Isolation and Identification of Polychlorinated Styrenes in Great Lakes Fish

D. W. Kuehl, H. L. Kopperman, G. D. Veith,
and G. E. Glass
U.S. Environmental Protection Agency
National Water Quality Laboratory
6201 Congdon Boulevard
Duluth, Minn. 55804

In 1969 an octachloro compound with mass number 376 was reported to be present in eider (Somaferia mollissima) and sandwich terns (Sterna sandvicensis) from the Rhine River in Europe and the Netherlands coastal area (KOEMAN et al. 1969). Further investigations revealed that this compound was also present in cormorant tissue, and it was identified by gas chromatography-mass spectrometry as octachlorostyrene (ten NOEVER de BRAUW and KOEMAN 1972/73). However, this chlorocarbon has not previously been reported in environmental samples from North America. This paper reports the confirmation of polychlorinated styrenes in fish from Lake Ontario, Lake Huron, and the Detroit River.

METHODS AND MATERIALS

Analysis of Fish Tissue

Composite fish samples from Saginaw Bay of Lake Huron, Lake Ontario, and the lower Detroit River were obtained from the 1972-73 collection of the U.S. Bureau of Sport Fisheries and Wildlife, Great Lakes Fisheries Laboratory. Extracts were prepared for pesticide analysis according to the procedure outlined by VEITH et al. (1975) and analyzed by gas chromatography-mass spectrometry (GC/MS) (KUEHL et al. 1974a).

Briefly, 500 g of fish tissue were blended with enough anhydrous Na_2SO_4 to completely dry the sample to a powder. The mixture was then extracted with hexane/ethyl ether (75:25) in a Soxlet extractor for 24 hours. Excess solvent was removed with a Kuderna-Danish apparatus fitted with a 3-ball Snyder column to yield approximately 30 g of oil. The oil was then thoroughly mixed with 15 g of prepurified Micro Cell-E (Johns-Manville)[1] to absorb the bulk of the lipids. A pesticide-enriched residue was separated from the Micro

---

[1] Mention of trade names does not constitute endorsement by the U.S. Environmental Protection Agency.

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 2

Cell-E by extraction with acetonitrile/acetone (95:5) to yield approximately 1 g of oil. This residue was diluted to 100 mg/ml with cyclohexane and placed on a 25- x 230-mm SX-2 (BIO-RAD Laboratories) gel permeation column for final cleanup. The column was eluted at 3.5 ml/min with cyclohexane, and the 100-200 ml fraction, which contained the pesticides, was collected. The sample was concentrated to 0.10 ml for GC/MS analysis.

Analysis by GC/MS of the residues was performed on a Varian MAT GC/MS/computer system previously described (KUEHL _et al._ 1974b). The GC was equipped with a 2 m x 2 mm glass column packed with 3% OV-101 on 80/100 mesh Gas Chrom Q. All residue work was done with a temperature program consisting of a 5 minute hold at 125° C followed by a 4° C/min rise to 235° C.

Preparation of Octachlorostyrene

Octachlorostyrene was prepared (by two independent synthetic routes) to be used as a standard for GC and GC/MS data.

Procedure A. Chlorine was bubbled through a solution of 500 mg of styrene in 20 ml $CHCl_3$ containing 5 mg $FeCl_3$ (Merck and Co.) to yield a mixture of di- and trichloroethylbenzenes. The CCl_4 was removed under reduced pressure, and the resulting residue was perchlorinated with $SbCl_5$ (ALFA Inorganics) at 175° C for 4 hours according to the procedure reported by BERG _et al._ (1972).

Procedure B. Chlorine was bubbled into a solution of 500 mg styrene in 20 ml CCl_4 until the solution was saturated with $Cl_2(g)$ (10 min). The solution was then irradiated with uv light (254 nm) for 30 minutes. This procedure was repeated until the electron capture/GC trace indicated no further change in the reaction mixture.

RESULTS AND DISCUSSION

An octachloro compound, molecular weight 376, was observed by GC/MS at the same retention time in residues of walleye (_Stizostedion vitreum vitreum_) from Lake Huron and the lower Detroit River and alewife (_Alosa pseudoharengus_) from Lake Ontario. The relative retention time (r.r.t.) of this component vs. p,p'-DDE was calculated and appeared to be similar to data presented by ten NOEVER de BRAUW and KOEMAN (1972/73) for octachlorostyrene (OCS). However, preparation of an OCS standard was necessary to confirm this chlorocarbon in Great Lakes fish.

