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Mass Spectral Confirmation and Analysis by the Hall Detector of Mirex and Photomirex in Herring Gulls from Lake Ontario

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Mirex, 1,2,3,4,5,5,6,7,8,9,10,10-dodecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane, and a monohydro Mirex, 1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane (photomirex), were discovered in the whole body lipid of Herring Gulls from Pigeon Island, Lake Ontario. PCBs, which were evident in high concentrations, coeluted with Mirex and photomirex in residue cleanup procedures, and interfered with their determination by electron-capture gas chromatography (EC-GC) using standard analytical columns. Samples were perchlorinated to remove this interference, and structures were confirmed by gas chromatography/mass spectrometry (GC/MS). The Hall electrolytic conductivity detector was shown to be useful as a specific detector to monitor residues of Mirex and photomirex in the presence of high PCB interference. Eggs of Herring Gulls proved to be useful as indicators of these compounds in Lake Ontario.

Mirex has recently been recognized by gas chromatography/mass spectrometry (GC/MS) as a contaminant of Lake Ontario fish (Kaiser, 1974). The adult Herring Gull is a widespread aquatic feeding carnivore which is relatively nonmigratory on Lake Ontario (Kaldec and Drury, 1968). Lake fish comprise the bulk of the bird's diet, although garbage is sometimes eaten. Herring Gulls on Lake Ontario have been found to contain high levels of organochlorines such as DDE and PCBs (Gilbertson, 1974; Frank et al., 1975); therefore we wished to determine if Mirex and other undiscovered compounds were also present.

It is difficult to determine Mirex and other organochlorine residues in the presence of high levels of PCBs and DDE because of interference in electron-capture gas chromatography (EC-GC) using standard analytical columns. The recently developed Hall electrolytic conductivity detector was reported to have a low specificity in the chloride mode for PCBs, while having a high specificity for highly chlorinated, nonaromatic compounds such as Mirex (Hall, 1974). The use of the Hall detector was therefore investigated for routine analysis of trace organochlorine pollutants in the heavily PCB-contaminated Herring Gull lipid.

Existing column chromatography procedures (Reynolds, 1971; Berg et al., 1972) were not effective in separating PCBs from Mirex and related compounds for confirmation of the latter by GC/MS. This was overcome by perchlorinating samples to convert interfering PCBs to decachlorobiphenyl.

EXPERIMENTAL SECTION

Reagents and Solvents. Florisil (60-100 mesh) obtained from Floridin Corp. was heated at 300 °C overnight to remove any electron capturing contaminants and used

in the activated form for extraction. For column chromatography it was deactivated with 1.2% by weight glass distilled water.

HCB (hexachlorobenzene), BHC (1,2,3,4,5,6-hexachlorocyclohexane), and *p,p'*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) were obtained from Chem Services Inc.

Mirex (1,2,3,4,5,5,6,7,8,9,10,10-dodecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane), *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene), heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-endomethanoindene), and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-*exo*-5,8-dimethanonaphthalene) were obtained from U.S. EPA, Research Triangle Park, N.C.

Decachlorobiphenyl was obtained from Analabs Inc. Aroclor 1260 was obtained from Monsanto Corp.

All solvents used were glass distilled, nanograde quality from Caledon Laboratories.

Preparation of Environmental Samples. Sixteen adult Herring Gulls were obtained in 1973 from a colony located on Pigeon Island, Lake Ontario. The gulls were plucked, macerated in a Hobart chopper, freeze dried, and extracted in a Soxhlet apparatus (extra-large size, Corning Glass Works) with hexane/acetone azeotrope. The solvent was removed, yielding approximately 1 kg of lipid. The lipid comprised approximately 10% of the whole body weight.

Herring Gull eggs were obtained from Scotch Bonnet Island, Lake Ontario in the spring of 1972, and from Muggs Island, Lake Ontario in the spring of 1974 and 1975. They were ground in precleaned sodium sulfate and extracted with hexane in a column. The lipid extracted comprised approximately 5% of the fresh weight of the eggs.

