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PROGRESS REPORT TO THE WISCONSIN CONSERVATION DIVISION DEPARTMENT OF NATURAL RESOURCES FOR THE RESEARCH CONTRACT ON CHLORINATED ORGANIC CONTAMINANTS IN THE MILWAUKEE RIVER

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Chlorinated Organic Contaminants in the Milwaukee River

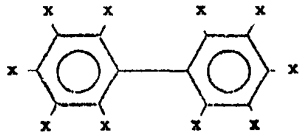
Introduction

The purpose of this study is to examine the chlorinated organic contaminants in the Milwaukee River fish, water and sediment with special consideration to possible contamination by the chlorinated biphenyls which are used as plasticizers. Before the results of the first three months of the study are summarized, however, it would be appropriate to reiterate the reasons this study is essential at this time and to discuss the chemistry of the chlorinated biphenyls in general.

Considerable effort has been expended in the monitoring of chlorinated hydrocarbon concentrations in fish, birds and water from Wisconsin and Lake Michigan. The results have described the widespread presence of the commonly used pesticides such as dieldrin and DDT, as well as the metabolic intermediates such as DDE and DDD. Except in isolated instances, these organochlorine compounds are found at what is considered to be sublethal concentrations. Accordingly, the major concern with the presence of these chemicals in fish and wildlife has been that of the "long-range" effects such as adaptability, reproduction and perhaps extinction.

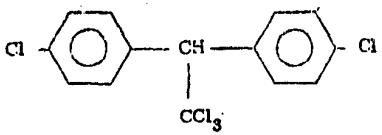
In the last few years, the concern over pesticides in the environment has developed a new dimension in the reports of environmental contamination by the chlorinated biphenyls used in industry. These compounds are widely used in the production of plastics, rubber, paints, dielectrics and packaging material throughout the world and are reportedly becoming widespread in the environment (Risebrough et al., 1968). The chlorinated biphenyls are prepared by chlorinating the biphenyl molecule as shown in Figure 1-a. The product is a complex mixture of chlorinated hydrocarbons containing between 1 and 10 chlorine atoms per molecule. Although over 200 compounds are theoretically possible, preliminary evidence indicates considerably fewer are present in commercial mixtures. The similarity of the chlorinated biphenyl structure to pp'DDT and pp'DDE is illustrated

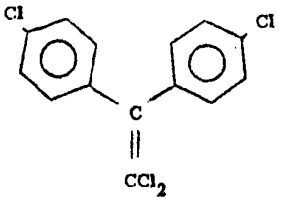
Figure 1. Structural similarities between the chlorinated biphenyls and common pesticides.



a. Chlorinated biphenyl (x indicates possible Cl positions for 1-10 Cl atoms).

b pp'DDT (2, 2-bis(p-chlorophenyl)-1, 1, 1-trichloroethane).





c. pp'DDE (2, 2-bis(p-chlorophenyl)-1, 1-dichloroethylene).

by comparing Figure 1a with Figures 1b and 1c respectively.

The chemical similarities of the chlorinated biphenyls with other chlorinated pesticides also create the possibility of interferences in pesticide determinations. Figure 2 is a chromatogram of a commercially available chlorinated biphenyl mixture, and the retention times for the common pesticides are indicated. It is evident that some interference would be experienced for many of the pesticides if this mixture were present in samples to be analyzed at sufficient concentrations. In addition, 7 chlorinated biphenyl mixtures, ranging from 21 to 62 percent chlorine, are prepared by Monsanto Company under the name "Arochlor."

Figure 3 is a simulated chromatogram indicating the retention times of the components of the chlorinated biphenyl mixtures. It should be pointed out that the compounds of the chromatograms in Figure 3 are not resolved completely by typical gas chromatography (GLC) conditions, but are partially superimposed such as in Figure 2. Also, since the mixtures are complex and unidentified, and since environmental samples may contain any combination of the 7 mixtures, there is no quantitative or qualitative method to examine the gross sample extracts.

Reports from Wisconsin (Degurse, 1969; Hickey, 1969) have indicated that fish and birds from the larger industrial centers contain many unidentified organochlorine compounds which behave chemically as the chlorinated biphenyls. Therefore, since the concentrations of these unidentified compounds are just becoming detectable, and it has been implicated that industry may be becoming a major source of these chlorinated hydrocarbons, chemical studies of the unidentified compounds are essential in order that early control measures can be exercised if necessary.

Environmental Sampling

As was previously stated, the purpose of this study is to investigate earlier reports that fish from the Milwaukee River contained many unidentified organochlorine compounds.

