts of methanol and isooctane were coinjected with ethyl acetate solutions of Kepone, no significant decrease in EC-GC response was observed. Ethyl acetate and

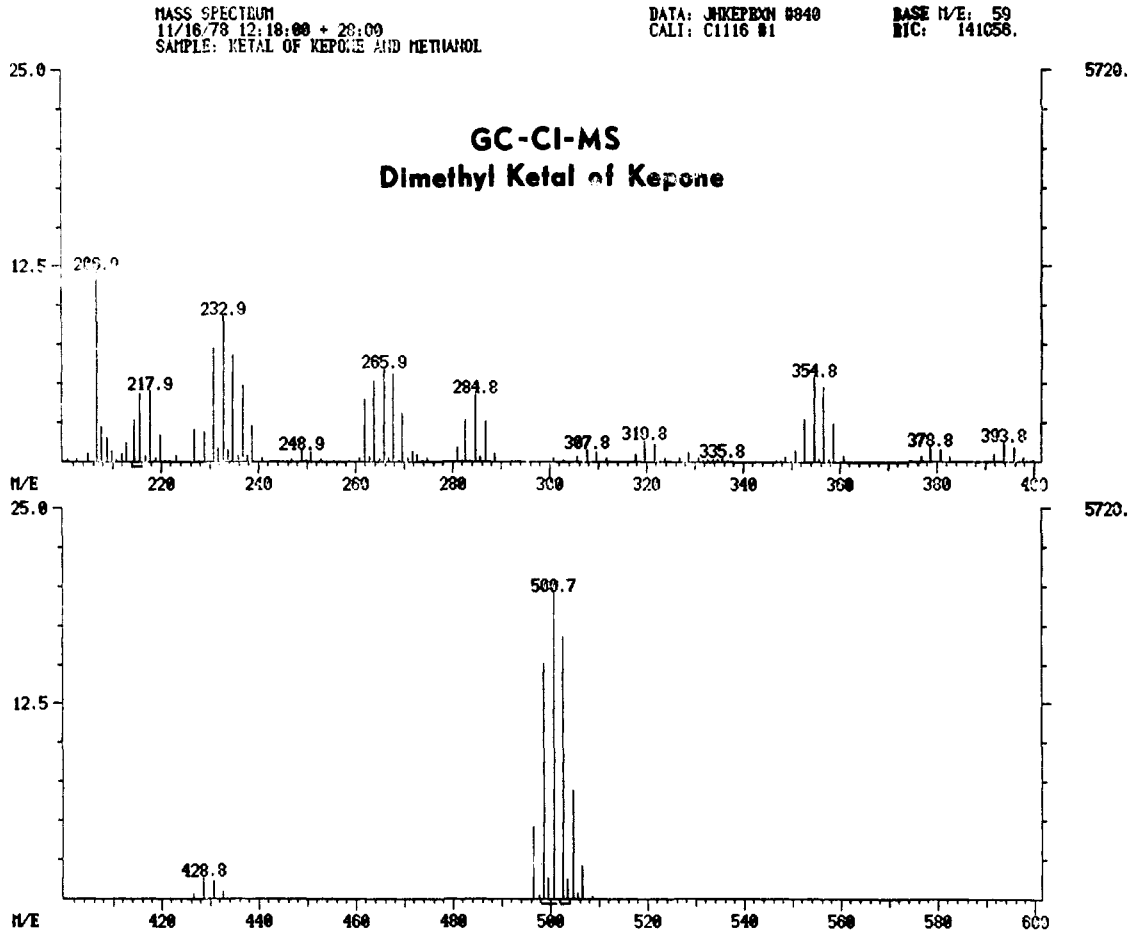

Figure 2. CI (methane)-MS of the dimethyl ketal derivative of Kepone. The GC column (6 ft × 2 mm i.d.) was 3% OV-101 on Chromosorb W HP (w/w). See Materials and Methods for instrumental conditions.

