that they were released in the center of the net. Therefore, the fish released prior to the set had to swim at least a distance equal to the radius of the nets' circle to escape enclosure, whereas untagged fish along the edge of the net had less distance to swim to avoid such entrapment.

CONCLUSION

The long-haul seine provides a means of estimating the abundance and standing crop estimates of adult and juvenile fish populations living in an open water habitat where more conventional techniques have failed. Major limitations of the gear are that samples may not be taken when a current is present, that optimal sampling sites are restricted to those having certain depth and bottom characteristics, and that an experienced crew of at least four men and a considerable amount of netting is required. Major advantages are that a large surface area may be sampled in a relatively short period of time, that fast swimming, pelagic and semi-demersal fishes not vulnerable to trawls may be sampled, and lastly, that estimates of fish vulnerability to long-haul capture can be obtained from mark-recapture experiments.

The need for more information on the relative and absolute sampling effectiveness of fish sampling gear has been stressed by Allen et al. (1960) and Watt (1968). Our approach provides such information, and hopefully will encourage other researchers to quantify the effectiveness of their sampling gears.

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HEXACHLOROBENZENE: EFFECTS ON SEVERAL ESTUARINE ANIMALS[1]

by

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ABSTRACT

Tests were conducted to determine (1) the acute (96-hour) toxicity of hexachlorobenzene (HCB) to pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*) and pinfish (*Lagodon rhomboides*) and (2) the rate of HCB uptake and depuration by pinfish. Hexachlorobenzene was not acutely toxic to any of the animals tested at measured concentrations in sea water to 25 ug/l. However, both species of shrimps in the highest HCB concentration were lethargic as compared to controls and exhibited an uncharacteristically white hepatopancreas at the end of the 96-hour exposure. Pinfish exposed to average measured HCB concentrations of 0.06, 0.15, 0.65, 1.87, or 5.2 ug/l for 42 days accumulated the compound throughout the exposure. Maximum residue in muscle (wet-weight) was 34,000X the measured concentration in test water. Pinfish retained most (>50%) of the HCB after a 28-day depuration period in HCB-free water.

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INTRODUCTION

We initiated research into the effects of hexachlorobenzene, a fungicide and industrial chemical, in mid-1973 at the request of Dr. John Buckley, U. S. Environmental Protection Agency, Office of Program Integration. High residues of hexachlorobenzene (HCB) had been detected in cattle in southern Louisiana, and it was feared that the compound was present in the highly productive estuaries of this Gulf state. Thus, we began to study its acute effects on four estuarine animals and to determine the rate of HCB uptake and depuration in an estuarine fish.

METHODS AND MATERIALS

Test animals

All animals except pink shrimp were collected near the Gulf Breeze Laboratory and acclimated to laboratory conditions for at least ten days before exposure. Pink shrimp (*Penaeus duorarum*), purchased from a local bait dealer, were acclimated similarly. If mortality in a specific lot of animals exceeded 1% in the 48 hours immediately preceding the test or if abnormal behavior was observed during acclimation, those animals were discarded. Pink shrimp tested were from 48 to 69 mm rostrum-telson length; grass shrimp (*Palaemonetes pugio*), 22 to 33 mm rostrum-telson length; sheepshead minnows (*Cyprinodon variegatus*), 18 to 35 mm standard length; and pinfish (*Lagodon rhomboides*), 52 to 83 mm standard length. Animals were not fed during acute toxicity tests, but they could obtain food (plankton and other particulate matter) from the unfiltered sea water in which they were maintained. In the uptake and depuration study, pinfish were fed commercial fish food that contained no pesticide or polychlorinated biphenyl contaminants detectable by gas-liquid chromatographic (GLC) analysis.

Test conditions

Acute toxicity of HCB was determined by exposing twenty animals for 96 hours to different concentrations in 68-l glass aquaria. Technical grade HCB (99.5% active ingredient) was dissolved in reagent grade acetone and metered by pump (Lowe *et al.*, 1972) at 60 ml/hr into unfiltered sea water which entered each aquarium at 150 l/hr. Control aquaria received the same quantities of water and solvent, but no HCB.

