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CADMIUM AND ENDRIN TOXICITY TO FISH IN WATERS CONTAINING MINERAL FIBERS.

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16. ABSTRACT

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ABSTRACT

Taconite tailings and their component asbestiform minerals in Lake Superior water had no demonstrable effect on the chronic toxicity of cadmium to the flagfish, *Jordanella floridae*. Maximum acceptable toxicant concentrations determined in life cycle tests, where effects on survival, growth, reproduction, and bioconcentration were used as endpoints, were between 3.3-7.4, 3.0-6.5, and 3.4-7.3 µg cadmium/liter at 0.004, 0.08, and 0.95 mg/liter taconite tailings concentrations, respectively. Similarly, in two tests (A and B) exposing recently hatched fathead minnows, *Pimephales promelas*, for 45 days to several concentrations of endrin, taconite tailings had no observable effect on survival, growth and bioconcentration. No observable effect concentrations (NOEC(s)) for endrin at 0.2 mg/liter taconite tailings concentration were between 0.30 and 0.60 µg/liter for group A fish and 0.075 and 0.15 µg/liter for group B fish. At 0.05 mg/liter taconite tailings concentration, the NOEC(s) were between 0.30 and 0.60 µg/liter for group A fish and 0.15 and 0.30 µg/liter for group B fish. At 0.95 mg/liter taconite tailings the NOEC(s) were between 0.15 and 0.30 µg/liter for group A fish and 0.30 and 60 µg/liter for group B fish.

INTRODUCTION

Lake Superior is the source of the experimental water supply at the Environmental Research Laboratory (ERL) of the U.S. Environmental Protection Agency located at Duluth, Minnesota. The suspended solids content of this water varies with climatological conditions. It contains diatom fragments, organic debris, quartz particles, some clay minerals, and amphibole particles ranging from blocky cleavage fragments to asbestiform fibers. The source of these amphibole particles has been traced to the Reserve Mining Company effluent to the lake at Silver Bay, Minnesota (Cook, et al. 1974). These materials are known as taconite tailings.

The amphiboles are hydrated silicates which include the commercially important asbestos minerals: amosite, crocidolite, anthophyllite, tremolite, and actinolite (Cook, et al. 1974). The predominant amphibole present in the tailings fraction of the suspended solids is cummingtonite-grunerite [$(Mg, Fe)_7 Si_8O_{22} (OH)_2$] with smaller amounts of tremolite-actinolite and hornblende present. Commercial amosite is an asbestiform cummingtonite-grunerite amphibole and is nondistinguishable by present methods of identification from many of the asbestiform mineral fibers found in the tailings fraction of suspended solids in the lake water.

In general, little is known about the effects of suspended fine particles, or, more specifically, the asbestiform amphibole minerals in the aquatic environment. It is not known how fine particles, such as those present in the tailings, influence the toxicity of a substance to fish, yet such information is important in establishing meaningful water quality criteria.

Daily analysis of amphibole (taconite tailings) and suspended solids concentrations in Duluth's water supply were begun in 1973 at the ERL-Duluth. Duluth's water intake is located approximately 4 km up-current from the intake of the ERL-Duluth experimental water supply and is similar in design and placement in the lake. Intermittent comparative measurements have indicated that the taconite tailings content of these water supplies were similar, even though at times suspended solid concentrations varied considerably. The taconite tailings concentration of the municipal water supply, measured daily from March 1973 to January 1974, was used as a guide in planning this study. The average tailings concentration during this period was 0.19 mg/liter and ranged from 0.04 to 0.8 mg/liter; the total suspended solid concentrations averaged 0.83 mg/liter.

The purpose of this study was to determine if the presence of the taconite tailings in Lake Superior water significantly alters the toxicity of an inorganic (cadmium) and organic (endrin) compound to fish.

MATERIALS AND METHODS

EXPOSURE SYSTEM

The exposure apparatus for both the cadmium and endrin studies consisted of three modified proportional diluters (Mount and Brungs, 1967) with independent cycling and toxicant delivery systems. Synchronization of the three diluter delivery cycles was achieved by wiring the diluters in series through the cycling micro-switches. Flows were split 4 ways with each chamber receiving approximately 500 ml per diluter cycle. Each diluter delivered five toxicant concentrations and a control to duplicate 30 x 60 x 30 cm glass spawning and offspring growth chambers each containing 43 liters of test water at a depth of 23 cm.

Prior to entering the spawning chambers, the water passed through a 28 x 30 x 9 cm glass chamber containing 6 liters of water which was used for embryo incubation. The intermittent flow rate to each chamber was 11 liters per hour and water temperatures were maintained near 25 C. Temperatures in the test chambers were usually within 1 C of each other; minimum and maximum daily temperatures over the test period were 21.0 and 26.6 C.

A combination of Sylvania Gro-Lux and Dura-Test Vita Lite flourescent lamps was the main source of illumination. The lamps were used in conjunction with incandescent bulbs controlled by a timer that regulated the photoperiod, 16 hours of light and 8 hours of dark, with simulated sunrise and sunset (Drummond and Dawson, 1970).

TEST WATER PHYSICAL-CHEMICAL CHARACTERISTICS

Suspended Solids and Taconite Tailings: Cadmium Study--The preheated test water in the cadmium experiments was unfiltered lake water which is routinely used in laboratory toxicity tests, and filtered lake water with nearly complete removal of suspended solids. The unfiltered lake water (ambient) was continually monitored and maintained at or below 3 Jackson turbidity units for the first 62 days of the cadmium experiment by using a 151,400 liter reservoir during periods of high turbidity. Thereafter, because of the danger of the reservoir freezing, its use was discontinued and instead lake water was passed through a 5 um prefilter and was used in the ambient or unfiltered test water and unfiltered plus tailings test water systems. Filtration of the lake water to remove suspended solids was accomplished by passing it through a sand filter (granite), a Filterite 5 um bleached white cotton prefilter, with final filtration through a Nuclepore 0.2 um "GO" membrane filter.