Extraction of Organochlorine Compounds. The acetonitrile-hexane partition method of Porter and Burke (1973) was modified as follows: The lipid (8 g) was mixed with Florisil as stipulated in the method, but the residues were eluted with 5% water in acetonitrile. This eluate was placed in a large separatory funnel and shaken with 100 ml of hexane. The sample was then partitioned with 800

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Table I. Response Comparison of PCBs, Mirex, and Organochlorine Pesticides by Least Detectable Amount (LDA)^a on EC and Hall Detectors

Compound	Retention time, min	LDA, pg/s		
		EC	Hall	Hall/EC
Aroclor 1260 (heptachlorobiphenyl)	20.0	1.83	3440	1880
Mirex	20.80	0.0129	0.910	70.5
<i>p,p'</i> -DDE	8.10	0.093	11.1	119
<i>p,p'</i> -DDT	10.70	0.022	2.96	132
Dieldrin	9.55	0.333	5.71	17.1
Heptachlor epoxide	6.35	0.107	1.68	15.7
α -BHC	2.60	0.051	0.814	16.1

^a LDA is calculated by dividing the amount of compounds giving a signal twice the noise level by the base width of the peak. GC conditions: A Tracor Model 220 GC fitted with both an EC and Tracor-Hall detector Model 310; column, 165 \times 2 mm i.d. glass column packed with 3% OV-1 on 80-100 mesh Chromosorb W; carrier gas, nitrogen; flow rate, EC, 60 ml/min; purge, 55 ml/min; Hall, 60 ml/min; hydrogen flow rate, 40 ml/min; solvent (50% 2-propanol-water), 0.2 ml/min; column temperature, 190 °C; Hall detector oven, 900 °C; EC detector, 225 °C; injector temperature, 230 °C; attenuation, EC, 16; Hall, 2.

ml of distilled water. Fifty milliliters of saturated sodium sulfate and 15 ml of methylene chloride were added. The aqueous layer was discarded and the organic phase was washed with two successive 100-ml portions of distilled water. The organic phase was finally filtered through anhydrous sodium sulfate. Recoveries of PCBs, DDE, HCB, and Mirex were improved to 99 \pm 0.5% at the 1-ppm level.

Column Separation. The extract was placed on a 3 \times 25 cm column containing deactivated Florisil. The column was eluted with 300 ml of hexane, followed by 300 ml of 30% methylene chloride/hexane. HCB, PCBs, DDE, and Mirex were recovered in total in the hexane fraction. The eluate was divided into two equal fractions: one for perchlorination, and one for direct analysis by EC and Hall detectors.

Perchlorination. Samples and standards were perchlorinated with antimony pentachloride as described by Armour (1973). Recoveries of PCBs as decachlorobiphenyl and Mirex in the parent form were 100% after perchlorination at the 200-ppm level.

Gas Chromatography. Three different gas chromatographs fitted with EC, FID, and Hall electrolytic conductivity detectors were used with specific conditions to suit each. Conditions are therefore described with figures for clarity.

Gas Chromatography-Mass Spectrometry. A Perkin-Elmer GC, Model 990, fitted with a flame ionization detector (FID) was connected to the mass spectrometer with a Watson-Bieman separator. The mass spectrometer was a Hitachi Perkin-Elmer RMU-6L interfaced to a Varian MAT 620L computer: GC column, 1850 \times 2 mm i.d. glass column packed with 3% OV-17 on 80-100 mesh Chromosorb W/HP; carrier gas, helium at a flow rate of 30 ml/min. Operating parameters of mass spectrometer were: emission current, 100 μ A; accelerating voltage, 3100 V; source temperature, 230 °C; manifold and separator temperature, 270 °C; connecting line to mass spectrometer, 230 °C.

RESULTS

Figure 1 shows an EC chromatogram of the hexane fraction containing organochlorine residues obtained from Herring Gull lipid. The major peaks evident are those attributable to PCBs present in Aroclor 1254, Aroclor 1260, and DDE (peak 1). Mirex (peak 3) was totally masked by PCB interference.