Analysis by GC/MS of the reaction products of synthetic procedures A and B (Methods and Materials) showed an octachloro compound at identical retention times. Spectra obtained for this compound were interpreted to be octachlorostyrene. In addition, a second compound with a mass spectrum identical to OCS and a GC retention time 1.68 times greater than OCS was observed in the reaction mixture obtained from procedure B. The second compound was confirmed as decachloroethylbenzene (DCEB), which loses Cl_2 in either the helium separator or MS ion source to yield OCS. BALLESTER and CASTANER (1960), BALLESTER _et al._ (1960), and SEIBER (1971) have shown that loss of a chlorine molecule can occur readily to relieve steric strain in perchloroalkylaromatics such as DCEB.

Table 1 lists GC retention times for OCS found in the fish extracts and for OCS standard with the temperature program described above. An average value of 0.852 (relative to p,p'-DDE) was obtained for the three residue samples, and a value of 0.850 was obtained for the standard.

TABLE 1 Relative retention times for octachlorostyrene (OCS) in fish extracts and OCS standard obtained with a temperature-programmed column

Sample	Relative retention time (DDE)
Lake Huron walleye (_Stizostedion vitreum vitreum_)	0.850
Detroit River walleye (_Stizostedion vitreum vitreum_)	0.855
Lake Ontario alewife (_Alosa pseudoharengus_)	0.851
OCS standard	0.850

The retention times of OCS relative to aldrin and DDE under GLC conditions used in conventional pesticide identifications (THOMPSON 1974) are presented in Table 2. The data indicated that OCS has a retention time 1.24 times greater than aldrin and 0.67 times as great as p,p'-DDE.

Table 2 Relative retention times obtained with an isothermal column (180° C)

Compound	r.r.t. vs. aldrin	r.r.t. vs. p,p'-DDE
Aldrin	1.00	0.54
OCS	1.24	0.67
DDE	1.85	1.00

Figure 1 shows mass spectra of OCS from m/e 200 to m/e 400 as observed in Lake Ontario alewife residue and OCS standard. Both spectra show an octachloro isotope pattern at m/e 376-386 followed by the losses of chlorine to a heptachloro pattern at m/e 341-351 and a hexachloro pattern at m/e 306-316. These three ion-cluster mass ranges are the most predominant and are characteristic of OCS. Table 3 shows the measured and calculated intensity of the OCS parent ion.

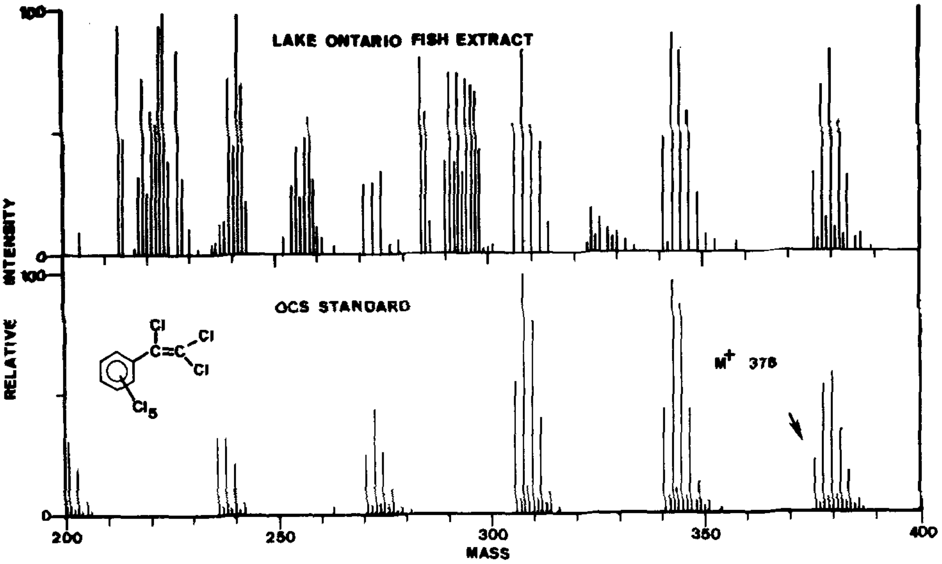

Fig. 1. Mass spectra of octachlorostyrene standard and fish extract.