Figure 2. Commercial Mixture of Chlorinated Biphenyls

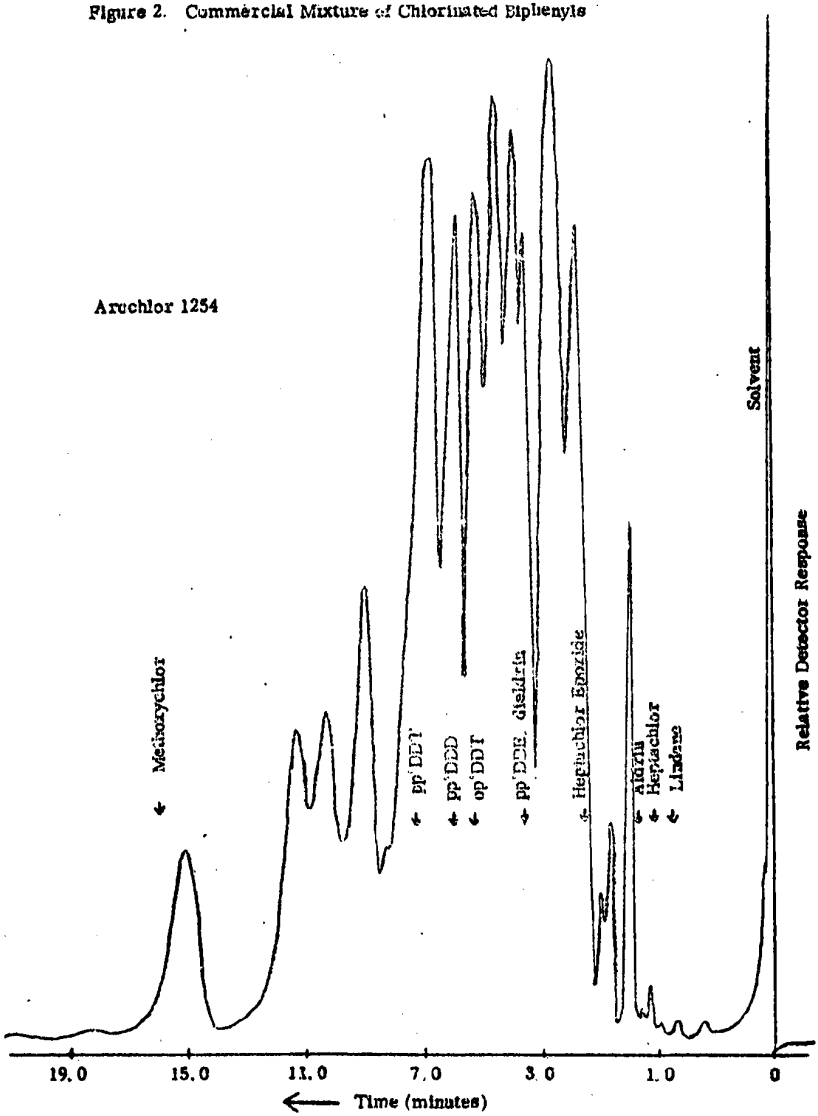


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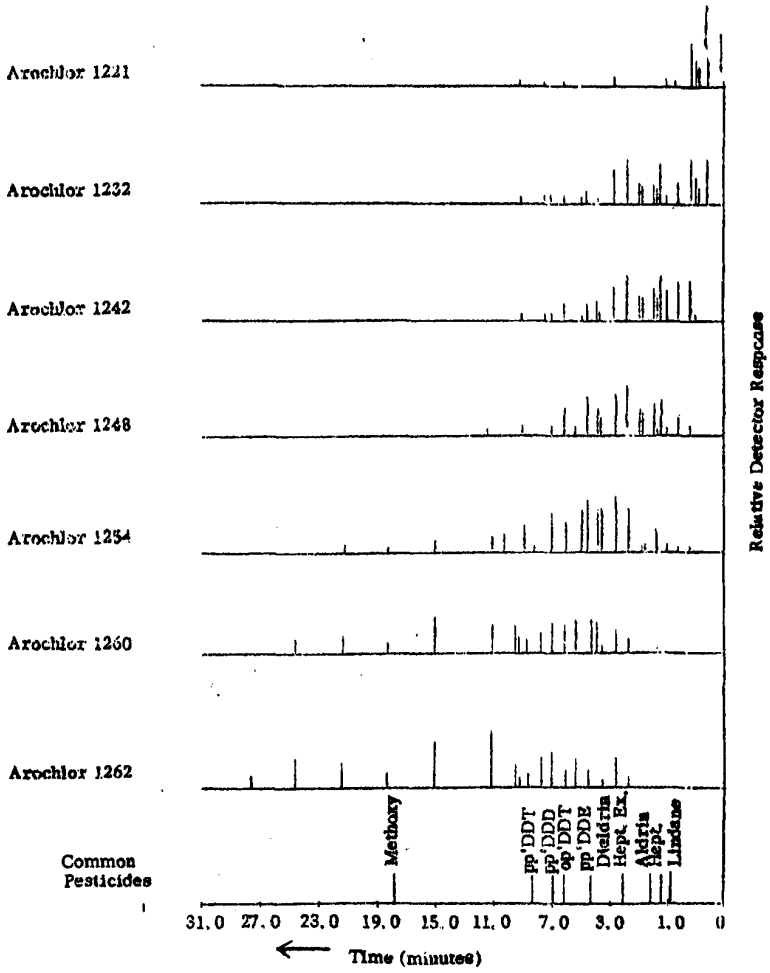
Figure 3. Simulated Chromatograms of the Arochlors



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Therefore, 11 sites on or near the river have been tentatively established for environmental sampling. Many of the locations were selected because fish samples were available from other WCD studies.

Figure 4 is a map of the study area with the sampling sites numbered 1 through 11. Site 1 is a tributary on County road "O" just below a plastics manufacturing firm. Sites 2 and 3 are located about 200 yards above the dams at Grafton and Thiensville, respectively. Site 4 is below the Thiensville dam and just above the Hwy. 167 bridge. Sites 5 and 6 are 200 yards above the dams at Glendale and Estabrook Park, respectively. Site 7 is just below the North Avenue dam and site 8 is at the Broadway Avenue bridge. Site 9 and 10 are located about 200 yards above the mouths of the Menomonee and Kinnickinnic rivers, respectively, where these rivers join the Milwaukee River. Finally, site 11 is located in the center of the harbor breakwater opening.

Preliminary Water Analysis

The sampling locations were sampled on August 13-15, 1969, for routine water analysis and pesticide analysis. Samples were obtained just below the surface at each site and in the center of the main channel. In addition, dissolved oxygen (DO) and temperature profiles were obtained where possible. Glass carboys (20 l.) were filled at approximately the 0.5 meter depth and were sealed with aluminum foil for pesticide analysis.