The rate of uptake and depuration of HCB by pinfish was determined by exposing 40 animals to 0.1, 0.32, 1.0, 3.2, or 10 ug/l for 42 days, then placing them in HCB-free water for 28 days. Test conditions were the same as for acute tests. Five fish were sampled from each concentration and control after 3, 7, 14, 28, and 42 days exposure and after 14 and 28 days depuration. HCB residue in pooled samples of edible flesh (all muscle above lateral line on left side of fish and overlying skin without scales), liver and remainder of fish was determined.

Chemical Analyses

Water samples. The approximate dilution volume for GLC analysis was determined from the nominal concentration, and each sample was spiked with o,p'-DDE as an internal standard at approximately the same concentration as that expected for HCB. One-liter samples were extracted twice with 100 ml of methylene chloride and the methylene chloride drained through sodium sulfate pre-washed with methylene chloride into a Kuderna-Danish (K-D)[1] evaporator. The methylene chloride was concentrated on a steam-bath to approximately 3 ml, 50 ml of petroleum ether was added and the extract again concentrated to approximately 1 ml to remove any remaining methylene chloride. The 1-ml extract was transferred to a 7-mm Chromoflex chromatographic column containing 1.6 g of Florisil activated at 130° C for 24 hours, then diluted with 20 ml of 1% ethyl ether in hexane. Each sample was then concentrated or diluted, as needed, to the pre-determined dilution volume for GLC analysis. Recovery of o,p'-DDE was calculated for each sample. When less than 70% of the o,p'-DDE was recovered, the sample was re-analyzed if possible. HCB concentrations were not corrected for recoveries.

Tissue samples. Tissue samples weighing less than 5 g were weighed into a Kontes tissue grinder and spiked with o,p'-DDE at approximately the same concentration as expected for HCB. The tissue was extracted 3 times by grinding with 5 ml of acetonitrile, centrifuging, and decanting into a 150 mm screw-capped test tube. The acetonitrile extract was then flooded with 75 ml of 2% sodium sulfate and extracted 3 times with 5 ml of hexane. The hexane extracts were concentrated and cleaned up in the same manner as were the water samples.

Other tissue samples (5-30g) were blended with sodium sulfate, spiked with o,p'-DDE at approximately the same concentration as that expected for HCB and extracted in a Soxhlet apparatus with petroleum ether for 4 hours. The extract was absorbed onto 10 cm of unactivated Florisil contained in a 400-mm X 20-mm chromatographic column, then eluted with 20% methylene chloride in hexane. The hexane was concentrated, adsorbed onto 10 cm of activated Florisil contained in a 400-mm X 20-mm chromatographic column, and eluted with 6% ethyl ether in petroleum ether.

GLC analyses were performed on a Varian model 1400 gas chromatograph equipped with an electron capture detector and a 185-cm X 2-mm ID glass column packed with 2% OV-101 on 80/100 mesh GAS CHROM Q. Operating parameters were: Nitrogen carrier gas 25 ml/min, detector temperature 210°C, injector temperature 250°C, and column temperature 160°C. Confirmation was performed on a183-cm X 2-mm glass column packed with 5% OV-210 on 80/100 mesh GAS CHROM Q.

RESULTS AND DISCUSSION

Chemical analyses

Hexachlorobenzene, because of its nonpolar character, is "insoluble in water" (Frear, 1969) and practically insoluble in polar organic solvents, such as acetone. We found that after filtering a saturated solution of HCB in acetone through a #1 Whatman filter, only 2.9 g/l of HCB remained in solution. The insolubility of HCB is reflected in the measured concentrations in test waters, which ranged from 26% to 96% of nominal concentrations (Tables 1 and 2).

Table 1. Acute (96-hour) toxicity of hexachlorobenzene to and uptake by pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*) and pinfish (*Lagodon rhomboides*). Whole-body residues are from animals alive at the end of the exposure.

