Distribution of Hexachlorobenzene and Hexachlorobutadiene in Water, Soil, and Selected Aquatic Organisms Along the Lower Mississippi River, Louisiana

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Hexachlorobenzene (HCB) is a fungicide and by-product of the chemical industry which has been reported in domestic and wild animals including sheep (AVRAHAMI and STEELE 1972a), chickens (AVRAHAMI and STEELE 1972b), terns (GILBERTSON and REYNOLDS 1972), fish (HOLDEN 1970, ZITKO 1971, JOHNSON et al. 1974) and several other organisms, including man (CAM and NIGOGOSYAN 1963). This compound has also been found in samples of ocean water and its persistence in the environment has been acknowledged (SELTZER 1975).

Excessive levels of HCB in adipose tissue and milk of cattle being raised in the vicinity of an industrialized region bordering the Mississippi River between Baton Rouge and New Orleans, Louisiana roused local concern in recent years. It therefore became of interest to study the distribution of the persistent chlorinated compound in the affected area and adjacent areas of the state. Hexachlorobutadiene (HCBD), another chlorinated compound is often found in association with HCB as an industrial by-product (LASKA, BARTELL and LASETER, unpublished results). HCBD as an environmental contaminant has received little attention, and since it has been demonstrated to have substantial toxic effects in experimental work (MURZAKAEV 1966), the levels of HCBD were monitored concurrently with HCB.

This report presents data resulting from gas chromatographic (GC) analysis of HCB and HCBD residues extracted from field samples of water, soil, and selected aquatic organisms taken at intervals along a 150-mile transect of the man-made levees of the Mississippi River, from Baton Rouge to Port Sulphur, Louisiana.

MATERIALS AND METHODS

Collections of samples were made between March and May, 1975, at five-mile intervals between Baton Rouge and New Orleans and greater intervals from New Orleans, south to Port Sulphur (Figure 1). At each site approximately 1 liter of water was taken from about 15 cm beneath the surface of the

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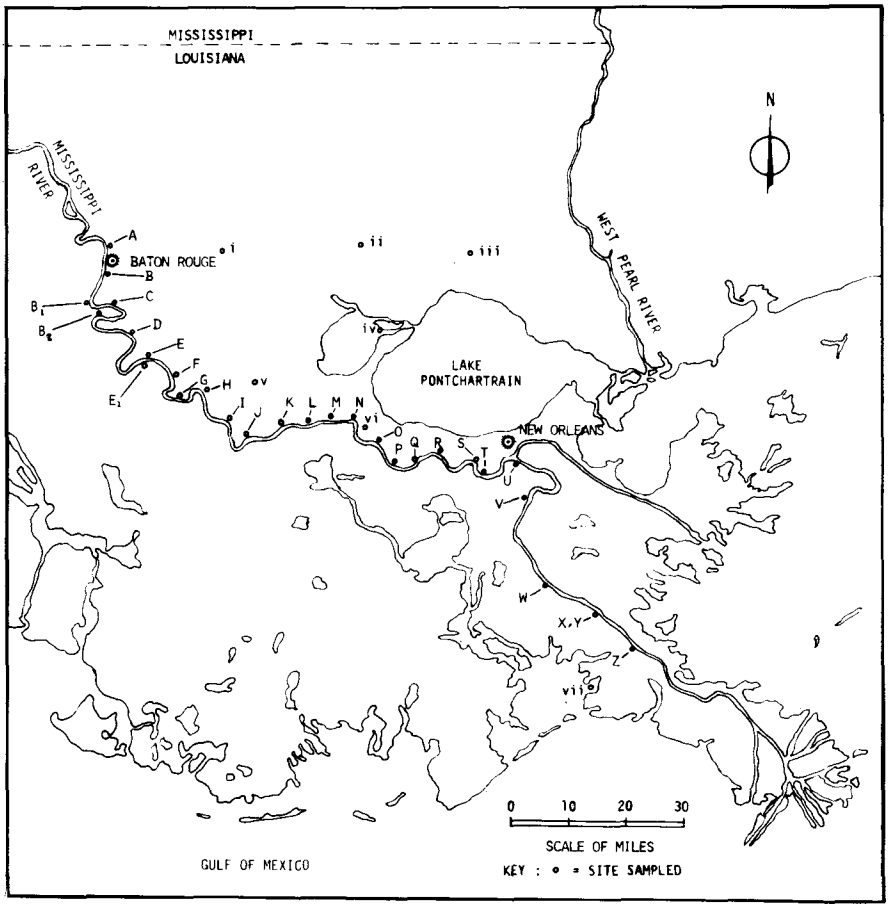
Mississippi River near the river's edge. A specimen of levee soil was collected at the same location also from beneath the water surface whenever possible. Mud samples were taken from the bottom of ditches running parallel to the levee and separated from the river by the levee. Access to sites was by public roads which run parallel to the levee. The drainage ditches are located between the road and the levee. Fish and aquatic invertebrates were collected at localities where they were present. Specimens were wrapped in aluminum foil and frozen immediately on dry ice for later analysis.

