Hexachlorobenzene (HCB) Residues in Fish

by
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Analysis of fish collected by the U.S. Fish and Wildlife Service for the fall 1970 National Pesticide Monitoring Program detected the presence of a compound eluting with, and subsequently identified incorrectly as β-benzenehexachloride. This compound was frequently observed when Florisil cleanup was used in the analysis of organochlorine residues in fish. Examination of selected sample extracts by gas chromatography-mass spectrometry (GC-MS), however, indicated that the compound was really hexachlorobenzene (C_6Cl_6 or HCB). After identification, residue levels were determined in fish collected from waters within the United States.

HCB residues have been reported previously in fish (HOLDEN 1970, ZITKO 1971) and wildlife (VOS et al. 1968), poultry products such as eggs (STANHOPE 1969), milk (GOURSAUD et al. 1972) and other dairy products (TUINSTRA and ROOS 1970) as well as in human milk and tissue (ACKER and SCHULTE 1970). However, little is known about its biological impact. VOS et al. (1971) fed HCB to adult Japanese Quail (Coturnix coturnix japonica) and reported impaired reproduction and liver damage. HCB is used as a fungicide and in organic synthesis.

This report presents data on GC-MS identification, silicic acid column separation, and gas-liquid chromatographic (GLC) analysis of HCB residues found in various species of freshwater and anadromous fishes from widely scattered areas in the United States. We also report HCB residues in fish eggs, fish fry, and fish oil.

Methods

Collection of samples took place throughout the United States during 1971 and 1972. Some samples were referred for crosscheck analysis from the fall 1971 National Pesticide Monitoring Program, whereas others were submitted to us for analysis by various state conservation agencies, a national fishery laboratory, and national fish hatcheries. Samples were stored frozen until analyses could be completed.

Bulletin of Environmental Contamination & Toxicology,
Vol. 11, No. 5 © 1974 by Springer-Verlag New York Inc.

Samples of fish tissues and other biological materials were extracted using the method of HESSELBERG and JOHNSON (1972). Lipid was removed from the samples by the gel permeation chromatography (GPC) method of STALLING et al. (1972). Liquid chromatography using Florisil® as outlined in the OFFICIAL METHODS OF ANALYSIS OF THE A.O.A.C. (1970) removed interfering substances that GPC was unable to remove. Further separation of HCB from pesticides was achieved using silicic acid column chromatography as outlined by ARMOUR and BURKE (1970). Two fractions of the sample extract are obtained, one contains primarily the polychlorinated biphenyls (PCBs) and HCB, while the other contains more polar organochlorine and organophosphorus compounds. The PCB fraction of the cleaned-up extracts was analyzed for organochlorine compounds using GLC. The GLC detector system consisted of an electron capture cell utilizing a ^{63}Ni foil. A GLC column 180 cm long x 2 mm ID was packed with OV-7 (0.3% w/w coated on 80-100 mesh Corning GLC-110 glass beads). Column temperature was 180° with a nitrogen carrier flow of 30 ml/min.

The identity of HCB residues for some samples was confirmed through the use of a Perkin-Elmer Model 270-B GC-MS interfaced to a PDP-12 LDP computer having parameters as described by STALLING et al. (1973).

Results and Discussion

HCB residues were found in many species of fish collected at widely separated locations throughout the United States (Table 1). Also, HCB residues were found in fish eggs, fish fry, and fish oil (Table 2).

In general, the concentration of HCB found in fish collected in the United States is comparable with the levels reported for fish collected in Canada (ZITKO 1971) and Europe (HOLDEN 1970). In some instances, however, fish collected in the United States had much higher HCB levels than fish from either Canada or Europe. For example, mean HCB residue levels in bigmouth buffalo (Ictiobus cyprinellus) and white perch (Morone americana) were about 10 times higher than mean residue levels reported for any Canadian fish. In addition, the highest residue level among the fish we analyzed was over 3,000 times higher than the highest level reported in fish from Canada.