PINK SHRIMPa 11-14 Sep 1973

Water concentrations (ug/l)		Mortality	Residue	Concentration factor	
Nominal	Measured	(%)	(ug/g)	Nominal	Measured
Control	NDb	0	0.009	-	-
0.1	0.08	0	0.27	2700	3375
1.0	0.87	0	1.7	1700	1954
3.2	2.3	0	4.1	1281	1783
10.0	7.0	13	13.0	1300	1857
32.0	25.0	33	21.0	656	840

a15 shrimp per test container.
bNot detectable; 0.01 ug/l.

	Minimum	Maximum	Mean
Temperature (°C)	29.0	30.0	29.8
Salinity (o/oo)	24.0	26.5	25.0

GRASS SHRIMP 13-17 Aug 1973

Water concentrations (ug/l) Nominal	Measured	Mortality (%)	Residue (ug/g)	Concentration factor Nominal	Measured
Control	ND	0	0.017	–	–
0.1	0.096	0	1.1	11000	11458
1.0	0.56	0	1.9	1900	3393
3.2	1.8	0	4.5	1406	2500
10.0	6.1	0	10.0	1000	1639
32.0	17.0	10	27.0	844	1588

	Minimum	Maximum	Mean
Temperature (°C)	30.0	32.0	31.4
Salinity (o/oo)	22.0	26.0	23.6

SHEEPSHEAD MINNOWS 20-24 Aug 1973

Water concentrations (ug/l) Nominal	Measured	Mortality (%)	Residue (ug/g)	Concentration factor Nominal	Measured
Control	ND	0	ND	–	–
0.1	0.072	0	0.024	240	333
1.0	0.33	0	0.028	28	85
3.2	1.49	0	0.69	216	463
10.0	4.06	0	15.0	1500	3695
32.0	13.3	0	89.0	2781	6692

	Minimum	Maximum	Mean
Temperature (°C)	29.5	32.0	30.5
Salinity (o/oo)	15.0	28.0	21.6

PINFISH 30 Jul-2 Aug 1973

Water concentrations (ug/l) Nominal	Measured	Mortality (%)	Residue (ug/g)	Concentration factor Nominal	Measured
Control	ND	0	0.021	–	–
1.0	0.3	0	6.3	6300	21000
3.2	--a	0	44.0	13750	--a
10.0	--a	0	47.0	4700	--a
32.0	8.4	0	79.0	2469	9405
100.0	--a	0	120.0	12000	--a

aNo chemical analyses performed.

	Minimum	Maximum	Mean
Temperature (°C)	30.5	32.0	31.0
Salinity (o/oo)	18.0	25.0	22.6

Table 2. Nominal and measured hexachlorobenzene concentrations in test water during an uptake and depuration study with pinfish (*Lagodon rhomboides*).

Nominal Concentration (ug/l, ppb)	Measured Concentration (ug/l, ppb)						Mean Measured Concentration
	26 Sep	2 Oct	10 Oct	16 Oct	24 Oct	6 Nov	
Control	NDa	ND	ND	ND	ND	ND	ND
0.1	0.05	0.04	0.09	0.06	0.08	-b	0.06
0.32	0.17	0.16	0.18	0.10	0.13	-b	0.15
1.0	0.65	0.63	0.77	0.50	0.67	0.69	0.65
3.2	1.73	1.93	2.04	1.90	1.62	2.00	1.87
10.0	5.8	5.4	3.3	4.4	8.9	3.3	5.2

aNot detectable; 0.01 ug/l.
bNo chemical analyses performed.

A number of methods have been reported for the determination and confirmation of HCB in various substrates (Taylor and Keenan, 1970; Collins *et al.*, 1972; Smyth, 1972; Baker, 1973; Holdrinet, 1974 and Johnson *et al.*, 1974). These authors point out that because of the solubility, volatility, and nonpolar character of HCB and its elution time on most standard pesticide GLC columns, the analysis and quantitation of HCB at low concentrations is hampered by the presence of the electron capturing co-extractives, polychlorinated biphenyls (PCBs) and chlorinated hydrocarbon pesticides. On the other hand, because of the high percentage of chlorine in the HCB molecule, this compound is very sensitive to electron capture detection.

