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NOTE

QUANTIFICATION OF OCTACHLOROSTYRENE AND RELATED COMPOUNDS IN GREAT LAKES FISH BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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ABSTRACT. Residues of octachlorostyrene (OCS) and related polychlorinated compounds including isomers of heptachloro-, hexachloro-, and pentachlorostyrene; hexachlorobenzene, pentachlorobenzene, isomers of tetrachloro- and trichlorobenzene; and hexachlorobutadiene have been quantitated by multiple-ion-detection gas chromatography-mass spectrometry in Great Lakes fish collected between 1974 and 1980. The results show that the two upper lakes, Superior and Michigan, do not appear to have residues of OCS greater than 5 ng/g, while residues in the lower lakes, Huron, Ontario, and Erie, are as high as 400 ng/g. A selected tributary to Lake Erie has been shown to contain very high levels of all of the chemicals studied which suggests one possible source of chlorostyrenes in the Great Lakes.

INTRODUCTION

In 1969, Koeman, ten Noever de Brauw, and de Vos (1969) observed an unusual peak in gas chromatographic - mass spectral (GC/MS) data obtained on residues of Sandwich terns (*Sterna sandvicen sis*) and Eider ducks (*Somateria mollissima*) collected from the Rhine River and the Netherland's coastal area in 1965/66. This peak, identified only as an octachloro compound at m/z 376, was later confirmed as octachlorostyrene (OCS), and also found in tissues of seals (*Phoca vitulina*) and fish (*Leucisus rutilus*) (ten Noever de Brauw and Koeman 1972/73). Other GC/MS peaks in these samples were tentatively identified as isomers of heptachlorostyrenes. Residues of octachlorostyrene have since been found in fish (Kuehl *et al.* 1976) and birds (Reichel, Pronty, and Gay 1977) from the Great Lakes, fish from Frierfjord and Eidangerfjord on the Norwegian coast (Lunde and Ofstad 1976, Ofstad *et al.* 1978), fish from the Ashtabula River, Ashtabula, Ohio (Kuehl *et al.* 1980), and sediments from Puget Sound near Seattle, WA (Burrows, MacLeod, and Brown 1981). In each case, residues of lower chlorinated styrenes, hexachlorobenzene (HCB), and hexachlorobutadiene (HCBD) were also often found in the same samples.

For the past 8 years this laboratory has been conducting GC/MS exploration studies of organic chemical residues in composite samples of fishes from major U.S. watersheds. These studies are an essential part of a hazardous assessment program by the U.S. Environmental Protection Agency for the regulation of industrial activity to ensure environmental protection (Veith *et al.* 1979a, and Veith *et al.* 1981). The identification and quantification of trace levels of hazardous chemicals is used to provide an accurate description of the actual chemical residues existing in our environment. A review of unusual polyhalogenated chemicals bioaccumulated in fish has recently been published (Kuehl 1981). This type of information can be used to establish priorities in regulatory and health-related research activities. As a result of these exploration studies, a large tissue bank of fish samples collected between 1974 and 1981 has been established. This study has drawn upon this tissue bank to evaluate levels of octachlorostyrene (and related polychlorinated hydrocarbons) in Great Lakes fish in order to alert researchers to their presence, and is not designed to be a monitoring study with great statistical significance. These samples also include fish from Lake St. Clair and the Ashtabula River and Fields Brook of Ohio.

METHODS AND MATERIALS

Sample Collection

Samples were collected by field crews of the U.S. Environmental Protection Agency, Region V Office, Chicago, IL, and the states of Minnesota, Michigan, Ohio, and New York. The samples were wrapped in aluminum foil and shipped frozen to the Environmental Research Laboratory–Duluth (ERL-D), where they remain frozen at -20°C.