The cleaned-up extract of a carp (Cyprinus carpio) from the Arkansas River in Kansas was examined by gas chromatography-mass spectrometry-computer (GC-MS-COM, STALLING et al. 1973). Spectra were acquired at 8 second intervals by the computer and stored on

TABLE 1

Hexachlorobenzene (HCB) residues in fish (whole body)

Species	Area collected	No.	HCB residues (µg/g wet wt)[1/]	
			Mean	Range
Paddlefish (Polyodon spathula)	Mo.	6	<0.001	<0.001 - 0.001
Coho salmon (Oncorhynchus kisutch)	Mich.	10	<0.010	<0.010 - 0.010
Lake trout (Salvelinus namaycush)	Mich.	13	0.017	<0.010 - 0.030
Carp	Calif., Kans., Ia.	4	16	0.006 -62
Bigmouth buffalo	Ia., La., Ill.	9	0.12	0 - 1.0
Channel catfish (Ictalurus punctatus)	Ark., Tenn., Ohio, Ind.	5	0.048	0 - 0.13
White perch	N.J., N.Y.	3	0.13	0.016 - 0.34
Striped bass (Morone saxatilis)	Md., Fla.	5	0.023	0.007 - 0.050

[1/] Residue data are uncorrected for extraction recovery which was about 60 percent.

TABLE 2

Hexachlorobenzene (HCB) residues in eggs, fry, and oil of fish (whole body)

Sample	Area collected	No.	HCB residues (µg/g wet wt)[1/]	
			Mean	Range
Eggs				
Paddlefish	Mo.	6	0.023	0.001 - 0.110
Chinook salmon (*Oncorhynchus tshawytscha*)	Ore.	1	0.002	-
Rainbow trout (*Salmo gairdneri*)	Mo., N.H.	3	0.002	<0.001 - 0.005
Striped bass	Md.	8	0.14	0 - 0.63
Fry				
Rainbow trout	Mo.	1	0.002	-
Striped bass	Fla.	1	0.090	-
Oil				
Cod (*Gadus* sp.)	-	3	0.26	0.060 - 0.38
Menhaden (*Brevoortia* sp.)	-	1	0.11	-

[1/] Residue data are uncorrected for extraction recovery which was about 60 percent.

magnetic tape. The 75 sequentially acquired spectra were then examined for the intensity of $m/e^+ = 282$, the parent ion of HCB, to create a mass fragmentogram (Figure 1). The resulting mass fragmentogram was plotted using an incremental plotter and the spectrum corresponding to the most intense ion fragment ($m/e^+ = 282$) was identical to that of C_6Cl_6. The GC-MS-COM system requires approximately 0.1 µg of C_6Cl_6 for confirmation and clearly eliminates any ambiguity in residue identification. However, satisfactory GLC analysis is possible when silicic acid is used for sample cleanup. HCB is found in the PCB fraction of the silicic acid column eluate and interference is minimal.

