Fate of Kepone and Mirex in a Model Aquatic Environment: Sediment, Fish, and Diet

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Laboratory studies of freshwater sediment and a daphnid–bluegill (*Daphnia magna–Lepomis macrochirus*) food chain indicated that the organochlorine insecticides Kepone and mirex resist microbial degradation, accumulate and resist metabolism in fish, and move through a food chain. We found no direct evidence of degradation of either compound after 56 d of incubation in freshwater sediments under aerobic or anaerobic conditions. Furthermore, we found no metabolism or co-metabolism of ^{14}C-labeled Kepone in sediments that had long-term preexposure to Kepone and by-products nor did we detect a selective process occurring in the sediments that changed the capacity of the autochthonous microbiota to metabolize Kepone. After 28 d of exposure to ^{14}C-labeled Kepone or mirex in water and natural food, bluegills accumulated Kepone and mirex equal to 10 606 and 12 274 times the respective exposure concentrations. Elimination patterns were dissimilar: mirex tended to persist in fish tissue but Kepone did not. Neither Kepone nor mirex was metabolized by bluegills. Retention of mirex introduced into bluegills by daphnids was about 3 times greater than that of Kepone. Accumulation of Kepone and mirex by bluegills was about half that of DDT.

Key words: Kepone, mirex, degradation, freshwater sediment, accumulation, elimination, bluegills, daphnids, residue dynamics, food chain, accumulation index

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D'après des études en laboratoire de sédiment d'eau douce et d'une chaîne alimentaire constituée par une daphnie (*Daphnia magna*) et du crapet arlequin (*Lepomis macrochirus*), les insecticides organochlorés Kepone et mirex résistent à la dégradation microbienne, s'accumulent dans le poisson, y résistent à la métabolisation et se transmettent le long de la chaîne alimentaire. Nous n'avons trouvé aucune preuve directe de la dégradation des deux composés après 56 jours d'incubation dans des sédiments d'eau douce, en conditions aérobies ou anaérobies. De plus, nous n'avons observé aucune métabolisation ni cométabolisation de la Kepone marquée au ^{14}C dans les sédiments qui avaient été préalablement exposés à long terme à la Kepone et à ses sous-produits et nous n'avons pas décelé non plus de processus sélectifs

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qui ont modifié l'aptitude des microbes indigènes des sédiments à métaboliser cet insecticide. Après 28 jours d'exposition à la Kepone ou au mirex marqués au ^{14}C, dans l'eau ou la nourriture naturelle, les crapets arlequins ont concentré la Kepone et le mirex, 10 606 et 12 274 fois leurs concentrations respectives de départ. Les formes d'élimination différaient: le mirex tendait à persister dans les tissus, contrairement à la Kepone. Les crapets n'ont métabolisé ni la Kepone ni le mirex. La rétention du mirex absorbé par les arlequins dans les daphnies a été 3 fois supérieure à celle de la Kepone. L'accumulation de la Kepone et du mirex par les crapets était inférieure de moitié à celle du DDT.

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KEPONE and mirex were found in water, sediments, and biota of freshwater and marine ecosystems (Saleh et al. 1978; Kaiser 1978; Borthwick et al. 1973) at levels disproportionately high to the small quantities released into the environment (Livingston 1978). The two organochlorine insecticides are structurally similar[2] (Fig. 1) but differ in chemical properties and in behavior within aquatic ecosystems (Livingston 1978).

Kepone has had only limited use in the United States as cockroach bait (Suta 1977); most of the compound has been synthesized for export. Therefore, the major sources of environmental contamination are at the manufacturing sites (Livingston 1978). In 1975 it was discovered that Life Sciences Product Company, Hopewell, Virginia, discharged about 45 000 kg of Kepone and by-products into water that eventually entered Chesapeake Bay, contaminating about 380 000 m^2 of sediment (Gregory 1976; Environmental Protection Agency 1976) and thus threatening an important estuarine fishery.

Mirex has been used extensively as an insecticide and fire retardant (Farm Chemical Handbook 1975). Since 1967 it has been sprayed at a rate of 4.2–8.6 g/ha on over 400 000 ha/yr to control the fire ant (*Solenopsis* sp.) in 11 southeastern states (Suta 1977). It was later detected in stream water and in estuarine biota (Baetcke et al. 1972; Borthwick et al. 1973; Pritchard et al. 1973; Tagatz et al. 1975).