The data from the first sampling are presented in Table 1. In general, the DO, pH and suspended solids concentration were much higher in the water above the dam than in the lower part of the river. For example, above the dam at Grafton, the water contained 10.0 mg/l DO, 60.5 mg/l suspended solids, and a pH of 8.7. On the other hand, the water at the Buffalo Avenue bridge in Milwaukee contained 4.2 mg/l DO, 14.7 mg/l suspended solids, and a pH of 7.7. The relatively high DO and pH can be explained by the photosynthetic processes of plankton, and the high suspended solids in the quiet waters behind the dams appeared to be essentially planktonic.

The water in the lower river was characterized by low DO and suspended solids

Figure 4. Milwaukee River Study Area

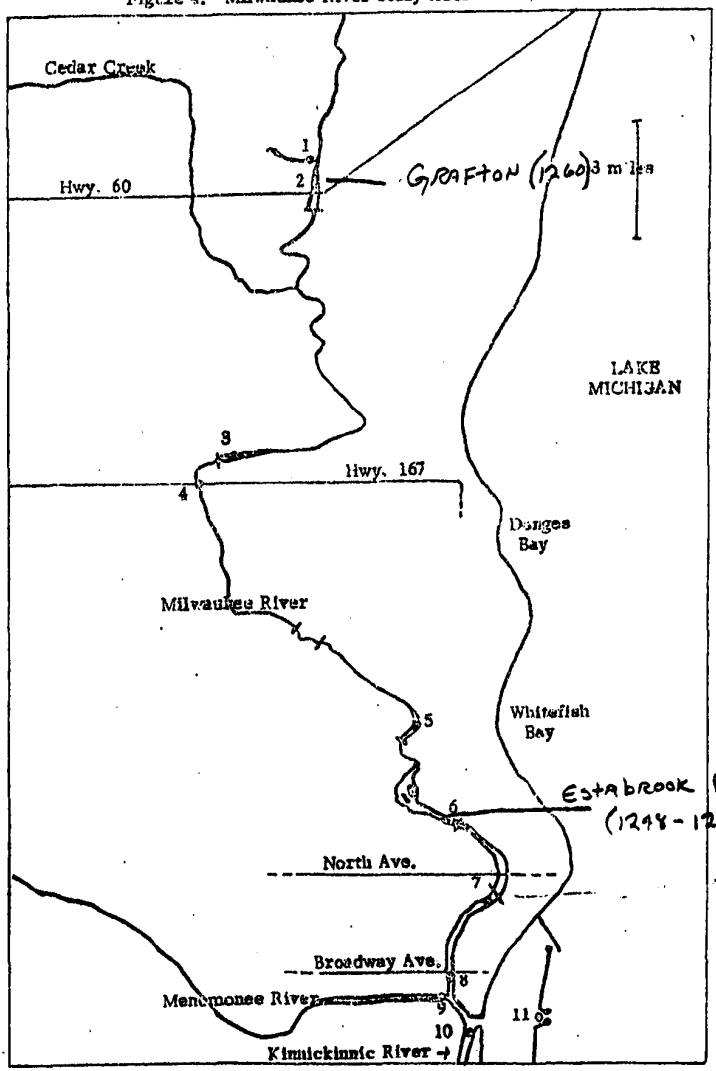

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Table 1. General Characteristics of Milwaukee River

Site	Depth (m)	Secchi (m)	Temp. (°C)	DO (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Conductivity (umhos/cm 23°C)	Suspended Solids (mg/l)	Turbidity (mg/l SiO_2)	Color (units)
1	0.3	--	24.5	10.1	8.8	242	670	12.2	13	25
2	1.7	0.4	26.0 (5)	10.0	8.7	257	720	60.5	21	40
			25.5 (1)	8.8						
3	2.1	0.6	27.5 (5)	13.6	8.9	245	720	30.0	21	35
			25.0 (1)	9.0						
4	0.5	--	27.2	10.2	9.2	251	740	24.0	21	35
5	0.8	--	27.0	15.4	--	--	--	--	--	--
6	2.4	0.3	30.0	15.6	9.0	232	745	50.0	17	35
7	5.4	0.8	26.0 (5)	8.5	8.6	196	642	14.6	18	30
			22.1 (5)	8.3						
8	7.0	1.0	28 (5)	4.2	7.7	145	505	14.7	12	20
			20 (5)	3.5						
9	9.6	1.1	27 (5)	3.4	7.2	131	565	10.6	14	15
			23 (5)	3.6						
10	10.0	0.7	23 (5)	4.1	7.3	127	478	5.0	16	10
			17 (5)	5.2						
11	10.4	1.7	19 (5)	8.7	8.0	118	420	5.6	19	5
			8.9 (10)	9.0						

concentrations, lower pH, and lower temperature. In addition, the alkalinity and conductivity decreased downstream from site 7 from 196 mg/l and 642 umhos/cm, respectively, to 118 mg/l and 420 umhos/cm at site 11. This decrease may be due to the input of Lake Michigan water near site 7. Blooms of algae and schools of goldfish were observed at site 7, but within a half-mile downstream, no algae or fish were evident and the DO dropped sharply. Oil films and floating objects were continuous in the lower river.

Development of Analytical Procedures

The analytical procedures used in this study are intended to include the general class of organochlorine molecules, and not to be selective for specific chlorinated pesticides. The basic methods are widely accepted, and only slight modifications as described by Degurse (1969) and Reynolds (1969) have been introduced.

Extraction. Fish are homogenized while frozen (-20°C) by grinding at the Nevin Fish Hatchery laboratory. Approximately 10 gm of the frozen sample are blended with 70 gm Na_2SO_4 (anhydrous) until the mixture appears dry. The sample is then extracted for 3.5 hours in an all-glass Soxhlet using a mixture of hexane and ethyl ether (1:1 v/v, 170 ml). The extract is concentrated to 15 ml in an air stream.