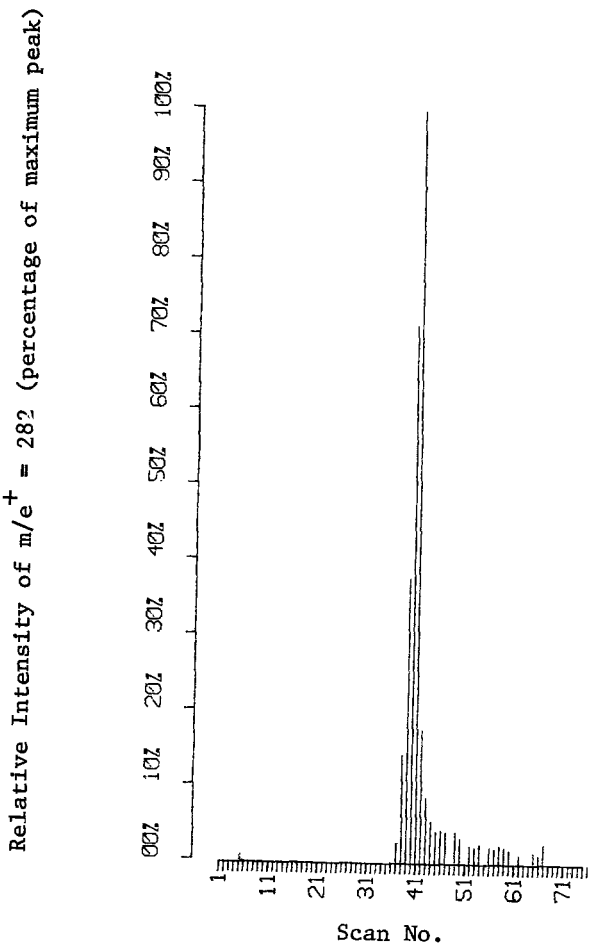

Figure 1. Mass fragmentogram of hexachlorobenzene

With one exception, the sources of HCB residues reported here have not been determined. The only exception being a carp that had HCB residues of 62 µg/g (Table 1). The source of HCB in that instance was tentatively identified as runoff from an industrial chemical storage area (DOWLIN 1972). A specific source may be difficult to locate for many residues since HCB is used in agriculture as well as in industrial processes.

Toxicological data pertaining to HCB residues in fish and wildlife are limited. However, the potential significance of HCB residues in fish or fish products should not be underestimated. For instance, HUNT and BISCHOFF (1960) suggested that organochlorine residues in fish are passed along to fish-eating predators and cause reduced reproduction or mortality among these predators. More specifically, GILBERTSON and REYNOLDS (1972) suggested that HCB residues in alewives (Alosa pseudoharengus) and smelt (Osmerus modax) may be the source of HCB residues in eggs of the common tern (Sterna hirundo). To date, however, this has not been proven.

Further experiments are necessary to elucidate: 1) the effect HCB residues have on fish and wildlife; 2) the geographical distribution of HCB residues, and 3) the specific sources of HCB contamination in the environment.

References

ACKER, L. and E. SCHULTE: Naturwissenschaften 57, 497 (1970).
ARMOUR, J. and J. A. BURKE: J. Assoc. Offic. Anal. Chem. 53, 761 (1970).
DOWLIN, L.: Personal communication (1972).
GILBERTSON, M. and L. M. REYNOLDS: Bull. Environ. Contam. Toxicol. 7, 371 (1972).
GOURSAUD, J., F. M. LUQUET, J. F. BOUDIER and J. CASALIS: Ind. Aliment. Agr. 89, 31 (1972).
HESSELBERG, R. J. and J. L. JOHNSON: Bull. Environ. Contam. Toxicol. 7, 115 (1972).
HOLDEN, A. V.: Pesticides Monit. J. 4, 117 (1970).
HUNT, E. G. and A. I. BISCHOFF: Calif. Fish and Game 46, 91 (1960).
OFFICIAL METHODS OF ANALYSIS OF THE A.O.A.C., 11th edition (edit. by HOROWITZ, W.), 475 (A.O.A.C., Washington, D. C., 1970).
STALLING, D. L., R. C. TINDLE and J. L. JOHNSON: J. Assoc. Offic. Anal. Chem. 55, 32 (1972).
STALLING, D. L., J. W. HOGAN, and J. L. JOHNSON: Environ. Health Perspec. 3, 159 (1973).
STANHOPE, W.: J. Agr. (Victoria, Australia) 67, 245 (1969).
TUINSTRA, L. G. M. Th. JR., and J. B. ROOS, JR.: Ned. Melk-Zuiveltijdschr 24, 65 (1970).
VOS, J. G., H. A. BREEMAN and H. BENSCHOP: Meded. Rijksfac. Landbouwwetensch. Gent 33, 1263 (1968).
VOS, J. G., H. L. VAN DER MAAS, A. MUSCH and E. RAM: Toxicol. Appl. Pharmacol. 18, 994 (1971).
ZITKO, V.: Bull. Environ. Contam. Toxicol. 6, 464 (1971).