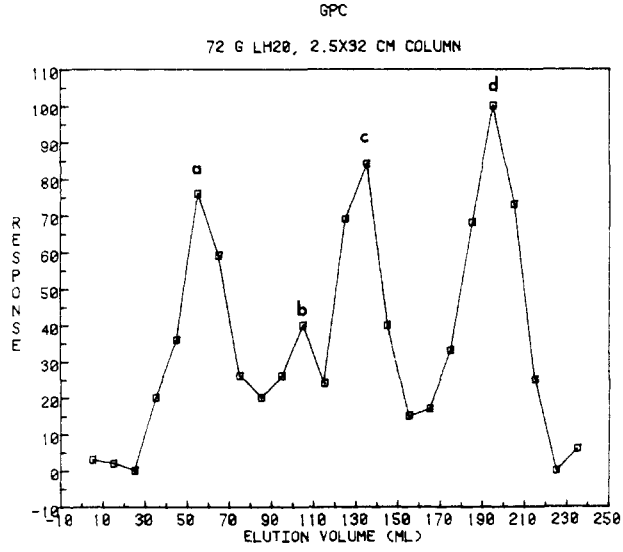

Figure 3. GPC of Kepone derivatives using a column of Sephadex LH-20. The solvent system was a mixture of toluene, methanol, and ethyl acetate (10/10/80 v/v/v), and the flow rate was set at 4.8 mL/min. Peak a, unknown Kepone derivative; peak b, dimethyl ketal derivative; peak c, hemiketal derivative, peak d, Kepone hydrate or *gem*-diol form (one to four H_2O molecules).

acetone solutions of Kepone appeared to optimize EC-GC responses to Kepone residues. Increasing the injector and column temperatures above 220 and 200 °C, respectively, did not appear to improve the reduced EC-GC response of Kepone in some solvents.

[^{14}C]Kepone Residues in Fish. Fathead minnows from the [^{14}C]Kepone flow-through dilutor exposure were analyzed for ^{14}C-labeled residues. Table I summarizes the whole body ^{14}C-labeled residue dynamics. ^{14}C-Labeled residues in fathead minnows increased throughout the 56-day partial chronic exposure. Whole body ^{14}C-labeled residues in fish exposed to 380 ng/L [^{14}C]Kepone for 56 days averaged 4.7 μg/g. However, the EC-GC analysis of three 56-day samples indicated that only 0.1–0.23 μg/g of the total ^{14}C-labeled residues in 56-day fathead minnows was [^{14}C]Kepone. Analysis of fathead minnow samples from a similar nonlabeled residue study, reported by Buckler et al. (1981), also revealed low concentrations of Kepone (0.38 μg/g) in 60-day samples. [^{14}C]Kepone concentrations in 28-day samples ranged from 0.2 to 0.64 μg/g, suggesting that the concentrations of [^{14}C]Kepone had reached a plateau before 56 days. The half-life of total ^{14}C-labeled residues accumulated by fathead minnows was greater than 7 days but less than 14 days whereas the half-life of free [^{14}C]Kepone, based on EC-GC analysis, was less than 7 days.

Composite fish samples (three to four fish) from a preliminary 9-day flow-through exposure (360 ng/L) to [^{14}C]Kepone and the 380 ng/L concentration of the partial chronic [^{14}C]Kepone exposure were examined for [^{14}C]-Kepone metabolites or derivatives. The GPC elution profiles of these radioactive extracts changed through time, suggesting alterations in residue compositions (Figures 4 and 5). Several major radioactive fractions (80–110) from the GPC cleanup of 28- and 56-day samples contained only small amounts or no recognizable residues of [^{14}C]Kepone (Figure 6). These unidentified ^{14}C-labeled residues represented as much as 52% of the total radioactivity in fathead minnows exposed to [^{14}C]Kepone for 56 days. They appeared to be relatively nonpolar and lipophilic in

Table I. Whole Body ^{14}C-Labeled Residues (Micrograms per Gram)[a] in Fathead Minnows Exposed to [^{14}C]Kepone in a Flow-Through Dilutor System

mean H_2O concn,[b] ng/L	days of exposure		days of elimination		
	28	56	7	14	28
4	0.04[c] ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
41	0.36 ± 0.09	0.68 ± 0.08	0.42 ± 0.05	0.28 ± 0.08	0.13 ± 0.02
380	2.8[d] ± 0.69	4.7[e] ± 0.31	3.5 ± 0.67	2.3 ± 0.38	1.1 ± 0.53

[a] Residue values were determined by direct counting of emulsified tissues and measuring $^{14}CO_2$ evolved from tissue combustion. [b] Water samples were analyzed weekly. [c] All values are mean of four to six individual samples (residues do not necessarily represent parent Kepone molecules). [d] Kepone represented 7–23% of this residue value. [e] Kepone represented 1–5% of this residue value.