Taconite tailings were obtained from the Reserve Mining Company plant at Silver Bay, Minnesota and transported to the laboratory in 19 liter polyethylene jugs and stored at ~10 C. Prior to use, the contents of the jugs were thoroughly mixed and then fractionated by sedimentation for 24 hours to remove particles with a settling size larger than 2 um (Cook 1975). The resulting suspensions were combined and stored in 380 liter Nalgene holding tanks equipped with recirculating pumps to keep the particles thoroughly mixed.

Tailings from the holding tanks were transferred by hand in 19 liter polyethylene jugs as needed to maintain a tailings supply in a 200 liter recirculating fiberglass reservoir (10 C) from which a peristaltic pump metered the tailings into head boxes feeding the appropriate diluter systems.

Three taconite tailings test concentrations were maintained. These test concentrations were selected to range from less than the minimum and greater than the maximum concentrations previously measured in the Duluth municipal water supply.

For the cadmium study, 0.2 µm filtered water was delivered to one of the three diluters, while a second diluter received the unfiltered lake water for the first 62 days and thereafter water filtered through the 5 µm Filterite filters. The third diluter system received water of the same quality as the second diluter system plus approximately 1 mg/liter taconite tailings.

Total suspended solids and taconite tailings concentrations of samples from a control in each diluter system were measured by gravimetric analysis and x-ray diffraction at least once a week using methods described by Cook (1975). The mean ± standard deviations and ranges of the measurements are presented in Table 1.

Suspended Solids and Taconite Tailings: Endrin Study--The test water used for the endrin study was essentially the same as that used for the cadmium experiment with the exception that the unfiltered (ambient) water was not treated by passing it through a 5 um prefilter as was necessary in the cadmium exposure. Filtration of the lake water was accomplished as described for the cadmium experiment except a Nuclepore 0.2 um "QR" membrane filter was used in place of the "GO" filter for the final filtration. Test water conditions were modified for the endrin study so that 0.2 micron filtered water was delivered to one of the three diluter systems, with the second diluter system receiving filtered water plus approximately 1 mg/liter tailings. The third diluter system received unfiltered lake water.

Measurement for total suspended solids and tailings concentrations of five day composite samples from each diluter system were made as described for the cadmium test and are presented in Table 2. Daily turbidity measurements were

Table 1. Taconite Tailings and Total Suspended Solids Concentrations (mg/L) in Test Water in Cadmium Study

		Test Water						
		Filtered (0.2 µm)			Unfiltered		Unfiltered plus Tailings (1 mg/L)	
Characteristic	Period of Test	No. of Analysis	Mean ± SD[a]	Range	Mean ± SD[a]	Range	Mean ± SD[a]	Range
Tailings Concentration (TC)	1-62 day[b]	14	-	-	0.08±0.07	0.05-0.32	1.06±0.37	0.6-1.57
Total Suspended Solids (TSS)	1-62 day	14	-	-	0.78±0.28	0.51-1.37	1.76±0.42	1.0-2.44
TC	62-90 day[c]	5	-	-	0.07±0.04	0.08-0.14	0.97±0.37	0.72-1.62
TSS	62-90 day	5	-	-	2.27±0.95	1.41-3.47	3.01±1.06	1.53-4.08
TC	Total-90 day	19	0.004±0.001	0.002-0.007	0.08±0.06	0.04-0.32	0.95±0.39	0.53-1.65
TSS	Total-90 day	19	-	-	1.17±0.84	0.51-3.47	2.09±0.83	1.04-4.08

[a]SD = standard deviation.

[b]During the first 62 days the test water was maintained below 3 Jackson turbidity units.

[c]After the 62nd day turbidity was not controlled as stated above but instead the water was filtered to 5 µm before use.

Table 2. Taconite Tailings, Total Suspended Solids Concentrations (μg/L) and Turbidity in Test Water in Endrin Study

	Test Water					
	Filtered (0.2 um)		Unfiltered		Filtered plus tailings (1 mg/L)	
Characteristic	Mean ± SD[a,b]	Range	Mean ± SD[a,b]	Range	Mean ± SD[a,c]	Range
Tailings Concentration (mg/l)	0.02±0.01	0.01-0.04	0.05±0.02	0.03-0.10	0.93±0.19	0.62-1.26
Total Suspended Solids (TSS)	0.30±0.25	0.06-0.38	0.72±0.48	0.33-1.59	1.43±0.52	0.83-2.32
Turbidity[d]	0.21±0.07	0.11-0.35	0.61±0.26	0.36-1.50	2.47±0.36	1.80-3.40

[a]SD = standard deviation

[b]N = 10

[c]N = 8

[d]Nephelometric turbidity units

also made in 2 chambers at each taconite tailings concentration in order to monitor suspended solid concentrations; results are presented in Table 3.

Problems were encountered with the filtration system during the entire testing period resulting in tailings concentrations in the filtered water approximately 5 times the mean level for the filtered water for the cadmium test (see Table 1).

Incoming lake water was passed through an Aquafine Model MP-2-SI (1200GPH) ultraviolet Water Sterlizer to minimize bacterial contamination of the filters and test chambers.

Water Chemistry: Cadmium Test--Water chemistry measurements were made at approximate 2 week intervals in at least one control and one high toxicant concentration in each diluter system. The results did not vary appreciably between systems or between experiments. Dissolved oxygen concentrations for the cadmium test ranged from 7.2-9.2 mg/liter. The ranges for total hardness, total alkalinity and acididty were 44-51, 37-49, and 0.2-2.0 mg/liter as $CaCO_2$, respectively. The pH measurements ranged from 7.4 to 8.2.

Water Chemistry: Endrin Study-- Analysis for chemical characteristics of the test water were made weekly using methods recommended by the American Public Health Association et al. (1971). The dissolved oxygen concentrations ranged from 7.3-8.5 mg/liter. Ranges for total hardness, total alkalinity and acidity were 40-44, 38-42, and 1.65-4.2 mg/liter as $CaCO_3$, respectively. The pH measurements ranged from 7.12 to 7.94.