Water Samples (20 l.) are batch-extracted with hexane in 2-liter separatory funnels. The samples are not filtered because the effect of filtering large volumes of turbid water on pesticide recovery has not been evaluated. Hexane (400 ml) is placed in 6 funnels, and the water (1600 ml) is introduced in the first 3 funnels. After repeated shaking and settling, the aqueous layers are drained into the 3 remaining funnels. The process is repeated until the 20-liter sample is extracted twice. The hexane portions are concentrated to 15 ml for separate cleanup before they are combined.

Air-dried plankton (less than 1 gm) and sediment samples (10-30 gm) are extracted for 30 hours in an all-glass Soxhlet using the azeotrope of hexane and acetone (41:59, 170 ml) as has been described by Veith (1968). The extract is evaporated to 15 ml in an air stream after adding an excess of hexane.

Extract Cleanup. The cleanup of extracts on Florisil has been studied at the Water Chemistry Laboratory (Hughes, 1968; Veith, 1968). In general, most pesticides can be separated from the bulk of the interferences on a Florisil column because fats, oils, pigments, etc., are retained on the column, waxes and relatively non-polar compounds pass with the hexane solvent front and can be discarded with the first 30 ml of eluate, and the majority of organochlorine pesticides can be eluted with 175 ml of 15 percent ethyl ether in hexane. Reynolds (1969) has reported that chlorinated biphenyls are quantitatively removed from Florisil by 200 ml hexane, along with heptachlor, aldrin, and DDE. Lindane (γ-BHC), heptachlor epoxide, DDD, DDT and dieldrin are not eluted with hexane but can be recovered by elution with 200 ml of 20 percent ethyl ether in hexane. Thus, this latter group of pesticides could presumably be analyzed without possible interference by the chlorinated biphenyls.

Florisil (Kensington Scientific) is extracted in an all-glass Soxhlet for 24 hours with the azeotrope of hexane and acetone in an effort to remove traces of organic impurities. The solvent is evaporated from the Florisil at 105°C and the solid is heated at 650°C for 2.5 hours. If not used immediately after heating, the Florisil is warmed to 105°C just prior to its use.

The Florisil (30 gm) is vibrated into a 25 mm O.D. glass column fitted with a glass frit and Teflon stopcock. The column is topped with 10 gm anhydrous Na_2SO_4 to prevent deactivation from water in the sample. Fish, water, plankton and sediment extracts (15 ml) are placed on the dry column and eluted with 250 ml of 15 percent ethyl ether in hexane. The eluant is concentrated in an air stream. With this procedure, only extracts from low-fat fish are suitable for GLC analysis.

Repeated chromatography on Florisil is necessary to remove the final traces of interferences and to obtain the separation of several pesticides as is described by Reynolds (1969). Florisil columns (19 gm) are prepared as above. The samples from the 30 gm Florisil column is concentrated to 15 ml and placed on the 19 gm column. Elution with

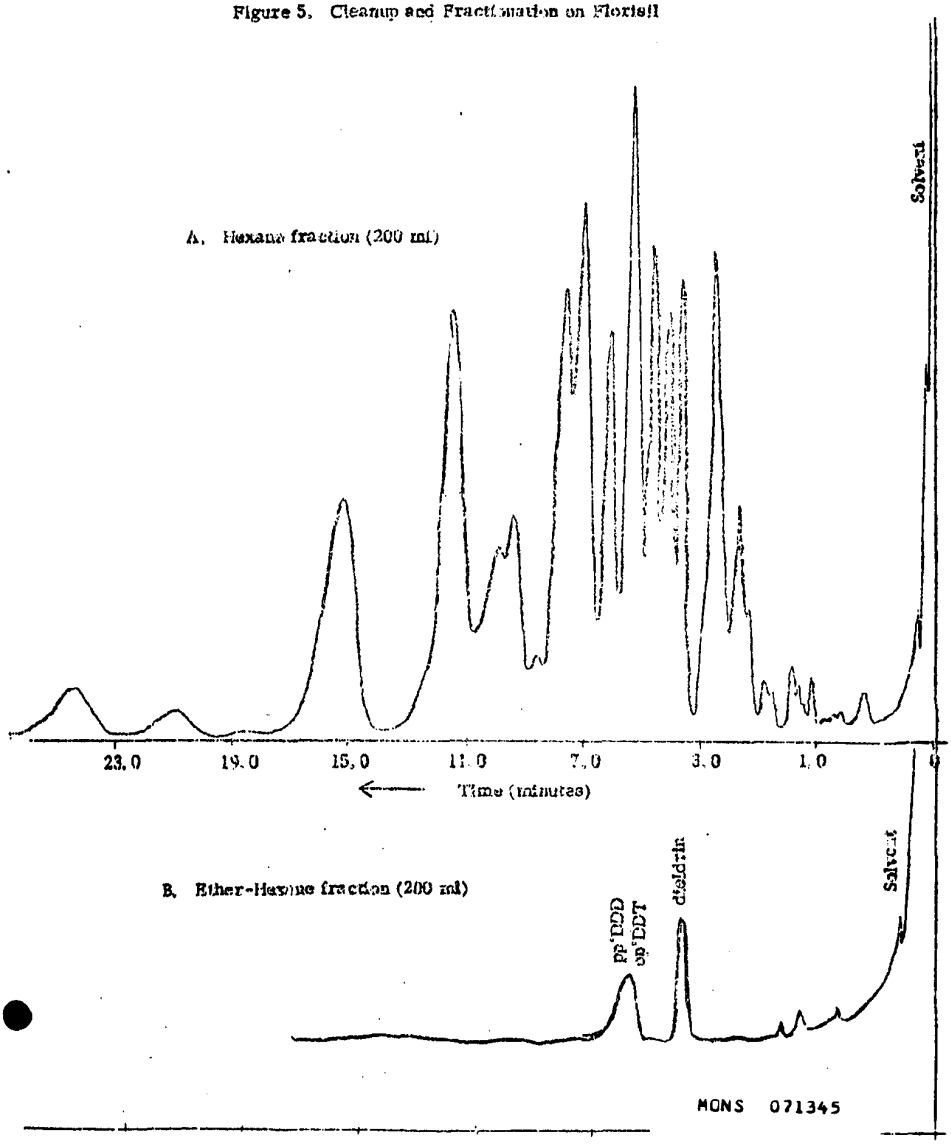
hexane (200 ml) is begun at 3-5 ml/min.to obtain DDE, heptachlor, aldrin, toxaphene and chlorinated biphenyls, if present. The receiving flask is changed and the column is eluted with 20 percent ethyl ether in hexane (200 ml) to obtain DDT, DDD, dieldrin, heptachlor epoxide, and lindane, if present. The samples are concentrated to appropriate volumes for GLC analysis.