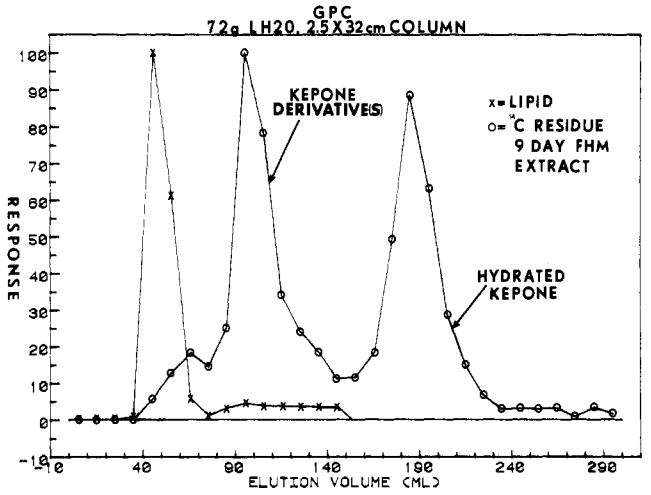

Figure 4. GPC of ^{14}C-labeled residues in fathead minnows exposed to [^{14}C]Kepone for 9 days. The column was Sephadex LH-20, and the solvent system was toluene, methanol, and ethyl acetate (10/10/80 v/v/v). The column flow rate was set at 4.8 mL/min.

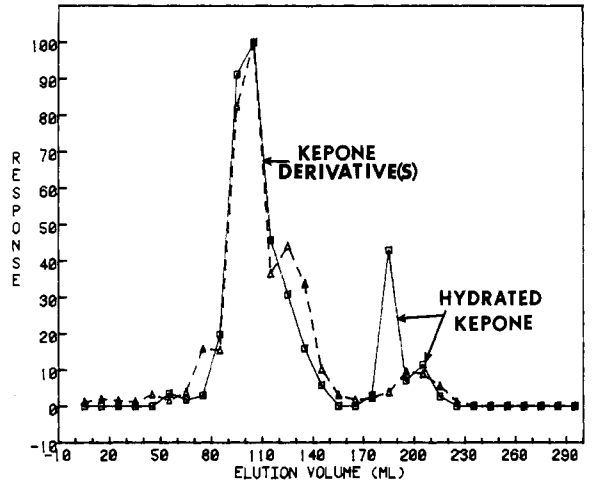

Figure 5. GPC of ^{14}C-labeled residues in fathead minnow samples exposed to [^{14}C]Kepone (380 ng/L) for 28 days. The column conditions were identical with those in Figure 4. The two lines plotted (□, △) represent separate samples.

nature based on their partition, adsorption, and size exclusion behavior during extraction and cleanup procedures. The presence of free [^{14}C]Kepone (*gem*-diol form) in these radioactive GPC fractions (80–110) appeared unlikely, because sample derivatization with an excess of diazomethane (40 °C, 1 h) failed to generate the dimethyl ketal derivative of Kepone. Acid hydrolysis and the Oleum cleanup method of Stafford et al. (1978) did not alter the analytical behavior or permit the identification by MS of the unknown ^{14}C-labeled residues in sample extracts. Although we were not successful in characterizing the

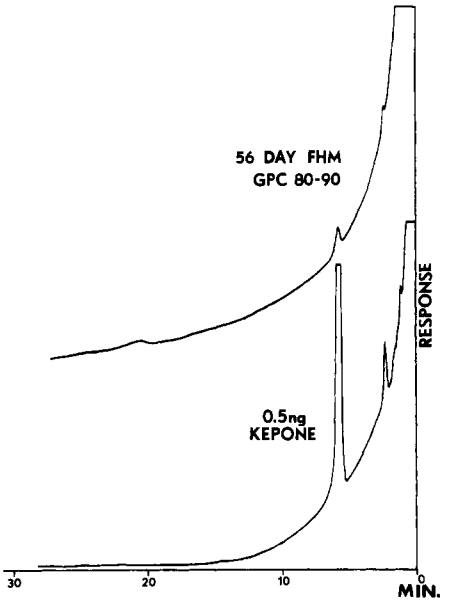

Figure 6. EC-GC analysis of a GPC fraction (80–90 mL) from the cleanup of fathead minnows exposed to [^{14}C]Kepone (380 ng/L) for 56 days. The top GC trace represents the injection of ^{14}C-labeled residues equivalent to the 0.5-ng [^{14}C]Kepone standard. The GC column was 3% OV-101 on Chromosorb W HP and the column temperature was set at 220 °C.

unknown ^{14}C-labeled residues, the data generated suggested that [^{14}C]Kepone may have undergone "in vivo" chemical reactions with fathead minnow lipids.