In this study, HCB measured in both water (1 ug/l nominal concentration) and tissue samples from animals exposed at this concentration was well above any background electron capturing compound. At higher concentrations, all interfering peaks were completely eliminated at the dilution volumes required to bring the HCB peak on scale. O,p'-DDE was an ideal internal standard. It eluted in the same Florisil fraction as did HCB and eluted just after HCB on the GLC column. Recoveries of HCB and o,p'-DDE in samples spiked with both compounds were greater than 85% for each.

Acute toxicity tests

Hexachlorobenzene, at the concentrations tested, was not acutely toxic to the animals tested (Table 1). Pink shrimp exposed to a measured concentration of 25 ug/l experienced the greatest mortality, 33%. Both pink shrimp and grass shrimp exposed to measured concentrations of 25 ug/l and 17 ug/l, respectively, were lethargic as compared to controls and exhibited uncharacteristically white hepatopancreases.

Uptake and depuration study

Pinfish exposed to average measured HCB concentrations of 0.06, 0.15, 0.65, 1.87, or 5.2 ug/l for 42 days accumulated the compound throughout the exposure (Table 3). Maximum residue in muscle was 34,000X greater than the measured concentration in test water, maximum residue in liver was 47,000X greater, and maximum residue in remainder of fish was 56,000X greater (Table 4). Considering previous experience with uptake of other organochlorine compounds by estuarine fish at this laboratory (Hansen *et al.*, 1971; Parish *et al.*, 1974), it is interesting that HCB was accumulated chiefly in the remainder of the fish, the liver being the usual site of greatest accumulation for the other compounds.

Table 3. Concentrations of hexachlorobenzene in tissues of pinfish (*Lagodon rhomboides*) during a 70-day uptake and depuration study.

Nominal Water Concentration (ug/l, ppb)		Tissue Concentration (ug/g, ppm)						
		Days Exposure					Days Depuration	
		4	7	14	28	42	14	28
Control	LIVER	0.01	0.06	0.1	0.15	0.06	0.03	0.05
0.1		0.39	0.98	2.8	2.8	1.5	1.6	0.75a
0.32		1.9	3.4	4.9	5.3	6.6	5.1	4.4b
1.0		8.8	11.2	22.4	20.2	28.0	19.0	17.0
3.2		22.7	22.2	63.5	54.7	73.0	45.0	48.0
10.0		61.4	90.3	202.5	202.6	245.0	131.0	234.0
Control	MUSCLE	0.02	0.02	0.04	0.05	0.02	0.03	0.02
0.1		0.33	0.28	1.75	1.31	1.5	1.4	1.1a
0.32		1.19	1.24	5.1	3.7	3.4	4.3	3.0b
1.0		3.3	3.5	16.5	11.5	15.0	10.0	12.0
3.2		9.4	10.0	36.9	38.0	39.0	36.0	34.0a
10.0		38.3	28.5	128.0	95.3	117.0	79.0	104.0
Control	REMAINDER	0.03	0.04	0.05	0.17	0.08	0.05	0.05
0.1		0.45	0.78	2.39	2.5	2.6	3.1	1.75a
0.32		1.9	3.5	8.3	4.3	7.8	8.7	6.65b
1.0		5.6	9.9	20.7	22.6	31.0	23.0	23.0
3.2		16.3	32.4	67.4	73.1	85.0	78.0	60.0a
10.0		52.8	141.7	265.0	202.1	274.0	236.0	184.0

aOnly four fish sampled.
bOnly two fish sampled.

Table 4. Ranges of concentration factors (based on nominal and measured water concentrations) in an uptake and depuration study with pinfish (*Lagodon rhomboides*).