We conducted laboratory studies to assess the potential hazard of these chemical contaminants due to their persistence and accumulation in the aquatic environment. We report on the ability of freshwater sediment to degrade Kepone or mirex and the ability of a freshwater fish to accumulate, metabolize, and eliminate Kepone or mirex from water or natural food.

Materials and Methods

^{14}C-labeled Kepone (UL, 1.158 MBq/mg) and ^{14}C-labeled mirex (UL, 0.492 MBq/mg) were purchased from Pathfinder Laboratories, Inc., St. Louis, Missouri. Purity exceeded 99% as determined by gas–liquid and thin-layer chromatography (TLC).

Freshwater Sediments and Microbial Degradation

Sediment samples were taken from Little Dixie Lake, Missouri, an 83-ha impoundment located in an agricultural watershed 16 km east of Columbia. Chemical characteristics of the water are similar to those of other surface waters in central Missouri (Jones 1977). The lake is slightly eutrophic; alkalinity is entirely bicarbonate (Heman et al. 1969); organic matter content of hydrosoil samples averaged 1.6% (dry weight). Samples were collected in April and October 1977 and April 1978 from the littoral zone (about 1.5 m in depth) with an Ekman dredge. An electron-capture gas chromatographic analysis of the sediment revealed no detectable Kepone or mirex (minimum detectable limit, 0.02 μg/g).

Sediment samples from Bailey Creek, Virginia, were obtained with a hand shovel in October 1977. This tributary of the James River drains several industrial sites and receives the effluent from the Hopewell municipal sewage plant. It has been contaminated with Kepone and Kepone by-products for years (Gregory 1976).

Possible microbial degradation of Kepone and mirex was tested in the upper 3–5 cm of sediment under aerobic and anaerobic conditions. For the Kepone microbial degradation studies only, we selected two sediments: one with known extensive continuous exposure to Kepone (Bailey Creek) and one without known or evident Kepone contamination (Little Dixie Lake).

We placed 10 g (wet weight) of sediment in a 50-mL Delong flask for aerobic studies or in a 30-mL screw-top glass tube for anaerobic studies, and added 20 mL of water from either Baily Creek (Kepone study only) or Little Dixie Lake. The contents were inoculated with 300 μL of acetone containing 3 μg of either ^{14}C-labeled Kepone or mirex (final concentration 0.1 μg/g). Control samples were autoclaved for 20 min at 121°C under 1.05 kg/cm^2 steam pressure; to ensure sterility 0.75 g of mercuric chloride was added to each container. All samples were incubated at 22°C in the dark, the aerobic group on a rotary shaker and the anaerobic group in a torsion jar evacuated under vacuum and overlaid with nitrogen. Samples taken in triplicate at intervals of 7, 14, 21, 28, and 56 d were frozen for later determination of degradation products of either Kepone or mirex by TLC autoradiography and $^{14}CO_2$ radiorespirometry (Johnson and Lulves 1975).

For TLC analyses, sediment samples were thawed to room temperature for extraction and inoculated with 2 mL of saturated KOH and 1.0 g of copper powder to remove sulfur. The mixture was acidified with 0.1 mol/L HCl and extracted with three 15-mL portions of ethyl acetate. The organic layer was

[2] Kepone (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one) and mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene) are both synthesized from hexachlorocyclopentadiene (Fig. 1). They differ in that two chlorine atoms in mirex are replaced with a double-bonded oxygen atom in Kepone; Kepone is about 2000 times more soluble in water than mirex (Livingston 1978).