A study was undertaken to accurately compare the electron capture detector and Hall conductivity detector for least detectable amounts (LDAs) of PCBs and various organochlorine pollutants suspected to be present in Lake Ontario (Table I). The PCB peak which interferes with

Table II. Organochlorine Residues (in Parts per Million) in Adult Herring Gull Lipid

	Nonperchlorinated ^c		Perchlorinated, EC
	EC	Hall	
PCBs (as Aroclor 1260)	2800 ^a		3530 (as decachlorobiphenyl)
DDE	310	330	
Mirex	nd ^d	210	220
Photomirex ^b	nd	70	84

^a Calculated by sum of peak areas after Webb and McCall (1973). ^b Calculated as equivalent to Mirex. ^c GC conditions were identical with Figure 1 for EC and Figure 2 for Hall. ^d nd, nondetectable.

the detection of Mirex by EC was a heptachlorobiphenyl present in Aroclor 1260 and, to a lesser extent, in Aroclor 1254. The specific response of Mirex compared to this heptachlorobiphenyl peak was 375 times greater with the Hall detector than with the EC detector. The LDA of the organochlorine pesticides was 10 to 100 times higher with the Hall detector than with the EC detector, whereas the LDA for PCBs was almost 2000 times higher. The Hall detector appeared, therefore, to be useful as a specific detector for Mirex and other trace organochlorine pesticides present in lipid highly contaminated with PCBs.

Figure 2 shows a Hall chromatogram of the same Herring Gull lipid fraction previously analyzed by EC (Figure 1). There was a considerable reduction in PCB interference and only three peaks were evident. The retention times of peaks 1 and 3 corresponded to DDE and Mirex, respectively. Peak 2, not previously detected in the EC chromatogram due to PCB masking, was suspected to be a metabolite or degradation product of Mirex because of its long retention time and high sensitivity with the chlorine-specific Hall detector.

In order to confirm the identity of Mirex and the unknown peak by GC/MS, it was necessary to eliminate the PCB interference. This was achieved by perchlorination of the sample, which has proven useful for the quantitation of PCB isomer as decachlorobiphenyl (Armour, 1973). The procedure did not degrade Mirex.

Figure 3 shows the FID chromatogram obtained during GC/MS analysis of the perchlorinated Herring Gull hexane eluate. The baseline shift was caused by temperature programming. The mass spectrum of peak 3 is shown in Figure 4. It was identified as Mirex based on the chlorine isotopic abundances for the *m/e* 540 molecular ion and the *m/e* 272 base peak of the hexachlorocyclopentadiene ion, characteristic of cyclodiene insecticides. Further fragmentation of the *m/e* 270 ion by loss of chlorine to yield the strong *m/e* 235 ion was consistent with a mass

