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Toxicity of Arsenic and PCBs to Fry of Deepwater Ciscoes (*Coregonus*)[1]

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The deepwater ciscoes (known commercially as chubs) are among the native fish of the Great Lakes that have declined or disappeared during this century (CHRISTIE 1974). The ciscoes of Lake Michigan, now primarily bloaters (Coregonus hoyi), are only about 1% as abundant as they were in 1960-61 (GREAT LAKES FISHERY LABORATORY 1977). Stress factors that may have interacted to cause the decline of ciscoes include overfishing, invasion of the lake by exotic species, and pollution. Compared with other fish species from Lake Michigan, ciscoes are relatively high in contaminants, especially arsenic and polychlorinated biphenyls (PCBs). Although the maximum concentration of PCBs in the open waters of Lake Michigan is only 10 ng/L (GREAT LAKES WATER QUALITY BOARD 1975), the average concentration of PCBs was 4.1 µg/g in ciscoes taken off Saugatuck, Mich., in 1976 (GREAT LAKES WATER QUALITY BOARD 1978). COPELAND et al. (1973) reported arsenic values of 0.26, 0.77, and 1.7 µg/g for three bloaters collected near Ludington, Mich., and 0.56, 1.1, and 2.5 µg/g for three ciscoes (Coregonus sp.) taken near Cook, Mich.

Information on the toxicity of arsenic and PCBs to ciscoes is needed to evaluate the relative importance of these potential stress factors. Because fry are usually the most sensitive life stage, toxicity data on the fry can often indicate threshold values for the entire life cycle (SCHIMMEL et al. 1974, MCKIM et al. 1978). Few data are available on the toxicity of arsenic and PCBs to fry of other fish species. The 96-h LC50 for newly hatched fathead minnows (Pimephales promelas) was 7.7 µg Aroclor 1254/L (NEBECKER et al. 1974). SPOTILA and PALADINO (1979) reported that 50 µg arsenic (As^{+3})/L caused 100% mortality in muskellunge fry (Esox masquinongy) during the swim-up stage. No other data are available for toxicity of arsenic to fish fry. Water quality criteria and objectives (U.S. ENVIRONMENTAL PROTECTION AGENCY 1976, GREAT LAKES WATER QUALITY BOARD 1975) have been established on the

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basis of such data as above, although possible additive effects of metals and organochlorines found in Lake Michigan are generally unknown.

As the initial step in determining the potential role of arsenic and PCBs in the decline of Lake Michigan ciscoes, our objectives were to measure the acute toxicity (96-h LC50) of arsenic and PCBs, singly and in combination, for cisco fry; to determine the additive index (MARKING 1977) for arsenic and PCBs; and to evaluate the adequacy of water quality criteria for these contaminants relative to protecting ciscoes.

MATERIALS AND METHODS

Mature ciscoes (Coregonus sp.) were captured by gillnet from a commercial fishing vessel, December 1976, at 130-150 m, 16 km NE of Marquette, Mich., in Lake Superior, because of the greater availability of ciscoes in Lake Superior compared with Lake Michigan. Four male Coregonus hoyi, four female C. hoyi, and one male C. zenithicus were stripped on board the vessel by personnel of the Michigan Department of Natural Resources and all eggs and sperm were mixed. After 80-84 days of incubation at the Great Lakes Fishery Laboratory in running well water (total hardness 400 mg/L as $CaCO_3$) at 4-5 C, 95% of the eggs hatched. Newly hatched fry were placed in floating baskets in flowing, 6-8 C well water and were fed brine shrimp (Artemia) nauplii, beginning when the fry were 1 week old. For 1 week prior to toxicity tests, fry were gradually acclimated to reconstituted soft water (total hardness 40-48 mg/L and alkalinity 30-35 mg/L as $CaCO_3$) (COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975).

Stock solutions of toxicants were prepared as follows. Arsenic trioxide (As_2O_3) was dissolved in warm water by adding 10 N NaOH and then neutralized with concentrated HCl. PCBs (Aroclor 1254 from Monsanto, St. Louis, Mo.)[3] were dissolved in 100% acetone.

Fry, randomly pipetted into battery jars containing 2.5 liters of reconstituted soft water, were acclimated overnight to the test chambers. The battery jars, each containing 10 fry, were randomly arranged in a trough of running water at about 7 C (6.8-7.8). Toxicants were then added to the battery jars and mixed by gentle stirring. A control containing 0.4% acetone, the highest concentration of acetone in the experimentals, was included. Toxicant concentrations were selected by progressive bisection of intervals on a logarithmic curve (AMERICAN PUBLIC HEALTH ASSOCIATION 1976, p. 715). After beginning the tests, we counted and removed dead fry at 3, 6, 12, 24, 48, 72, and 96 h. At 96 h, we also noted any

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[3]Use of trade names or manufacturers' names does not imply U.S. Government endorsement of any commercial product.

moribund individuals. During the extended bioassay, the water and toxicants were renewed at 96 h and daily observations of mortality were made until day 14.