Based on preliminary data, the above procedure results in excellent cleanup of fish, water and suspended solids analysis. Figure 5a is a chromatogram of a fish extract from Estabrook Park after cleanup with hexane. Figure 5b is the corresponding chromatogram of the 20 percent fraction. Both chromatograms have a rapid return to baseline following the solvent peak which suggests that cleanup is complete. In 5b, it is indicated that dieldrin is present by the first peak, and either op'DDT and/or pp'DDD is present by the second peak. Confirmation of op'DDT could be made by dehydrohalogenation with KOH/EtOH since op'DDT is converted to op'DDE and pp'DDD would be converted to op'DDMU.

As is seen in Figure 5a GLC chromatograms of the extracts from Milwaukee River samples are difficult to interpret because of the numbers of components present. A variety of GLC conditions have been studied in an effort to gain maximum resolution in a practical analysis time. Liquid phases which have been used include DC-200, OV-101, OV-17, FFAP, XE-60 and QF-1. It is evident that OV-101 is a very suitable non-polar solvent. Also, more polar mixtures of FFAP/XE-60 (1:1) and QF-1/OV-17 (2:1) resulted in good separation but the analysis time was increased to over 65 minutes. Therefore, to obtain the satisfactory resolution in less time, it was necessary to employ low-loaded, etched-glass beads as supports for the polar phases.

The glass beads (GLC-110, 120-140 mesh) were coated with 0.05 and 0.18 percent of the FFAP/XE-60 (1:1) and QF-1/OV-17 (2:1) mixtures. The evaluation of these phases has not been completed. Nonetheless, all chromatograms presented in this report were obtained from 7 ft.x 1/8 in. columns packed with the 0.05 percent QF-1/OV-17 mixture

Figure 5. Cleanup and Fractionation on Florisil



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on the glass beads. A N_2 flow of 27 ml/min was maintained in an Aerograph 1520-B gas chromatograph which is equipped with concentric tube electron capture detectors. The column, detector, and injector temperatures were 180°C, 215°C and 230°C, respectively.

Experimental Results

The initial objectives of this study have been to determine the relative amounts of chlorinated organic compounds in fish and water from the Milwaukee River. The research approach has been to attempt to duplicate existing procedures for fish analysis, and then to investigate the occurrences of unidentified compounds which have been found by Degurse (1969). Degurse has noted that "obscure peaks" begin to appear in chromatograms of fish extracts as sampling becomes nearer to industrial centers such as Milwaukee. Indeed, in some of the fish near Milwaukee, the pesticide peaks are not detectable in large numbers of unidentified peaks. These observations, along with the fish samples provided, have enabled this laboratory to establish a study area immediately rather than monitoring many areas of Wisconsin to find the more frequently occurring unidentified compounds.

Fish Extracts. To achieve the initial objectives, fish from along the river from County road "H" bridge north of West Bend to Milwaukee were analyzed. It immediately became apparent that the complexity of the organochlorine mixtures in the fish was so great that quantitative analyses were not realistic. Consequently, the data are presented in the form of chromatograms which represent equal amounts of fish. In this manner, the relative amounts of organochlorine compounds can be compared directly. Figures 6a, 6b and 7 are chromatograms of fish extracts from County road "H" bridge (Washington County), Grafton and Estabrook Park, respectively. It should be pointed out that the response in 6a is multiplied by a factor of 4 to observe the peaks; so a direct comparison with 6b or 7 would require the peaks first be reduced by a factor of 4. It is evident that these figures substantiate the work of Degurse in that the numbers and relative magnitudes of the unidentified peaks increases about 20-fold in fish from Milwaukee in comparison to fish from Washington County. These results also suggest that the contamination may be of industrial