Sample Preparation

Whole fish from each sampling site were composited and ground frozen. A 20-g sample was blended with enough anhydrous sodium sulfate to dry it. It was then packed into a 2.5 × 50-cm glass column which was eluted with 200 mL hexane. The extract was concentrated to 20 mL using a Kuderna-Danish apparatus fitted with a three ball Snyder column. Ten percent of the extract was taken for percent fat determination. Two milliliters from the 20-mL extract were transferred to a weighed aluminum pan, heated to 100°C for 1 hour, and reweighed to calculate percent fat. The remaining extract was transferred to a 40-g Florisil column, eluted with 200 mL hexane, and finally concentrated as before to 10 mL. A 1-mL aliquot was spiked with 100 μL p-diiodobenzene, the internal standard, to yield a final concentration of 200 pg/μL.

Blanks and Spikes

Three composite fathead minnow samples (20 g) from the ERL-D culture unit were spiked with HCBD, HCB, and OCS at 25, 50, and 100 ng/g. Recoveries calculated for each compound exceeded 95% in each trial. Solvents, glassware, sodium sulfate, and Florisil were analyzed before use to assure no interferences were present, and one complete procedural blank was included with each group of five samples.

Reagents

Hexane was pesticide grade (Burdick and Jackson) which was redistilled in our laboratory. Sodium sulfate (Baker) and Florisil (Floridin Corp.) were extracted with 1:1 hexane/acetone in a large Soxhlet extractor, vacuum-oven dried at 100°C for 24 hours, and heated to 600°C for 24 hours. The Florisil was activated for > 12 hrs at 100°C before use.

Analytical grade standards of the chlorinated benzenes and HCBD were obtained from the Health Effects Research Laboratory, USEPA, Research Triangle Park, NC. OCS was obtained from Dow Chemical Co., Walnut Creek, CA. *p*-Diiodobenzene (99%) was obtained from Aldridge Chemical Co.

Mass Spectrometry

Analyses were accomplished by multiple-ion-detection (MID) of two ions for each compound on a Finnigan 4000 GC/MS/computer system using standard INCOS peak-finding and quantification software. Linear standard curves for each compound were established with standards ranging from 25 pg/μL to 1 ng/μL. Samples were concentrated or diluted as required to keep values within the range of linearity. Quantification was base dupon *p*-diiodobenzene as an internal standard with relative response factors calculated from a 200 pg/μL external standard of each compound. The external standard was also run as a sample after every fifth sample to check for accuracy and precision. The mean of the percent deviation of actual pg found to pg expected, and the standard deviation, for four GC/MS runs monitoring two ions for each chemical are given in Table 1. Verification that the chemicals being quantified were identified correctly was accomplished by full mass range scanning GC/MS on a more concentrated aliquot of each sample.

Instrumental Parameters

Scan time: MID - 3.0 sec., Full scan - 1.0 sec.
Electron energy: 70 eV
GC column: 0.25 mm × 15 m SE-30 fused quartz capillary (J & W Scientific)
Carrier flow: 30 cm/sec, Helium
Temperature program: 60–120°C @ 3°/min, 120–250 @ 10°/min
Injection: 2 μL, Grob technique, 30 sec purge delay

TABLE I. Multiple ion detection GC/MS quantification variance results. Data calculated from four GC/MS analysis monitoring two ions for each compound. Quantification Internal Standard used was p*-diiodobenzene.*

Chemical MID[a] Ions	1,3,5-Trichloro-benzene 180/182	1,2,4-Trichloro-benzene 180/182	1,2,3-Trichloro-benzene 180/182	1,2,4,5-Tetrachloro-benzene 214/216	1,2,3,4-Tetrachloro-benzene 214/216	Pentachloro-benzene 248/250	Hexachloro-benzene 282/284	Hexachloro-butadiene 258/260	Octachloro-styrene 378/380
pg injected on to GC/MS	206.3	203.3	208.8	208.2	207.6	203.4	194.0	224.0	226.0
Mean % deviation of pg found	0.6	2.1	2.0	1.7	1.7	3.8	7.2	3.0	6.5
Standard Deviation	1.87	2.00	2.38	1.97	4.05	7.29	18.8	3.95	17.9