Table 3 Measured and calculated intensities of the octachlorostyrene parent ion

Ion mass intensity	376	378	380	382	384	386
Measured	39.39	87.12	100.00	62.12	26.51	7.57
Calculated	33.55	87.58	100.00	65.24	26.62	6.93

In addition, mass spectral data show three heptachloro compounds at m/e 342 and three hexachloro compounds at m/e 308 with the same spectral pattern as observed in the OCS spectrum. It appears that these compounds are heptachlorostyrene (MW 342) and hexachlorostyrene (MW 308) isomers. A mass chromatogram of mass range 306-310 is plotted for the Lake Ontario residue data (Fig. 2). The 306-310 mass range includes the molecular ion of hexachlorostyrene isomers, M+-Cl ions from heptachlorostyrene, and M+-2Cl ions from OCS. Peaks observed in Fig. 2 at spectra 68, 77, and 85 are hexachlorostyrene isomers; at 91, 104, and 114 are heptachlorostyrene; and at 125 is OCS.

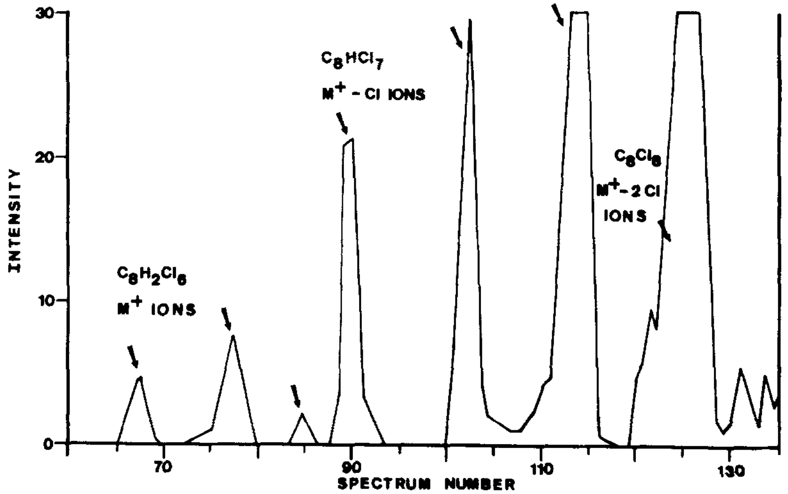

Fig. 2. Mass chromatogram of Lake Ontario fish extract. Mass range 306-310.

SUMMARY

Octachlorostyrene was prepared by two independent synthetic routes, and GC retention time data as well as a mass spectrum of the standard were obtained. A comparison of these data with those observed for a component in fish from Lake Huron, Lake Ontario, and the lower Detroit River indicates that octachlorostyrene is present in fish residues from these sources. In addition, mass spectral data indicated the presence of several hepta and hexachlorostyrene isomers in these fish. Further studies should be concerned with quantification of OCS and evaluation of possible sources of these compounds, such as impurities in products produced by exhaustive chlorination or as by-products in the electrolytic production of chlorine.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Robert West, University of Wisconsin, Madison, for his assistance and discussions concerning OCS.

REFERENCES

BALLESTER, M., and J. CASTANER: J. Am. Chem. Soc. 82, 4259 (1960).

BALLESTER, M., C. MOLINET, and J. CASTANER: J. Am. Chem. Soc. 82, 4254 (1960).

BERG, O. W., P. L. DIOSADY, and G. A. V. REES: Bull. Environ. Contam. Toxicol. 1, 338 (1972).

KOEMAN, J. H., M. C. ten NOEVER de BRAUW, and R. H. DE VOSS: Nature 221, 1126 (1969).

KUEHL, D. W., G. E. GLASS, and G. D. VEITH: Paper presented at 22nd Annual Meeting of the American Society for Mass Spectrometry, May, 1974a.

KUEHL, D. W., G. E. GLASS, and F. A. PUGLISI: Anal. Chem. 46, 804 (1974b).

SEIBER, J. N.: J. Org. Chem. 36, 2001 (1971).

ten NOEVER de BRAUW, M. C. and J. H. KOEMAN: Sci. Total Environ. 1, 427 (1972/73).

THOMPSON, J. F., ed.: Analysis of Pesticide Residues in Human and Environmental Samples. Prepared by Pesticides and Toxic Substances Effects Lab., U.S. Environmental Protection Agency, National Environmental Research Center, Research Triangle Park, North Carolina. Section 4, A, (5,6). Revised December 1974.

VEITH, G. D., D. W. KUEHL, and J. ROSENTHAL: J. Assoc. Off. Anal. Chem. 58, 1 (1975).