Figure 6. Fish Chromatograms from Upper Milwaukee River

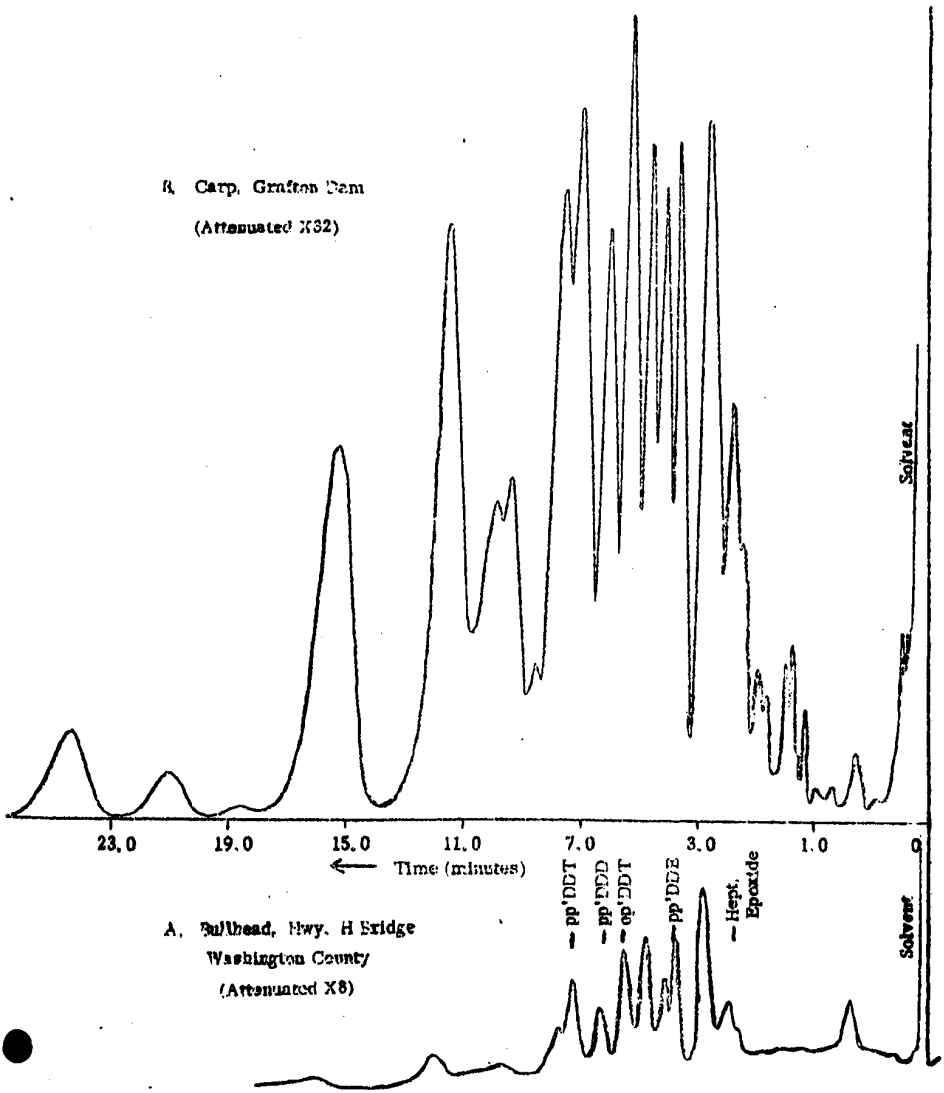
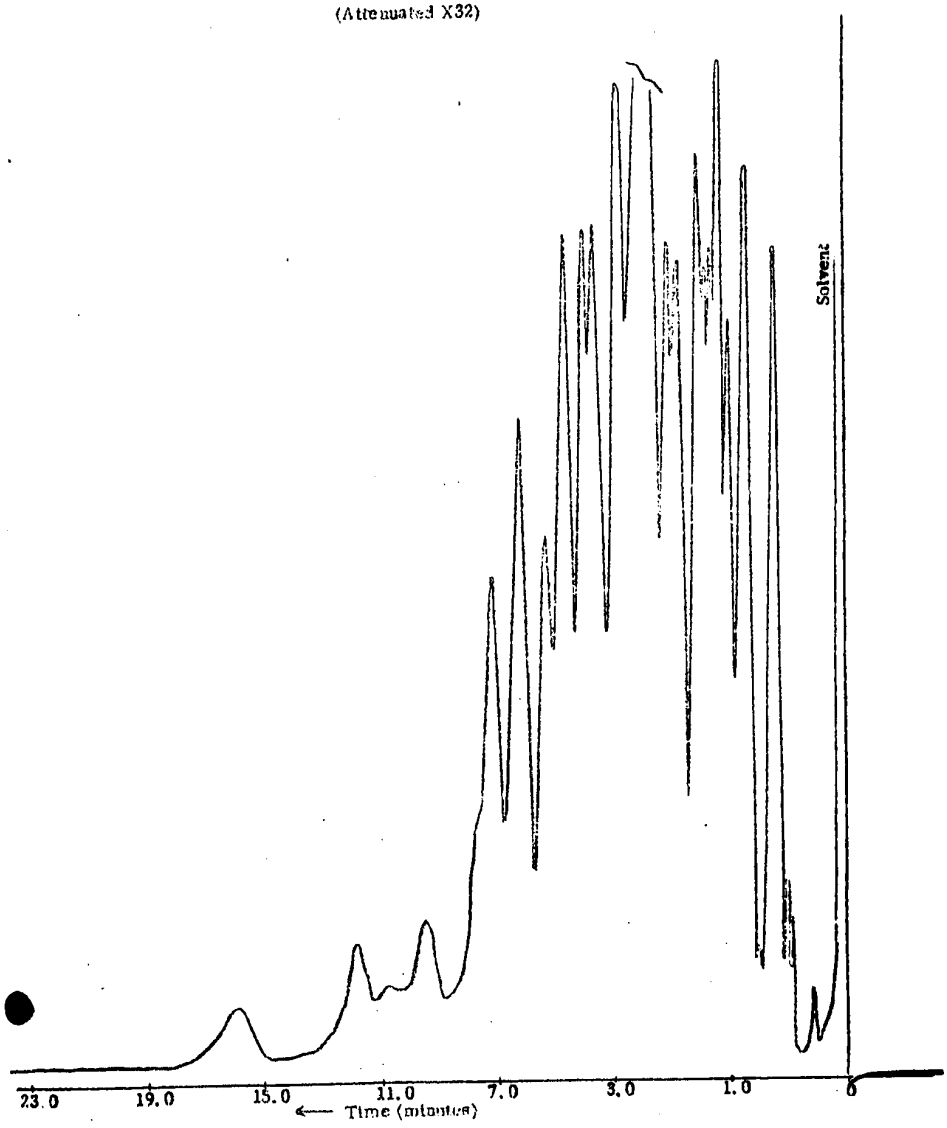


Figure 7. Goldfish from Estabrook Park

(Attenuated X32)



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origin, or at least associated with metropolitan centers.