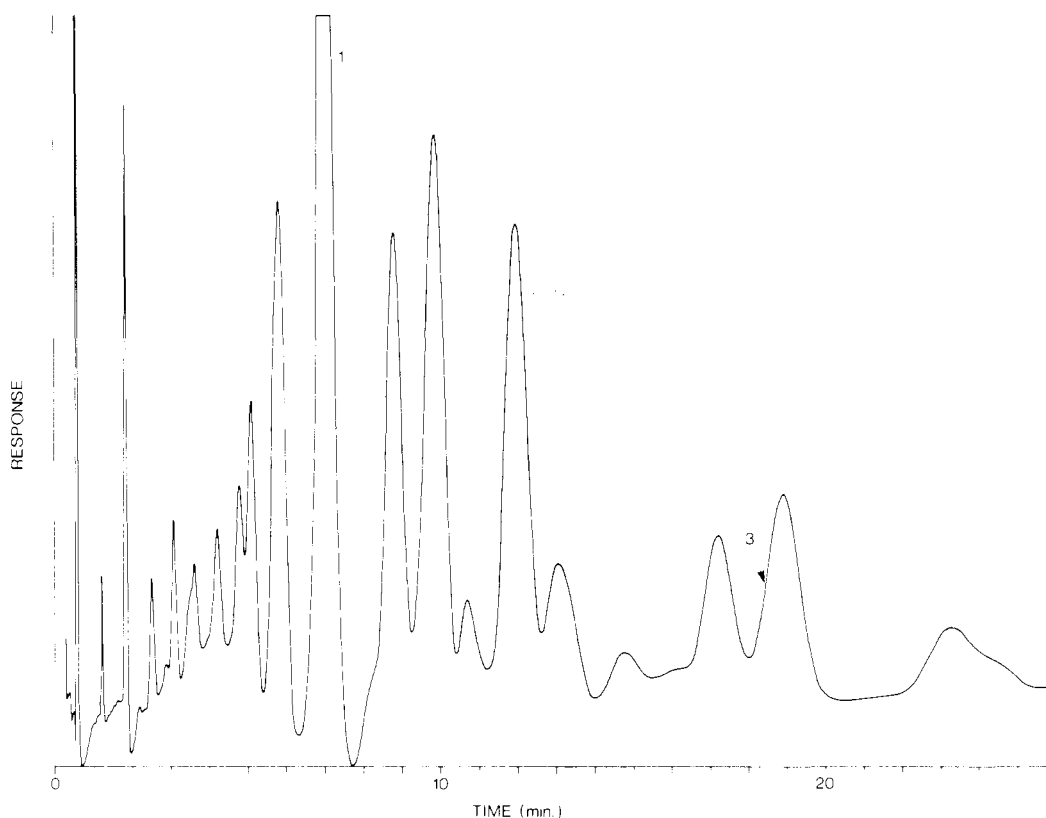


Figure 1. Chromatogram obtained with an electron capture detector of adult Herring Gull lipid extract: peak 1, DDE; peak 3, Mirex and heptachlorobiphenyl. GC conditions: A Hewlett Packard Model 5750 GC fitted with a Ni-63 EC detector; column, 185 × 3.5 mm i.d. glass column packed with 3% OV-101 on 80/100 mesh Chromosorb W(HP); carrier gas, 90% argon-10% methane; flow rate, 50 ml/min; column temperature, 205 °C; detector temperature, 300 °C; injector temperature, 280 °C.