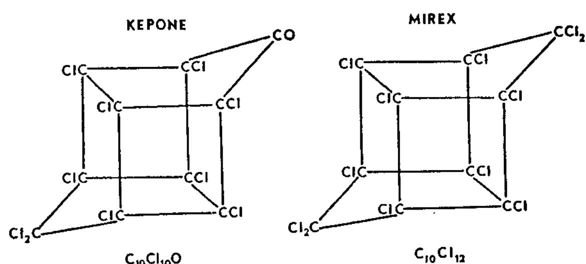

FIG. 1. Chemical structures of Kepone and mirex.

removed by centrifugation. The ethyl acetate extractions were combined, concentrated, and purified on an AMOCO px-21 carbon/foam column (Huckins et al. 1978) to remove interfering biological material. The ^{14}C-labeled Kepone extracts were eluted from the carbon/foam column with sequential washes: 50 mL of petroleum ether, 100 mL of ethyl acetate, and 50 mL of methanol; ^{14}C-labeled eluates of Kepone were spotted on a silica gel plate (0.25 mm in thickness) and developed 3 times in a 4:1 (v/v) mixture of benzene and acetone. Autoradiograms were made by exposing the TLC plate in the dark to Kodak no-screen X-ray film for 7 d. R_f values and tentative identification of separated extracts were made by comparing cochromatographed nonlabeled standards with the radiogram. Residues of ^{14}C-labeled mirex were extracted from sediment as described for Kepone. The TLC plates were developed in 5% diethyl ether in petroleum ether (v/v).

EVOLUTION OF $^{14}CO_2$ FROM SEDIMENTS

Sediments of Little Dixie Lake were incubated with labeled Kepone and the effluent gas (air in the aerobic test and nitrogen gas in the anaerobic test) was measured with a radiorespirometer (Johnson and Lulves 1975). The gas was bubbled slowly through a trapping solution of monoethanolamine–ethylene glycol (3:7, v/v). Samples (1 mL) of the trapping solution were added to 15 mL of a 1:1 mixture (v/v) of methanol and Fluoralloy (Beckman Instrument Co.). In triplicate these samples were quantified using a Beckman LS-200 liquid scintillation counter (LSC) and corrected for quench and background radiation. The ^{14}C counting efficiency was 85 ± 5%.

Evolution of $^{14}CO_2$ from sediments of Little Dixie Lake was measured by a method modified from Gledhill (1975). Sediment was incubated with mirex in 250-mL Erlenmeyer flasks. Glass vials suspended from rubber stoppers in each flask contained 10 mL of trapping solution of monoethanolamine–ethylene glycol (3:7, v/v). Aerobic conditions were maintained by aerating the samples for several minutes semiweekly, and anaerobic conditions by overlaying the sample with gaseous nitrogen. Triplicate 1-mL aliquots were counted by the LSC, as described for Kepone. Anaerobiosis was monitored with methylene blue (Manual of Microbiological Methods 1957).

MODULAR FOOD CHAIN

Residue dynamics of Kepone and mirex were examined in bluegills (*Lepomis macrochirus*) fed daphnids (*Daphnia magna*) according to the methods of Johnson (1974, 1980). Accumulation studies were conducted in an intermittent flow-through diluter system modified after Mount and Brungs (1967) and McAllister et al. (1972). A flow-splitting chamber, modified after Benoit and Puglisi (1973), was used to mix and divide the toxicant for delivery to the exposure tanks (78 L). Photoperiodicity was 16-h light:8-h dark.

Well water (pH of 7.4 and total hardness 270 mg/L as $CaCO_3$) was aerated and maintained at 22 ± 1°C for all experiments. Stock solutions of each of the ^{14}C-labeled toxicants were made up in acetone. We detected no deleterious effect on daphnids or bluegills in control chambers exposed to 0.33 mL/L acetone. Toxicant concentrations in the exposure chambers were monitored 3 times per week by the radiometric method described. We removed 500 mL of water from the exposure chamber and extracted it twice in a separatory funnel with 15 mL of analytical grade methylene chloride. Exactly 5 mL of toluene (scintillation grade) was added to the methylene chloride extract and the mixture was evaporated in a porcelain evaporation dish to 1–2 mL. This volume was transferred with 15 mL of Triton-X–Fluoralloy (6:9, v/v) into scintillation vials. Radioactivity was quantified in a Beckman LS-200 LSC. Corrections were made for extraction efficiency (85% Kepone; 82% mirex) and quench.

Bluegills were obtained from Federal hatcheries and maintained by the methods of Brauhn and Schoettger (1975); daphnids were maintained by the method of Dewey and Parker (1964) at our laboratory.