Cadmium Dosing and Measurement--The cadmium stock solution consisted of 106 mg $CdCl_2$ (reagent grade) and 0.1 ml concentrated HCl per liter of filtered (0.2 um) lake water. This stock was contained in a Mariotte bottle which provided a constant hydrostatic head delivering the toxicant to all three diluter systems where the dilution ratio was adjusted to provide a control and five cadmium concentrations with a 0.5 diluter factor. Each diluter was equipped with a toxicant metering device (McAllister et al., 1972), providing a means of independent adjustment of toxicant flow to the diluters.

Measurements for cadmium in test water samples were made weekly on an alternating basis between duplicate tanks (Table 3). Analysis for cadmium were conducted on a Perkin-Elmer Model 403 atomic absorption spectrophotometer using the flameless method for detection. The mean percent recovery and standard deviation (SD) for 45 spiked cadmium water samples was 93.39 ± 11.08. Analysis of cadmium reference standards with known concentrations of 1.8 and 16 μg/liter cadmium were run concurrently with the unknowns. Mean recovery and SD for the standard were 1.84 ± 0.08 with 17.8 ± 1.23 (n = 21) respectively.

Whole fish, that survived from test initiation to test end (90 days), in the controls and the nominal cadmium concentrations of 3.7 and 7.5 μg/liter were analyzed for cadmium using a wet digestion, atomic absorption spectrophotometer method described by Leonard (1971). Cadmium tissue concentration of duplicate composite samples of offsprings which were exposed through embryo development and for 30 days post hatch were also determined.

<u>Endrin Dosing and Measurement</u>--A saturation apparatus (Veith and Comstock 1975) was utilized to maintain a stock solution of endrin which was delivered to each diluter by a chemical metering device (Mount and Warner 1965). Water containing five concentrations of endrin with a 0.5 dilution factor and a control were delivered to duplicate test tanks. Endrin concentrations in test water samples were analyzed using gas chromatographic procedures for chlorinated pesticides (Thompson, 1974). Measurement of toxicant concentration in one of each of the duplicate tanks was made each week on an alternating basis. The mean ± standard deviation (SD) and ranges for endrin in test water are shown in Table 4. The mean ± SD recovery of endrin from spiked control water samples was 99.81% ± 3.02 (n = 19).

Table 3. Measured Cadmium Concentrations (μg/L) in Test Water at Three Taconite Tailings Concentrations

Nominal concentration (μg/L)	Mean Taconite Tailings (mg/liter): 0.004, Mean ± SD[a]	0.004, Range	0.03, Mean ± SD[a]	0.03, Range	0.95, Mean ± SD[a]	0.95, Range
	n = 24		n = 24		n = 24	
30.0	31.2±3.9	25.7-41.9	29.9±2.5	26.5-33.9	32.3±4.6	23.7-45.4
15.0	16.9±1.8	14.7-19.5	15.5±1.4	14.0-18.0	16.9±2.2	12.8-21.6
7.5	7.4±1.5	5.4-11.2	6.5±1.2	4.3-9.0	7.3±1.3	5.1-9.5
3.7	3.3±0.3	2.7-3.8	3.0±0.3	2.5-3.5	3.4±0.3	2.1-4.0
1.8	2.0±0.3	1.5-2.7	1.6±0.2	1.2-2.1	1.8±0.2	1.4-2.5
0.0 (control)	0.0	0.0-trace	0.0	0.0-trace	0.0	0.0-trace

[a]SD = standard deviation

Table 4. Measured Endrin Concentrations (μg/L) in Test Water at Three Taconite Tailings Concentrations

Nominal concentration (μg/L)	Number of Analysis	Mean Taconite Tailings (mg/liter)					
		0.02		0.05		0.93	
		Mean and ± SD[a]	Range	Mean and ± SD[a]	Range	Mean and ± SD[a]	Range
0.60	14	0.57±0.39	0.52-0.66	0.60±0.04	0.55-0.70	0.57±0.05	0.52-0.66
0.30	12-14	0.25±0.012	0.23-0.28	0.28±0.02	0.25-0.33	0.23±0.03	0.20-0.30
0.15	5-7	0.13±0.01	0.12-0.15	0.15±0.03	0.13-0.21	0.14±0.02	0.11-0.16
0.075	6-7	0.078±0.05	0.065-0.80	0.082±0.004	0.077-0.087	0.074±0.008	0.063-0.088
0.037	8-9	0.044±0.04	0.037-0.51	0.042±0.003	0.037-0.045	0.045±0.002	0.042-0.051
0.00 (control)	14	0.0		0.0		0.0	

[a]SD = standard deviation

Endrin tissue concentrations were determined by grinding the fish in a blender with sufficient anhydrous sodium sulfate to produce a dry powdery mixture. This mixture was extracted in a Soxhlet extractor for six hours with a 50-50 acetone-n-hexane solvent mixture. The extract was evaporated to near dryness then diluted to 20 ml with n-hexane. Eight ml (40%) of the extract was placed on a 20 g Florisil column and eluded with 15% Ethyl-ether in n-hexane. After discarding the first 50 ml of eluant the next 200 ml was collected in a volumetric flask. This sample was analyzed, by gas chromatography, "as is" or diluted to bring the endrin concentration into the linear range of the analytical standard (concentrating samples was not required). The mean $\pm$ standard deviation recovery of endrin was 97.6% $\pm$ 7.14 for endrin spiked tissue samples. A determination of the percent fat (lipids) was carried out on 2 ml (10%) of the extract. Fat was defined as the residue remaining after 15 min in a 130 C drying oven.

BIOLOGICAL METHODS

Cadmium Study--Flagfish *Jordanella floridae* brood stock was obtained from the University of Michigan and held in 0.2 um filtered water and used as a source of young test fish for the following experiment.

The experiment was initiated by placing 25 flagfish larvae up to 2 days old (group 1) into the spawning chambers. The next day 20 larvae (group 2) from the same lot were placed into the growth chambers (replicates of the spawning chambers) of each of the three exposure systems. In the system receiving the additional taconite tailings, group 1 and 2 control fish were combined after the second week of exposure and the rest of group 1 discarded. To obtain reproductive information, the number of fish was reduced on the 30th and 45th day of exposure so that each chamber contained no more than 15 or 2 males and 5 females, respectively. Additional larvae (20 per chamber) were placed in the vacated chambers and exposed for 14 days. For details of the test procedure see Spehar (1976).