The recovery of [^{14}C]Kepone residues from spiked fathead minnow samples varied considerably and was also suggestive of "in vitro" Kepone–biogenic binding. The average recovery of spiked samples based on radiometric analysis was significantly higher (84%) than the average recovery of the same spiked samples analyzed by EC-GC (53%). Additional cleanup of these spiked samples did not increase the EC-GC detectable residues of [^{14}C]Kepone, and the aldol condensation product of Kepone and acetone was not detected in purified spikes.

A significant portion of the unknown ^{14}C-labeled residues may have been generated during sample cleanup, as indicated by lower EC-GC recoveries of spiked tissues than radiometric recoveries of the same tissues. However, in vivo reactions of [^{14}C]Kepone and biogenic compounds also appeared to occur in exposed fish. This observation was supported by a decrease of free Kepone in fish relative to accumulated radioactive residues (excluding the Kepone–acetone aldol condensation product) as exposure time increased.

Little literature exists on in vivo or in vitro xenobiotic bonding to lipids, lipoproteins, and proteins. Skalsky et al. (1979) reported that Kepone residues in human plasma were bound to high-density lipoproteins and were not separated from these blood constituents by gel filtration

Table II. Whole Body ^{14}C-Labeled Residues (Micrograms per Gram)[a] in Fathead Minnows Exposed to [^{14}C]Mirex in a Flow-Through Dilutor System

mean H_2O concn,[b] µg/L	days of exposure		days of elimination		
	28	56	7	14	28
0.04	11.9[c] ± 1.9	19.0 ± 2.5	17.5 ± 2.5	18.6 ± 3.1	12.3 ± 1.2
3.8	32.3 ± 15.4	47.6 ± 24.8	70.3 ± 12.8	63.6 ± 8.7	42.5 ± 8.8
33	45.3 ± 24.0	122 ± 33.2	139 ± 18.0	120 ± 23.4	88.6 ± 20.9

[a] Residue values were determined by direct counting of emulsified tissues and measuring $^{14}CO_2$ evolved from tissue combustion. [b] Water samples were analyzed weekly. [c] Mean of three to five samples.

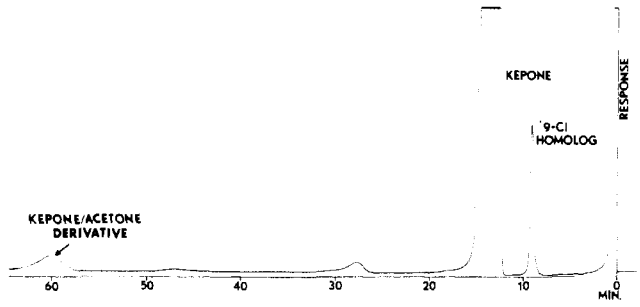

Figure 7. EC-GC analysis of a concentrated [^{14}C]Kepone stock. The aldol condensation product of [^{14}C]Kepone and acetone represented <1% of the 5 ng of [^{14}C]Kepone stock injected. The early eluting peak was tentatively identified as a 9-Cl dechlorination product (*) of [^{14}C]Kepone. The GC column was 3% OV-101 on Chromosorb W HP and the column temperature was set at 180 °C.

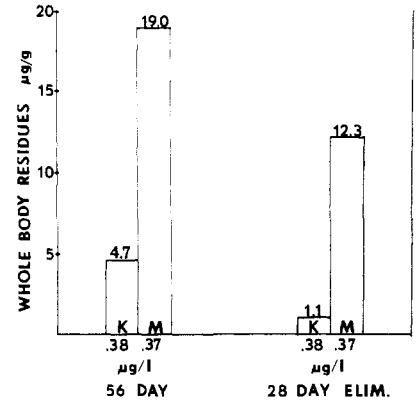

Figure 8. Comparison of the accumulation and elimination rates of similar concentrations of [^{14}C]Kepone (K) and [^{14}C]mirex (M) residues by fathead minnows. Kepone residues shown are only 1–5% molecular Kepone.