Residue dynamics of Kepone and mirex in fish were determined after exposure in water and natural food. We placed 40 bluegills (0.5–1.5 g) in each of four 78-L chambers. Three chambers were continuously exposed to a constant water concentration of Kepone (or mirex) at 150 ng/L for 28 d. Daphnids were exposed for 24 h to the same ^{14}C-labeled insecticide in the same tanks within a screened chamber. Fish were fed treated daphnids daily at either 10% (Kepone) or 7.5% (mirex) of their body weight (wet). Five 50-mg samples of daphnids were frozen for residue analyses semiweekly by the radiometric method of Johnson et al. (1971).

Seven fish were removed from each exposure chamber for the determination of total body ^{14}C-labeled residue after 7, 14, 21, and 28 d of exposure to either ^{14}C-labeled Kepone or ^{14}C-labeled mirex. Total body residues were determined by the radiometric method of Johnson (1980) and measured with an LSC. Data were corrected for quench (15 ± 5%) and background radiation. Accumulation factors were determined by dividing the toxicant concentration in the fish by the toxicant concentration in the water.

On day 28, four or five fish from each aquarium (about 15 fish) were frozen for metabolite analyses; the rest were transferred to compound-free water to monitor elimination after 1, 3, 7, 14, 21, and 28 d (4 fish assayed per date). Fish were fed untreated daphnids at the previous rations.

To determine the accumulation of Kepone and mirex from daphnids, we exposed 40 bluegills to compound-free water in the flow-through diluter, as described in the previous experiment. Fish were fed daphnids exposed for 24 h to 400 ng/L Kepone (or mirex) at 10% of their body weights each day. Seven fish were removed for LSC analyses of total body residue at 7, 14, 21, and 28 d.

Data were subjected to statistical analyses at $\alpha = 0.05$ by linear regression, analysis of variance, and multiple means analysis (least significant difference).

Metabolism of Kepone

Analytical methods for Kepone analysis were modified after the work of J. N. Huckins (Columbia National Fisheries Research Laboratory, U.S. Department of the Interior, Fish and Wildlife Service, Columbia, MO, unpublished data). The frozen fish were ground in a Sorvall Omni-mixer, blended with 4 times their weight of anhydrous Na_2SO_4, and poured into an extraction column (29 cm × 2 cm ID). The fish homogenate in the column was washed sequentially with three solutions: 150 mL of 5% diethyl ether in petroleum ether, 150 mL of ethyl acetate–acetone (1:1 v/v), and 50 mL methanol. We concentrated and chromatographed the second eluate on a silica gel column with sequential washes: 50 mL of hexane, 50 mL of 5% methanol in ethyl acetate, and 50 mL of methanol. The second eluate from the silica gel column was concentrated to 2 mL and chromatographed on an Autoprep 1001 gel permeation chromatograph (GPC). The GPC column (30.5 cm × 2.5 cm ID) was packed with 70 g of Sephadex LH-20 (25–100 μm). The solvent system was ethyl acetate–methanol–toluene (80:10:10, v/v/v). We examined fractions containing radioactive residues by gas chromatography (GC), using a Tracor MT-220 or MT-222 equipped with a ^{63}Ni electron capture detector. The GC columns (15.3 cm × 2 mm ID) were packed with 3% OV-7 on 80–100 mesh Chromosorb W HP (w/w). Carrier gas (N_2) flow was 30 mL/min; oven and injector temperatures were set at 190 and 220°C, respectively.

The analysis of mirex was similar to that of Kepone. Fish tissue was extracted in a column with 150 mL of 5% diethyl ether in petroleum ether, followed by 50 mL methanol. The first column eluate was concentrated to about 2 mL and chromatographed on a silica gel column with 43 mL of 0.5% benzene in hexane, 15 mL of 4% ethyl acetate in benzene, and 25 mL methanol. The first eluate of the silica gel column was concentrated to 2 mL and chromatographed on GPC with a column packed with 50 g Bio-Beads S-X3 (200–400 mesh) using a mixture of cyclohexane–methylene chloride (85:15, v/v). Fractions containing radioactive residues were analyzed for percent recovery of mirex by GC analysis, as described above.