The fish were fed live San Francisco brine shrimp, nauplii Atemia salina, for the first 30 days and frozen brine shrimp supplemented with a commercial trout food thereafter. The fish were not fed for 24 hours prior to sacrifice.

Measurement of the effects of cadmium on survival, growth, reproduction, and bioconcentration was used to determine a maximum acceptable toxicant concentration (MATC) (Mount and Stephan, 1967) at the three levels of taconite tailings.

Endrin Study--Fathead minnow Pimephales promelas embryos used were produced by a brood stock maintained at the University of Minnesota, St. Paul, and were hatched in the filtered Lake Superior water. Twenty-five larvae, 2-3 days post-hatch (Group A), were placed into each chamber, and exposed to the various endrin-tailings mixtures for 45 days. The next day 20 larvae (B Group--up to 4 days old) were placed into replicate chambers within the same exposure system and exposed for 45 days. Feeding and care of the young fathead minnows was the same as reported for the flagfish.

TACONITE TAILINGS TEST

An additional 6 day exposure starting with flagfish larvae up to 2 days old was conducted. Twenty larvae were placed in each chamber. Conditions were similar to the previous flagfish tests, with the exception that no cadmium was added and higher concentrations of taconite tailings were tested. The three diluter systems previously described were modified to provide taconite tailing concentrations ranging from about 0.004 mg/liter to 4 mg/liter.

DATA ANALYSIS

Survival, growth, and reproduction data were statistically analyzed using a one-way analysis of variance classification. Dunnett's test for comparing all means with a control was used with 5 or more treatments. Duncan's multiple range test was used when treatments compared were four or less (Steel and Torrie, 1960). A significance level of P=0.05 was established for all statistical tests. Nominal toxicant and mean taconite tailings concentrations will be used hereafter for discussion purposes.

RESULTS AND DISCUSSION

Exposure of flagfish to 30.0 μg/liter cadmium at the three taconite tailings concentrations caused high mortality within the first 30 days of exposure and all were dead by test end (Table 5). At the 15 μg/liter cadmium concentrations, effects on survival were evident after 30 days of exposure and only a few survived to test end. Exposure at this cadmium concentration caused fish behavioral aberrations which consisted of apparent muscle spasms followed by a period of unconsiousness, then recovery. This aberrant behavior appeared to be initiated by disturbances such as chamber cleaning and was first observed 17 days after testing began. Only one spawn occurred at this cadmium concentration (Table 6) and was in the 0.004 mg/liter taconite tailings concentration. At 7.5 μg/liter cadmium, survival was reduced during the spawning period (day 45-90) at the high taconite tailings concentration (0.95 mg/liter). Mean egg production per female (Table 6) was reduced by exposure to 7.5 μg/liter cadmium at the two lower taconite tailing concentrations (0.004 and 0.08 mg/liter) and the percentage hatch was lessened by exposure to 7.5 μg/liter cadmium at the high taconite tailings concentration (0.95 mg/liter). Survival and growth of offspring in the control at the 0.95 mg/liter taconite tailings concentration was less than at the 1.8-7.5 μg/liter cadmium concentrations (Table 7).

The growth measurements for parental fish were highly variable between many duplicates and treatment levels (Table 8) and only one difference from

Table 5. Survival of Fish (Group 2) Exposed to Several Concentrations of Cadmium at Three Concentrations of Taconite Tailings

Nominal cadmium concentration (μg/L)	Duplicate chambers	Percentage survival to 30 days[a]			Percentage survival from 30th to 45th day[b]			Percentage survival from 45th to 90th day[b]		
		Mean taconite tailings (mg/L)								
		0.004	0.08	0.95	0.004	0.08	0.95	0.004	0.08	0.95
30	A	5^c	5^c	0	0(1)	0(1)	-	-	-	-
	B	0^c	0^c	5	-	-	0(1)	-	-	-
15	A	50^c	55	30	40.0(10)^c	27.3^c(11)	33.3^c(6)	50.0^c(4)	0^c(3)	0^c(2)
	B	60^c	45	30	33.3(12)^c	44.4^c(9)	50.0^c(6)	50.0^c(4)	25.0^c(4)	0^c(4)
7.5	A	90	95	100^c	100.0(15)	100.0(15)	93.3(15)	100.0(7)	100.0(7)	57.1^c(7)
	B	95	90	85^c	93.3(15)	100.0(15)	86.7(15)	100.0(7)	71.4(7)	71.4^c(7)
3.7	A	100	95	85	100.0(15)	100.0(15)	100.0(15)	100.0(7)	83.3(6)	100.0(7)
	B	95	70	75	100.0(15)	93.3(15)	100.0(15)	85.7(7)	88.7(7)	100.0(7)
1.8	A	95	65	85	100.0(15)	100.0(15)	100.0(15)	85.7(7)	100.0(7)	100.0(7)
	B	85	90	50	100.0(15)	100.0(15)	100.0(15)	85.7(7)	100.0(7)	100.0(6)
0.0 (control)	A	95	90	15^b	100.0(15)	100.0(15)	100.0(15)	85.7(7)	100.0(7)	100.0(7)
	B	100	75	45^b	100.0(15)	100.0(15)	100.0(15)	100.0(7)	85.7(7)	100.0(6)

[a] Initially 20 fish were placed in each chamber.

[b] N is in parentheses.

[c] Mean of duplicates significantly different from control mean (Dunnetts' test, P = 0.05).

[d] Data deduced. No mortality occurred after combining group 1 and 2 fish on the 14th day of testing.