LIVER		MUSCLE		REMAINDER	
Nominal	Measured	Nominal	Measured	Nominal	Measured
21,000 to 28,000	39,000 to 47,000	12,000 to 18,000	21,000 to 34,000	26,000 to 31,000	41,000 to 56,000

The pattern of loss of HCB by pinfish was erratic. After 14 days of depuration in HCB-free water, the loss rate appeared high (Table 3), but samples taken after 28 days of depuration showed a much lower loss rate. Decrease in HCB residue in liver ranged from 4% to 50% after 28 days of depuration; in muscle, from 11% to 27%; and in remainder of fish, from 15% to 33%.

In another study (Parrish *et al.*, 1974), we found that spot (*Leiostomus xanthurus*) lost all detectable dieldrin residues after a 13-day depuration period. Hansen and Wilson (1970) found that after 56 days of depuration pinfish lost 87% of DDT residues and Atlantic croaker (*Micropogon undulatus*) lost 78% of accumulated DDT. Thus, the rate of loss of HCB by pinfish appears similar to that of DDT.

It has been shown that HCB is a widespread aquatic contaminant. HCB residues have been reported in fish eggs, fish fry and fish oil from the U. S. (Johnson *et al.*, 1974), in fish from Canada (Zitko, 1971), and in fish from Europe (Holden, 1970). Although our study showed that HCB is not acutely toxic to four estuarine animals, the compound is accumulated by an estuarine fish. Further work is needed to determine chronic effects of HCB on estuarine animals, particularly fishes.

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[1]Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

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ENDRIN: EFFECTS ON SEVERAL ESTUARINE ORGANISMS[1]

by

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ABSTRACT

Acute (96-hour) bioassays were performed with endrin and the following estuarine organisms: American oyster (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sailfin molly (*Poecilia latipinna*) and sheepshead minnow (*Cyprinodon variegatus*). Endrin was acutely toxic to all organisms tested, except oysters, whose shell growth was appreciably inhibited by 56 ug/l (parts per billion) of the chemical. Pink shrimp were the most sensitive animal tested, but significant numbers of both species of shrimps and fishes died when exposed to concentrations of one ug/l or less. In a separate test, embryos and fry of the sheepshead minnow were exposed to concentrations of endrin ranging from 0.046 to 1.0 ug/l (nominal) for 33 days in an intermittant-flow bioassay. Embryos were not affected by the concentrations to which they were exposed, but the estimated LC50 (probit analysis, a=.05) of fry was 0.27 ug/l.

INTRODUCTION

Widespread use of the organochlorine insecticide, endrin, has prompted numerous investigations to determine the effects of this compound on aquatic organisms. Several studies involving marine organisms have shown that endrin is acutely toxic at low levels. Eisler (1969) found endrin acutely toxic to sand shrimp (*Crangon septemspinosa*), grass shrimp (*Palaemonetes vulgaris*), and hermit crabs (*Pagurus longicarpus*). The 96-hour LC50's were 1.7 ug/l, 1.8 ug/l, and 12 ug/l, respectively. Davis and Hidu (1969) assessed the effects of endrin on oysters by (1) determining the number of fertilized eggs that developed into normal larvae after 48-hours exposure to a given concentration of endrin, and (2) observing survival and growth of larvae over a period of 12 days. At concentrations greater than 0.025 mg/l, endrin reduced the number of eggs developing, survival, and growth of larvae. Katz (1961) and Katz and Chadwick (1961) found the 96-hour LC50 of endrin to threespine stickleback (*Gasterosteus aculeatus*) ranges from 0.5 to 1.5 ug/l. Salinity had little effect on toxicity, but temperature markedly affected toxicity - the higher the temperature the greater the toxicity. Eisler and Edmunds (1966) found that acute exposure to sublethal concentrations (1 ug/l or less) of endrin impaired liver function in northern puffers (*Sphoeroides maculatus*).

Few data have been published concerning the effects of endrin on larval marine fishes. One such study by Johnson (1967) on the threespine stickleback (*Gasterosteus aculeatus*) demonstrated that endrin immobilizes hatching fry at 15.0 ug/l and

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