The recovery of radioactive residues from fish for both ^{14}C-labeled compounds was verified by complete combustion of whole body samples and measurement of $^{14}CO_2$ after completion of solvent extractions.

Results and Discussion

Toxicant Degradation in Sediments

We found no direct evidence that Kepone or mirex was degraded in these freshwater sediments under either aerobic or anaerobic conditions. After 56 d of incubation we could detect no significant ($P > 0.05$) evidence of either primary or ultimate degradation of either Kepone or mirex. Concentrations ≤10 mg/kg of either Kepone or mirex did not impair respiration in sediments (Johnson 1981).

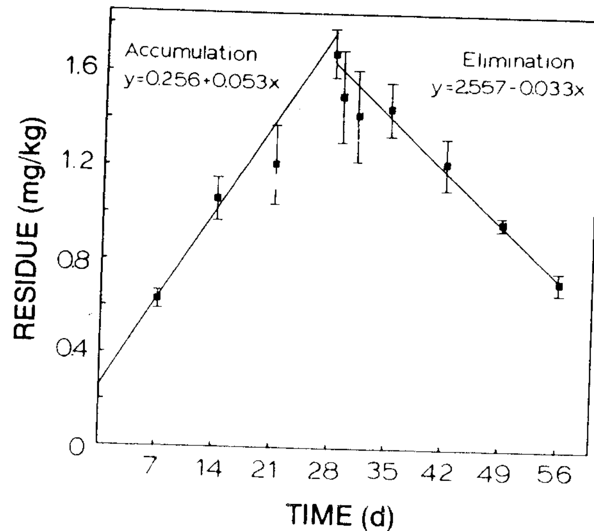

FIG. 2. Mean accumulation and elimination of Kepone by bluegills from food and water over a 56-d period. Vertical bars show standard deviation.

Radiorespirometric and TLC autoradiographic analyses of sediments from Little Dixie Lake (Missouri) and Bailey Creek (Virginia) indicated that no degradation of Kepone had occurred. Recovery of ^{14}C-labeled Kepone from sediments ranged from 75 to 94%, which compared favorably with recoveries of 83–103% from spiked control sediments. Kepone was tightly bound to the sediment and required vigorous treatment to effect chemical extraction. Although sediments in the Bailey Creek area had been contaminated with Kepone and its by-products for years (Gregory 1976), this preexposure did not increase the microbial capacity to degrade Kepone. We detected no natural selective process toward enzymatic induction with the autochthonous microbiota. The Bailey Creek sediments exhibited the same inability to degrade Kepone. We were thus unable to corroborate the findings reported by Orndorff and Colwell (1979) of Kepone degradation in the Bailey Creek sediments.

We did detect significantly higher ($P > 0.05$) $^{14}CO_2$ evolution from aerobic sediments treated with ^{14}C-labeled mirex in one of four sampling dates (day 7). This difference represented 0.9% of the total labeled mirex inoculated and occurred only during the 1st week. We suspect a low-level ^{14}C contaminant rather than a metabolite of mirex. No significant difference ($P < 0.05$) was detected between treated and control sediments under anaerobic conditions. Autoradiograms revealed only mirex. These results support the findings of Jones and Hodges (1974), who found no evidence of biological degradation of mirex in soil.

Modular Food Chain

After 28 d of exposure to ^{14}C-labeled Kepone in food (0.046 ± 0.01 mg/kg) and in water (158 ± 13 ng/L), bluegills contained a mean total body residue of 1.68 ± 0.11 mg/kg Kepone. A plot of body residue versus time indicated that Kepone accumulation from water and food was

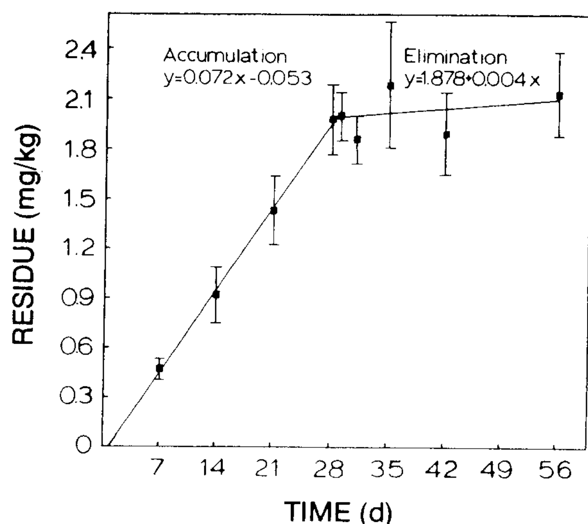

FIG. 3. Mean accumulation and elimination of mirex by bluegills from food and water over a 56-d period. Vertical bars show standard deviation.