Table 6. Surviving Spawning Adult Flagfish, Egg Production and Percentage Hatch (Cadmium study)

Nominal cadmium concentration (µg/l)	Duplicate chambers	Number of fish surviving at test end (male/female)			Total egg production			Adjusted mean number of eggs per surviving female[a]			Percentage hatch[b,c]		
		Mean taconite tailings (mg/L)											
		0.004	0.08	0.95	0.004	0.08	0.95	0.004	0.08	0.95	0.004	0.08	0.95
15	A	0/2[d]	0/0	0/0	95	0	0	45[e]	-	-	39.2(1)[f]	-	-
	B	1/1	0/1	0/0	0	0	0	0[d]	-	-	-	-	-
7.5	A	2/5	2/5	1/3	2,040	1,620[f]	4,598	392[e]	348[e]	995	59.1(9)	67.8(5)	40.5(11)[e]
	B	2/5	2/3	1/4	4,404	4,314	3,469	880[e]	935[e]	772	67.3(13)	69.8(11)	33.6(8)[e]
3.7	A	2/5	2/3	2/5	4,065	4,810	8,425	813	1,008	1,682	49.7(14)	59.2(10)	40.3(13)
	B	2/4	2/4	2/5	4,705	5,941	9,984	968	1,277	1,984	66.9(11)	70.8(18)	55.4(16)
1.8	A	1/5	2/5	2/5	3,863	11,216	9,543	960	2,243	1,901	60.2(12)	74.2(19)	53.7(15)
	B	2/4	2/5	2/4	4,326	9,699	6,799	865	1,940	1,691	58.5(13)	77.2(13)	58.7(14)
Control	A	1/5	2/5	2/5	5,106	8,617	2,651	1,101	1,670	530	65.3(14)	75.3(19)	61.5(8)
	B	2/5	2/4	2/4	5,357	6,166	6,843	1,071	1,311	1,704	51.4(12)	56.9(18)	60.6(18)

[a] The adjusted mean was calculated from adjusted production which was obtained by subtracting the mean number of embryos per female from total production on the date a female died.

[b] N is in parentheses.

[c] All embryos were treated with 5 mg/liter malachite green (zinc free) for 3 minute periods, once a day, during the first three days of incubation.

[d] One male survived to spawn but died shortly after.

[e] Mean of duplicates significantly different than control mean (Duncan's test, P = 0.05).

[f] Because of low sample number, this value was not compared to others, also it is within the range of control samples.

Table 7. Growth and Survival Data of Flagfish Offspring After 30 Days of Exposure (Cadmium Study)

		Percent survival[a]			Mean total length and weight (mm/gm)		
		Mean Taconite Tailings (mg/liter)					
Nominal cadmium concentration (ug/l)	Duplicate chambers	0.004	0.08	0.95	0.004	0.08	0.95
15	A	82[b]	-	-	20.2/17.5[b]	-	-
	B	-	-	-	-		-
7.5	A	85	55	95[c]	24.6/36.4[d]	19.9/14.9	23.7[c]/23.6
	B	95	50	95[c]	22.9/24.6	22.0/21.3	24.6[c]/26.3
3.7	A	90	75	85[c]	22.3/22.8	20.1/13.6	23.3[c]/24.2
	B	95	95	80[c]	22.5/22.6	22.1/18.1	23.1[c]/27.3
1.8	A	100	85	90[c]	24.5/26.7	22.8/24.5	23.0[c]/22.8
	B	85	70	100[c]	22.4/24.0	20.4/16.0	23.3[c]/26.3
Control	A	90	80	55	20.1/14.8	20.8/16.9	21.3/17.8
	B	75	95	55	22.2/20.1	18.9/11.9	21.7/21.1

[a] Twenty larvae were placed in each chamber shortly afeter hatch, except where noted below.

[b] Eleven larvae were placed in this chamber.

[c] Mean of duplicates is significanlty different than control mean (Ducan's test, P = 0.05).

[d] Data based on 10 fish. Ten others were removed by mistake before 30 days of exposure.

Table 8. Mean Total Length (mm) and Live Weight (gm) of Spawning Adult Flagfish at Test End (Cadmium Study)

Nominal cadmium concentration (µg/l)	Duplicate chambers	Mean total length and weight of females (mm/gm)			Mean total length and weight of males (mm/gm)		
		Mean Taconite Tailings (mg/liter)					
		0.004	0.08	0.95	0.004	0.08	0.95
15	A	43.5/1.8[a]	-	-	-	-	-
	B	29.0/0.8[a]	-	-	45.0/1.7[a]	-	-
7.5	A	43.0/1.7	39.2/1.4	37.0/1.0	58.0/3.6	47.0/1.9[b]	54.0/3.0
	B	42.4/1.5	38.0/1.2	39.7/1.3	58.5/4.3	51.5/2.7[b]	59.0/4.3
3.7	A	41.4/1.6	42.4/1.1	42.4/1.6	56.3/3.5	57.0/3.8	54.0/3.4
	B	43.7/1.7	39.0/1.3	42.2/1.5	55.0/3.8	60.5/4.5	52.5/2.8
1.8	A	43.0/1.7	44.8/1.9	41.2/1.5	57.0/3.8	57.5/4.1	55.5/3.5
	B	42.7/1.7	41.2/1.4	44.7/1.9	60.5/4.6	56.0/2.4	55.5/3.4
Control	A	41.8/1.6	42.0/1.6	39.2/1.2	58.0/4.4	56.5/3.4	56.0/3.7
	B	44.0/1.8	40.0/1.4	41.0/1.6	53.6/3.0	59.0/3.7	59.0/4.0

[a]These values were not included in the statistical analsyes.

[b]Mean of duplicates significantly different from control (Duncan's test, P = 0.05).

the control could be demonstrated. This was reduced weight of males exposed to 7.5 ug/liter cadmium at 0.08 mg/liter taconite tailings.

The uptake of cadmium by the parental fish and offspring at 3.7 and 7.5 ug/liter was not demonstrably affected by taconite tailings concentration (Table 9).