[a] Multiple Ion Detection

RESULTS AND DISCUSSION

Sampling location, date of sampling, fish species, percent lipid, and GC retention time, as well as results of quantification, are listed in Table 2. Residues of OCS were only found in fish from the three lower Great Lakes (Huron, Erie, and Ontario, including Lake St. Clair which connects lakes Huron and Erie) and not in the two upper lakes, Michigan and Superior. In all, OCS was found in 13 of 17 samples, ranging in concentration from 2.0 ng/g (whole fish wet weight) in 1977 Saginaw Bay Lake Huron lake trout to 405 ng/g in northern pike taken from the Ashtabula River in Ashtabula, Ohio, where the river empties into Lake Erie. Levels of OCS seem to be changing the greatest in fish from Lake Huron, increasing from less than 1 ng/g in 1977 Mackinac chub, to 2.0 ng/g in 1979 Saginaw Bay trout, to 110.7 ng/g in 1980 Saginaw Bay trout. After correcting for percent lipid, this represents a 35–40-fold increase of OCS levels in fish from Saginaw Bay within a 4-year period. OCS has previously been detected in 1974 Lake Huron samples from Goderich, Ontario (Kuehl and Kopperman 1976), however no quantitative data are available. Although OCS levels dropped in Lake Ontario between 1977 and 1979, the samples originated from different areas of the lake, and therefore are not readily comparable.

HCB was found in 16 of 17 samples, ranging in concentration from a high of 1,600 ng/g in 1976 Ashtabula River fish down to 5 ng/g in a 1980 Saginaw Bay Lake Huron fish composite. 1,2,4-trichlorobenzene was the most frequently found lower chlorinated benzene, being found in 11 of 17 samples ranging in concentration from 1.2 ng/g in 1980 Lake Superior lake trout to 124 ng/g in 1979 Fields Brook suckers. Other chlorobenzenes were found in nine or fewer samples, and only where levels of HCB exceeded 100 ng/g. Each sample containing OCS also contained HCB; however, their levels, and those of other chlorobenzenes, did not appear to follow any pattern. In 5 of the 13 samples containing both HCB and OCS, the level of OCS exceeded HCB. A ratio of OCS to HCB of 20:1 was observed for 1980 fish from Saginaw Bay Lake Huron.

HCBD was found in 7 of 17 samples each containing OCS, although their concentration could not be correlated. 1980 Saginaw Bay Lake Huron fish contained 110.7 ng/g OCS and less than 5 ng/g HCBD, while 1979 Fields Brook suckers contained 3,250 ng/g HCBD and only 256 ng/g OCS. It was observed, however, that HCBD levels were detectable when HCB levels exceeded 100 ng/g and increased as HCB levels increased. The one exception was the 1979 Fields Brook river sample where almost twice as much HCBD, 3,250 ng/g, was found than HCB, 1,530 ng/g.

In addition to the compounds listed in Table 1, the mass spectrometer also monitored ions for isomers of heptachloro-(m/z 342/344), hexachloro-(m/z 308/310), and pentachlorostyrene (m/z 272/274). The elution patterns for the many isomers of pentachloro- and hexachlorostyrenes was very complex and therefore no quantification was attempted (Figure 1). Three isomers of heptachlorostyrene were consistently found in samples containing high levels of OCS. Although no standards were available for quantification, an estimate of concentration was made based upon the assumption that the response factor for each isomer to the internal standard was the same as OCS. This assumption was made based upon the small change in response factor observed between HCB and pentachlorobenzene, and has probably resulted in values that are within ±25%. OCS levels were

TABLE 2. Results of quantification of octachlorostyrene and related chemicals in Great Lakes fish. All values are in parts-per-billion whole fish wet weight and are mean values of duplicate analyses using two ions for each chemical.