Water collections were refrigerated at 4°C whenever short term storage was necessary. Samples of 350 ml were shaken with 20 ml of benzene for 3 h on an Eberbach reciprocating action shaker. Following passage through a separatory funnel an aliquot

Figure 1. Localities sampled for presence of HCB and HCBD in soil and water.



from the benzene layer was ready for injection into the gas chromatograph. In preparing refrigerated mud samples aliquots of approximately 20 g of mud were shaken with 20 ml of acetone for 20 minutes, after which 20 ml of benzene was added. Following 24 h of shaking, the benzene-acetone extract was injected into the GC.

Tissue samples were weighed and homogenized with anhydrous sodium sulfate and acetone. The liquid was filtered into a separatory funnel and the residue homogenized twice with acetone which was then added with filtration to the separatory funnel. After adding sodium chloride to the combined acetone extracts in a separatory funnel, the acetone-sodium chloride mixture was extracted three times with hexane and the hexane evaporated to near dryness on a rotary evaporator. This residue was dissolved in hexane and placed on a Florisil column washed previously with 50 ml of elution solvent (95% hexane, 5% ether). Following elution with 100 ml of elution solvent the eluent was evaporated on a rotary evaporator. The residue was dissolved in 10 ml of benzene and an aliquot was prepared for injection into the gas chromatograph.

The gas chromatograph used in this study was a Hewlett-Packard model 5710A with an electron capture detector utilizing ^{63}Ni foil. A chromatographic column having dimensions of 91.44 cm x 4 mm ID was packed with 10% OV-1 stationary phase. The column temperature was maintained at 165°C for tissue and water samples and was adjusted to 145°C for mud samples. Argon-methane (95:5) was employed as the carrier gas at a flow rate of 35 ml/min. The injection port temperatures were held at 250°C and the detector was set at 300°C.

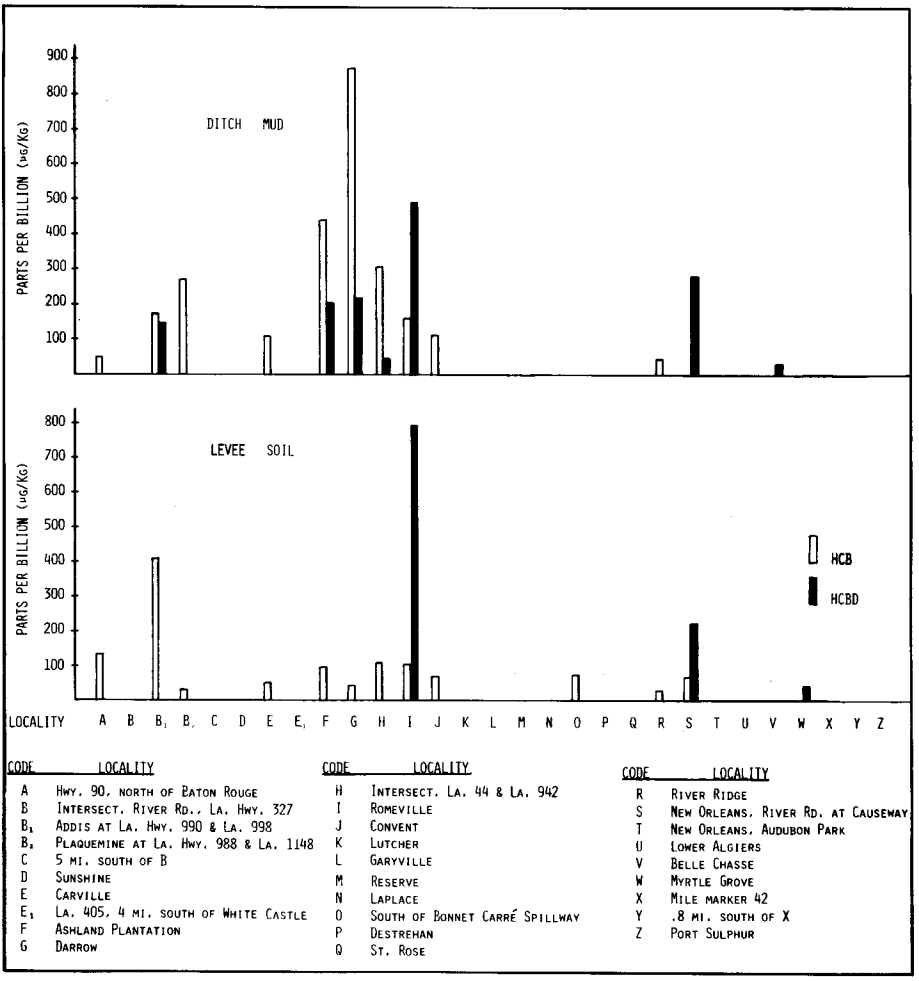
Quantitation was accomplished using external standards of Hexachlorobenzene (zone refined; B. Pauric, Philadelphia, Pa. 19120) and 1,3-Hexachlorobutadiene (Chem Service, West Chester, Pa. 19380) at concentrations of 1 ppm and 0.1 ppm in a benzene solvent. Concentrations of compounds in water were computed in terms of µg/g of sample. The concentrations in µg/g of the compounds in the wet samples (mud and soil) are expressed both in terms of wet weight and dry weight of the samples (Table I). Concentrations are tabulated in parts per billion (ppb) for individual analyses of single specimens.