The maximum acceptable toxicant concentrations (MATC) for cadmium and flagfish, based on the above survival, growth, reproductive, and bioconcentration data for Lake Superior water with 0.004, 0.08, and 0.95 mg/liter concentrations of taconite tailings, are between 3.3-7.4, 3.0-6.5, and 3.4-7.3 ug Cd/liter, respectively. These MATC values are similar to those determined by Spehar (1976). He found the MATC for flagfish to lie between 4.1 and 8.1 ug Cd/liter under ambient conditions (unmeasured) of suspended solids in Lake Superior water. Mean concentrations of cadmium measured in the tissues of the parental fish exposed for 90 days at cadmium concentrations near 3.7 and 7.5 ug/liter in the present study ranged from 5.6 to 8.2 and 9.2 to 12.1 ug/g of fish tissue, respectively. Spehar (1976) found similar cadmium tissue concentrations (means near 6 and 10 ug/g) after 100 days of exposure at 4.1 and 8.1 mg/liter cadmium. Offspring tissue concentrations were also similar between studies.

The percentage survival of flagfish after two weeks of testing was markedly less in control chambers receiving the additional taconite tailings (Table 10) but was not affected thereafter (Table 6). In two of these chambers, where accurate cumulative counts of dead larvae were made, most deaths occurred within the first three days of testing (2-5 days post-hatch). Several moribund fish were removed from these tanks and were examined under a microscope and found to be infected with fungus. Generally fish exposed to cadmium at concentrations up to 15 ug/liter survived in greater numbers than in the disease affected controls. This indicates that the addition of taconite

Table 9. Mean and Standard Deviation (N in Parentheses) of Cadmium in Whole Body Tissue Samples (ug/g Wet Weight) for Flagfish Exposed to Two Concentrations of Cadmium at Three Taconite Tailings Concentrations. Parents were Exposed for 90 Days, and Offspring for 30 Days After Hatch.

Nominal cadmium concentration in water (ug/l)	Mean Taconite Tailings (mg/liter) 0.004 — Cadmium tissue concentration (ug/g)	0.08 — Cadmium tissue concentration (ug/g)	0.95 — Cadmium tissue concentration (ug/g)
		Parents	
7.5	10.1 ± 1.4 (3)	9.4 ± 0.9 (8)	12.1 ± 2.7 (5)
3.7	5.9 ± 1.6 (8)	5.6 ± 1.7 (8)	8.2 ± 2.9 (8)
Control	0.2 ± 0.05 (4)	0.3 ± 0.09 (4)	0.8 ± 0.8 (4)
		Offspring	
7.5	12.7 ± 1.8 (4)	18.0 ± 3.6 (4)	16.3 ± 6.1 (4)
3.7	10.0 ± 1.0 (4)	13.7 ± 0.5 (4)	11.8 ± 3.4 (4)
Control	0.1 ± 0.03 (4)	0.2 ± 0.04 (4)	0.1 ± 0.03 (4)

Table 10. Survival of Fish to 13 or 14 Days of Exposure to Several Cadmium Concentrations at 3 Concentrations of Taconite Tailings

Nominal cadmium concentration (µg/L)	Duplicate chamber	Group 1 fish Percentage survival Taconite tailings (mg/L)			Group 2 fish Percentage survival Taconite tailings (mg/L)			Group 3 fish Percentage survival Taconite tailings (mg/L)		
		0.004	0.08	0.95	0.004	0.08	0.95	0.004	0.08	0.95
30	A	4[a]	16	0	5[a]	20[a]	0	35	30[a]	35
	B	12[c]	24	8	0[a]	0[a]	5	20	25[a]	20
15	A	99	88	84	90	90	60	100	95	90
	B	52	88	84	80	80	45	90	100	100
7.5	A	96	72	84	90	95	100	75	85	95
	B	100	88	84	95	90	85	100	100	100
3.7	A	76	100	76	100	95	85	100	100	95
	B	92	88	16	95	70	75	85	90	95
1.8	A	92	88	96	95	65	85	75	85	90
	B	84	60	88	85	90	50	90	55	85
0.0	A	88	68	48[b]	95	90	15[b]	80	80	15[d]
(control)	B	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a]Mean of duplicates is significantly different than the control mean (Dunnetts' test, $P = 0.05$)

[b]The fish from the controls of group 1 and 2 were combined with respective duplicates and used to produce growth and reproductive data.

[c]Twelve dead larvae were removed on the third day of testing.

[d]Thirteen dead larvae were removed on the third day of testing.

tailings to the water increased the incidence of infection and cadmium exposure reduced the incidence of infection. A causal disease organism was not identified but may have been fungus which can be a primary or secondary source of infection. The disease is probably not due to the direct effect of taconite tailings because later testing spanning the 2-5 day post-hatch life stage at approximately 4 times the taconite tailings concentrations (Table 11) did not elicit similar disease symptoms or affect survival.

ENDRIN STUDY

No fish survived in groups A or B fish at 0.60 μg/liter endrin (Tables 12 and 13). Survival was not affected by endrin at 0.30 μg/liter or lower concentrations.

Fish growth in group A fish (Table 12) was reduced at 0.30 μg/liter endrin only in water containing the highest concentrations of taconite tailings (0.93 mg/liter). Contrasting results were evident in group B fish (Table 13). Growth was reduced at 0.30 μg/liter endrin concentrations in water containing 0.02 and 0.05 mg/liter taconite tailings, respectively, but was not reduced in water containing 0.93 mg/liter taconite tailings. Growth also was reduced at the 0.15 μg/liter endrin concentration in water containing 0.02 mg/liter taconite tailings.

The uptake of endrin in the fathead minnow tissues, whole body (Table 14) and fat (Figure 2), was directly proportional to the endrin concentration in the test water and was not demonstrably affected by taconite tailings concentration.

The no observable effect toxicant concentration (NOEC) for endrin-exposed minnows at the 0.02 mg/liter taconite tailings concentration was between 0.30 and 0.60 μg/liter for group A fish and between 0.075 and 0.15 μg/liter for

Table 11. Survival of Flagfish Larvae After Six Days of Exposure to Several Concentrations of Taconite Tailings

Nominal taconite tailing concentration (mg/L)	Mean measured conc. (mg/L)		Turbidity (N.T.U. Units)		Percentage survival[a]	
	Taconite tailings	Suspended solids	Mean	Range	Mean	Range
	n = 2[b]		n = 14			
4.0	3.78	3.82	5.8	(5.1-6.3)	92.5	90-95
3.0	-	-	4.4	(4.0-4.8)	86.2	80-100
2.0	-	-	3.2	(2.8-3.8)	95.0	90-100
1.0	-	-	1.9	(1.6-2.2)	95.0	90-100
0.5	-	-	1.1	(0.9-1.4)	93.7	80-100
0.25	-	-	0.7	(0.6-0.8)	90	85-90
0.02	0.012	0.44	0.4	(0.3-0.5)	83.7	65-95
0.004	0.010	0.18	0.2	(0.1-0.2)	90.0	65-100

[a] Twenty larvae were placed in each of 4 replicate chambers at each concentration.