Watershed	Date (year)	Fish Species	1,3,5-Trichlorobenzene[1]	1,2,4-Trichlorobenzene[1]	1,2,3-Trichlorobenzene[1]	1,2,4,5-Tetrachlorobenzene[2]	1,2,3,4-Tetrachlorobenzene[2]	Pentachlorobenzene[2]	Hexachlorobenzene[2]	Hexachlorobutadiene[2]	Heptachlorostyrene[1]	Heptachlorostyrene[1]	Heptachlorostyrene[1]	Octachlorostyrene[1]	
Relative Retention Time*			0.196	0.296	0.379	0.737	0.869	1.329	1.687	0.429	1.860	1.927	1.975	2.033	
Lake Superior Apostle Islands, WI	1974	Lake trout	ND**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	12.2
Lake Superior Copper Harbor, MI	1980	Lake trout	ND	1.2	ND	ND	ND	ND	6.5	ND	ND	ND	ND	ND	23.8
Lake Michigan Grand Traverse Bay, MI	1980	Lake trout	ND	2.0	ND	ND	ND	ND	6.3	ND	ND	ND	ND	ND	26.4
Lake Huron Mackinac, MI	1974	Chub	ND	1.4	ND	ND	ND	ND	11.5	ND	ND	ND	ND	ND	20.2
Lake Huron Saginaw Bay, MI	1977	Lake trout	ND	NQ***	ND	ND	ND	ND	NQ	ND	ND	ND	ND	2.0	16.9
Lake Huron Saginaw Bay, MI	1980	Composite Catfish Carp	ND	NQ	ND	ND	ND	ND	5.0	ND	ND	ND	ND	110.7	11.3
Lake Erie Woodtic, MI	1980	Carp	ND	ND	ND	ND	ND	ND	20.9	ND	ND	ND	ND	33.8	9.6
Lake Ontario Cape Vincent, NY	1977	Lake trout	NQ	36.0	9.5	12.0	17.0	17.0	230	2.5	46.0	30.0	27.0	281	17.1
Lake Ontario Oswego, NY	1978	Brown trout	NQ	2.8	1.1	2.5	5.0	11.0	115	1.7	4.5	NQ	NQ	35	10.1
Lake Ontario Stony Island, NY	1979	Lake trout	NQ	2.4	NQ	2.9	4.5	9.1	117	NQ	28	NQ	NQ	86	20.0
Lake St. Clair Anchor Bay, MI	1980	Carp	ND	NQ	ND	ND	ND	ND	48.8	ND	NQ	NQ	ND	227	11.0
Ashtabula River Ashtabula, OH	1976	Composite Alewife Shad Bass Perch Bullhead	16	4.7	NQ	81	17	221	1,600	323	124	62	NQ	144	x
Ashtabula River Ashtabula, OH	1978	Composite Sucker Roller Carp	2.1	NQ	3.9	7.0	1.3	33	466	61	105	NQ	NQ	152	x
Ashtabula River Ashtabula, OH	1979	Smelt	2.1	2.1	NQ	15	3.7	19	48	NQ	NQ	NQ	NQ	NQ	5.0
Ashtabula River Ashtabula, OH	1979	Northern pike	11.0	7.5	NQ	96.0	20.0	157	1,140	445	335	171	4.9	405	5.4
Fields Brook Ashtabula, OH	1979	Sucker	75	124	20	1,150	205	789	1,530	3,250	12	6.9	NQ	256	x
Fields Brook Ashtabula, OH	1980	Composite Carp Bass	31	14	1.9	193	43	352	2,210	811	117	104	NQ	184	x

- Relative to p,p'-diiodobenzene; **ND = not detected; ***NQ = detected but not quantifiable; x = not determined; 1. Detection limit 1 ppb wet weight whole fish. 2. Detection limit 5 ppb wet weight whole fish. All values are in ppb wet weight whole fish.