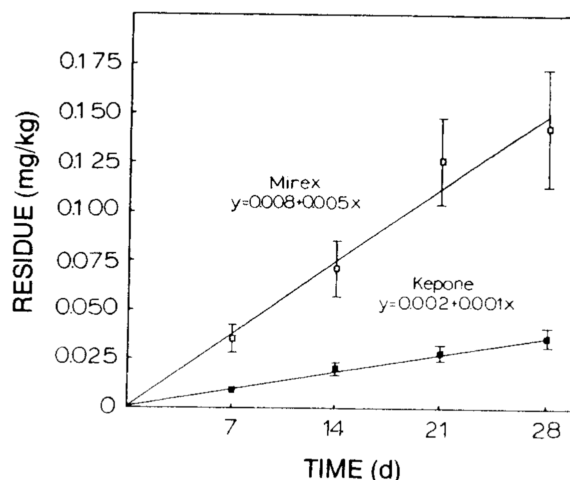

FIG. 4. Mean accumulation of Kepone and mirex by bluegills from food (*Daphnia*) over a 28-d period. Vertical bars show standard deviation.

constant ($r = 0.99$) between 7 and 28 d. The accumulation factor (AF = concentration in fish divided by concentration in water) was 10606. The equilibrium concentration of ^{14}C-labeled Kepone in bluegills did not appear to be approached during the 28 d of continuous exposure (Fig. 2). However, determination of the theoretical accumulation equilibrium factor (after Branson et al. 1975) suggests that bluegills were approaching steady state. Estimation of the rate constant for accumulation and elimination and the theoretical accumulation factor of Kepone at equilibrium was calaculated from rate constants modified after the method of Branson et al. (1975) from the equation:

$$C_f / C_w = k_1 / k_2 = K_{cf}$$

where C_f = concentration in fish, C_w = concentration in water, k_1 and k_2 are rate constants for accumulation and elimination, and K_{cf} = the accumulation factor at equilibrium. The estimated rate constant for accumulation and elimination for Kepone was $15.6 \cdot h^{-1}$ and $0.0012 \cdot h^{-1}$. The K_{cf} value of 13000 was slightly higher than the 10606 accumulation factor we determined after 28 d of exposure to Kepone. These values obtained for bluegills agree with the accumulation factor reported by Bahner et al. (1977) for young estuarine sheepshead (*Archosasgus probatocephalus*) (exposure period, water concentration, and fish size were similar).

Elimination of Kepone by bluegills in Kepone-free water followed a decreasing, linear pattern described by the equation: $y = 2.5 - 0.03x$ ($r = 0.85$). The estimated half-life of Kepone in bluegills was 25 d. Similarly, Bahner et al. (1977) reported that elimination of Kepone from crustaceans and estuarine fish was low, body residues decreasing 30–60% in 24–28 d.

After 28 d of exposure to ^{14}C-labeled mirex in food (0.24 ± 0.05 mg/kg) and water (161 ± 11 ng/L), bluegills contained a mean total body residue of 1.98 ± 0.2 mg/kg. When these total body residues were plotted against time (Fig. 3), the accumulation of mirex by fish tended to be linear ($r = 0.96$). Equilibrium had not been reached after 28 d of continuous exposure to mirex (Fig. 3). This accumulation factor for mirex (12274) was about 60 times greater than that reported by Metcalf et al. (1973) in a static, multicomponent model ecosystem. No significant ($P > 0.05$) elimination of mirex by bluegills was detected after 28 d in mirex-free water (Fig. 3).