[b] Number of analysis.

Table 12. Survival and Growth Data for Fathead Minnows Exposed to Several Concentrations of Endrin at Three Taconite Tailings Concentrations for 45 Days (Group A).

Nominal endrin concentration (μg/l)	Duplicate chambers	Percentage survival[a]			Mean total length ± SD[b] (mm)			Mean weight ± SD[b] (gm)		
		Mean Taconite Tailings (mg/liter)								
		0.02	0.05	0.93	0.02	0.05	0.93	0.02	0.05	0.93
0.60	A	0[c]	0[c]	0[c]	-	-	-	-	-	-
	B	0[c]	0[c]	0[c]	-	-	-	-	-	-
0.30	A	92	100	92	35.5±2.3	36.4±4.2	36.7±2.7[c]	.43±.09	.51±.22	.49±.09[c]
	B	88	76	96	36.4±3.8	37.0±3.9	37.1±3.7[c]	.47±.14	.52±.20	.51±.15[c]
0.15	A	100	92	88	37.1±3.4	38.2±4.1	37.9±4.3	.51±.14	.60±.19	.58±.19
	B	100	92	60	35.3±4.1	36.6±3.2	38.2±4.7	.44±.15	.50±.12	.58±.23
0.075	A	96	84	68	36.4±3.1	39.4±2.8	38.9±3.6	.47±.14	.63±.12	.61±.17
	B	100	88	84	36.5±2.8	38.3±3.7	37.1±4.6	.47±.12	.58±.19	.52±.20
0.038	A	100	84	84	36.6±3.2	38.8±2.7	38.5±3.0	.48±.12	.60±.12	.58±.15
	B	96	92	88	36.9±3.0	38.1±3.4	38.4±3.7	.50±.14	.56±.16	.57±.17
Control	A	96	100	72	37.5±3.7	37.3±3.7	39.2±4.1	.50±.14	.51±.16	.64±.23
	B	100	88	28	36.9±3.1	39.0±1.8	39.1±3.8	.47±.12	.58±.17	.60±.09

[a]Twenty-five larvae placed in each test chamber.

[b]SD = standard deviation.

[c]Mean of duplicates is significantly different from control mean (Dunnetts' test, P = 0.05).

Table 13. Survival and Growth Data for Fathead Minnows Exposed to Several Concentrations of Endrin at Three Taconite Tailings Concentrations for 45 Days (Group B).

		Percentage survival[a]			Mean total length ± SD[b] (mm)			Mean weight ± SD[b] (gm)		
		Mean Taconite Tailings (mg/liter)								
Nominal endrin concentration (ug/l)	Duplicate chambers	0.02	0.05	0.93	0.02	0.05	0.93	0.02	0.05	0.93
0.60	A	0[c]	0[c]	0[c]	-	-	-	-	-	-
	B	0[c]	0[c]	0[c]	-	-	-	-	-	-
0.30	A	75	65	90	36.3±3.8[c]	34.8±5.1[c]	35.3±2.7	.44±.13[c]	.39±.18[c]	.43±.12
	B	90	75	100	36.5±3.2[c]	35.5±3.3[c]	34.9±3.5	.46±.13[c]	.43±.12[c]	.43±.13
0.15	A	50	90	90	36.1±4.6[c]	37.6±4.2	37.3±3.7	.45±.17[c]	.53±.19	.54±.15
	B	100	95	80	36.3±4.2[c]	36.2±3.5	38.7±2.8	.48±.20[c]	.47±.13	.57±.11
0.075	A	100	95	100	37.6±3.2	38.4±3.9	37.6±3.2	.51±.14	.60±.19	.54±.13
	B	85	95	70	36.7±2.9	37.4±3.3	38.4±2.7	.48±.12	.51±.15	.57±.12
0.038	A	95	90	75	35.6±3.4	39.6±3.2	37.5±2.9	.50±.17	.62±.17	.54±.14
	B	95	95	80	37.2±3.2	39.1±3.8	35.6±3.3	.51±.10	.60±.19	.45±.13
Control	A	100	80	85	37.7±5.7	38.2±4.0	36.6±2.6	.52±.20	.58±.18	.48±.09
	B	100	100	60	38.0±2.7	39.8±2.6	38.0±3.1	.52±.10	.65±.13	.61±.15

[a]Twenty larvae placed in each test chamber.

[b]SD = standard deviation.

[c]Mean of duplicates is significantly different from control mean (Dunnetts' test, P = 0.05).

Table 14. Concentrations of Endrin in Duplicate (A and B) Composite Whole Body Tissue Samples (µg/g Wet Weight) and Percent Fat (in Parentheses) for Fathead Minnows Exposed to Several Concentrations of Endrin at Three Taconite Tailings Concentrations

Nominal endrin concentration in water (µg/l)	Mean Taconite Tailings (mg/liter)								
	0.02			0.05			0.93		
	Endrin tissue concentration (µg/g)[a]		Mean	Endrin tissue concentration (µg/g)[a]		Mean	Endrin tissue concentration (µg/g)[a]		Mean
	A	B		A	B		A	B	
0.30	4,120(8.9)	3,760(8.7)	3,940	3,290(6.5)	4,130(8.8)	3,710	3,400(9.4)	3,590(8.2)	3,495
0.15	1,950(7.7)	2,240(10.2)	2,095	2,400 (9.6)	2,240(10.1)	2,320	1,950(10.6)	2,110(10.0)	2,030
0.075	1,050(11.1)	1,100(9.1)	1,075	1,240(11.1)	1,160(10.1)	1,200	998(10.8)	1,110(10.6)	1,054
0.037	552(8.0)	578(10.0)	550	573(11.2)	522(11.0)	548	570(10.7)	461(8.2)	515
0.00 (control)	70(9.1)	55(10.8)	61	36(10.7)	45(11.3)	40	78(9.4)	85(10.3)	82

[a]Percent fat in parentheses.