RESULTS AND DISCUSSION

Both HCB and HCBD residues were found in soils at several sites along the transect studied (Figure 2). HCB was detected more frequently than was HCBD. Peaks for samples collected in the vicinity of Plaquemine and Darrow coincide with the presence of large chemical plants having HCB as one of their

byproducts. At Myrtle Grove, the soil sample was taken from spaces in a cement levee immediately downstream from a grain storage elevator which may have been the source of the HCBD that was found there.

Figure 2. Distribution of HCB and HCBD in soil along the Mississippi River, Louisiana, Baton Rouge - Port Sulphur Transect.



CODE	LOCALITY
A	Hwy. 90, north of Baton Rouge
B	Intersect. River Rd., La. Hwy. 327
B_1	Addis at La. Hwy. 990 & La. 998
B_2	Plaquemine at La. Hwy. 988 & La. 1148
C	5 mi. south of B
D	Sunshine
E	Carville
E_1	La. 405, 4 mi. south of White Castle
F	Ashland Plantation
G	Darrow
H	Intersect. La. 44 & La. 942
I	Romeville
J	Convent
K	Lutcher
L	Garyville
M	Reserve
N	Laplace
O	South of Bonnet Carré Spillway
P	Destrehan
Q	St. Rose
R	River Ridge
S	New Orleans, River Rd. at Causeway
T	New Orleans, Audubon Park
U	Lower Algiers
V	Belle Chasse
W	Myrtle Grove
X	Mile marker 42
Y	.8 mi. south of X
Z	Port Sulphur

In addition to the samples taken along the Mississippi River, collections were made at seven sites removed from the transect. The locations and concentrations of HCB and HCBD are given in Table I. Influence of the load carried by the Mississippi River is reflected in elevated levels at site vi. At the time of sampling, the gates of the Bonnet Carré Spillway were open to permit a portion of the river's waters to pass through Lake Pontchartrain on the way to the Gulf of Mexico. This is part of a flood protection system constructed by the U. S. Army Corps of Engineers.

TABLE I

Concentrations of HCB and HCBD in Sites Removed from Mississippi River Transect

Location	(Code)	Water (ppb) HCB	HCBD	Soil (ppb)** HCB	HCBD
Walker	(i)	.8	.7	*	*
Hammond	(ii)	.9	1.2	*	*
Covington	(iii)	*	*	*	*
Pass Manchac	(iv)	*	1.5	*	*
Sorrento	(v)	*	.7	*	*
Spillway	(vi)	1.5	1.5	171.7 (231.2)	321.5 (433.0)
Lake Grand Ecaille	(vii)	*	1.0	*	21.6 (62.6)

*: < .7 ppb
**: Figures in parentheses are corrected for dry weight of sample.