(Sephadex G-200) or electrophoresis on argose gels. However, treatment of the lipoprotein–Kepone complex with sulfuric acid resulted in the dissociation of Kepone from lipoproteins, permitting GC analysis. Irreversible covalent binding of phenolic metabolites of microsomal protein was observed by Hesse and Mezger (1979). Semiquinones and quinones are also known to react with nucleophilic sites of proteins (Marks and Hecker, 1969). Bose and Fujiwara (1979) indicated that pentachlorophenol was covalently bound to lipids in hepatopancreatic tissues of blue crabs. More recently, Hutson (1980) found that rats exposed to 3-phenoxybenzoic acid formed (3-phenoxybenzoyl)dipalmitin in the skin. Hutson also reported that hydroxycannabinols are conjugated with endogenous fatty acids.

Analysis of additional GPC fractions with an EC-GC fitted with a Tenax column for trapping volatiles, revealed the [^{14}C]Kepone/acetone aldol condensation product in 28- and 56-day fish (380 ng/L, uptake) extracts. As much as 47% of the total ^{14}C-labeled residues present in 56-day fish (380 ng/L, uptake) was the aldol condensation product. However, less than 1% of the radioactivity in [^{14}C]-Kepone dilutor stocks for partial chronic exposure was associated with the Kepone–acetone aldol condensation product (Figure 7).

Degradation of [^{14}C]Kepone in Hydrosoils. Selected weekly anaerobic and aerobic samples from Little Dixie Reservoir and the James River tributary were analyzed in duplicate. We found that most [^{14}C]Kepone residues in hydrosoil samples could be extracted into ethyl acetate under either basic or acidic conditions. This observation was surprising because Kepone hydrate (*gem*-diol) is slightly acidic, and Carver et al. (1978) found it to be readily soluble in aqueous bases. Recoveries of [^{14}C]-Kepone residues from 56-day hydrosoil samples ranged between 75 and 94% and compared favorably with similarly spiked control samples, which ranged between 84 and 103%. Carbon/foam column chromatography of these extracts permitted the removal of most interfering biological materials and procedural contaminants without major losses of radioactivity (<3%).

Most of the radioactivity in the 56-day samples appeared to be associated with [^{14}C]Kepone. The TLC R_f value of the [^{14}C]Kepone standard and [^{14}C]Kepone in hydrosoils was 0.50. We observed another very small band of radioactivity in several 56-day samples (<3% of the radioactivity) having a R_f value of 0.89 that appeared to be the Kepone–acetone aldol condensation product.

EC-GC analysis of the remaining 56-day anaerobic and aerobic James River samples failed to reveal peaks that were not present in corresponding controls. We did not detect Kepone dechlorination products like those reported by Borsetti and Roach (1978). However, close examination of the [^{14}C]Kepone stock (refrigerated at 5 °C in acetone) suggested the presence of small amounts (<1%) of an EC-GC peak that appeared to be the 9-chloro Kepone homologue (Figure 7). This peak was not observed in similar stock checks 6 months earlier.

[^{14}C]Mirex Residues in Fish. Whole body ^{14}C-labeled residues in fish exposed to [^{14}C]mirex were determined by direct counting of emulsified tissues and by counting $^{14}CO_2$ evolved from the combustion of tissues. Table II summarizes the results of whole body sample determinations. [^{14}C]Mirex was rapidly accumulated by fathead minnows and residue levels did not appear to plateau after 56 days. The half-life of mirex residues was greater than 28 days. Comparison of the residues in fathead minnows resulting from exposure to similar concentrations of [^{14}C]Kepone and [^{14}C]mirex showed a marked dissimilarity in the accumulation rate and half-life of the two compounds (Figure 8). Fish exposed to 0.37, 3.8, and 33 µg/L [^{14}C]mirex for 56 days concentrated ^{14}C-labeled residues 51 300, 12 400, and 3700 times, respectively.