Bluegills accumulated ^{14}C-labeled Kepone or ^{14}C-labeled mirex from Kepone- or mirex-free water by consuming live daphnids that had previously been exposed to the two insecticides. During separate 28-d feeding experiments, bluegills accumulated 0.036 ± 0.005 mg/kg Kepone and 0.143 ± 0.29 mg/kg mirex from live daphnids containing a mean residue of 0.078 ± 0.020 mg/kg for Kepone and 0.125 ± 0.037 mg/kg for mirex, respectively (Fig. 4). A plot of the data indicated that the accumulation of Kepone and mirex from food was increasing and linear over time: Kepone, $r = 0.91$; mirex, $r = 0.89$ (Fig. 4). Bluegills tended to accumulate more mirex than Kepone from ingested live food. The food transfer factor (consumer residue per food residue (w/w)) was 0.45 for Kepone and 1.14 for mirex. Calculations[3] of the total percent of Kepone and mirex retained by the bluegills indicated that after 28 d, 34% of the mirex and 14% of the Kepone introduced in the daphnids remained in the fish.

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[3]The bluegills were fed daily an average of 0.87 g live daphnids which contained 0.78 μg/g Kepone; thus the total Kepone introduced was 0.190 μg per daphnid. All daphnids were rapidly consumed. After 28 feedings the average fish weighed 0.754 g and contained 0.036 μg/g Kepone; therefore the total Kepone retained was 0.027 μg per fish. Percent retention of Kepone by bluegills was determined by the equation:

$$\% \text{ retention} = \frac{\text{total residue (consumer)}}{\text{total residue (prey)}} \times 100.$$

Therefore Kepone retention equals $\frac{0.027\ \mu g}{0.190\ \mu g} \times 100 = 14\%$. Mirex values were calculated similarly.

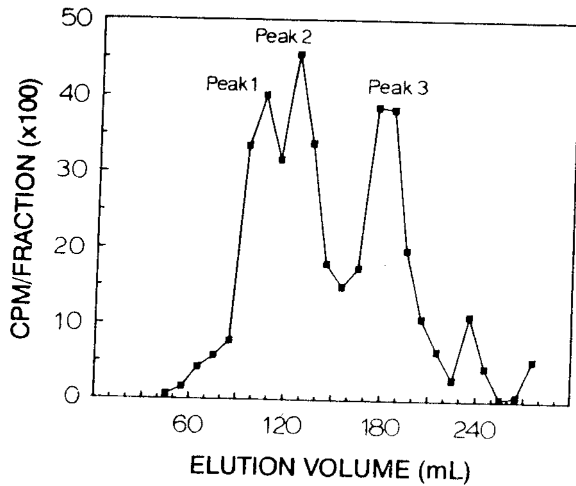

FIG. 5. GPC elution profile of extract from bluegills that had been exposed to Kepone for 28 d before analysis. CPM = counts per minute.

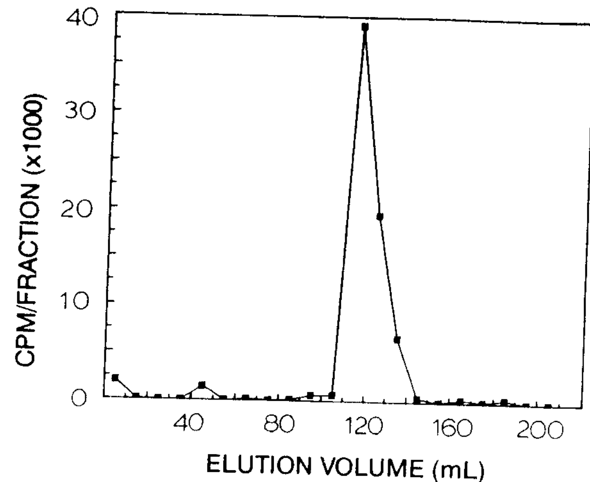

FIG. 6. GPC profile of extract from bluegills that had been exposed to mirex for 28 d before analysis. CPM = counts per minute.