Water Extracts. Approximately 20 liters of water from the Milwaukee River sampling sites were extracted and concentrated about 2,000-fold. The highest levels of organochlorine compounds (on a comparative basis) were found near Estabrook Park. Figure 8 presents a chromatogram of the water extract from Estabrook Park which illustrates that many unidentified compounds are present, although at very low levels. Once again, the complexity of the mixtures makes quantitative and/or qualitative analysis impossible at this time.

A number of the peaks in the chromatogram in Figure 8 have retention times similar to heptachlor epoxide, dieldrin and pp'DDE. However, these same peaks and others also have retention times similar to components of technical chlordane and several chlorinated biphenyl mixtures. Thus, the possible serious errors which are introduced into an analysis become evident when the analyst interprets retention time data in GLC chromatograms as qualitative data. It may be much more meaningful to interpret chromatograms of unidentified compounds in terms of what chemicals are not present, rather than assign identities to peaks which are present.

Characterization of Unidentified Compounds. Although it is apparent that chromatograms cannot be interpreted as a means of identification in many cases, the appearance of general peak patterns such as peak shoulders, doublet peaks and relative peak heights of major peaks is of interest to the analyst. For example, Figure 9a is a chromatogram of a carp extract from the Grafton area and shows a very strikingly similar pattern to Figure 9b, which is a chromatogram of 10 ng of the chlorinated biphenyl mixture, Arochlor 1260. If the total areas of each chromatogram are calculated, it might be concluded that the carp near Grafton contain approximately 12 ug/gm of equivalent Arochlor 1260 (the concentration of each individual component would be substantially less), and that Arochlor 1260 is being used and discharged into the river in or near Grafton. However, while these comparisons are interesting and provide the analyst with a "best guess" in directing further

Figure 8. Chromatogram of Water Extract from Estabrook Park (X 1, 940)