While concentrations of the compounds in levee and ditch soil samples ranged from undetectable to nearly 900 ppb, levels in most river water residues were usually much lower, remaining below 2 ppb. It should be noted, however, that at least one of the compounds was detected in every water collection between Baton Rouge and New Orleans. Highest levels were at locality A, above Baton Rouge and downstream from a heavily industrialized area, where 2.2 ppb HCB and 1.9 ppb HCBD were measured. At Reserve, 2.3 ppb HCB and 1.5 ppb HCBD were found in river waters 0.1 mile downstream from the landing of the Edgard-Reserve Free Ferry. Concomitant with higher levels in soil samples as shown in Figure 2, a river water sample taken at Plaquemine yielded 90.3 ppb HCB and 1.4 ppb HCBD. Such a level may be attributed to nearby dumping of wastes into the river.

Organisms frequently collected from the river and ditch sites included the mosquitofish (Gambusia affinis) and crayfish (predominantly Procambarus clarki, the red swamp crayfish). Concentration of the compounds in whole body tissue extracts of mosquitofish from the river ranged from 71.8 ppb HCB and 112.8 ppb HCBD at site L (Garyville) to a maximum of 379.8 ppb HCB and 827.3 ppb HCBD at site A above Baton Rouge (Table II). At site A this concentration represents a biomagnification of 172 times the concentration of HCB and 435 times the concentration of HCBD measured in river water at the time of sampling. Levels of the compounds in crayfish reflect their presence in the environment as well (Table III). A better correlation is seen between concentrations of HCB in the ecosystem and in crayfish than for HCBD. This may be the result of the greater volatility of HCBD.

TABLE II

Mean concentrations of HCB and HCBD in Mississippi River mosquitofish in comparison with levels measured in water and soil

Location	(Code)	Water (ppb)		Soil (ppb)**		Fish (ppb)	
		HCB	HCBD	HCB	HCBD	HCB	HCBD
Garyville	(L)	*	.9	*	*	71.8	112.8
Romeville	(I)	*	1.4	107.0(135.0)	793.4(1001.3)	136.8	197.4
Baton Rouge	(A)	2.2	1.9	134.9(167.0)	*	379.8	827.3

*: < .7 ppb.
**: Figures in parentheses are corrected for dry weight of sample.

TABLE III

Mean concentrations of HCB and HCBD in crayfish from ditches in comparison with levels measured in soil

Location	(Code)	Soil (ppb)**		Crayfish (ppb)	
		HCB	HCBD	HCB	HCBD
Walker	(i)	*	*	*	10.6
Romeville	(I)	160.6 (217.7)	489.9 (665.3)	22.2	24.1
Ashland	(F)	440.3 (884.5)	204.9 (410.1)	192.3	70.1
Darrow	(G)	874.4 (1676.8)	219.4 (420.2)	194.3	22.9

*: < .7 ppb.
**: Figures in parentheses are corrected for dry weight of sample.

The wide distribution of the compounds suggests various means of dispersal. Soil on the river side of the levee accumulates HCB and HCBD from the load carried in solution and suspension in river waters. Ditches on the levee's edge away from the river receive runoff from the slopes of the levee and the adjacent roadway. While some of the material may be lost from passing vehicular traffic hauling chemical and agricultural products, the contribution from air-borne sources, particularly in the vicinity of industrial complexes, may be important.

Accumulation of the compounds in mud samples to levels far in excess of those in water is to be expected. Concentrations of HCB in fish analyzed in our study are far above those observed in fish from Canadian rivers and marine fishing grounds reported by ZITKO (1971). The highest level found in ZITKO's analyses was 19 ppb in American eel (Anguilla rostrata) in fresh-water and 6 ppb in herring (Clupea harengus) from a bay in Nova Scotia.

HCB levels in fish tissue in Mississippi River samples in the present study also exceeded all but the highest one reported by JOHNSON et al. (1974). Their data on fish taken from various parts of the United States range below 150 ppb HCB with the exception of a single reference to carp, exposed to possible industrial chemical storage, which contained 62 ppm HCB. Since the work of JOHNSON et al. included some fishes received from national fish hatcheries, some of the source of HCB contamination in these instances may be due to feeding with contaminated fish diets. Our laboratory recently observed a concentration of 88 ppb HCB and 60 ppb HCBD in a sample of commercial fish food used nationally in game fish culture (unpublished). Care must therefore be exercised in toxicological studies with HCB and aquatic species when commercial fish food must be employed.

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