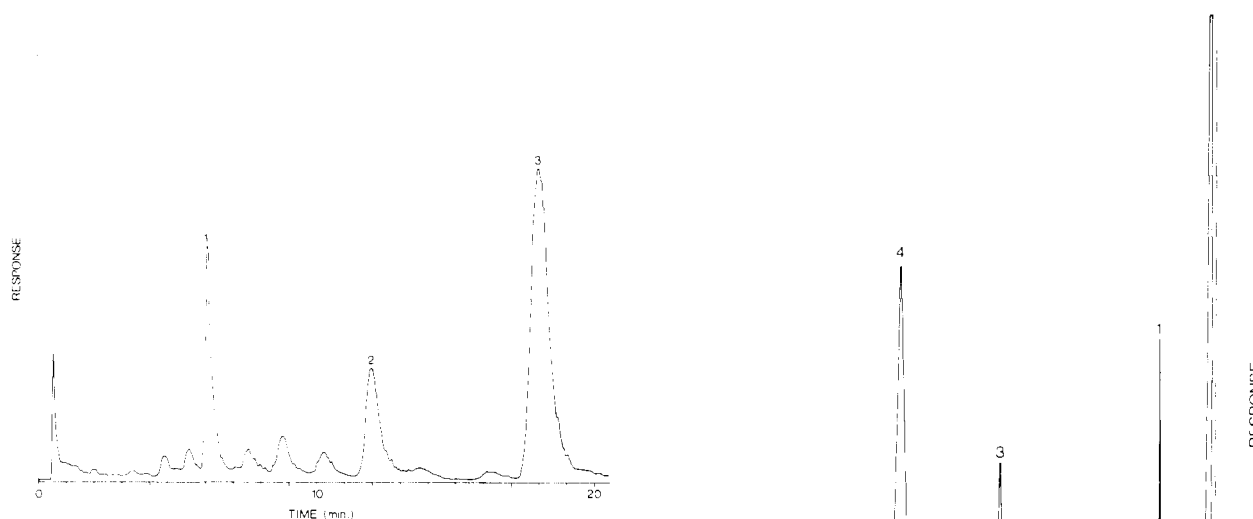


Figure 2. Chromatogram obtained with Hall conductivity detector of adult Herring Gull extract: peak 1, DDE; peak 2, photomirex; peak 3, Mirex. GC conditions: A Tracor Model 220 GC fitted with a Tracor-Hall detector Model 310; column, 165 × 2 mm i.d. glass column packed with 3% OV-1 on 80/100 mesh Chromosorb W(HP); carrier gas, nitrogen; flow rate, 60 ml/min; reaction gas, hydrogen; flow rate, 40 ml/min; solvent, 50% 2-propanol-water; flow rate, 0.2 ml/min; column temperature, 190 °C; Hall detector furnace, 900 °C; injector temperature, 230 °C.

spectrum recorded for Mirex and with spectra previously reported (Uk et al., 1972).

The mass spectrum of the unknown peak (peak 2), which was also detected with the Hall detector in the nonperchlorinated eluate, is shown in Figure 5. It exhibited strong ion abundances at m/e 270 and 235 indicative of

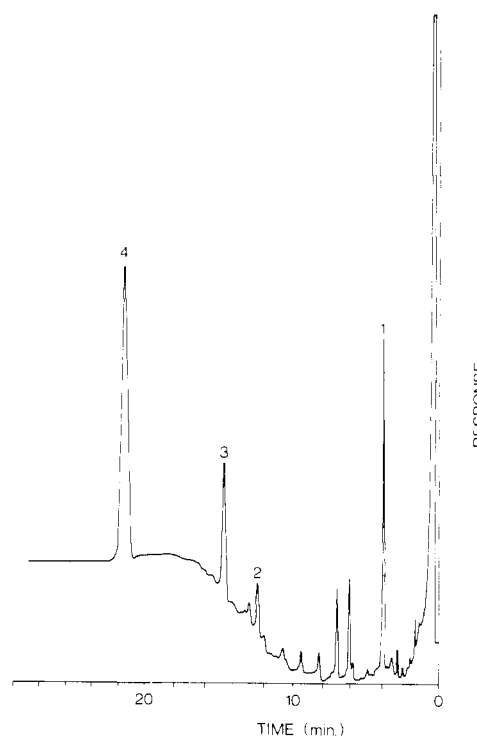


Figure 3. Chromatogram obtained with FID detector of adult Herring Gull lipid extract after perchlorination: peak 2, photomirex; peak 3, Mirex; peak 4, decachlorobiphenyl. GC conditions: A Perkin-Elmer GC, Model 990, fitted with a flame ionization detector (FID); column, 185 × 2 mm i.d. glass column packed with 3% OV-17 on 80/100 mesh Chromosorb W(HP); carrier gas, helium; flow rate, 30 ml/min; temperature program, 90 to 270 °C at 6 °C/min with 8-min hold.