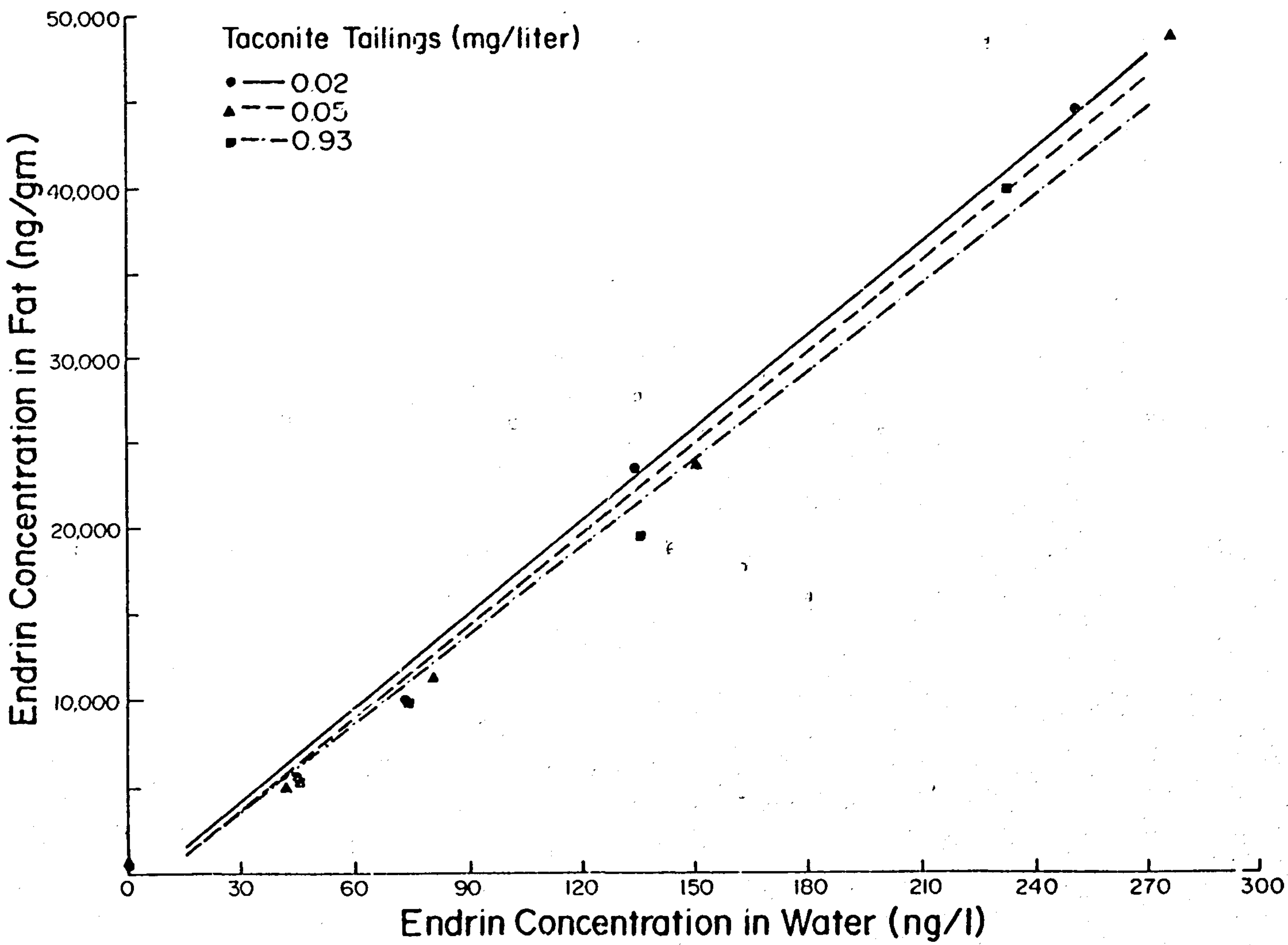

Figure 1. Linear relationship of endrin in fat of fathead minnows exposed to several concentrations of endrin at three taconite tailings concentrations.

group *B* fish. At 0.05 mg/liter taconite tailings concentration, the NOEC was between 0.30 and 0.60 μg/liter for group *A* fish and between 0.15 and 0.30 μg/liter for group *B* fish. At the 0.95 mg/liter taconite tailings concentration the NOEC for endrin was between 0.15 and 0.30 μg/liter for group *A* fish and between 0.30 and 0.60 μg/liter for group *B* fish. Although these values are quite variable, they are similar to the following results obtained in previous endrin toxicity tests at this laboratory. The NOEC for fathead minnows exposed to endrin for 300 days was between 0.14 and 0.25 μg/liter (Jarvinen and Tyo, 1978). A MATC determined for endrin exposed flagfish was between 0.22 and 0.30 μg/liter (Hermanutz, 1978).

CONCLUSION

The differences in toxicant responses measured for fish in these experiments could be caused by inherent biological variability of the fish and/or experimental error. However, these experiments do reflect the presence of effect threshold concentrations unaffected by the range of taconite tailings concentrations maintained in these studies. It is concluded, under the conditions of these experiments, that mean taconite tailings concentrations ranging from 0.004 to 0.95 mg/liter had no demonstrable effect on the chronic toxicity of cadmium to the flagfish. Similarly, mean taconite tailings concentrations ranging from 0.02 to 0.93 mg/liter had no demonstrable effect on endrin toxicity to the fathead minnow. There were also no effects of taconite tailings on the bioconcentration of cadmium or endrin by the fish studied. The information gained from these toxicity tests in regard to the direct effects of taconite tailings are inconclusive. Therefore, it is our conclusion that taconite tailings at the concentrations tested did not demonstrably alter the effect threshold concentrations for cadmium and endrin determined by previous experience in this laboratory.

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