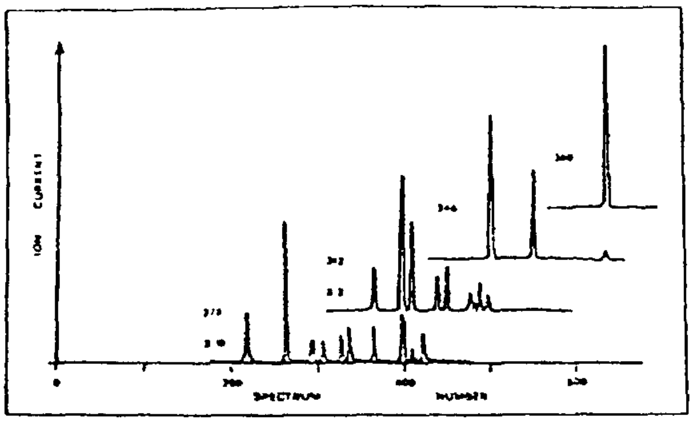

FIG. 1. Selected ion current profiles for pentachloro- (m/z 278), hexachloro- (m/z 312), heptachloro- (m/z 346), and octachlorostyrene (m/z 380) as found in northern pike from the Ashtabula River, Ashtabula, Ohio.

higher than those of any individual heptachlorostyrene isomer. However, the summation of the levels for all three isomers exceeded the level of OCS in the 1979 Ashtabula River northern pike.

The potential of OCS to bioaccumulate in fish has been previously estimated using the correlation between the n-octanol/water partition coefficient (P) and the bioaccumulation factor (BCF), log BCF = 0.85 log (P) - 0.70 (Veith, DeFoe, and Bergstedt 1979b). The log BCF value obtained for OCS is 4.52, or a concentration in a fish 33,000 times greater than in the water in which it swims. In comparison, the BCF for hexachlorobenzene, p,p'-DDE, and mirex are 4.27, 4.71, and 4.26, respectively.

OCS is not a commercial product; instead it appears to be formed when high temperature carbon/chlorine reactions take place. No source for OCS in the Rhine River samples was discussed in the Dutch papers (ten Noever de Brauw and Koeman 1972/73). However, the source of OCS in fish from the Norwegian fjords was identified as an industrial process involving the electrolysis of magnesium chloride ($MgCl_2$) using graphite electrodes (Lunde and Bjorseth 1977). The random distribution of OCS and the inconsistency of the ratios of OCS to other related polychlorinated chemicals seems to indicate that several sources of OCS exist on Lakes Huron, St. Clair, Erie, and Ontario, and that these sources are of differing industrial processes. This may account for the high levels in the Ashtabula River/Fields Brook samples from near Ashtabula, Ohio, where several industrial processes using chlorine were used.

Quantification of OCS was not done on the Dutch samples (ten Noever de Brauw and Koeman 1972/73), but mean values of OCS in the Norwegian whole fish samples ranged from 5.8 - 94 ppm in Frierfjord cod (Ofstad *et al.* 1978). Concern for the health of the human population in the Frierfjord/Eidangerfjord area resulted in the monitoring of OCS in the blood of three groups workers: those from a magnesium plant, a vinyl chloride-producing plant, and the mercantile division of a company with no occupational exposure to chlorinated hydrocarbons (Lunde and Bjorseth 1977). The mean value of OCS for each group respectively was 1.24 ng/g, 0.24 ng/g, and 0.20 ng/g. No explanation was given for why OCS was found in the blood of the non-exposed group, but it is most interesting to observe that it indeed was found. Since no human toxicological data exist for OCS, no further assessment of these data could be made.

SUMMARY

Octachlorostyrene (OCS) has been identified as a significant xenobiotic contaminant in Great Lakes fish, with levels as high as 280 ng/g in one Lake Ontario trout sample. This observation is unusual as OCS is not a known commercial product, or a by-product from any specific industrial process. It is known that OCS residues have a bioaccumulation factor as great as similar polyhalogenated chemicals like polychlorinated biphenyls (PCBs); that OCS has been found in environmental samples from various parts of Europe as well as North America; and that it has also been found in the blood of people living and working near sites of discharge of OCS. It is suggested, therefore, that analysis for polychlorinated styrenes be included in any comprehensive characterization of environmental contaminants, and that studies of aquatic and potential human toxicity of those chemicals be conducted.

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