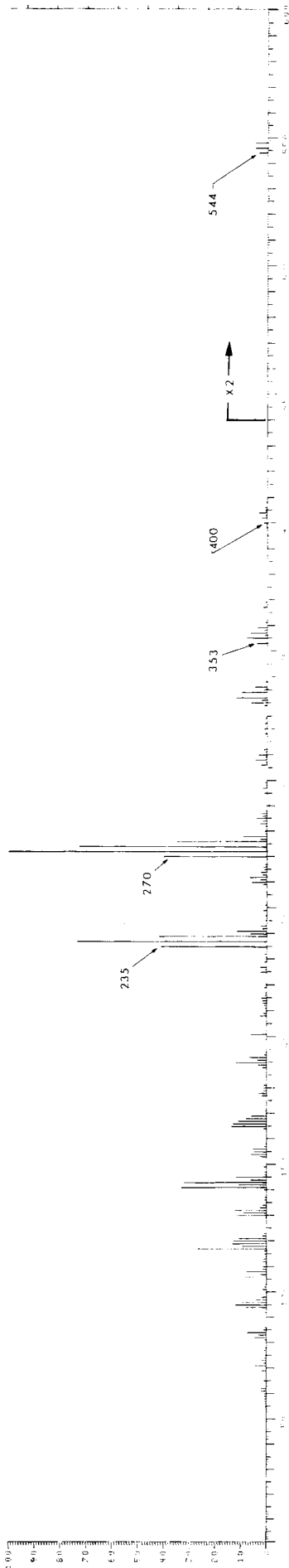


Figure 4. Mass spectrum of Mirex obtained from perchlorinated fraction of adult Herring Gull lipid extract (peak 2, Figure 3).

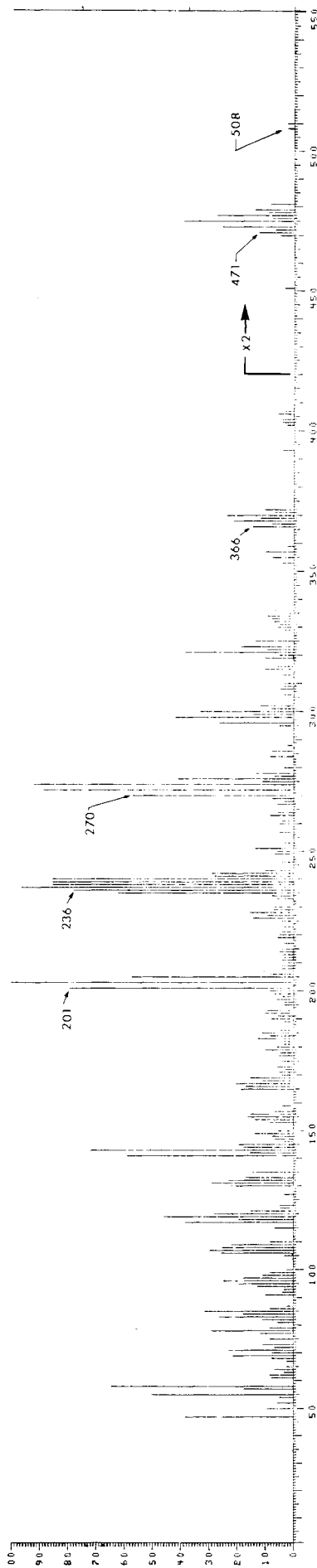


Figure 5. Mass spectrum of photomirex obtained from perchlorinated fraction of adult Herring Gull lipid extract (peak 2, Figure 3).

Table III. Organochlorine Residues (in Parts per Million)^d in Herring Gull Eggs

Year	n	Mean ± standard deviation			
		Mirex	Photomirex	Photomirex/Mirex	PCB ^c
1972 ^a	10	7.2 ± 3.5	3.8 ± 2.0	0.51 ± 0.07	204 ± 50
1974 ^b	9	6.6 ± 2.8	3.7 ± 1.7	0.56 ± 0.09	126 ± 36
1975 ^b	10	5.5 ± 2.9	2.9 ± 1.6	0.51 ± 0.09	82 ± 16

^a Eggs from Scotch Bonnet Island, near Kingston, Ontario. ^b Eggs from Muggs Island, Toronto Harbour. ^c Calculated as Aroclor 1260 according to the method of Reynolds and Cooper (1975). ^d Micrograms of residue/gram fresh weight of egg.

the hexachlorocyclopentadiene fragmentation characteristic of Mirex. The cluster at m/e 236 occurs from the concurrent retro Diels-Alder rearrangement ion of the molecule leading to the formation of a pentachlorocyclopentadiene moiety ($C_5HCl_5^+$). Subsequent expulsion of a chlorine atom yielded the m/e 201 ion abundance. Weak ion fragments are observed at m/e 508 and 510, with a stronger ion abundance at m/e 471 for the ($M - 35$) loss, which supported a molecular composition of $C_{10}HCl_{11}^+$. The mass spectrum was consistent with that of a monohydro Mirex formed by irradiation of Mirex in cyclohexane and isooctane (Alley and Layton, 1974). Only one major monodechlorinated isomer of a possible three was formed on photolysis of Mirex. This monohydro derivative was assigned the structure 1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo[5.3.0.0^{2,6}.0^{3,9}.0^{4,8}]decane. We will refer to this compound as photomirex. The retention time of the monohydro Mirex from the Herring Gull lipid relative to Mirex was 0.75 on an OV-210 column at 180 °C, identical with that of photomirex (Gibson et al., 1972) and different from the other two possible isomers. Therefore, it was concluded that peak 2, Figures 2 and 3, was photomirex.