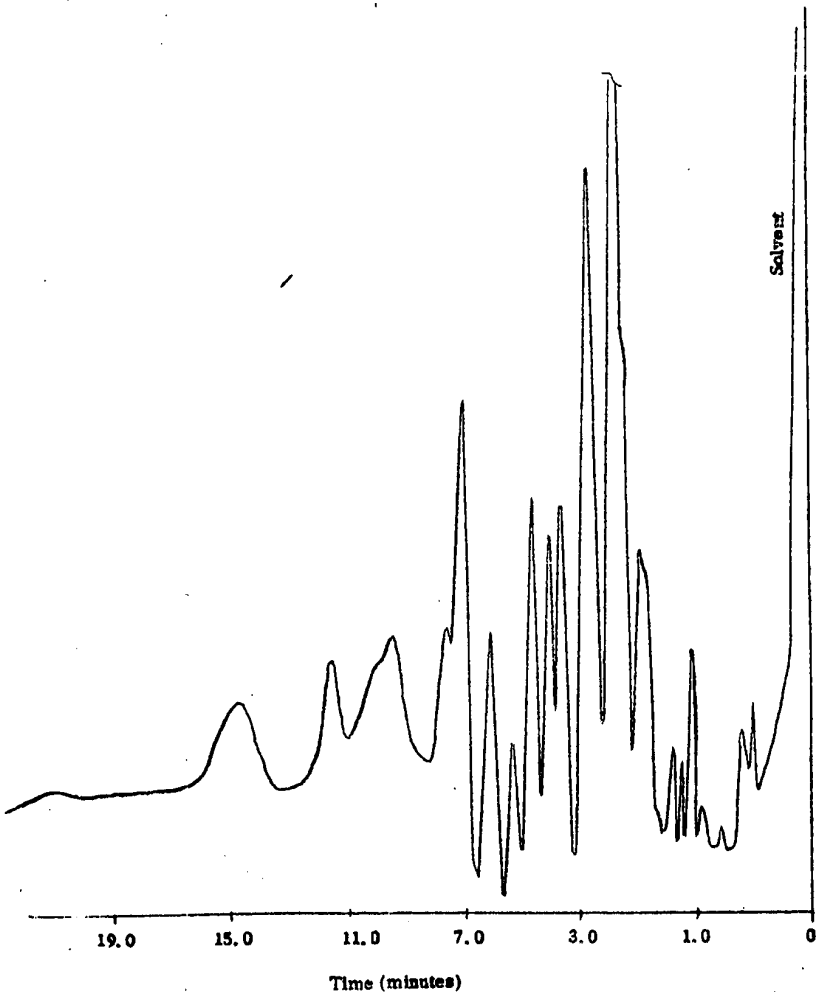

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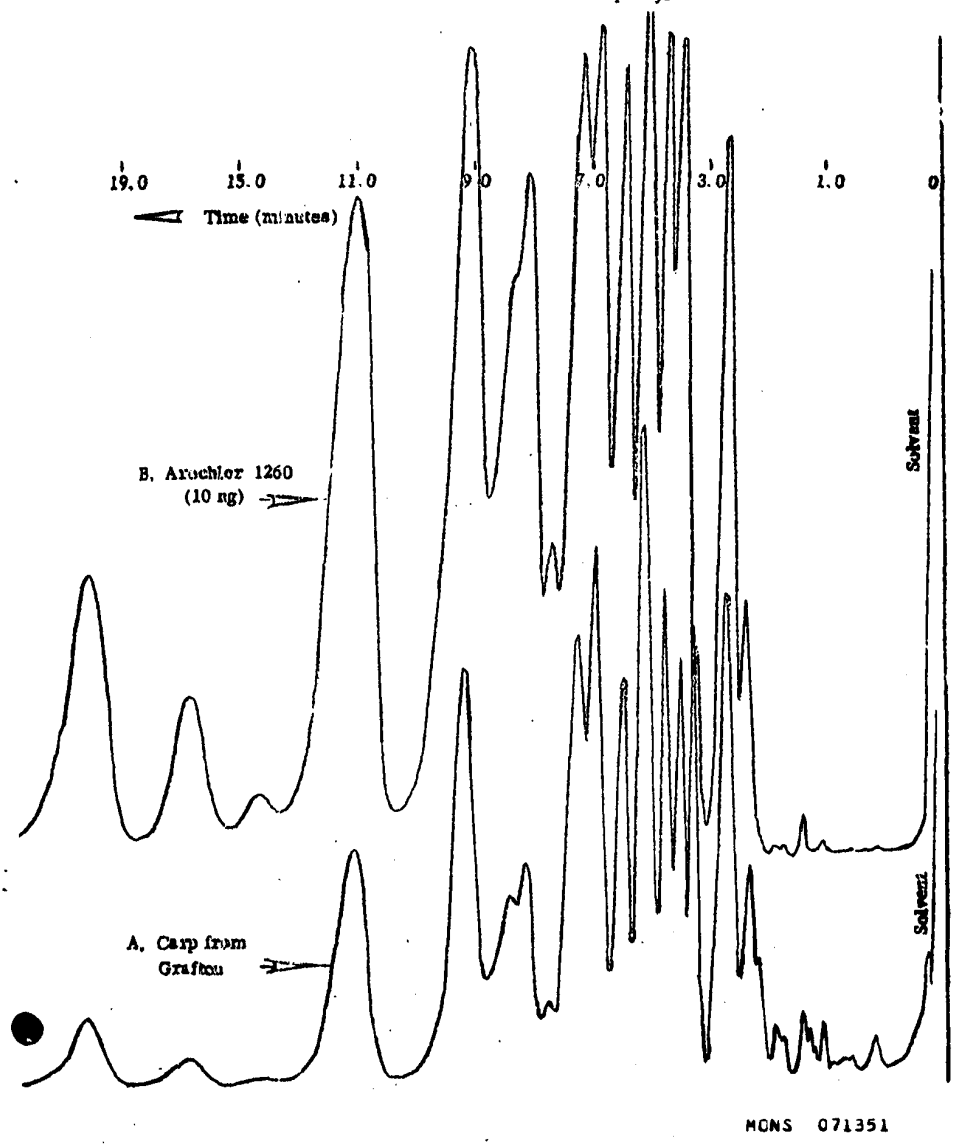
Figure 9. Comparison of Fish Extract Chromatogram with Arochlor 1260 Chlorinated Biphenyl



research, they are only presumptive evidence and it would be improper to assign a chemical identification to any of the components with the information available.

It is also interesting to compare the chromatograms of fish extracts from Estabrook Park to those from Grafton. For example, in comparing Figure 9a with Figure 7, it is apparent that earlier eluting major peaks are more prominent as the samples are obtained from further downstream in the river. By the same analogy used above, it might be concluded that the goldfish at Estabrook Park contained approximately 15 ug/gm of an equivalent Arochlor (chlorinated biphenyl mixture) with an average chlorine content of slightly less than that predominant at Grafton. By comparison, the fish extract is similar to either Arochlor 1248 or 1254 (containing 48 and 54 percent chlorine, respectively). If this were true, it might be concluded either that lower chlorine-content Arochlor mixtures are used and discharged in or near Estabrook Park and the Arochlor 1260 from upstream at Grafton was removed by the river sediments, etc., or that the Milwaukee River has received contamination from a source above Grafton and the mixture of chlorinated organic compounds is being selectively degraded as it is carried downstream. (Toxaphene is thought to be selectively degraded, presumably by removing chlorine atoms; environmental samples show a similar shift to earlier eluting major peaks in chromatograms,(Hughes and Lee, 1967).) However, all of the above reasoning is derived from presumptive data, and it is not the intention of this report to conclude that the unidentified compounds are chlorinated biphenyls, that they are of industrial origin or that the numbers presented in the discussion are realistic outside the context of use.

Conclusion

The data in the form of GLC chromatograms indicate that unidentified compounds are present in fish and water extracts after routine pesticide analysis of samples from the Milwaukee River downstream

from Grafton. Similar data indicated that the unidentified compounds were not present (or at least were present at much lower levels) in samples from the West Bend area. These

conclusions are consistent with previous reports of "obscure peaks" in fish chromatograms from the study area (Degurse, 1969).

On a very presumptive basis, the data also suggests that the possibility of chlorinated biphenyl contamination cannot be dismissed. Future work in this laboratory will be aimed at evaluating this possibility.

From the chromatograms presented, it is evident that the complexity of the mixtures would cause any monitoring studies aimed at quantitative analysis of these mixtures in fish and water to be meaningless and misleading. This is compounded by the fact that the history of the samples is essentially unknown except for sampling location when studying a system such as the Milwaukee River. Thus, it is concluded that qualitative analyses of the fish extracts will have to be performed before additional environmental work is begun. Accordingly, this laboratory has directed research to that end.

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APPENDIX

To supplement this report, a study of the present state of knowledge of chlorinated biphenyl compounds as it appears in the literature is presented. This review is intended for publication by the principal investigators.