To gain a better perspective of the bioaccumulation hazard of Kepone and mirex to freshwater fish, we calculated an accumulation index value for both compounds. The accumulation index (AI), in this instance from food plus water ($f + w$), is a comparison of the accumulation factor of the test chemical ($AF(X)$) with the accumulation factor of p,p'-DDT (AF(DDT)) from a similar source under standard conditions in the protocol described by Johnson (1980). We used the equation:

$$AI_{f+w} = (AF_{f+w}(X))/(AF_{f+w}(\mathrm{DDT})) \times 100.$$

AF_{f+w}(DDT) = 24 323 for bluegills (0.5–1.5 g) after 28 d of continuous exposure to p,p'-DDT (300 ng/L) at 22°C (Johnson 1980). The calculated AI_{f+w} values for Kepone and mirex were 44 and 51. If DDT was given the arbitrary index value of 100, we thus estimated that the propensity for Kepone and mirex to bioaccumulate in bluegills was about half that of DDT.

We found no evidence to suggest that either Kepone or mirex was metabolized by bluegills. However, there was some evidence of Kepone–lipid binding which may or may not be metabolically mediated. A more extensive investigation of metabolic incorporation into lipids has been reported by Huckins (unpublished data).

When extracts from Kepone-treated fish were chromatographed on GPC, the radioactive profiles derived appeared to consist of three main peaks (Fig. 5). Peak 1 (from an elution volume of 90–120 mL) made up 26.7% of the total radioactivity, peak 2 (120–160 mL) 28.3%, and peak 3 (160–220 mL) 31.7%. In analysis of the GPC eluates by GC, 8% of the radioactivity in peak 1, 33% in peak 2, and 80% in peak 3 were identified as Kepone. The minimum detection limit of Kepone was 0.04 μg/g. The lack of an adequate GC response for radioactivity in peak 1 may represent ^{14}C-labeled Kepone–lipid binding products that were probably too large for adequate GC analyses. The concentration of possible lipid-bound ^{14}C-labeled residue in Fig. 5 was 0.7 μg/g. The radioactive residues of peaks 2 and 3 represent Kepone and Kepone derivatives. It appeared that peak 2 was the hemiketal analog of Kepone and methanol, and peak 3 was a combination of Kepone hydrate and an aldol condensation product of acetone and Kepone. The identification of these compounds was based on previous studies (Huckins unpublished data). The Kepone solvent derivatives may be mistaken for degradation products because differences in chromatographic behavior occur during cleanup procedures. Therefore, care must be taken in selecting a solvent for the analysis of Kepone residues.

Although we anticipated possible dechlorination or hydroxylation of Kepone in bluegills, we found none. Acidification of samples did not enhance extraction efficiency. Slight differences of recovery values (8–10%) for Kepone did not necessarily suggest metabolism in the fish.

Chromatography of extracts from mirex-treated fish by GPC yielded only one radioactive peak (Fig. 6). Individual fractions from this peak analyzed by GC showed only mirex. The minimum detection limit of mirex was 0.04 μg/g. About 81% of the radioactive residue from the GPC eluate was recovered and identified as mirex.

The persistence of these compounds in fishes was also shown by Borsetti and Roach (1978), who detected only a trace amount of Kepone metabolites, and by Pritchard et al. (1973), who found no degradation of mirex.

The general history of organochlorine compounds (PCBs, DDT, etc.) entering aquatic ecosystems is well known; they sorb from water to organic matter in sediments, resist microbial degradation, are rapidly accumulated and retained by animals, and move throughout food webs (Woodwell 1967; Hamelink et al. 1971; Metcalf 1977). Our study indicated that Kepone and mirex also resisted microbial degradation, accumulated in fish from food and water, and resisted metabolism in freshwater fish.

In our studies with bluegills, the accumulation potential (as indicated by the accumulation factors) was similar for Kepone and mirex when exposure through water and food was concurrent. However, the retention of mirex introduced to fish only in food was about 3 times greater than that of Kepone. Also,

mirex tended to persist in fish tissue whereas Kepone did not. Presumably, bluegills continuously exposed to mirex would accumulate the compound until death, without reaching an equilibrium. This difference is probably related to the chemical structures of the compounds. Kepone is less lipophilic than mirex and contains a potentially reactive carbonyl group whereas mirex has a strong affinity for fatty tissues and lacks reactive groups that are more easily eliminated (Baetcke et al. 1972). The high accumulation and retention of mirex in fish tissues could be potentially dangerous to higher trophic level consumers. Although the use of these two chemicals has been significantly curtailed, their continued presence in aquatic ecosystems remains a threat to important fishery resources.

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