Peak 4 was confirmed as decachlorobiphenyl on the basis of the mass spectrum obtained. Other peaks evident in this chromatogram represented trace amounts of other organochlorine residues. Trace amounts of hexachlorobenzene, the first major peak in the chromatogram, are known to be present in the gull lipid, but the large amount evident here may well be the result of breakdown of other residues during perchlorination. Unfortunately, the perchlorination procedure forms thermal decomposition products of perchloro DDE, evident as the second and third major peaks in the chromatogram, and the reaction is not quantitative (Hutzinger et al., 1972).

Chromatograms obtained when the same perchlorinated extract was examined with the Hall detector showed strong response for peak 2, photomirex, and peak 3, Mirex.

Quantitative results for PCBs, DDE, Mirex, and photomirex obtained from chromatograms using both EC and Hall conductivity detectors before perchlorination and EC after perchlorination are shown in Table II. The values were comparable for all methods.

Extracts from a series of Herring Gull eggs collected between 1972 and 1975 were then subjected to analysis for DDE, Mirex, and photomirex with the Hall detector, and for PCBs with the EC detector (Table III). No chemical reactions or column separations of PCBs from the pesticide residues were necessary to quantitate these residues. The ratio of Mirex to photomirex remained relatively constant over the 4-year span. The apparent decrease in the levels of all residues over this period is not statistically significant.

DISCUSSION

Two methods are described in this paper for determining Mirex in environmental samples containing high levels of PCBs. Perchlorination of the extracts provided an excellent means for quantitating residues of Mirex and PCBs with the standard EC detector, and for confirming their identity by GC/MS. The procedure is, however, tedious for routine analysis.

Analysis of extracts with the Hall detector provided a more useful method for routinely monitoring the residues of Mirex and DDE in the Herring Gull samples, and led to the detection of photomirex. Removal of PCBs by column chromatography was not necessary. In our hands, the Hall detector in the reductive chloride mode was capable of detecting residue levels of organochlorine

pesticides in the 100-pg range. It was extremely useful for these compounds in the presence of PCB interference, and would prove useful as a confirmatory procedure when identifying trace organochlorine contaminants.

Based on a Mirex standard, >30% of the total Mirex-related residues in the Herring Gulls and eggs was photomirex. This monohydro derivative has been shown to be the primary product of Mirex photolysis (Alley and Layton, 1974; Gibson et al., 1972). Mirex is readily accumulated in both terrestrial and aquatic ecosystems (Mehendale, et al., 1972; Metcalf et al., 1973; Pritchard et al., 1973; Collins et al., 1974), but no metabolites have been detected. Therefore, it can be concluded that the photomirex found in the Herring Gulls was formed by photodegradation of Mirex prior to accumulation in the food web.

The importance of the discovery of photomirex in Lake Ontario is difficult to assess at this time, since virtually nothing is known about its toxicity. The existence of photomirex as an environmental pollutant has hitherto been unreported, even in the southern United States where Mirex has been used extensively. The concentration of photomirex is the fourth highest of any organochlorine residue present in Herring Gulls (after PCBs, DDE, and Mirex). Levels of other pesticides such as dieldrin, chlordane, and their decomposition products, for which there is a history of usage in the Lake Ontario region, are an order of magnitude lower (D. J. Hallett and R. J. Norstrom, unpublished data). An intensive study of Mirex and photomirex in Lake Ontario biota should be made, particularly in fish, to determine whether residue levels and distribution of these compounds constitute a potential environmental hazard in the Great Lakes region.

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