Examination of [^{14}C]mirex residues in purified (GPC and silica gel) 56-day extracts with EC-GC, TLC, and column chromatography did not reveal the presence of mirex derivatives or metabolites. Mirex residues in a 56-day composite fish sample (3.8 µg-L, Table II) were mea-

sured with EC-GC and then compared with the concentration of [^{14}C]mirex, found by dividing the amount of radioactivity in the sample with the specific activity of [^{14}C]mirex (556 cpm/μg). On the basis of these determinations, the area under the mirex peaks represented 102% of the radioactivity in the injected sample.

Degradation of [^{14}C]Mirex in Hydrosoils. Before beginning the [^{14}C]mirex exposure, we developed a benzene extraction method for recovery of mirex spikes from hydrosils. Recovery of [^{14}C]mirex spikes from triplicate hydrosoil samples ranged between 82 and 97%. However, the application of this method to sterilized hydrosoils exposed to [^{14}C]mirex for 56 days resulted in poor recoveries (≤40%) of mirex. Recovery values were improved (96–104%) after adjusting the pH of these samples to 1.0 with concentrated HCl and partitioning with ethyl acetate.

Extracted ^{14}C-labeled residues were purified by carbon/foam chromatography and examined on TLC and EC-GC. No evidence of degradation products was observed in autoradiograms or TLC scans of 56-day [^{14}C]-mirex samples. The amount of [^{14}C]mirex present in purified extracts was computed on the basis of specific activity and compared to EC-GC response of the same samples. These results indicated that 95% of the radioactivity in anaerobic samples and 104% of the radioactivity in aerobic samples were mirex.

CONCLUSIONS

[^{14}C]Kepone represented only a small portion of the ^{14}C-labeled residues reported in Table I. Unknown ^{14}C-labeled residues represented 48–52% of the radioactive residues in fathead minnows exposed to 380 ng of [^{14}C]-Kepone/L for 56 days. The relatively nonpolar nature and high resistance to acid hydrolysis suggested that the unknown ^{14}C-labeled residues were not the products of well-known detoxification mechanisms. The chromatographic behavior of the unknown ^{14}C-labeled residues, coupled with the lack of identifiable MS spectra using techniques adequate for [^{14}C]Kepone, indicated that the *gem*-diol moiety of Kepone hydrate had covalently bonded to specific fish lipids forming a large nonpolar conjugate. The propensity of Kepone to react with compounds containing alcohol, ketone, or secondary amine groups may play a role in the formation of these unknown ^{14}C-labeled residues.

The formation of Kepone derivatives with some commonly used solvents complicates the analysis and the interpretation of sample residues and requires monitoring for artifacts during sample cleanup and analysis. Thermally mediated GC injection port reactions are also important in the detection of Kepone. Conversion of Kepone residues to the parent carbonyl structure in the injection port is necessary for adequate column chromatography. This reaction may be enhanced or inhibited by specific solvents. These problems require careful attention to the solvent composition of sample solutions and the use of control samples spiked with Kepone.

Fathead minnows did not metabolize [^{14}C]mirex residues under the experimental conditions of this study and [^{14}C]mirex residues did not plateau during the 56-day exposure. Concentration factors of [^{14}C]mirex from water appeared to vary inversely with mirex water concentrations. This observation suggests that a high body burden of mirex induced increased rates of excretion or that the rate of transport across the gill membrane has a maximal limit for very hydrophobic compounds regardless of water concentrations.

[^{14}C]Kepone and [^{14}C]mirex were not degraded by aerobic or anaerobic hydrosoil microorganisms under the test conditions described in the experimental section. Although some difficulties were encountered in extracting [^{14}C]-Kepone from hydrosoil sediments, no evidence of Kepone biogenetic reactions was observed. The difficulty encountered in extracting mirex from hydrosoils may be explained by hydrophobic diffusion-limited interactions of nonpolar mirex with sediment organics and clays.

ACKNOWLEDGMENT

We thank Don Skaar, Ginger Gibson, Philip Giles, George Tegerdine, Thomas Durham, Don Schumacher, and Darryl Hartke for their technical assistance during the project.

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Received for review May 26, 1981. Revised manuscript received March 17, 1982. Accepted August 17, 1982. This research was partly sponsored by the U.S. Environmetal Protection Agency through Interagency Agreement No. EPA-IAG-D6-0126 [J. I. Teasley (USEPA) and F. L. Mayer (USFWS), Project Officers].