Water samples were taken from the battery jars at 96 h for analysis of Aroclor 1254 and total arsenic to determine possible losses during that time. For Aroclor 1254 analysis, 100-mL samples were extracted twice with petroleum ether and dried over anhydrous Na_2SO_4. The volume of the combined extracts was reduced to about 1-2 mL by heating on a hot plate. The sample was allowed to cool before transferring to a test tube, and the volume was adjusted to 10.0 mL with isooctane. After additional dilutions in isooctane, the samples were injected into a gas chromatograph (WILLFORD et al. 1976). The samples were quantitated against standard solutions of Aroclor 1254. Recovery after 96 h in the battery jars was 73% for 1.0 mg /L and 80-89% for 1.8 to 10 mg/L. All of the concentrations were above the solubility of Aroclor 1254 in water (WIESE and GRIFFIN 1978), even in the presence of 0.4% acetone. The 10 mg PCBs/L "solution" was a cloudy suspension.

We saponified (REINERT 1970) 10-g samples from homogenized whole ciscoes before analysis for PCBs (WILLFORD et al. 1976) and p,p'DDE (saponified p,p'DDT plus p,p'DDE) (HESSELBERG and SCHERR 1974). We quantitated PCBs with standards containing a 1:1:1 mixture of Aroclors 1248, 1254, and 1260, the Aroclors most common in environmental samples. Based on isomer analysis of fish from Lakes Michigan and Superior, typically the p,p'DDT represents about 25% of the saponified mixture, which includes at least 80% of the total DDT complex present (HESSELBERG 1979). The p,p'DDE for the standards was obtained from Analabs, New Haven, Conn.

Using nondestructive neutron activation (DESOETE et al. 1972), the Phoenix Memorial Laboratory, University of Michigan, analyzed water samples for arsenic. The analysis included 10-h irradiation in the Ford Nuclear Reactor, counting by lithium drifted germanium detector, and data reduction by a computerized analyzer system (NICHOLSON and RENGAN 1979). After 96 h, the recovery was 104 ± 0.9(SE)% for 19 samples containing 10 to 100 mg arsenic/L.

Analysis of arsenic (NICHOLSON and RENGAN 1979) in tissue samples of eggs and adult ciscoes required additional steps to remove bromine, which is normally present in environmental samples and which interferes with determination of arsenic. After passage of irradiated and acid-digested samples through a tin dioxide column, we counted the radioactivity of the arsenic-76 with the tin dioxide, using a Ge(Li) detector.

We analyzed bioassay data by probit analysis (FINNEY 1952) with a computer program (a modified version of IBM SSP package) from the Dow Chemical Company, Midland, Mich. The probit slope, b, is related to the classical slope function, S (LITCHFIELD and WILCOXON 1949), as follows: $b = (\log S)^{-1}$. The additive indexes for mixtures of arsenic and PCBs were calculated by the method of MARKING (1977).

TABLE 1

PCBs, DDT + DDE, and Arsenic in Lake Superior Ciscoes

Species	Sex	Number[a]	Total length mm $\bar{x} \pm SE$	PCBs[b] µg/g tissue[c] $\bar{x} \pm SE$	DDT + DDE µg/g tissue[c] $\bar{x} \pm SE$	Arsenic µg/g tissue[c] $\bar{x} \pm SE$
Coregonus hoyi	M	4	304 ± 7	2.3 ± 0.2	1.4 ± 0.3	0.75 ± 0.11
C. hoyi	F	4[d]	295 ± 14	1.2 ± 0.3	0.67 ± 0.20	0.84 ± 0.05
C. zenithicus	M	1	276	1.8	0.74	0.81
Chub eggs		e		0.51	0.25	0.26

[a]The fish were analyzed individually.
[b]Quantified by using a mixed standard of 1:1:1 Aroclor 1248:1254:1260.
[c]Wet weight.
[d]Eggs were obtained from four females, but tissue sample of one female was lost during analysis for PCBs and DDT + DDE. The mean length for the three females was 298 ± 20 mm.
[e]Ten grams were used for analysis of PCBs and DDT + DDE, and 2.2 g for analysis of arsenic. Eggs were analyzed shortly before hatching.

RESULTS AND DISCUSSION

The difference in organochlorine concentration between male and female C. hoyi was highly significant ($P<0.01$) for PCBs and significant ($P<0.05$) for DDE (Table 1). In view of the lower concentrations of both PCBs and DDE in the females and eggs, these contaminants are probably not preferentially stored in the eggs. Concentrations of PCBs tended to be lower in Lake Superior ciscoes than in those from Lake Michigan, but DDE concentrations were similar in 1976 (GREAT LAKES WATER QUALITY BOARD 1978).

Other subsamples of the same fish homogenates and eggs yielded the data for total arsenic shown in Table 1. The difference in arsenic concentration between male and female C. hoyi was not significant, and arsenic was not preferentially stored in the eggs.

Duplicate acute toxicity tests (96 h) with arsenic (As_2O_3) were performed when the fry were 15 to 19 days old. The mean LC50 was 26 mg/L and the mean slope 5.6 probit/log (mg/L).

Acute toxicity tests (96 h) of arsenic and PCBs (Aroclor 1254), singly and in combination, when the fry were 22 to 26 days old, yielded the results shown in Table 2. Only 20% of the fry died in 10 mg PCBs/L; however, all living fry were abnormally lethargic. Accurate acute toxicity values for PCBs could not be determined because of the low solubility of these compounds.

The LC50 values from Table 2 show that PCBs in the presence of arsenic (LC50 = 3.5 mg PCBs/L) were more toxic than arsenic either singly (17 mg As_2O_3/L) or in combination (11 mg As_2O_3/L). The slopes of these three toxicity curves were not significantly different (i.e., the 95% confidence limits overlapped). The presence of hybrid C. hoyi x C. zenithicus in the experimental population probably contributed to the variability in the data.

TABLE 2

LC50 Values and Slopes of Toxicity Curves (95% confidence limits in parentheses) for Arsenic and PCBs, Singly and in Combination, after 96 Hours (22-Day-Old Fry)

Toxicant	LC50 mg/L	Slope probit/log (mg/L)
Arsenic	17 (13-22)	5.39 (2.61-8.18)
Arsenic (PCBs)	11 (8-15)	3.90 (2.17-5.64)
PCBs	>10	
PCBs (Arsenic)	3.5 (2.6-4.7)	3.92 (2.16-5.68)

To determine the additive toxicity of the mixture according to the method of MARKING (1977), we needed an LC50 value for PCBs individually. Because 20% mortality occurred and all the fry appeared moribund at 10 mg PCBs/L and because the low solubility of PCBs made tests at higher concentrations unrealistic, the value of >10 mg PCBs/L was substituted in the equation below to estimate the sum of biological activity:

$$(A_m/A_i) + (B_m/B_i) = S$$

where A and B = chemicals, i and m = toxicities (LC50s) of the individual chemicals and mixtures, respectively, and S = sum of biological activity. With our data, $S \leq 1.01$, indicating simple additive toxicity. Estimating the additive index by $(S)(-1) + 1.0$, yields ≥ -0.01, again indicating simple additive toxicity.

When the above toxicity test (Table 2) was extended to 5 days after renewal of solutions at 96 h, a marked dose-related increase in mortality occurred between days 4 and 5, especially for PCBs (Table 3). Apparently handling stress contributed to the increased sensitivity of the fry. Based on data in Table 3, the sum of biological activity, S, was 1.07, and the additive index was 0.07, with 95% confidence limits of -1.73 to 1.95. Since these confidence limits overlap zero, simple additive toxicity is assumed, confirming the estimated results at 96 h. When the exposure of these fry to arsenic was extended to 14 days, the LC50 value was 6.7 mg/L (C.L. = 5.31 to 8.46), and the slope was 7.71 probit/log concentration (C.L. = 3.29 to 12.12). After 14 days' exposure, 100% mortality was observed for all fry at 1.0 to 10 mg PCBs/L, alone and in combination with 3.2 to 32 mg arsenic/L.

TABLE 3

LC50 Values and Slopes of Toxicity Curves (95% confidence intervals in parentheses) for Arsenic and PCBs, Singly and in Combination after 5 Days (22-Day-Old Fry)

Toxicant	LC50 mg/L	Slope probit/log (mg/L)
Arsenic	14 (11-19)	4.56 (2.30-6.82)
Arsenic (PCBs)	6.3 (3.1-10)	2.32 (0.73-3.90)
PCBs	3.2 (1.9-5.5)	1.98 (0.83-3.13)
PCBs (Arsenic)	2.0 (0.96-3.3)	2.27 (0.69-3.85)

Since additive toxicity was indicated for the mixture of PCBs and arsenic, we selected the 96-h LC50 values for the mixture to calculate safe concentrations (WARREN 1971). Using an application factor of 0.01 (NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF

ENGINEERING 1973), we estimated safe concentrations to be 35 µg PCBs/L and 110 µg arsenic/L. These values are well above the reported levels of <10 ng PCBs/L and 1 µg arsenic/L for Lake Michigan waters (GREAT LAKES WATER QUALITY BOARD 1975, COPELAND and AYERS 1972). Hence, the water quality criteria and objectives for PCBs (1.0 ng/L) and arsenic (50 µg/L) recommended by the U.S. ENVIRONMENTAL PROTECTION AGENCY (1976) and the GREAT LAKES WATER QUALITY BOARD (1975) would appear to be adequate to protect ciscoe populations in Lake Michigan. Subacute tests at ambient (Lake Michigan) and calculated safe concentrations should be performed to test the no-effect hypothesis for arsenic and PCBs on ciscoes.

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